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Comparative Analysis of Functional and Nutritive Values of Amla (*Emblica officinalis*) Fruit, Seed and Seed Coat Powder

Poonam Mishra and Charu Lata Mahanta

Department of Food Engineering and Technology, School of Engineering, Tezpur University, Tezpur Napaam, Assam, 784028, India

Corresponding Author: Poonam Mishra, Department of Food Engineering and Technology, School of Engineering, Tezpur University, Tezpur Napaam, Assam, 784028, India Tel: 9103712267007, 267007 (5705) Fax: 9103712267005, 267006

ABSTRACT

In present investigation amla seed and seed coat was investigated separately for their compositional, functional property. Water retention capacity and swelling capacity of seed coat powder was higher than seed powder. Among the three, fruit powder showed strongest free radical (DPPH) activity followed by seed coat whereas, seed had only 30% of DPPH* scavenging activity. Both seed and seed coat powder were found to be good sources of P, K, Mg, Fe etc. Seed powder had high value of P(395.44 mg/100 g) in comparison to fruit powder and seed coat powder while seed coat was found to be a good source of Ca, Cr and Mn. The Total Phenolic Content (TPC) of seed coat and seed powder was lower than fruit powder. The 9, 12, 15, octadecatrienoic acid (z, z, z) and tetradecanoic acid were the major fatty acids present in seed and seed coat.

Key words: Amla, antioxidant, amla seed coat

INTRODUCTION

Amla (Emblica officinalis L.) as a plant of Euphorbiaceous family is widely distributed in subtropical and tropical areas of India, Indonesia, China and Malaysia (Liu et al., 2008). Amla is a good source of polyphenols, flavones, tannins and other bioactive compounds (Zhang et al., 2003). These substances being strong antioxidants might contribute to the health effects of amla. Several active compounds have been extracted from amla (Zhang et al., 2003; Habib-ur-Rehman et al., 2007; El-Desouky et al., 2008; Luo et al., 2011). These bioactive components have anticancer, hypolipidemic, expectorant, purgative, spasmolytic, antibacterial, hypoglycaemic (Liu et al., 2012; Jamwal et al., 1959) hepatoprotective, hypolipidemic activities and also can attenuate dyslipdaemia (Thakur and Mandal, 1984; Yokozawa et al., 2007). Several amla based products i.e., amla candy (Devi and Mishra, 2010), ready to serve beverages (Deka et al., 2001), spray dried amla powder (Mishra et al., 2013), ready to eat amla chutney (Mishra et al., 2011) etc., have been developed. Though the functional properties of amla have been reported but the seed and seed coat of amla have never been investigated for their functional properties as well as compositional analysis. In this chapter the physicochemical properties of different varieties of amla are presented. Further, amla seed and seed coat of Chakaiya variety (major processing waste of amla based industries) were separately analyzed for their proximate composition, antioxidant properties, total phenolic contents, major/micronutrients and fatty acid profile.

MATERIALS AND METHODS

Raw material: Amla of *Chakaiya* variety was procured from local market of Allahabad, India. The amla fruits were thoroughly cleaned under tap water to remove adhering dust and wiped with muslin cloth. Fresh fruits of different varieties were evaluated for their physical, chemical and functional properties. The fleshy part of *Chakaiya* variety of amla was grated and seed was separated manually from adhering amla. Grated amla shreds were dried in tray drier at 40°C. The dried amla shreds were ground in laboratory grinder and passed through 0.5 mm screen sieve. Whole amla seeds were dried in tray drier at 40°C. As the whole seeds dried they broke along the ridges with a crackling sound. The seed coat was separated from the brown seed from each of the broken units. Seed coat and seed were separated manually and both were converted into powder separately as done for amla shreds. The powder of fruit, seed and seed coat were stored at refrigerated temperature (4°C) for further analysis.

Analysis of physical properties of amla: Different varieties of amla were measured for their height and width with varnier callipers with least square of 0.02 cm. Number of fragments and shape were analyzed visually. Ten readings were taken for each physical property.

Proximate analysis: The moisture, crude fat, protein, crude fiber content of the samples was estimated as per the procedures of AOAC (2010). Acidity and pectin were determined by the method of (Ranganna, 1986). Available carbohydrate was calculated by balance method.

Functional properties: The Water Retention Property (WRC) and Swelling Water Capacity (SWC) of amla seed and seed coat powder were analysed by the methods given by Robertson *et al.* (2000). Vitamin C content of the sample were evaluated by the method described in Indian Pharmacopoeia (1996). Diphenyl picryl hydrazil free radical (DPPH*) scavenging activity of the sample were evaluated by the method of Luo *et al.* (2009).

Total phenolic content estimation by high performance liquid chromatography (HPLC) analysis: The total phenolic content by HPLC was estimated by the method given by Seruga et al. (2011) with some modifications. Gallic acid standard was used for total phenolic content estimation. Calibration curves were made by diluting stock solutions with methanol to give concentration of the standards in the range of 1-100 mg L^{-1} of gallic acid. The components in the sample were separated by HPLC (Waters, model Breeze 2) column C₁₈ binary system. For separation, 0.1% orthophosphoric acid as solvent A and 100% methanol (HPLC) grade as solvent B were used. The elution conditions were 0-30 min from 5% B to 80% B; 30-33 min 80% B; 33-35 min from 80% B to 5% B and 35 to 40 min 5% B. Flow rate was 0.8 mL min^{-1} . The operating condition of temperature was 20°C and injection volume was 20 µL. The detection wavelength for gallic acid was 280 nm. The total phenolic was determined from the total area of RP-HPLC chromatogram at 280 nm and expressed as GAE g⁻¹ of sample. For the sample preparation, 250 mg sample was extracted in mobile phase (HPLC grade methanol containing 0.1% of orthophosphoric acid) for 30 min. The mixture was centrifuged for 10 min at 8000 rpm and 4°C temperature. Supernatant was filtered through 0.45 μm filter paper. Supernatant (20 μL) was injected for HPLC analysis.

GC-MS analysis: Fatty acid profile analysis of the fat extracted from amla seed and seed coat was analyzed by GC-MS (Perkin Elmer Clarus 600). For derivatization of fatty acid in the form of

methyl esters, 40 mL of dried methanol was taken into a 500 mL round bottom flask and cooled for 30 min on ice bath. During cooling, 14 mL of acetyl chloride was added to the dried methanol in a drop wise manner with constant shaking followed by addition of cold oil. To this, 20 mL of chloroform was further added to dissolve the oil completely. The whole mixture was refluxed for 2 h, after which the mixture was cooled and subsequently 4.0 N NaOH was added to make the reaction mixture alkaline. Distilled water (100 mL) was added and the whole mixture was transferred to a 500 mL separating funnel to which diethyl ether (50 mL×3) was added in order to extract fatty acid methyl esters (FAMES). The organic layer was dried with anhydrous sodium sulphate, filtered and solvent was evaporated in vacuum. A complete removal of solvent was achieved by flushing with nitrogen. The sample was analyzed by using GC-MS with capillary column Elite-5 (Ge column of 30 m×0.25 mm; phase thickness 0.25 μm) and the temperature was first held at 40°C (2 min) and then raised to 250°C (5 min) at a rate of 10°C/min (Lepage and Roy, 1984). Flow rate of helium was 1 mL min⁻¹. The compounds were confirmed by comparison of their retention time with that of the reference compounds of NIST libraries.

Mineral analysis: Major and micro minerals were analyzed by the method given by Food and Agriculture Organization of the United Nations (FAO, 1983) with slight modifications. Briefly, 2 g of sample was placed in Kjeldahl tubes and 25 mL of freshly prepared nitric acid-sulphuric acid-perchloric acid mixture (3:1:1) was added. The sample as digested at 250°C for 2-3 h until a clear solution was obtained. After cooling the solution was diluted with 100 mL with deionized water and the residue was filtered through an ash less filter paper. The nutrient mineral content of the sample was determined by Atomic Absorption Spectroscopy (AAS) (Thermo, ICE 2000) with air acetylene flame for Ca, P, Fe Zn, Mg while graphite mode was used to analyze the Cu, Mn at ppb level.

Spectral analysis: To find out the functional characteristics the samples were scanned by Fourier Transform Infrared (FTIR) spectrophotometer (Perkin Elmer, Spectrum 100) in the range of 4000-600 cm with a resolution of 4 cm. Spectra were collected at ambient temperature by averaging three scans and coupling the attenuated total reflection ATR accessory to an FTIR spectrometer (Mayachiew and Devahastin, 2010).

Statistical analysis: All the samples were analyzed in duplicate. Results are shown as Mean±standard deviation. The significant difference was analyzed by using Analysis of variance.

RESULTS AND DISCUSSION

Proximate analysis, pectin, vitamin C and functional properties of amla seed and seed coat powder: The amla fruit, seed and seed coat powder were analyzed for proximate content. The results are presented in Table 1. The protein content of seed was significantly higher than fruit powder and seed coat powder at 5% probability level (Table 1). High % of protein in seed powder suggests that the level of carbohydrates in seed powder is less than that in amla fruit powder and seed coat powder.

Fat content was high in seed with a mean value of 8.84%. The present finding does not match with findings of earlier workers who have reported 18% of fat in amla (Arora *et al.*, 2010). Seed coat had comparatively less amount of ash than fruit powder and seed powder. Seed powder and amla fruit powder showed higher concentration of ash with a mean value of 3.81 and 3.54% respectively whereas, seed coat powder showed only 1.8% of ash (Table 1). The low % of ash in seed

Table 1: Chemical composition and functional properties of amla fruit, seed and seed coat powders

Particulars	Amla fruit powder	Amla seed powder	Amla seed coat powder
Ash (%)	3.54±0.2ª	3.81±0.1 ^b	1.48±0.1°
Fat (%)	0.51±0.1 ^a	8.84 ± 0.2^{b}	$2.45{\pm}0.1^{\circ}$
Protein (%)	6.04 ± 0.1^{a}	14.03 ± 0.4^{b}	$7.04\pm0.2^{\circ}$
Crude fiber (%)	2.78 ± 0.1^{a}	3.42 ± 0.1^{b}	$4.47{\pm}0.2^{\circ}$
Carbohydrates (%)	82.91±1.3ª	73.35 ± 1.5^{b}	81.94±1.1ª
Pectic substances (%)	ND	1.51 ± 0.1^{a}	$3.56\pm0.1^{\rm b}$
WRC (g water/g dwb)	ND	1.21 ± 0.2^{a}	$9.50{\pm}0.2^{b}$
SWC (mL water/g dwb)	ND	$2.21{\pm}0.1^{a}$	12.86 ± 0.1^{b}
% DPPH* scavenging activity/mg of powder	1.88 ± 0.0^{a}	$0.74{\pm}0.1^{b}$	$1.85{\pm}0.2^{a}$

Values in the same row with different superscripts differ significantly at 5% probability level, WRC: Water retention capacity, SWC: Swelling water capacity, ND: Not detected

Table 2: Comparative mineral analysis of amla fruit, seed and seed coat powders (mg/100 g)

Minerals	Amla powder	Seed coat powder	Seed powder
Ca	129.78±1.21ª	73.68±1.43 ^b	23.60±1.56°
P	159.02±4.44ª	89.61±2.53 ^b	395.44±9.63°
K	2544.00±1.24ª	$1314\pm3.40^{\mathrm{b}}$	2542.50±2.50ª
Fe	11.70 ± 0.08^{a}	14.00 ± 0.08^{b}	11.30±0.09ª
Mg	46.30±0.65ª	23.60±0.65 ^b	53.25±0.30°
Zn	0.23 ± 0.02^{a}	$0.14{\pm}0.01^{\rm b}$	0.20±0.01ª
Cr	0.82±0.01ª	0.86 ± 0.01^{a}	0.56 ± 0.01^{b}
Co	0.69±0.01ª	$1.46\pm0.01^{\rm b}$	1.42 ± 0.01^{b}
Mn	0.19± 0.01ª	0.21±0.01ª	0.42 ± 0.01^{b}
Cu	0.22±0.01ª	0.19 ± 0.01^a	0.14 ± 0.00^{b}

Value are Mean±SD, Values in the same row with different superscripts differ significantly at 5% probability level

coat powder can be justified from Table 2 also, which shows that the major minerals like Ca, P, K were significantly low in the seed coat powder than the fruit powder and seed powder. The % crude fiber was comparatively higher in seed coat powder than other samples tested. Water retention capacity is the quantity of the water that remains present in bound form along with hydrated fiber after application of an external force (pressure or centrifugation) (Raghavendra et al., 2006). The water retention capacity and swelling capacity of both seed and seed coat are presented in Table 1. Seed coat powder showed higher water holding capacity and swelling capacity with mean value of 9.5 g water/g dwb and 12.86 mL water/g dwb, respectively than the seed powder that were significantly different at 5% probability level. The high water retention capacity of seed coat may be related to the high dietary fiber content (Grigelmo-Miguel and Martin-Belloso, 1999). High pectic substances in seed coat might account for its high water holding capacity and swelling capacity. The same trend was observed during the analysis of the swelling capacity of seed and seed coat powder. While amla seed coat and amla powder showed 1.85 and 1.88% DPPH* scavenging activity respectively, seed powder had only 0.74% of DPPH* scavenging activity.

Mineral analysis of amla fruit, seed and seed coat powders: Minerals are inorganic nutrients which may be present both as single atom and in singlet form (Palmer, 2001). Processing wastes of amla were analyzed for some of the major and micro minerals and results are shown in Table 2. High value of Ca was obtained in amla fruit powder (129.77 mg/100 g) followed by seed coat and seed powder. Seed powder and amla fruit powder contained higher amount of P with mean value of 395.44 mg/100 g and 159.02 mg/100 g respectively whereas, in seed coat the mean value of P was found to be 89.61 mg/100 g. All three samples showed higher concentration of K

with mean values of 2543.70 mg/100 g, 1314 mg/100 g and 2542.50 mg/100 g in fruit powder, seed coat and seed powder, respectively. Magnesium levels in all three samples were lower than the recommended RDA (420 mg/100 g) (Palmer, 2001). Seed powder and amla fruit powder had comparatively higher value of Mg with mean value of 53.25 mg/100 g and 43.6 mg/100 g than seed coat (23.60 mg/100 g). The concentration of Mn obtained in seed powder was maximum with content of 41.98 µg/100 g. The studies also revealed that Mn was the most abundant trace mineral among the tested micro minerals followed by Fe, Co and Cr. K is the most abundant intracellular cation which is known to activate number of enzymes which are responsible to catalyze the transfer of phosphoryl groups or elimination reactions (Misenberg and Simmons, 1998). According to RDA, 2000 mg of potassium is required (Palmer, 2001). It thus implies that 20 g of amla fruit powder or seed powder will satisfy one fourth requirement of K whereas, 38.5 g of seed coat will be required to fulfill one fourth RDA of K. Phosphorous is involved in energy transfers during cellular metabolism. A number of enzymes and vitamin B becomes activated in presence of phosphate group (Palmer, 2001). Calcium is important for bone formation. The mean concentration of Ca of amla powder and seed coat powder shows that less than 50 g of powder may complete the RDA of Ca. Amla fruit powder, seed powder and seed coat powder had good amount of iron. Iron can bind a variety of ligands including cyanide, carbon monoxide, oxygen binding proteins such as haemoglobin, myoglobin and cytochrome oxidase etc (Anderson and Fitzgerald, 2010). But the form of iron in fruits and vegetables is of non heme form and the absorption of this form is low (Shilf et al., 2006). Co is the mineral in Vit B_{12} and its deficiency is rare. Cr aids the metabolism of carbohydrates by increasing the insulin function and recommended RDA of Cr is 35 µg and no toxicity of Cr is reported when consumed in amount greater than recommended value (Swaminathan, 2010). Consumption of 5 g of amla fruit powder or seed coat powder will satisfy the RDA of Cr whereas 7 g of seed will fulfill the requirement of Cr. Cu is responsible for hemoglobin and melanin production, electron transport, phospholipids synthesis, collagen synthesis and helps to maintain healthy bones, nerves and immune system. The RDA of Cu is 2-3 mg and too much Cu is poisonous and can lead to nausea, vomiting etc (Lalitharani et al., 2009). All three samples tested had very low level of Cu and consumption of 100 g of seed coat powder will only give 0.19 mg of Cu hence no toxicity of Cu will be experienced after consumption of large quantity of seed coat and seed powder. Zn is a very crucial micronutrient and is the mineral compound in a number of enzymes. Zn is also involved in protein and CO₂ metabolism and also involved in healing of cuts and wounds. The RDA of Zn is 15 mg (Maruthupandian and Mohan, 2011). Intake of amla fruit, seed coat and seed powder will provide substantial amount of Zn.

Fatty acid profile analysis of amla seed and seed coat: Through GC-MS it was revealed that the major components in seed coat oil were 9, 12, 15 octadecatrienoic acid, tetradecanoic acid and linoleates, whereas benzoic acid, 6 tetradecansulfonic acid, hydroquinone, dodecane 1-fluoro, phthalic acid 2-cyclohexylethyl iso butyl ester were the minor components (Fig. 1a). The major fatty acids in seed oil of amla were 9, 12, 15, octadecatrienoic acid (z, z, z) and tetradecanoic acid while benzoic acid, octenoic acid, 6 tetradodecane sulfonic acid, octadecanoic acid, 11 methyl ester, hydroquinone, octadecane 1, 1 dimethoxy were present in very minute quantities (Fig. 1b). In seed coat each component had different retention time except for tetradecanoic acid 10,13 dimethyl which was detected at two different retention times i.e., 14.22 and 15.52 min. Tetradecanoic acid was common in both seed and seed coat and this acid has antioxidant activity whereas 9, 12, 15, octadecatrienoic acid (z, z, z), the major component of seed oils have anti-inflammatry and antiarthritic properties (Lalitharani et al., 2009; Maruthupandian and Mohan, 2011). Omega-3 fatty acid is essential for normal growth and may also be important in preventing coronary artery

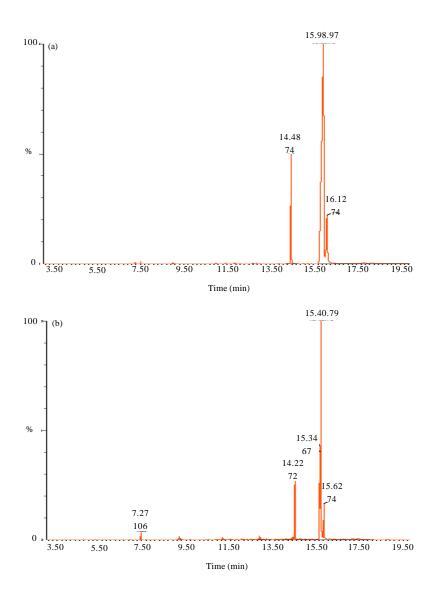


Fig. 1(a-b): Fatty acid profile analysis by GC-MS amla, (a) Seed and (b) Seed coat powder

diseases. Omega-3 fatty acid is the major component of seed oil and the present finding was in agreement with the finding of the Arora *et al.* (2010). Dodecane and phthalic acid were present in very minute quantities but they are reported to have antibacterial properties as reported by Adeleye *et al.* (2011).

HPLC analysis: HPLC analysis of amla fruit, seed coat and seed for total phenolic content and gallic acid content were carried out. UV detector with wavelength 280 nm was used to detect gallic acid. By comparing the spectra of the standard gallic acid was found to be one of the major phenolics in all three samples tested; the present finding was in agreement with the Kumar *et al.* (2006) that gallic acid and tannic acids are the major phenolic acids of amla. Total phenolic content and gallic acid content in amla fruit powder were 8738.00 mg GAE/100 g and 3000 mg/100 g

Table 3: Analysis of total phenolic content by HPLC

Sample powder	Total phenolic content GAE mg/100 g	Gallic acid mg /100 g
Amla fruit	8738.00±5.38 ^a	3000.00±2.1ª
Seed coat	593.06±3.98 ^b	240.00±1.21 ^b
Seed	51.53±4.10°	17.03±0.37°

Values in same column with different superscript differ significantly

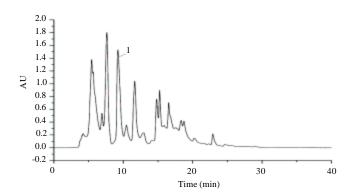


Fig. 2: HPLC chromatogram of amla fruit powder at 280 nm, 1: Gallic acid

(Fig. 2), respectively. Total phenolic content and gallic acid content in amla fruit powder was approximately 15 times more than that in seed coat powder (Fig. 3a, Table 3). On the other hand, seed powder had very negligible levels of total phenolic content and gallic acid (Table 3, Fig. 3b). Kumaran and Karunakaran (2007) also reported that gallic acid is the major component of ethyl acetate extract of amla. These results do not agree with the findings of Mayachiew and Devahastin (2008) who observed that gallic acid is not the main component of amla.

From the HPLC analysis, it might be inferred that though the total phenolic content of seed coat powder is significantly less than amla fruit powder, DPPH* scavenging activity of the seed coat powder is comparable with fruit powder which suggested that seed coat powder has very good potential in terms of bioactive properties. The lower value of total phenolic content in seed and seed coat may be because of the presence of high ratio of bound phenolics whereas in amla fruit the major portion of phenolics is present in free form (Kumaran and Karunakaran, 2007).

FTIR analysis: FTIR spectroscopy was performed to obtain the finger prints of all three samples (Fig. 4). On comparison the IR spectra of amla fruit, seed coat and seed powder it was found that the seed coat and seed powders showed drop in relative peak intensities of 2924, 2854, 1461.71 and 1162.85 cm⁻¹. Seed coat and seed powder showed no relative peak at 666.26 and 509.09 cm⁻¹ which were present in fruit powder. Broad signal in the range 3200-3420 cm⁻¹ indicates the presence of O-H. The presence of C-O stretching in the range of 1200-1000 cm⁻¹ in all three samples confirms the presence of phenolic content. Presence of two weak bands in the range of 1230-1030 cm⁻¹ may also indicate the presence of tertiary amines in all three samples tested. The peak intensity of the seed powder was significantly less than seed coat and amla fruit powder which is supported by the poor DPPH* radical scavenging activity and HPLC analysis.

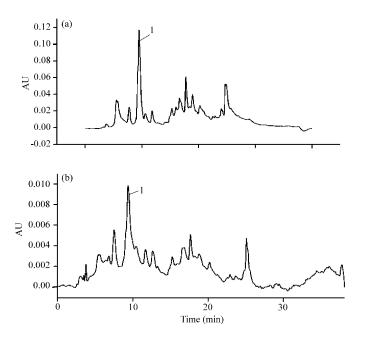


Fig. 3(a-b): HPLC chromatogram of (a) Seed coat and (b) Seed powder at 280 nm, 1: Gallic acid

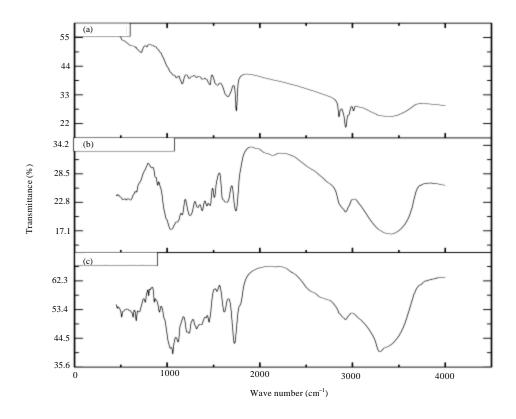


Fig. 4(a-c): FTIR spectra of amla fruit powder, (a) Seed, (b) Seed coat powder and (c) Fruit powder

CONCLUSION

The fruit, seed coat and seed of *Chakaiya* variety of amla were analyzed for their functional characteristics by GC-MS and HPLC and major and micro mineral analyses were conducted by AAS. Seed coat powder was found comparatively more potent than seed powder in terms of hydration property. The % DPPH* scavenging activity of seed coat was observed to be similar to fruit powder. Both seed and seed coat had good amount of major and micro minerals. Seed powder had very good amount of P, K, Mn and Co whereas seed coat was a good source of Ca, Cr, Co and Fe but comparatively poor source of P and K when compared to fruit powder. Fatty acid profile of seed and seed coat showed that the major portion of fatty acid is unsaturated in nature and ω -3 fatty acid is the major fatty acid of the seed. So it can be concluded from the present study that seed coat may be a good source of antioxidants and may be used for value addition of products alone or in combination with seed. Seeds being very good source of protein, minerals, ω -3, ω -6 fatty acids can be used to enrich foods. Combined utilization of amla seed and seed coat with better hydration and water retention properties and higher P, Cr, Co, Fe, Mn and ω -3 and ω -6 fatty acids levels than fruit powder will be more fruitful.

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