



Characterization of seed kernel oil of Bangladeshi mango and its evaluation as cosmetic ingredient

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Abstract

The physicochemical properties, fatty acid composition, aflatoxin contamination and heavy metals of extracted mango kernel oils of four Bangladeshi varieties (Langra, Gopalbhog, Khirshapat & Amrupaly) were studied. The results were compared with published values of 'CIR expert panel', 'Indian Standard of Mango Kernel Fat', 'Scientific Commission of the European Community' and 'US Food and Drug Administration (FDA)'. The estimated values of aflatoxin B1 and total aflatoxins were 1.45809 ppb and 5.14761ppb respectively which were lower than the limit levels of 'European Community (2 ppb for aflatoxin B1)' and 'US FDA (20ppb for total aflatoxins value)'. Heavy metals like arsenic & lead concentration were found 0.034 & 0.45 ppm respectively which were lower than the value of 'Indian Standard of Mango Kernel Fat; 9231 (1979)' (0.5 & 5.0 ppm respectively). In fatty acid composition; palmitic acid, stearic acid, arachidic acid, oleic acid and linoleic acid were within the range of 'CIR expert panel' reported values.

Key words: Mango kernel oil; physicochemical properties; fatty acid composition; aflatoxin contamination; heavy metals; CIR Expert Panel

Introduction

The mango (*Mangifera indica*, Linn), a member of the Anacardiaceae family is one of the most common, important and popular fruits in Bangladesh and is considered as the "king of fruits". Bangladesh is one of the major mango producing countries along with India, Pakistan, Mexico, Brazil, Philippines, etc (Alexander, 1989). In Bangladesh, 32011 hectares of land are used for the cultivation of mango with an annual production of 1047849 metric tons estimated by Bangladesh Bureau of Statistics, 2011 (Barua *et al.*, 2013). In the year 2014, the mango production estimated by the agriculture extension department of Bangladesh was 945000 tons. There are nearly 100 cultivars of mango available in Bangladesh. Besides the fresh fruit, processed mango products such as juices, nectars, concentrates, jams, jelly powders, fruit bars, flakes and dried fruits have become increasingly popular in the world. The seed content of different varieties of mangoes ranges from 9% to 23% of the fruit weight (Palaniswamy *et al.*, 1974) and the kernel content of the seed ranges from 45.7% to 72.8% (Hemavathy *et al.*, 1988). Byproducts of industrial mango processing may amount to 35% to 60% of the total fruit weight.

The physicochemical properties and fatty acid composition of mango kernel oil were studied from the varieties of Thailand, Congo, Northwestern part of Madagascar island and Bangladesh (Kittiphoom and Sutasinee, 2013; Nzikou *et al.*, 2010; Gaydou and Bouchet, 1984; Ali *et al.*, 1985). The four Kenyan varieties were compared with the Borneo illipe, shea butter, tallow and cocoa butter and suggested to be used in the food industry as a substitute for these fats (Muchiri *et al.*, 2012). Bangladeshi variety kernel oil compared with cotton seed oil and utilized in butterscotch toffee (Moharram and Moustafa, 1982). Rukmini and Vijayaraghavan studied on nutritional and toxicological evaluation of mango kernel oil by multigeneration breeding in Weanling Albino rats (Rukmini and Vijayaraghavan, 1984). All these works evaluated the mango kernel fat as edible oil.

The present study emphasizes on the investigation of aflatoxin contamination in kernel oil of Bangladeshi mango cultivars. Aflatoxins (B₁, B₂, G₁, G₂), which are potent carcinogens, their contamination must be controlled in

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cosmetics and the value should not cross the limit. For this reason, aflatoxins were investigated in Bangladeshi mango kernel oil. Moreover, characterizing its physicochemical properties and fatty acid composition and the values of those were compared with the 'CIR expert panel' and 'Indian Standard of Mango Kernel Fat', reported values as a cosmetic ingredient. This study also promote utilizing Bangladeshi mango kernel oil in cosmetics instead of mineral & petroleum oil which can clog pores of skin, leading to breakouts.

Materials and methods

Materials

Ripe mangoes were collected from the local market 'Shaheb Bazar, Rajshahi, Bangladesh'.

Methods

Extraction of oil

The mango kernel powder was used for oil extraction with n-Hexane as solvent by using soxhlet apparatus. First of all, seeds of ripe mango (5 kg) were taken. Then kernels were brought out from outer hard shell. Then they were cleaned, crushed and dried (using a solar dryer, drying loss 80%). After then, making powder and sieving (sieve-mesh no. 100) and the powder was ready for oil extraction where sample and solvent ration was 1:5(g/ml). The yield was 65.7 g (11.5% on dry basis and 1.314% on wet basis).

Physicochemical properties investigation

Physicochemical properties of the kernel oils of four different mango cultivars have been investigated together (1:1:1:1). Iodine absorption number of oils was determined by Hanus Method (AOAC 920.158) Peroxide value was determined by Titration Method (AOAC 965.33). Saponification number was determined by Titrimetric Method (AOAC 920.160). Free fatty acid value was determined by Titration Method (AOAC 940.28). Unsaponifiable matter of oil was determined by Ether Extraction Method (AOAC 933.08). Refractive Index was determined using analog refractometer at 40°C (Analog Abbe-Refractometer, Model: AR4, Origin: Germany). Melting point was determined by Capillary Tube Method (AOAC 920.157). Moisture and volatile matter (total) in oil was determined by Vacuum Oven Method (AOAC 926.12). Relative Density was determined by Digital Density Meter at 30°C (Anton Paar, Model: DMA 35N, Origin: Austria).

Fatty acid composition analyses

Fatty acid composition was analyzed by Gas Chromatograph (Model 14B SHIMADZU, Japan, loaded with software Class GC-10, version-2.00) fitted with Flame Ionization Detector (GC-FID), and capillary column (length

15m and ID 0.25 mm). The operating condition was programmed at oven temperature 150°C (hold time 5 min), 8 C/min - 190°C, 2 C/min - 200°C (hold time 10 min), injection port temperature 250°C and detection temperature 250°C. Nitrogen was used as carrier gas, flow rate 20 ml/min and aliquots of 1 µl FAME (formed by esterification of oil samples following the AOAC 969.33) was injected and the peaks of fatty acids were recorded for their respective retention time and areas comparing with known fatty acid standards.

Heavy metal determination

Arsenic (As) and Lead (Pb) sample were digested following acid digestion procedure. 5g of sample was heated with HCl and HNO₃ (1:1) mixture in a beaker until white fume came out. Then it was filtered and made a volume in a 100 ml volumetric flask. After then it was analyzed using Atomic Absorption Spectrometer (model: AA220) by cold vapor hydride generation technique and applying method APHA 3114.C for As whereas Pb was analyzed using Zeman Atomic Absorption Spectrometer (model: AA240Z) and applying method APHA 3113.B Standard Methods for the Examination of (Water and Waste water, 1998).

Determination of aflatoxins

The standard aflatoxins were dissolved in benzene and acetonitrile (98: 2) to obtain a concentration of 0.5 µg/ml aflatoxin B₁ and G₁ and 0.25 µg/ml for B₂ and G₂. Appropriate aliquots were taken to give specific concentration of the individual aflatoxins.

The AOAC method (970:40, 1990) was followed for the purification of aflatoxins from extracted mango kernel oil of four varieties together (1:1:1:1) and were determined by High-Performance Liquid Chromatography (HPLC). Purification procedure: 250 ml of methanol-water (55:45) were added to oil sample (50g), then 50 ml 0.1 N HCl were added, the mixture was blended and then centrifuged, and filtered through filter paper Whatman No 1. Fifty milliliter of filtrate was transferred into a 250 ml separating funnel; 50 ml 10% NaCl solution were added and swirled; 50 ml of hexane were added and gently shaken for 30 s. The separated lower aqueous layer phase was drained into another 250 ml separator; then extracted by 3x25 ml dichloromethane and were added to aqueous phase and shaken vigorously for 30 s. Then the phases separated and the lower dichloromethane layer was drained, collected and evaporated on a boiling water bath to dryness.

Hexane (200 µL) was added to the extract and 50 µL Tri-Floro-Acetic acid (TFA) and mixed on a Vortex for 30s; allowed to stand for 5 min then 1.950 µL water-acetonitrile (9+1) was added. Mixed vigorously on Vortex for 30s, and

allowed layers to separate for 10 min; and lower layer of acetonitrile-water phase was taken in vial for HPLC determination. The HPLC equipment was a Agilent reverse phase HPLC system (series 1100, Germany) with FLD (fluorescence detector). The mobile phase was (methanol: acetonitrile: de-ionized water; 22.5: 22.5: 55) and the flow rate was 1.0ml/minute. The aflatoxin concentrations in the sample extract were determined and quantified by the retention time and peak areas, respectively.

Statistical analysis

The extractions and all analyses were carried out at least in triplicate and data were expressed as means \pm standard deviation.

Results and discussion

Physicochemical properties

Chemical properties including iodine number, peroxide value, saponification value, unsaponifiable matter, free fatty acid value and other physical properties such as refractive index, relative density, moisture etc were shown in Table-1. The chemical properties represent the present condition of fat.

Acid value is the measure of free fatty acids (FFA) present in the fat or oil, and define as the weight of KOH in mg needed to neutralize the organic acids present in 1g of fat and the causes of increasing the FFA value are high temperature, relative humidity, tissue damage etc. The FFA

regard to FFA value, it can be concluded that the crude mango kernel fat could be applicable for industrial non-edible uses only.

Iodine value (number) is the mass of iodine in grams that is consumed by the amount of unsaturation in 100 grams of fatty acid. Oil with lower iodine number can be used for making soap whether higher iodine number containing oil can be used as drying oil. In this study, the iodine value was 49 which were within the range of 'CIR expert panel' accepted value 32-93 (Table-I); therefore, it can be suitable for use in cosmetics. The value was also comparable to some other varieties cultivated in Kenya (Muchiri *et al.*, 2012).

Saponification value is the number of milligrams of potassium hydroxide required to saponify 1g of fat under a specific condition. In this investigation, it was 196 (Table-I) where 'CIR expert panel' accepted value and 'Indian Standard of mango kernel fat; 9231 (1979)' values are 190-195 and 185- 198 respectively. Therefore, mango kernel fat of the present investigation is comparable with these two standard values and it could be recommended to be used as cosmetics ingredient. It is also comparable with the Kent variety (195.9) from Kenya (Muchiri *et al.*, 2012).

Peroxide value gives the initial evidence of rancidity in unsaturated fats and oils and the value should be less than 10 milli-equivalents/ kg for fresh oils. In this study, this

Table I. Comparison of extracted Mango Kernel Oil (means \pm SD) with "CIR expert panel reported Mango Seed Oil" and "Indian Standard of Mango Kernel fat; 9231 (1979)"

Parameters	Extracted mango kernel oil	"CIR expert panel reported Mango Seed Oil" (Burnett and Fiume, 2011)	"Indian Standard of Mango Kernel fat; 9231 (1979)" (Kisan <i>et al.</i> , 1979)	
			Raw Grade1 (not for direct edible use)	Raw Grade2 (for industrial non-edible uses only)
Appearance	Pale yellow to ivory cream color	Pale yellow to ivory cream color	Greyish white	Greyish white
Odor	Characteristics odor	--		
Moisture (%)	0.84 \pm 0.045	--	0.5	0.5
Relative Density at 30°	0.905 \pm 0.002	--	--	--
Melting point (°C)	34	34 to 43	--	--
Refractive Index at 40°C	1.4610 to 1.4620	1.456 at 20°C	1.4394 to 1.4498 at 60°C	--
Iodine Number	48.95 \pm 0.05	32 to 93	32 to 57	32 to 57
Saponification value as KOH(mg/g)	196	190-200	185 to 198	185 to 198
Unsaponifiable mater (%)	2.3 \pm 0.1	0.8-2.9	2.5	2.5
Peroxide value (meq O2/kg)	1.73 \pm 0.03	--	--	--
Free Fatty Acid Value as KOH (mg/g)	11.85 \pm 0.05	--	6	20
Solidity at 20 °C (68 °F)	Semi solid	Semi solid	Semi solid	Semi solid

value of extracted oil was 11.85 \pm 0.05 mg/g (Table I); according to the 'Indian Standard of mango kernel fat, 9231 (1979)', the value of FFA for indirect edible use and direct industrial use are 6 mg/g and 20 mg/g respectively. So with

value was 1.7 (Table-I). The peroxide values of other investigations on Bangladeshi varieties Fazli and Langra was 0.5 each (Ali *et al.*, 1985).

Unsaponifiables are components of an oily (oil, fat, wax)

mixture that unable to form soaps when blended with sodium hydroxide and the percentage limits are <1% is low & 6-17% is high. In this research, this value was found 2.3 for crude mango kernel fat whereas 'Indian Standard of mango kernel fat; 9231 (1979)' of raw grade1 (indirect edible) and 'raw grade2' (non-edible use only) is 2.5 for each grade (Table-I). The amount of unsaponifiable matters from Congo variety is 4.58% which guarantees the use of mango kernel oil in cosmetics industry (Nzikou *et al.*, 2010). The value of this investigation was also relevant to Kenyan varieties (2.26 – 2.74) (Muchiri *et al.*, 2012).

The refractive index of Bangladeshi mango kernel fat was 1.4610 to 1.4620 at 40°C where 'CIR expert panel' established value is 1.456 at 20°C and 'Indian Standard of Mango kernel fat; 9231 (1979)' value is 1.4394 -1.4498 at 60°C. The value of the present investigation was also comparable to the Zairean varieties Gedong (1.4600), Haden (1.4601), M'Vuazi 1 (1.4600) and Tumba (1.4604) (Van, Foma and Boni, 1980).

The melting point of the Bangladeshi four varieties together was found 34°C which was comparable to the 'CIR expert panel' accepted value 34-43°C.

Fatty acid composition

The fatty acid composition of kernel oils of four Bangladeshi mango cultivars were compared with the 'CIR

mainly used in the production of soaps, detergents and cosmetics such as shampoos and shaving cream. In this investigation, Amrupaly which is the hybrid variety had the highest composition of stearic acid (40.70%) whereas Langra had the lowest (37.91%). Gopalbhog and Khirshapat had 38.71% and 38.94% respectively. The values of stearic acid of these four varieties were within the range of 'CIR expert panel' reported value (33-48). Stearic acid of Langra variety was also comparable to Congo variety (37.9%) (Nzikou *et al.*, 2010). Arachidic acid which is a minor saturated component found highest in khirshapat (1.74%) and the lowest found in Gopalbhog (0.86%). Langra and Amrupaly had almost the same concentration; 1.35% and 1.38% respectively. The values of Arachidic acid in all varieties were within the range of 'CIR expert panel' reported value (1-7%). Behenic acid which is often used for making hair conditioner found in Amrupaly (0.32%), Langra (0.25%) and Khirshapat (0.29%) but Gopalbhog contained trace amount. Oleic acid, the monounsaturated fatty acid was found highest amount in Langra (49.66%), whereas Gopalbhog, Khirshapat, and Amrupaly contained almost the same amount; 47.97%, 47.26% and 47.37% respectively which were within the range of 'CIR expert panel' reported value'. The sodium salt of oleic acid is used as an emulsifying agent in soap and also used as an emollient. Another monounsaturated fatty acid was Eicosenoic acid which found highest in Gopalbhog

Table II. Comparison of fatty acid composition in mango kernel oil with "CIR expert panel reported Mango Seed Oil" (Burnett and Fiume, 2011)

Fatty Acids (%) ± SD	Langra	Gopalbhog	Khirshapat	Amrupaly	"CIR expert panel report of Mango Seed Oil"
Palmitic Acid (C16:0)	5.96±0.007	6.25±0.005	6.32±0.005	5.38±0.008	5-8
Stearic Acid (C18:0)	37.91±0.009	38.71±0.011	38.94±0.014	40.70±0.018	33-48
Arachidic Acid (C20:0)	1.35±0.009	0.86±0.006	1.74±0.001	1.38±0.015	1-7
Behenic Acid (C22:0)	0.25±0.005	Trace	0.29±0.002	0.32±0.003	--
Total Saturated Fatty Acids	45.47	45.83	47.29	47.78	--
Oleic Acid (C18:1)	49.66±0.017	47.97±0.033	47.26±0.005	47.37±0.05	35-50
Eicosenoic Acid (C20:1)	0.08±0.001	0.52±0.002	Trace	0.08±0.004	--
Linoleic Acid (C18:2)(Omega-6)	4.51±0.06	5.28±0.023	5.11±0.006	4.46±0.005	4.0-8
Linolenic Acid (C18:3)(Omega-3)	0.28±0.002	0.40±0.005	0.34±0.003	0.31±0.002	--
Total Unsaturated Fatty Acids	54.53	54.17	52.71	52.22	--

expert panel' reported values (Table-II). Eight fatty acids; Palmitic acid (C16:0), Stearic acid (C18: 0), Arachidic acid (C20:0), Behenic acid (C22:0), Oleic acid (C18:1), Eicosenoic acid (C20:1), Linoleic acid (C18:2) and Linolenic acid (C18:3) were detected in oils of all the four varieties studied. Among them, stearic acid which is a saturated fatty acid has antiviral and anti-inflammatory activities. It is

(0.52%), Langra and Amrupaly contained 0.08% and 0.07% respectively, whereas Khirshapat had trace amount. Linoleic acid is a polyunsaturated essential fatty acid, which is used in the biosynthesis of prostaglandins and cell membranes. It was found highest in Gopalbhog (5.28%); the other varieties, Khirshapat, Langra and Amrupaly contained (5.11%), (4.49%) and (4.46%) respectively. The values of all these

varieties are within the range of 'CIR expert panel' reported value (Figure-II). Linolenic acid, a polyunsaturated fatty acid is involved in the formation of prostaglandins. In this study, the highest Linolenic acid found in Gopalbhog (0.40%); Khirshapat, Amrupaly, and Langra contained 0.34%, 0.31% and 0.28% respectively. In total, saturated and unsaturated fatty acids found in Bangladeshi four mango cultivars were 45.47 – 47.78 % and 52.22 – 54.53 % respectively. Similarity was observed in Iranian variety, where saturated and unsaturated fatty acids were 44-48% and 52- 56 % respectively (Jusof and Hamid, 2013). Bangladeshi varieties contained slightly higher amount of stearic and oleic acid but lower amount of palmitic, linoleic, arachidic and linolenic acids compared to the another investigation where stearic acid 32.7-44.0%, oleic acid 43.7-53.4%, palmitic (6.7-9.7%), linolic (3.6-6.9%), arachidic (1.1-2.5%) and linolenic (0.3-1.0%) acids (VanPee *et al.*, 1981).

Heavy metals contamination

Heavy metals and pesticide residues may be present in botanical ingredients like fatty acid oils and should be continued to use the necessary procedures to limit these impurities in the ingredient before blending into cosmetic formulations (Burnett and Fiume, 2011). In this study, heavy metals like Pb & As present in mango kernel oil were 0.45 & 0.034 mg/kg respectively (Table-IV), which were below the limit of Indian Standard (Specification for mango kernel fat) where the maximum limit of Pb and As are 5.0 & 0.5 mg/kg respectively.

Table III. Comparison of aflatoxins in mango kernel oil with the limit level of European Community and US FDA

Name of aflatoxins	Estimated value (ppb) in Extracted oil	Limit level (ppb) in food material by European Community	Limit level (ppb) in food material by US FDA
Aflatoxin B ₁	1.45809	2.00	--
Aflatoxin B ₂	0.28136	--	--
AflatoxinG ₁	--	--	--
Aflatoxin G ₂	3.40816	--	--
Total	5.14761	--	20.00

Table IV. Comparison of toxic metals in the mango kernel oil and "Indian Standard of Mango kernel Fat; 9231 (1979)" (Kisan *et al.*, 1979)

Minerals/ Heavy Metals (ppm)	Extracted Mango Kernel Oil (means ± SD)	"Indian Standard of Mango kernel Fat; 9231 (1979)
Lead(Pb),	Max 0.45±0.007	5.0
Arsenic(As), Max	0.034±0.003	0.5

Aflatoxin contamination

Aflatoxins are metabolic products of the molds *Aspergillus flavus* and *Aspergillus parasiticus*, may occur in stored agricultural crops (such as peanuts and other nut crops) when growth conditions and genetic requirements are available (Wood, 1989). Humid condition and heavy draught (while harvesting plant) both can also cause the growth of these mycotoxins. The common aflatoxins are B₁, B₂, G₁, and G₂. Among these mycotoxins, the aflatoxin B₁ is most toxic followed by G₁, the toxicities of B₂ and G₂ are relatively weak. The International Agency for Research on Cancer (IARC) concluded that there was sufficient evidence in humans for the carcinogenicity of naturally occurring aflatoxins (Report on Carcinogens, Thirteenth Edition; Aflatoxins CAS No. 1402-68-2). In this study, the value of aflatoxin was (B₁= 1.45809 ppb, G₁= not detected, B₂= 0.28136 ppb and G₂= 3.40816 ppb) in total 5.14761ppb (Table-III). Depending on the degree of development and economic consideration, the legal limits of aflatoxins may vary from one country to another. The Scientific Commission of the European Community has regulated the maximum allowable level of 2 ppb for B₁ (Commission of the European Communities, 2001). In the US, the FDA has set a maximum acceptable level of 20 ppb for total aflatoxins in all foods for human consumption (Creppy, 2002). The limit value reported in 'Indian Standard for mango kernel fat IS 9231 (Kisan *et al.*, 1979)' is 5ppm. The detected B₁ level in this study was found to be lower than the legal limits that regulated by European Community. The estimated total aflatoxins' level was also observed lower than the limits established by FDA in US and Indian Standard. This indicated that the mango kernel oil in Bangladesh is safe for use. But as there is no legal limit by our country's regulatory body so it would not be wise to use this crude oil as edible use. Moreover unrefined oils are more susceptible to aflatoxin contamination than refined oils (Idris *et al.*, 2010), so it may safer to apply this crude oil as cosmetic ingredient.

Conclusion

The crude mango kernel oil of these four varieties (Langra, Gopalbhog, Khirshapat & Amrupaly) in Bangladesh are suitable for use as a cosmetic ingredient. The estimated aflatoxin B₁ at level of 1.45809 ppb and in total of 5.14761ppb, which are within the safe limit according to European Community (2 ppb) and US FDA (20 ppb) respectively. On the perspective of our country's humid condition, it is safer to use this crude oil in cosmetics rather than edible use. For being a cosmetic ingredient the heavy metals concentration must be controlled and the estimated values are 0.45 ppm for Pb and 0.03 ppm for As which are

within the limit values (Pb, 5.0 and As, 0.5 ppm) in 'Indian Standard'. The physicochemical properties and fatty acid composition revealed that the values are within the range of 'Indian Standard' and 'CIR expert panel' reported values which also permit it to use in cosmetics.

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