Original article

Exploring varietal differences in nutritional and antioxidant potential of mango kernel and its use for enrichment of pasta

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Summary

Introduction **– Mango kernels contribute to a major segment of waste from mango processing industry. Utilization of these resourceful kernels can alleviate problems of environment and sustainability.** *Materials and methods* **– Kernels sourced from 14 mango varieties were analyzed for their protein, fat, tannins, antioxidant activity and minerals. Mango kernel flour (MKF) after pre-treatment was incorporated at 5 to 10% in semolina-based fusilli pasta.** *Results and discussion* **– The protein content amongst the varieties ranged from 5.40 to 7.29%. Dehydrated mango kernels were found to possess very high phenolics (309.66–535.16 mg GAE 100 g-1) and antioxidant activity (5.00–27.28 µmol TE g-1). They were found to be a rich source of potassium (519.0–912.4 mg 100 g-1), magnesium (114.0–198.0 mg 100 g-1), iron (3.8–12.8 mg 100 g-1) and calcium (106.6–263.6 mg 100 g-1). Hydrothermal treatment was found effective in minimizing the bitter taste perceived on consuming the mango kernel flour (MKF). Incorporation of MKF in pasta was found acceptable on sensory, textural and cooking parameters. Ten percent MKF pasta was enriched in both phenolics (220 mg GAE 100 g-1) and antioxidant activity (100.68 µmol TE g-1).** *Conclusion* **– Hydrothermally treated mango kernel can be successfully used as functional ingredient for enhancing phenolics and antioxidants in food.**

Keywords

India, mango, *Mangifera indica*, biochemical composition, phenolics, by-product management

Introduction

India is the leading producer and consumer of mango with 42.19% share in global mango production, followed by China, Thailand, Indonesia and Mexico (Evans *et al*., 2017). Mango processing facility in India is well developed in cluster-based manner. Ripe mangoes are processed through canning and pulping. The pulp is used as base material in drinks, beverages, desserts, ice-creams, *etc*., throughout the year because of its universal appeal. Unripe mangoes are used as ingredients in pickles and chutneys. Mango kernels amount for considerable waste of the processing industry in India, generating more than 5 lakh tons of kernels every year (Bung and Chachadi, 2002; Anon., 2019). Kernels constitute 17 to 22% of mango fresh weight (Kittiphoom, 2012). Abundance

Significance of this study

What is already known on this subject?

- Mango kernels are predominant by-products from mango processing industry.
- High phenolics and antioxidant potential of mango kernels is already known.

What are the new findings?

- Huge inter-varietal variation in kernel nutritive and antioxidant contents were found amongst 14 prominent mango varieties.
- Mango kernels were successfully utilized as source of phenolics and antioxidant for functional food, such as enriched pasta.

What is the expected impact on horticulture?

• Mango kernel utilization as source of nutrients and phenolics in functional food industry shall serve purposes of environmental issues as well as sustainability.

of micronutrients, polyphenols, lipids and good quality proteins in mango kernels has been previously documented (Kittiphoom, 2012; Torres-Leon *et al.*, 2016). Mango kernels are rich in protein and all important amino acids such as leucine, valine, lysine, glutamate (13 g 100 $g⁻¹$ protein) but low in methionine, threonine and tyrosine (Abdalla *et al.*, 2007; Jahurul *et al.*, 2015).

The kernels have been found to be non-toxic based on acute toxicity studies as per OECD 423 guidelines. Ashalatha *et al.* (2015) reported that mango kernel powder is safe for longer use even as high as 500 mg kg-1 body weight, based on the absence of any histopathological changes or abnormal behaviour with a dose of $5,000$ mg kg⁻¹ body weight. Low levels of hydrogen cyanide, phytic acid and trypsin inhibitors within safe levels has also been reported by Okpala and Gibson-Umeh (2013) for Indian origin mangoes. Kernel extract also has antimicrobial properties which can inhibit growth of both gram negative and gram positive bacteria (esp. *Pseudomonas aeruginosa*, *Escherichia coli*, *Campylobacter jejuni* and *Yersinia enterocolitica*, at 100 and 50 ppm). In addition, tyrosine inhibitory and metal chelating properties of the mango kernel extracts are important in the cosmetics segment. The extract of mango kernel exhibits strong antioxidant activities and has strong hepatoprotective properties (Torres-Leon *et al.*, 2016). The ethanolic extract from mango seed (*Mangifera indica* L.) was identified as one of four extracts with the highest antioxidant capacity in addition to the ethanolic extracts of *Punica granatum* (peel), *Syzygium aromaticum* (bud) and *Phyllanthus emblica* (fruit) (Saito *et al.*, 2008; Torres-Leon *et*

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al., 2016). Antioxidant activity of mango kernel extract has been demonstrated by several researchers for improving oxidative stability of buffalo ghee, potato chips frying sunflower oil and mortadella sausages and found equivalent or better than synthetic antioxidants (Pereira *et al*., 2011; Jafari *et al*., 2014). Other uses of mango by-products include use of shell as biosorbent for dyes (Alencar *et al*., 2012).

Use of mango kernels as source of nutraceuticals and bioactive compounds can be a viable alternative to reduce the burden on environment for degradation by dumping them as such. A wide range of variation is seen amongst the mango cultivars throughout the world. As their quality traits vary, the composition of kernels also differs (Lakshminarayana *et al*., 1983). Despite India being a major hub for mangoes, there is lack of information on composition and antioxidant activity of mango kernels available. Actually the astringent taste of mango kernel due to presence of high tannins limits its use as food ingredient (Menon *et al*., 2014). However some pre-treatments such as autoclaving, heating, roasting can transform bitter tannins into free form so that the bitter taste and astringency is reduced (Legesse and Emire, 2012). Keeping this background in mind, the present investigation was undertaken to identify varietial variations in nutritional and functional components of mango kernels. In addition, hydrothermally treated mango kernels were evaluated for their potential as a functional ingredient in a popular convenience food, pasta. The choice of pasta for fortification was sought as this product is gaining wide popularity amongst the young generation. However, being based on semolina or refined wheat, it is rated low on nutritional content. Fortification of pasta with antioxidants shall enhance its acceptability amongst the health-conscious population segment.

Materials and methods

Ripe mangoes of 14 varieties were procured from the Division of Fruits and Horticulture Technology, ICAR-IARI, New Delhi. The kernels were separated from the fruit with the help of a guillotine-like hand-held device. The separated kernels were dehydrated in tray drier (60 °C) and powdered using a hammer mill.

Chemical analysis

Proximate analysis (fat, fibre, ash and protein) was done using AOAC methods. The protein content of MKF was determined using micro Kjeldahl method (AACC, 2000), using fully automatic digester and distillation unit (Velp Scientifica, Italy). The crude fat was estimated by using method 30-10 (AACC, 2000) with the help of a J.P. Selecta-make fat extractor. Ash content was determined using muffle furnace (method No. 08-01, AACC, 2000). Standards for antioxidants analyses were procured from Sigma-Aldrich. Total phenolic content of kernels was estimated spectrophotometrically using Folin-Ciocalteu reagent (Singleton *et al*., 1999). Results were expressed as Gallic acid equivalent (mg GAE 100 g^{-1}). Antioxidant activity was determined measured using three *in vitro* assays, namely: Ferric Reducing Antioxidant activity was (FRAP) assay according to the method of Benzie and Strain (1996); Cupric ion reducing antioxidant capacity was determined according to Apak *et al.* (2004), and 2,2-diphenyl-1-picryl hydrazyl (DPPH) through assay specified by Apak *et al.* (2008). The results were expressed as μmol Trolox equivalents 100 $g¹$. Tannins were determined using the method of Saxena *et al.* (2013) using Folin-Denis reagent. Minerals analysis was done using MPAES (Agilent Technologies, USA).

Kernel pre-treatment and pasta preparation

Mango kernel flour (MKF) was subjected to steam treatment at 100 °C for 20 min in a vertical laboratory autoclave (10 psi) for the purpose of de-bittering. Pasta were formulated with semolina at three levels (5, 10 and 15%) of replacement by MKF. Pasta elaboration into fusilli shape was done using laboratory scale Dolly La Montiferrina, Italy.

Pasta quality evaluation

Cooking quality

Cooking quality of pasta (cooking time, solid loss, weight gain and volume gain) was determined using standard protocols (Schoenlechner *et al*., 2010; D'Amico *et al.*, 2015). Gruel loss on cooking pasta was evaluated according to AACC method 16-50 (AACC, 2000). Briefly, 25 grams of pasta were heated in a beaker (500 mL capacity) containing 300 mL of boiling distilled water on gas burner. Optimum cooking time (OCT) for each sample was determined by removing units at regular intervals and pressing between two petri-plates until the central core disappeared. The pasta was subsequently drained using a stainless steel sieve and cooled by keeping under running water for a few seconds. Once the OCT of each pasta sample was determined, the pasta sample was optimally cooked and the weight gain and solid loss during cooking were determined in duplicates. Weight gain and volume gain after cooking were determined by noting an increase in weight of 10 g pasta after cooking while volume gain was evaluated by toluene displacement method.

Solids loss

Twenty-five grams of the sample was cooked in a pan containing 250 mL of boiling water for OCT. The cooking water was drained into beaker and evaporated to dryness in hot air oven at 100 °C to determine the solids loss in the gruel and expressed as % gruel loss using AACC method 16-50 (AACC, 2000).

Textural analysis of pasta

Textural analysis of cooked pasta was conducted with a texture analyser (TA-XTplus, UK). The firmness and stickiness of pasta was determined using P/30 probe under compression mode with test speed of 0.5 mm $s⁻¹$, compression strain 90% and post-test speed of 10 mm s-1. The peak positive force was considered as firmness (g) and peak negative force as stickiness (g) of cooked pasta. The cut force (g) for cooked pasta was determined using knife blade set with pretest speed of 2 mm $s⁻¹$, test speed 3 mm $s⁻¹$ and compression distance of 20 mm. Ten semi-trained panelists (6 females and 4 males), aged between 21 to 45 years, ranked the freshly cooked pasta served in random order two hours post meal. Sensory evaluation of cooked pasta was done based on 9-point hedonic scale (0=not acceptable; 9=highly acceptable). Sensory evaluations were conducted in the isolated booths at the room temperature (30 \pm 1 °C). Between two samplings, the panelists were provided with plain water to rinse their mouth.

All experiments were conducted in triplicate. Values are presented as mean ± standard deviation (SD) of three replicates. The results were statistically analysed by ANOVA (SPSS version 10.0). Statistical significance was accepted at a level of 5%. Selected data were subjected to correlation analysis.

Results and discussion

Mango kernel

Proximate composition

There was a significant variation in protein and crude fat content in different varieties (Figure 1). The protein content ranged from 5.4 to 7.2%, while fat content varied from 6.4 to 10.5%. Highest protein content was found in 'Neelam' followed by 'Bombay green' (Figure 1). In terms of fat, 'P. Arunima' and 'St. Alexandrina' were found richest, while 'Amrapali' recorded minimum fat percentage. High fat content in mango kernels has food and pharmaceutical value and is used as a cocoa butter substitute and in cosmetics (Torres-Leon *et al.*, 2016). The unique fatty acid composition of mango kernel constituted by stearic, lauric and palmitic fatty acids, which are responsible for the peculiar melting profile at body temperature, makes it suitable for use in chocolates with typical melt in the mouth characteristic (Jahurul *et al.*, 2014). Our findings are in conformity with Jahurul *et al.* (2015) who have reported protein and crude fat contents of 6.75% and 7.1 to 15.0% dry weight basis in their review.

Total phenolics

Mango kernels from different mango varieties were found to be high in total phenolics content and the content ranged from 309.66 to 535.16 mg GAE 100 $g⁻¹$ in 'Bombay green' and 'Gulabkhas green', respectively (Figure 2). Total phenolics recorded in Indian varieties were found to be generally higher than those reviewed and reported by Torres-Leon *et al.* (2016) and Soong and Barlow (2006). Variation in phenolic content can be attributed to the differential accumulation of phenolics in crops, depending on climatic conditions, varieties, stress, *etc*. Since all the mango cultivars used in this study were grown in the same location, following similar horticultural practices, the variation in total phenolics demonstrate the genetic variability probably owing to differential regulation in the biosynthesis of phenolic secondary metabolites.

Figure 1. Total protein and fat contents in kernels of different mango varieties.

Figure 2. Total phenolic and flavonoid contents in kernels of different mango varieties.

Torres-Leon *et al.* (2016), in their review on mango kernels, have reported phenolic content ranging widely from 21 to 447 mg $g⁻¹$ dry weight, indicated vast differences in the phenolics amongst varieties and agro-climatic origins. The major phenolics reported in mango kernels are mangiferin, isomangiferin, homomangiferin, quercetin, kaempferol, anthocyanins, phenolic acids (gallic, protocatechuic, ferulic, caffeic, coumaric, ellagic, 4-caffeoylquinic acids) (Ribeiro and Schieber, 2010). Content of total phenolics present in mango is equivalent to that found in plums and blueberries (292–672 mg GAE 100 g-1 dry weight) (Cevallos-Casals *et al*., 2006; Prior *et al.*, 1998). This content is also at par with phenolics reported in bitter-gourd, finger-millet and black gram (Hani *et al.*, 2017; Rudra *et al.*, 2015). Phenolics are the major health promoting compounds and have been associated with anti-cancerous and anti-diabetic properties, are known to scavenge reactive oxygen species (ROS), and act as effective antioxidants. Considering high phenolic content found in the mango kernel, there is immense potential of their use as a valuable ingredient for development of functional foods.

Flavonoids

Flavonoids are important antioxidant compounds known to possess hepato-protective, anti-inflammatory, antiviral, and anti-proliferative activities and help protect from many cardiovascular, cancer and age-related diseases (Calzada *et al*., 2017). The flavonoids content of mango kernel was 88.11 to 136.17 mg QE 100 $g⁻¹$ (Figure 2). 'Mallika' and 'Sensation' recorded highest flavonoids content, whereas 'Gulabkhas green' and 'Bombay green' registered the minimum values (88.11 to 91.02 mg QE 100 $g⁻¹$). High flavonoids content in varieties is at par with content reported in sweet orange, pomelo and grapes (Kumar and Kaur, 2017). A wide variation for flavonoid content in mango kernel exists in literature for, *e.g.*, 10.98 mg rutin equivalents g-1 by Bakar *et al.* (2009); 46.5 mg 100 g-1 dry weight by Ribeiro *et al*. (2008), while Dorta *et al*. (2012, 2014) have reported flavonoid content of mango kernels to be in range of 0.72–1.13 g 100 g^{-1} dry weight basis. Considering moisture content in mango kernel to be 6.34%, these values come out to be $4.30-1.058.36$ mg 100 g⁻¹ and in a range exhibited by our cultivars.

In vitro **antioxidant activity**

The antiradical capacity of the mango kernel extracts was estimated in terms of three *in vitro* assays namely, CUPRAC (cupric reducing antioxidant power), DPPH assay (2,2-diphenyl-1-picrylhydrazyl) and FRAP (ferric reducing antioxidant power). The CUPRAC values ranged from 9.62 to 37.46 μmol TE g^{-1} . DPPH activity ranged from 7.77 to 27.28 µmol TE g^{-1} and FRAP ranged from 11.05 to 22.56 μ mol g⁻¹ (Figure 3a).

Figure 3. Antioxidant activity of kernels of different mango varieties and their correlation with total phenolic content.

 $F_{\rm eff}$ and $F_{\rm eff}$ and their correlation with total phenolics correlation with total phenolics content

Significant variation in antioxidant activity was observed in kernels amongst the cultivars and maximum antioxidant activity was recorded in mango kernels from varieties 'Bombay green' and 'Langra'. Such high antioxidant activity is on par with activity reported in plums, grapes, pomegranate and even blue berries which exhibit antioxidant activity in range of 13.9 to 45.9 µmol TE $g¹$ (Kumar and Kaur, 2017; Prior *et al*., 1998). Our results are in agreement with Dorta *et al.* (2012) who reported activity in the range of 18–24 g 100 g^{-1} catechin equivalents for significant high correlation (*p*= 0.00) was found between total phenolic content and antioxidant activity $(R^2 > 0.8)$ (Figure 3b).

Recently, Torres-Leon *et al*. (2017) have reported microwave application for extracting the phenolics from mango kernels. They have reported ethyl gallate, penta-*O*-galloyl-glucosides as major gallotannin compounds in mango seeds kernel, while rhamnetin-3-[6″-2-butenoil-hexoside] was found to be the major flavonoid compound.

Tannins

Mango kernels are known to possess high amounts of hydrolysable tannins, which are easily hydrolyzed by the action of digestive acid and/or enzymes, releasing Gallic acid or ellagic acid units (Bernardini *et al.*, 2004; Scalbert and Williamson, 2000). Tannins are primarily responsible for bitterness or astringency of flours and low amount of tannins is highly desirable for food fortification. Tannin content in mango kernels ranged from 5.6 to 21.89 mg $g⁻¹$ (Figure 4). In this context, varieties with low amounts of tannins, such as 'Pratibha', 'Dussheri', and 'P. Pitamber', were found to be better for edible purposes compared to other varieties. Varieties 'Surya', 'Neelam' and 'Gulabkhas green' showed highest content of tannins (18.63 to 21.89 mg g^{-1}). High content of tannins in these varieties can be exploited for their anti-microbial effects. Antibacterial property of Indian mango kernel extracts against human pathogenic bacterial strains like *Bacillus subtilis*, *B. cereus*, *Salmonella typhimurium*, and *Escherichia coli* has been reported (Alok *et al.*, 2013). The antimicrobial activity of gallotannins is attributed to their iron binding capacity and ability to form extra stable complexes with proteins and some carbohydrates of bacterial cell membrane (Chung *et al*., 1998; Engels *et al*., 2011). Methanolic extract of mango kernel has reported to have anti-diarrheal activity and also affects intestinal transit (Sairam *et al*., 2003).

Minerals content

Iron content in mango kernels was found to vary widely amongst the varieties and ranged from 4.2 to 12.8 mg 100 $g⁻¹$. Maximum iron content was observed in 'Amrapali' and 'Bombay green' (Figure 5). This is in agreement with content (11.9 mg 100 g⁻¹) reported by Fowomola (2010). Calcium is an important mineral responsible for bone development and muscle contraction in human body. Mean calcium content in varieties was 154 mg 100 g⁻¹, however, inter varietal variation amongst varieties was very high (106 to 263.6 mg 100 g⁻¹). Kernels from 'Bombay green' and 'Langra' were found to possess the highest calcium content ($>$ 216 mg 100 g⁻¹). Zinc content ranged from 1.8 to 5.2 mg 100 $g⁻¹$ with mean content of 2.2 mg 100 $g⁻¹$. Magnesium content ranged from 114 to 265 mg 100 g⁻¹ with a mean of 164.2 mg 100 g⁻¹. Magnesium is an important micronutrient and plays an important role in regulating of body's acid-alkaline balance. Our results are in agreement with values reported by Yatnatti *et al.* (2014); calcium (170 mg 100 g⁻¹), zinc (5.6 mg 100 g⁻¹); magnesium $(210 \text{ mg } 100 \text{ g}^{-1})$ and potassium $(368 \text{ mg } 100 \text{ g}^{-1})$. Copper content of kernels was recorded as 1.15 mg 100 g⁻¹ on an average with varietal variation ranging from 0.8 to 1.8 mg 100 g⁻¹. Potassium ranged from of 519 to 912 mg 100 g⁻¹ while sodium content ranged from 30 to 113 mg 100 g^{-1} . Besides neuron transmission, sodium and potassium are also known to play an important role in protein synthesis in the human body. Recommended levels of magnesium and potassium are 310–340 mg day⁻¹ and 10 g day⁻¹ respectively (Nair and Augustine, 2018). Large variation in potassium content ranging from 60.0 to 540.5 mg 100 $g⁻¹$ was found in several reports (Elegbede *et al*., 1995; Odunsi, 2005; Nzikou *et al*., 2010; Ifesan, 2017). This could be attributed to differences in plant variety, cultivation climate, ripening stage, harvesting time of seed kernels and the extraction method used (Kittiphoom, 2012).

Utilization of mango kernel in pasta

In order to utilize mango kernel for food purposes powdered mango kernel flour (MKF) was autoclaved at 100 °C for 20 min for de-bittering. Autoclaving has been reported to reduce bitterness and transform the flour into sweet flour with light brown coloration in line with previous work (Legesse and Emire, 2012). The autoclaved MKF was analysed for phenolics and antioxidant activity. In general, total phe-

Figure 4. Total tannin content (mg g^{-1}) in kernels of different mango varieties.

nolics were found to decrease by 4 to 17% upon autoclaving. The only exception was the 'Chausa' variety, where 20.5% increase in phenols was observed upon autoclaving. Jambrec *et al*. (2015) have also reported reduction of free and increase of bound phenolic forms in autoclaved buckwheat grains and enhancement of sensory quality of tagliatelle pasta prepared from them. Treated MKF was then incorporated in fusilli pasta at three levels namely 5, 10 and 15%. There was significant decrease in protein content from 11.97 to 10.47% and increase in ash content in formulated pasta. This was expected due to difference in protein content in respective semolina and mango kernel. Also there was substantial decrease in expansion volume of cooked formulated pasta as compared to control pasta (Table 1).

FIGURE 5. Minerals content (mg 100 g^{-1}) in kernels of different mango varieties.

Sensory attributes of MKF pasta

The degree of openness of the gluten network in pasta would influence the extent of water absorption by starch granules during cooking (Sun-Waterhouse *et al*., 2013). The starch-gluten interactions would be different in the presence of MKF, and this ultimately determines the quality attributes of the pasta including the firmness, solid loss and stickiness of pasta upon cooking. For all formulations, leached solids were low due to formation of networks insulating the starch and restrained impregnation of water for expansion and also limited solids loss in gruel. Generally, leached solids in commercial pasta should not exceed 10%, and for all formulations of pasta, the leaching loss was within this range. As expected, firmness and stickiness of cooked pasta increased with increasing levels of mango kernel in formulated pasta. Overall acceptability score of control pasta was 8.6, whereas formulated pasta scored slightly lower (8.4) which was due to low colour score and brownish coloration. However, in terms of taste and flavour, the formulated pasta with MKF rated high (8.0). This was probably due to sweetness imparted by autoclaved mango kernel. Incorporation of mango kernel flour in pasta was found acceptable up to 10%. For 15% MKF formulation, there was decline in scores due to brownish appearance of formulated pasta (Figure 6).

Phenolics content and antioxidant capacity of pasta

In terms of phenolics, while control pasta made from semolina had a total phenolics content of 163.9 ± 2.9 mg GAE 100 g⁻¹, the 15% MKF recorded phenolics content of 294.4±7.8 mg GAE 100 g⁻¹. The 10% MKF pasta which showed maximum acceptability had phenolics amounting to 220.5 ± 2.9 mg GAE 100 g^{-1} while its antioxidant activity in terms of DPPH was recorded to be 100.68 ± 8.9 µmol TE g⁻¹, which was 13.45 times higher than control pasta. The antioxidant content thus achieved lies in the range of açai berries with 87.4 to 114 μmol trolox equivalents (TE) $g⁻¹$ (Pacheco-Palencia *et al*., 2009). Sun-Waterhouse *et al.* (2013) have incorporated fettuccine pasta with elderberry juice to enrich them with phenolics and antioxidants. They have reported maximum antioxidant activity in uncooked pasta < 2 mg TE g^{-1} and phenolic content of 115–211 mg catechin equivalents 100 $g¹$. Similarly, Sant'Anna *et al*. (2014) have incorporated grape marc powder to enrich fet-

Figure 6. Fusilli pasta enriched with mango kernel flour (MKF).

tuccine pasta with phenolics to possess 100 mg GAE 100 g^{-1} phenolics, and antioxidant activity of 336 mM TEC 100 g^{-1} . Comparably better phenolics and AOX activity in MKF justify the use of MKF for enrichment purposes, while providing a means for value addition of by-products from mango processing industry.

Conclusion

Mango kernels are a reasonably good source of proteins and minerals. Vast varietal differences exist in the nutrients and functional profile of the mango kernels. MKF can be easily and effectively debittered using autoclaving treatment. The debittered MKF can be used as functional ingredient in various foods like pasta. In case of pasta, the phenolics were improved by 34.5 to 79.6% over control while antioxidant activity was enhanced by 13.5 to 17.7 times over control pasta, without hampering sensory and cooking quality. Hence, the findings of this study suggest the potential of mango kernel for use as a promising ingredient in functional food industry for enhancing antioxidant content and micronutrients.

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