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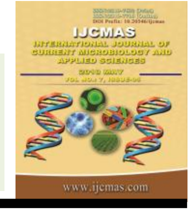

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Extraction of Total Polyphenols and Dietary Fiber from Mango Peel - As Potential Sources of Natural Phytonutrients

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A B S T R A C T

Mango is "King" of fruits. Its production and consumption has gradually increased. As mango peel is not currently being utilized, discarded as waste and becoming source of pollution. The edible pulp makes up to 33 to 85 per cent of the fresh fruit, while the peel and the kernel amounts to 7–24 and 9–40 per cent respectively. It contains total polyphenols (TPP) and total dietary fiber (TDF) which needs to be exploited as natural phyto-nutrients. Hence the present study sought to determine the processing and extraction of total polyphenols and dietary fiber from mango peel. The results revealed that mango peel powder is good source of total dietary fiber, insoluble dietary fiber and soluble dietary fiber was 69.86, 44.23 and 24.63g/100g of MPP; β-carotene 5600 µg/g; total polyphenols 4.5 mg GAE/100g and antioxidant activity 76.96 per cent. These extracts can be incorporated at different levels in formulation of bakery and extruded products which enhanced nutrition and shelf life of food products. Hence the TPP and DF can be commercially exploited as natural anti-oxidants which is useful in nutraceutical formulation in the management of life style disorders

Introduction

Mango (*Mangifera indica* L., Anacardiaceae) is truly a "King" of fruits has been cultivated for about 4,000 years and its production and consumption has gradually increased as its popularity has grown. At least 87countries grow over 26,286,255 MT per annum.

Mangoes are consumed as fresh fruits and after processing into pickles, chutneys, canned or dried products, juices or nectars. During processing of mango, by-products such as peel and kernel constitutes about 17-22 per cent of the fruit (Pitchaon, 2011).

As a result of rapid advancement made in the field of agriculture and food processing industry, significant quantities of agricultural products are subjected to processing to make them suitable for consumption, increased storage stability and improved nutrition and sensory quality.

This has resulted in the generation of huge amount of waste products causing environmental pollution. Pollution has not only scientific aspects but also sociological, economic, affecting adversely all human beings and the environment. Therefore, currently attention has been given for utilization of by-products of different food processing industry. Since many agricultural wastes are good source of nutrients and phytonutrients and isolation of value added products from agro-waste are widely used (Ajila, 2007).

Mangoes peel is not only rich in nutrients, but also high in non-nutrient phytochemical compounds. Recently attention is being given to phytonutrients and the distinctive roles they play in anti-inflammatory and anti-cancer properties. Mango peels are thus deemed to be a promising raw material for recovery of phytonutrients like polyphenols, dietary fiber, pectin and other products. With respect to value-added by-product utilization in mango processing, strategies were developed and evaluated to reduce waste volumes and to make high-value compounds, especially those located in the peels, available for the use as food and feed ingredients. At the food processing level, various types of prototype processing lines for food-grade by-product stabilization were assessed for the application to fresh peels of full-ripe mango fruits (Nagel *et al.*, 2012).

The characteristic feature of mango peel is that it has relatively high content of polyphenols and dietary fiber, which is reported to have more health benefits compared to apple peel, orange peel, wheat bran and oat bran. It has been found that dietary fiber and associated non-fibrous compounds reduce the rate of starch digestion in vitro and in vivo, resulting in low metabolic responses. Mango dietary fiber may exert this effect by means of potentially high viscosity of its soluble dietary fiber fraction or through polyphenol-mediated amylase inhibition. In the digestive tract, dietary fiber exerts a buffering effect that reduces excess of acid in the stomach, increases the fecal bulk and stimulates the intestinal evacuation; besides, providing a favourable environment for the growth of the beneficial intestinal flora. Fiber has also been found to bind diverse substances including cholesterol. It has been reported that these specific properties of dietary fiber play an important role in the prevention and treatment of obesity, atherosclerosis, coronary heart diseases, colorectal cancer and diabetes. Furthermore, within fruit fibers, the water and oil holding capacities and colonic fermentability, as well as lower phytic acid and caloric value contents besides their associated bioactive components namely, carotenoids, flavanols, and polyphenols, which exert higher health promoting effects than the dietary fiber alone. As peel is not currently being utilized for any commercial purpose, it is discarded and becoming a source of pollution. Earlier methods have been developed for the isolation of pectin from peel. Recently Ajila *et al.,* (2007) reported that mango peel contains polyphenols and dietary fibers which need to exploited as natural phytonutrients. Hence the objective of the present study was under taken to process the mango peel, nutrient analysis, extraction of total polyphenols and dietary fiber from mango peel powder.

Materials and Methods

Procurement of mango sample

The Mango peel for research was procured from a local mango processing Safal, industry, Bengaluru, Karnataka, India. The analysis of mango sample for microbial and pesticide residue and heavy metals, microbial study of mango peel sample-serial dilution plate count technique (Tate, 1995) was carried out.

Microbial study of mango peel sample-serial dilution plate count technique (Tate, 1995)

Ten grams of mango peel sample was mixed in 90 ml sterile water blank to give 10^{-1}

dilution. Subsequent dilutions up to 10^{-4} were made by transferring serially 1 ml of the dilution to 9 ml of sterile water blanks. The populations of bacteria, molds and yeasts were estimated by transferring 1 ml of 10^{-2}, 10^{-3} and 10^{-4} dilutions respectively to a sterile petridish and approximately 20 ml of media viz., Nutrient Agar, Martins Rose Bengal Agar and Davis Yeast Extract Agar for bacteria, molds and yeasts respectively was poured into plates. The plates were rotated twice in clockwise and anticlockwise direction for uniform distribution of the inoculums. After solidification of the media, plates were kept for incubation in an inverted position at 30 ± 1^0 C for 2-4 days and emerged colonies were counted.

Analysis of mango peels sample for heavy metals

Analysis for pesticide residue and heavy metals in mango peel was out sourced at Bengaluru test house

Processing of the mango peel

The samples was cleaned, blanched and dried in hot air oven at 50^0 C. The material was ground to fine powder passed through a 60 mesh sieve and stored at in air tight container in refrigerator conditions for further use.

Sensory evaluation of mango peel powder

Criteria for selection of the panel members was their familiarity with the mango peel powder selected for value addition.

Organoleptic evaluation of mango peel powder: Mango peel powder were processed in laboratory and organoleptic evaluation was carried out. A nine-point Hedonic Scale was adopted for the evaluation by semi trained panelists. Mango peel powder submitted to sensory evaluation by fifteen semi-trained panel member of Department of Food Science and Nutrition. The panellists were asked to rate each sensory attribute using the mango peel powder as the basic for evaluation mango peel powder were evaluated for appearance, texture, colour, aroma, taste and overall acceptability on a 9-point hedonic scale.

Nutrient composition of Mango Peel Powder (MPP)

The mango peel samples were finely powdered and were subjected for chemical analysis. The nutrients analyzed were moisture, protein, fat, crude fiber, total dietary fiber (soluble and insoluble), and total polyphenols and β-carotene, ash, calcium, phosphorous, iron and zinc in mango peel powder by AOAC methods. Carbohydrate and energy were computed.

Estimation of insoluble dietary fiber, soluble dietary fiber and total dietary fiber (AOAC, 1995)

Defatted foods are gelatinized and proteins and starch are removed by enzymatic digestion. The residue is quantitated gravimetrically. The soluble fiber is estimated in the filtrate obtained after enzymatic digestion of protein and carbohydrates of defatted sample. The insoluble fiber is precipitated and estimated gravimetrically. The total dietary fiber is the sum of the insoluble and soluble dietary fiber, estimated as follows;

Total Dietary Fiber = IDF+SDF values

Extraction of total polyphenols (TPP)

Extraction of bulk quantities of total polyphenols from stabilized mango peel powder. 100 gm of mango peel powder was extracted with 1000ml of 80% acetone for 4-5 hr by stirring with magnetic stirrer. The slurry was then strained through muslin cloth to separate the extract. The total polyphenols extracts were then subjected to vacuum evaporation (Rotary evaporator) at 45^0C to remove acetone completely and concentrated to get total polyphenols extract in liquid form. Stored in air tight container and kept in cool place. The extraction was repeated to obtain crude total polyphenols extract and was used for product development. Sadasivam and Manickam, (1991)

Estimation of total polyphenols

Weigh exactly 0.5 to 1.0 gm of the sample and grind with a pestle and mortar in 10 times volume of 80 percent ethanol. Centrifuge at 10000 RPM for 10 min collect supernatant. Re-extract the residue with 5 times volume of 80 percent ethanol centrifuge and poor the supernatants.

Evaporate the supernatants to dryness over water bath. Dissolve the residue in a known volume of distilled water (3ml). Pipette out 0.5 and 1.0 ml into test tube. Add distilled water to make up the volume to 3 ml. Add 0.5 ml FCR to each test tube. After 30 min, add 2 ml of 20 percent NaCO3 solution to each test tube. Mix thoroughly, place the tube in boiling water bath for exactly 1 min, cool and measure OD at 650 nm (Sadasivam and Manickam, 1991).

Estimation of β- carotene

The β- carotene content contents of the mango peel powder was estimated. The carotenes present in the sample were first extracted using acetone and then the carotene was brought to the petroleum ether phase. The concentration of β- carotene in the solution was determined by measuring the optical density of the solution using a spectrophotometer/colorimeter at 452nm (Ranganna, 1995).

Estimation of antioxidant activity by DPPH method

The 2, 2- diphenyl (DPPH) radical was the oxidizing radical to be reduced by the antioxidant (AH) present in the given sample. The disappearance of the DPPH radical absorption at 517nm by the action antioxidants is measured spectrophotometrically in a methanol solution until the absorbance remains constant.

The antioxidant activity was expressed in terms of ascorbic acid equivalents; so ascorbic acid is taken as standard. Various concentrations of ascorbic acid were prepared and added to DPPH solution. The decrease in O.D is plotted against concentration of ascorbic acid. The concentration of sample was calculated using the standard curve Sadasivam and Manickam (1991).

Functional properties of mango peel powder and extracted dietary fiber

Water absorption capacity and oil absorption capacity, swelling power and solubility index were studied for mango peel powder samples. All the analysis was carried out in triplicates.

Water and oil absorption capacity (Rosario and Flores, 1981)

One-gram sample was mixed with 10ml of distilled water and also in 15 ml oil for 30minutes.

The contents were allowed to stand at 30°C in a water bath for 30 minutes and then centrifuged at 3000 to 5000rpm for 20 to 30 minutes.

After centrifuging the volume of the supernatant was recorded and used for determination of water and oil absorption and the results were expressed as g/g sample.

Swelling power and solubility index (Adebooye and Singh, 2008)

About 500 mg (dry basis) (db) of sample was cooked in about 20 ml of water at about 100°C for 30 min. They were weighed and made equivalent to 25 g by the addition of water and were centrifuged at 3000 rpm for 15 min. Supernatant was decanted carefully and kept, and residue was weighed for swelling power determination.

10 ml of the supernatant was pipetted out to a wide-mouth petri dish (of known weight) and kept on a boiling water bath for evaporation. Afterwards, the dish was dried at 105 °C for 3 h, cooled, and weighed. Solubility and swelling power was estimated with the following

$$\text{Swelling power} = \frac{\text{Weight of the wet residue} - \text{Weight of the dried sample}}{\text{Weight of sample on dry basis (mg)}}$$

$$\text{Solubility index} = \frac{\text{Weight of the dry residue (mg)} \times 2.5 \times 100}{\text{Weight of sample on dry basis (mg)}}$$

Results and Discussion

Analysis of fresh mango peel sample for microbial load, heavy metals, antibiotics and pesticide residue.

Microbial load of fresh mango peel sample

Microbial study for total count, molds and yeast count was carried out for fresh mango peel sample that was procured from mango processing industry and the values are depicted in Table 1.

It was observed that there was no microbial load in all the three group of microorganisms in the fresh sample.

Analysis of fresh mango peel sample for heavy metals and antibiotics

Analysis of fresh mango peels sample for heavy metals, antibiotics and pesticide residue was carried by out sourcing at Bangalore test house and the results are presented in Table 2 and 3 respectively. The results obtained shows that the contents for lead, cadmium, arsenic, mercury were less than 0.01ppm and antibiotics streptomycin was not detected and analysis for pesticide residue endosulfan was below detection limit of 0.05, dimethoate not detected, and carbendazim below detection limit of 0.01. Hence the freshly obtained mango peel sample was safe for the further processing, i.e. for the extraction of total polyphenols and dietary fibers and these extracts were used for food product formulation.

According to Table 4 per cent recovery of fresh mango peel to powder was loss of sample due to moisture. Peel contains about 80 per cent moisture. On heat at 60^0 C most of it is lost due to evaporation in each step of processing and 20 per cent recovery was obtained, which is lower as compared to the reported values by Ruiz *et al.,* (2011) who obtained 40 per cent after processing.

The MPP subjected to sensory evaluation, scored 8.2 for appearance, 7.8 texture, 8.1, colour, 8.5, flavor, 8.4 taste and 8.3 for overall acceptability as presented in Table 5.

Nutrient compositions of dry sample mango peel powder (Table 6**)** indicates that the moisture, protein, fat, crude fiber, carbohydrate, energy and ash were 3.93 per cent, 3.78, 2.61, 8.87, 86.39, 384 Kcal and 3.29g/100 respectively. The β-carotene content was 5600 µg/g. The mineral content in the peel was 4.15 mg for calcium, 0.45 mg phosphorous, 4.62 mg iron and 2.85 mg zinc respectively.

Table.1 Microbial load of fresh mango peel sample after blanching

	Group of microorganisms		
Sample	Bacteria	Molds	Yeast
	Population (x 10^2 CFU/10g sample)		
Mango peel sample	Nil	Nil	Nil

Table.2 Analysis of fresh mango peel sample for heavy metals and antibiotics

Tests	Contents
Description of mango peel	Yellow coloured paste
Lead (Pb)	Less than 0.01ppm
Cadmium(Cd)	Less than 0.01ppm
Arsenic(As)	Less than 0.01ppm
Mercury(Hg)	Less than 0.01ppm
Antibiotics	
Streptomycin	Not detected

Source: Bangalore test house, Rajajinagar, Bangalore

Table.3 Analysis of fresh mango peel sample for pesticide residue

Pesticide Residue	Result (mg/Kg) sample	Detection limit
Endosulfan	Below detection limit	0.05
Dimethoate	Not detected	0.05
Carbendazim	Below detection limit	0.01

Source: Bangalore test house, Rajajinagar, Bangalore

Table.4 Per cent recovery of fresh mango peel to powder

Weight of peel taken for blanching (g)	Weight of peel after oven drying (g)	Weight of peel after milling (g)	Milling loss (g)	Per cent recovery (%)
1000	203.2	200	3.13	20

Table.5 Mean sensory scores of mango peel powder

N = 15

Sample	Sensory attributes					
	Appearance	**Texture**	**Colour**	**Flavour /Aroma**	**Taste**	**Overall Acceptability**
Mangopeel powder	8.2	7.8	8.1	8.5	8.4	8.3

(Nine-point hedonic scale)

Table.6 Nutrient compositions of mango peel powder (per 100g)

Nutrients	Composition
Moisture%	3.93
Protein (g)	3.78
Fat (g)	2.61
Crude fiber (g)	8.87
Carbohydrate(g)	86.39
Energy (K.cal)	384
β-carotene (μg/g)	5600
Ash (g)	3.29
Calcium (mg)	4.15
Phosphorous(mg)	0.45
Iron (mg)	4.62
Zinc (mg)	2.85

Table.7 Per cent recovery of total dietary fiber, total polyphenols extract and antioxidant activity of MPP

Components	Contents
Total dietary fiber (TDF) %	69.86
Insoluble dietary fiber (IDF) %	44.23
Soluble dietary fiber (SDF) %	24.63
IDF/SDF ratio	1.80
Total Polyphenols (g GAE/100g)	4.50
Antioxidant activity (Vit-C Eq. μg/g)	76.96

Table.8 Functional properties of mango peel powder and dietary fiber

Properties	Mango peel powder	Dietary fiber
Water absorption capacity(g/g)	5.26	8.69
Oil holding capacity (g/g)	1.97	2.26
Solubility index %	7.58	9.01
Swelling power %	3.23	4.98

In the present study Table 3 presents the nutrients; moisture, protein, fat, crude fiber, ash, (3.93, 3.78, 2.61, 8.87 and ash 3.29 g per 100 respectively), which are similar to the values reported by Ashoush and Gadallah, (2011) for moisture, protein, fat, crude fiber and ash (4.92, 3.6, 1.23, 9.33, 3.88 g/100 respectively) and also to that reported by Ajila *et al.*, (2007).

Extraction of total polyphenols and dietary fibers from MPP**,** Table 7 presents the per cent recovery of total polyphenol and dietary fiber from MPP. The total dietary fiber, insoluble dietary fiber and soluble dietary fiber in the present study was 69.86, 44.23 and 24.63g/100g of MPP, total polyphenols 4.5 mg GAE/100g and antioxidant activity was 76.96 per cent. These values are in concurrence with Wachiriasiri *et al.*, (2009) who reported that the yield of 41.9 g per 100g dry weight of banana peel on wet milling processing method. Palmeira *et al.*, (2012) reported that a maximum extraction yield of 33.7 per cent of phenolics was obtained for the espada variety with ethanol concentration. The total dietary fiber, insoluble dietary fiber and soluble dietary fiber in the present study was 69.86, 44.23 and 24.63g/100g of MPP; β-carotene 5600 µg/g; total polyphenols 4.5 mg GAE/100g and antioxidant activity 76.96 per cent. Which were on par of values reported by Ajila *et al.*, (2007). Similar values were also reported by Larrauri *et al.*, (1997) that dried mango peel contained 28.1 per cent of soluble dietary fiber and 43.4 per cent of soluble dietary fiber. For health benefits, it is reported that 30 to 50 per cent soluble dietary fiber and 50 to 70 per cent insoluble dietary fiber are considered to be well- balanced proportions.

Functional properties were determined and presented in Table 8. The results revealed that significant difference exists for functional properties of mango peel powder (MPP) and extracted dietary fiber (DF). The water absorption capacity, Oil holding capacity, solubility index and swelling power of extracted dietary fiber was significantly more than mango peel powder. The water absorption capacity (8.69 g/g), oil holding capacity (2.26 g/g); solubility index and swelling power (9.01 and 4.98 per cent respectively) of extracted dietary fiber (DF) was significantly higher compare to mango peel powder. The water absorption capacity 5.26 g/g, Oil holding capacity 1.97 g/g; solubility index and swelling power were 7.58 and 3.23 per cent respectively. Hydration property of dietary fiber refers to its ability to retain within its matrix. Fiber with strong hydration properties would increase stool weight and potentially slow the rate of nutrient absorption for the intestine. It can enhance viscosity of added foods as reported by Gallaher and Schneeman (2001). Water absorption capacity, oil holding capacity g/g, solubility index per cent and swelling power percent were observed to be high in dietary fiber extract 8.69, 2.26, 9.01 and 4.98 respectively compared to mango peel powder which was found to be 5.26, 1.79, 7.58 and 3.23 respectively. This shows that processed samples have higher water absorption capacity. These results are in harmony with the results obtained by Ashoush and Gadallah (2011). Wachirasiri *et al.,* (2009) reported that washing after wet milling could enhance the concentration of TDF in banana peel by improving the removal of protein and fat which increased the water holding capacity. Swelling power is the volume occupied by a known weight of sample under controlled conditions. The sample is hydrated with water for a particular time with no external stress except gravity. Decrease in particle size increases the swelling capacity, swelling power is particularly important in the products where the granules are gelatinized but remain essentially intact. The softness of cooked product is an important attribute. This is impaired by ability of starch granules to swell and maintaining the integrity among the granules of food. Eating quality is often connected with retention of water in the swollen starch granules. Swelling power was found to be high in DF extract 4.98 per cent compared to MPP 3.23 per cent which were comparable to the values reported by Ajila *et al.,* (2007) and Ashoush and Gadallah (2011). Slight variation could be due to difference in processing methods, particle size, and texture and storage materials. Through in terms of health benefits, both insoluble dietary fiber and soluble dietary fiber complement each other; each fraction has different physiological effect. Insoluble dietary fiber helps in absorption and intestinal regulation whereas soluble dietary fiber associates with cholesterol in blood and diminished its intestinal absorption. The characteristic feature of mango peel is that it has high content of soluble dietary fiber, which is reported to have more health beneficial effects.

This study showed that mango peel is a rich source of bioactive compounds such as polyphenols, carotenoids and dietary fibers. Consumption of mango either as whole fruit or in some processed form will increase their intake in the diet. Mango peel, by-product of mango processing industry, could be a rich source of bioactive compounds. This new source will be potential as a functional food or value added ingredients in future in our dietary system. Mango peel if conveniently processed, could furnish useful products that may balance out waste treatment costs and also decrease the cost of main product. Therefore, there is a scope for the isolation of these active ingredients and also use of mango peel as an ingredient in processed food products such as bakery products, breakfast cereals, pasta products, bars and beverages.

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