J Am Oil Chem Soc (2016) 93:823–836
DOI 10.1007/s11746-016-2825-6

ORIGINAL PAPER

Lipids and Fatty Acid Profiling of Major Indian *Garcinia* Fruit: A Comparative Study and its Nutritional Impact

Mahesh Mansing Patil[1,2] · Ali M. Muhammed[3] · K. A. Anu-Appaiah[1,2]

Received: 11 January 2016 / Revised: 16 February 2016 / Accepted: 26 March 2016 / Published online: 8 April 2016

Abstract The effects of plant oils on health are being intensively studied. Many fatty acids have attracted significant scientific attention since the studies pointed them as potential nutrients. An attempt was made to analyze the variation in three major Indian *Garcinia* fruits for their oils, lipid sub-classes, fatty acids and total amino acids. Solvent extraction and chromatographic techniques were used for the isolation, purification, separation and detection of these compounds. Three major *Garcinia* fruits *G. gummi-gutta*, *G. indica* and *G. xanthochymus* varied in their chemical composition. Oil content was significantly higher ($p < 0.05$) in *G. xanthochymus* seeds (16.9 %) when compared to 11.21 % in *G. gummi-gutta* seeds. Fatty acids observed were mainly capric, undecanoic, lauric, palmitic, stearic, oleic and linolelaidic acid. Monounsaturated fatty acids (MUFA) were predominant in both pericarp and seeds with oleic acid being the major fraction (29.24-58.6 %). The lipid classification of *Garcinia* oils showed the varying percentage of neutral lipids, glycolipids and phospholipids. Oleic acid (32.91–71.54 %) was found to be the major fatty acid in neutral-, glyco- and phospho-lipids. Alanine, leucine, proline and phenylalanine were the predominant amino acids found in *Garcinia* fruits. The study has broadened our understanding related to the different biochemical composition of *Garcinia* fruits, thereby providing the groundwork that may lead to the production, utilization and application of products from *Garcinia* in a more efficient way.

Keywords *Garcinia gummi-gutta* · *G. indica* · *G. xanthochymus* · Fatty acid composition · Neutral lipids · Glycolipids · Phospholipids · Amino acids

Introduction

Consumer resistance to synthetic additives is increasing with a growing interest in natural product use for human food and animal feed industries [1]. *Garcinia* fruits have been used in Ayurveda to medicate various pathophysiological disorders. *Garcinia* species belong to the family Clusiaceae and are known as a rich source of metabolites having therapeutic properties [2–4]. Throughout the years, *Garcinia* trees have considerable value as sources of food medicines, pigments, gums, waxes, resins, fuel and lumber [5]. The fruits have been used for centuries in Asian countries, for culinary purposes. It is used as a condiment in places of common souring agents like tamarind or lemon to give flavor, taste, and it also improves the shelf life of the product [6, 7].

The syrup formulated from *G. indica* fruits locally known as 'Kokum' has a beneficial effect on skin damage and allergies from the tropical climate [8]. Soup from kokum fruits known as 'birindi saar' and 'kokum kadi' are digestive and relieve gastric problems [9]. Kokum juice is a favorite cold drink during summer months. *G. gummi-gutta* fruits are acidic in taste and used as a substitute for polishing of gold and silver, as a replacement for acetic or formic acid and in coagulating rubber latex [7]. Flavonoids

✉ K. A. Anu-Appaiah
anuapps@yahoo.com

[1] Department of Microbiology and Fermentation Technology, CSIR-Central Food Technological Research Institute, Mysuru 570 020, India

[2] Academy of Scientific and Innovative Research (AcSIR), CSIR-CFTRI, Mysuru 570 020, India

[3] Department of Meat and Marine Sciences, CSIR-Central Food Technological Research Institute, Mysuru 570 020, India

from *G. gummi-gutta* were found to exert hypolipidemic activity *in vivo* [10]. Phenolic compounds isolated from *G. xanthochymus* fruits, bark and leaves exhibited antioxidant, anticancer and anti-inflammatory activities [7]. Fermented beverages from *G. xanthochymus* fruits were well accepted and have the right nutritional attributes [11].

Currently, only a small number of *Garcinia* species are cultivated for food and commerce. *G. gummi-gutta*, *G. indica* and *G. xanthochymus* are the most widely cultivated species in India [12]. *G. gummi-gutta* is largely distributed in Southeast Asia, India, West and Central Africa [13]. *G. indica* is mainly found in India [14] while *G. xanthochymus* is endemic to India, Malaysia, Myanmar and Thailand [15]. *G. gummi-gutta* and *G. indica* are the rich sources of (-) hydroxycitric acid (HCA) and are beneficial in obesity-related complications such as inflammation, oxidative stress, and insulin resistance [16]. In India, the dried seeds of *Garcinia* often yield a protein and fat-rich butter, known as 'uppage tuppa'. *Garcinia indica* fat (kokum butter) is commercially available and is mainly used as an edible fat and also as substitute for cocoa butter [4, 17]. Refined and deodorized fat is white and compares favorably with hydrogenated fats. Kokum (*G. indica*) fat with cocoa butter finds application in chocolates and confectionery [18–20]. The present study dwells on the comparison of the fatty acid composition of fruit pericarp (pulp) and seeds from *G. gummi-gutta*, *G. indica* and *G. xanthochymus* fruits.

The nutritional quality and health benefits of edible oil are ideally determined by the presence of more unsaturated fatty acids (e.g. oleic acid, α-linolenic acid) and lower level of saturated fatty acid (e.g. palmitic acid, stearic acid). The knowledge about the biochemical composition of *Garcinia* fruits cultivated in Indian is limited. Very few studies have been carried out to understand the difference between these fruits in detail on the physical and chemical properties of oils. The aim of the study is to explore the variability in nutritional components of *Garcinia* fruits with special reference to fatty acids. Thus, it is necessary, and useful, to investigate how these fruits differ from each other based on their nutritional attributes and health benefits.

Materials and Methods

Fruit Materials

G. gummi-gutta and *G. xanthochymus* fresh fruits were procured from three cultivars of Kodagu district, Karnataka (India). *G. indica* fresh fruits were collected from Dandeli forest of Uttar Kannada district, Karnataka (India). The fruits were collected for three consecutive seasons of 2012, 2013 and 2014 to account for year to year variability in composition. It was expected to generate a clear understanding about the variability in biochemical composition as these varieties are widely distributed in geographic location.

Chemicals Used

The standard supelco 37 component FAME mix was purchased from Sigma-Aldrich Company Ltd. (Bangalore, India). Chromatography grade *n*-hexane was obtained from Merck Chemicals, Mumbai. Standard solutions of the amino acids, in addition to the eluting and derivatization agents, were all provided in an inclusive amino acid analysis kit (EZFaast GC-FID) purchased from Phenomenex (Torrance, CA, USA). All other chemicals, which were purchased from Merck Chemicals, Mumbai (India), were of analytical grade unless otherwise specified.

Determination of Moisture Content

Moisture content (MC) of the fruit part was determined gravimetrically, according to the method of the American Oil Chemist's Society [21, 22]. Fresh fruit sample was weighed before and after drying in an air circulating oven (Serwell Instruments Incorporation, Bangalore, India). Finely crushed 100 g of the sample was weighed into a clean and dried glass Petri plate (W_1) and dried in the oven at 105 °C until a constant weight was achieved (W_2).

$$\text{MC}\ (\%) = (W_1 - W_2/W_1) \times 100$$

Determination of Oil Content

Seeds and pericarp were subjected to freeze-drying (LT5S, Lyophilization Systems, Incorporation, USA) at a temperature of −90 °C. The dried sample was ground into a powder, and a known quantity was soaked in a chloroform:methanol (2:1) solvent for 24 h with continuous agitation. The clear colored solution was filtered through Whatman No. 1 filter paper. The residue was repeatedly dissolved in the solvent until decolorization had occurred. The combined extract was dried by using nitrogen gas flow.

$$\text{Oil content}\ (\%) = (\text{weight of solvent extract/weight of the dried sample}) \times 100$$

The oil sample of the fruit thus obtained was further used for fatty acid profiling and lipid classification.

Determination of Wax Content

The wax analysis was carried out using a wax precipitation procedure [23] with some modifications. Oil extracted was dissolved in *n*-hexane (1:5 w/w) and stirred for 30 min. Acetone (acetone/*n*-hexane ratio 3:1) was added to the mixture and cooled down to −20 °C for 24 h. The liquid phase

present in the oil was separated using a micropipette. The solid phase was re-dissolved in *n*-hexane to remove non-dissolving materials. After solvent removal, the wax content was analyzed gravimetrically.

Determination of Protein Content

Protein was determined by the Kjeldahl method [24] with minor modifications. A dried powder (0.5 g) sample was digested in 20 mL of sulfuric acid at 380 °C using 1 g of catalyst mixture [potassium sulfate:copper sulfate:selenium dioxide (5:2:1)]. Digested sample was distilled using 40 % NaOH in Kjeldahl distillation equipment (Vapodest 30S, Gerhardt, Germany). Ammonia absorbed in excess of 2 % boric acid solution was titrated with 0.014 N HCl, to estimate total nitrogen content. Protein content was evaluated by using the factor 6.25 [25].

$$\text{Protein content (\%)} = [(V_S - V_B) \times 0.014 \times 14.01/W \times 10] \times 6.25$$

Here, V_S = volume (mL) of standardized acid used to titrate a test; V_B = volume (mL) of standardized acid used to titrate reagent blank; W = weight (g) of test portion or standard.

Determination of Ash Content

The defatted dried powder was placed in a clean and pre-heated crucible (W_1) and heated in a muffle furnace for about 6–8 h at 550 °C until a constant weight of greyish white ash (W_2) was obtained [26]. Desiccator was used for cooling the crucible while weighing and monitoring the weight.

$$\text{Ash content (\%)} = (W_2/W_1) \times 100$$

Total Amino Acid Composition Analysis by Gas Chromatography (GC)

Amino acids were analyzed using a commercial EZ:faast™ amino acid analysis kit (Phenomenex, Torrance, CA, USA). Lyophilized 100 mg *Garcinia* powder (in flame seal tube) was hydrolyzed (liquid phase hydrolysis) at 110 °C for 22 h in 100 µL 6 N HCl containing 4 % thioglycolic acid. The hydrolyzed mixture (100 µL) further derivatized at pH 1.5–5.0 as described in the manual. Amino acid analysis was carried out by GC system (Shimadzu GC 2014; M/s Shimadzu, Kyoto, Japan) fitted with a flame ionization detector (FID) for identifying individual amino acids. Amino acid esters were dissolved in chloroform and analyzed on a Column ZB-AAA-10 m (10 m × 0.25 mm × 0.25 µm; Phenomenex, Torrance, CA, USA) with a split ratio of 1:10. The temperatures of the injector, column and detector were set at 300 °C, 110–320 °C at a gradient rate of 35 °C/min, and 320 °C, respectively. The amino acids were identified by comparing them with authentic standards (Fig. 1).

Column Chromatography Fractionation of the Lipid Classes

The lipid classes of extracted *Garcinia* oils were determined by column chromatography [27, 28]. Briefly, a Borosil glass column (30 cm × 20 mm ID) was packed with activated silica gel (100–200 mesh) by applying a slurry of the adsorbent in chloroform (1:5, w/v). *Garcinia* oil was mixed with 20 mL of chloroform and applied to the column, which was eluted sequentially with 20 washes of chloroform (for neutral lipids), with acetone (for glycolipids), and with methanol (for phospholipids). The solvent was evaporated by rotary evaporation, and the lipid percentage of each fraction was determined gravimetrically. The lipids were dissolved separately in their respective solvents and stored at −20 °C until required for fatty acid profiling.

Preparation of Fatty Acid Methyl Esters (FAME)

The extracted oil was used for complete fatty acid profiling of *Garcinia* fruits. One milliliter extract of each sample was used to obtain FAME [29, 30]. The gas chromatography (GC) method was followed for fatty acid profiling [31]. The experiments were conducted in triplicates. The lipids were transmethylated using 0.2 mL methanolic sodium hydroxide (2 M) followed by 2 M methanolic hydrochloric acid (0.2 mL) to obtain FAME residues.

Fatty Acid Profiling

FAME residues were dissolved in hexane and precaution was taken to maintain concentrations of FAME. The analysis was carried out by GC (Shimadzu GC 2014, Japan). FAME (1 µl) were analyzed using Omegawax™ 320 fused silica capillary column (30 m × 0.32 mm ID × 0.25 µm film thickness) with nitrogen as a carrier gas. The conditions used for GC analysis were; injection temperature of 250 °C, flame ionization detector (FID) temperature of 260 °C and column temperature of 240 °C. The peaks were identified by comparing the retention time with authentic standards (Fig. 2). The unknown FAME peak areas were computed, and relative percentage of individual fatty acid was calculated. The data is presented as the mean of three analyses.

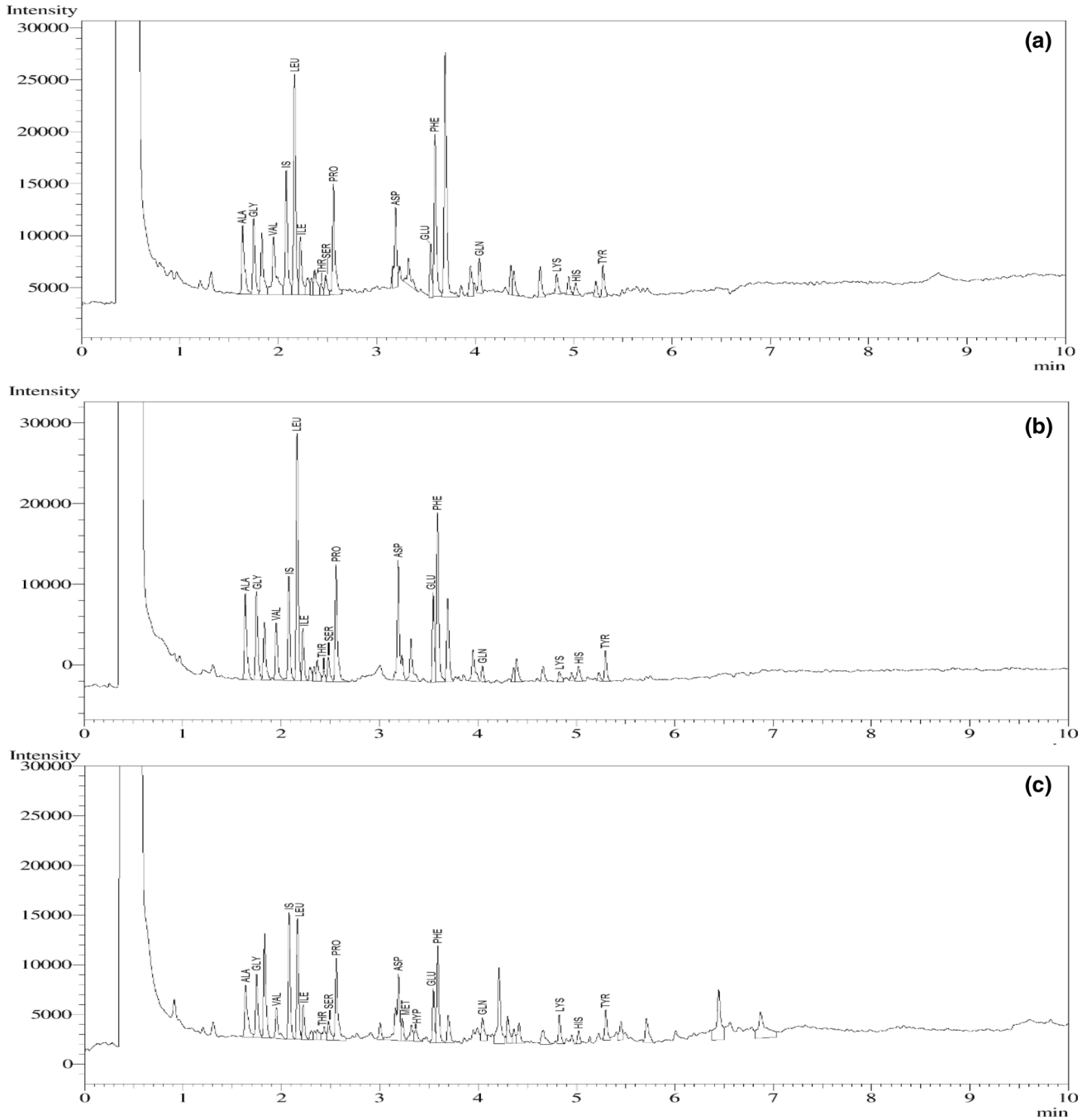

Fig. 1 Representative chromatograms showing amino acids of *Garcinia* seed determined by GC: **a** *G. gummi-gutta*, **b** *G. indica*, and **c** *G. xanthochymus*

Gas Chromatography–Mass Spectrometry (GC–MS) Analyses of FAME

FAME were analyzed by GC–MS (PerkinElmer, Turbomass Gold, Mass spectrometer) equipped with FID. The separation was carried out using a cross bond polyethylene glycol elite-wax column (PerkinElmer, 30 m, 0.32 mm ID and 0.25 μm film thickness). The injector port and detector temperatures were set up 250 and 260 °C, respectively. Helium gas was used as the carrier gas at a flow rate of

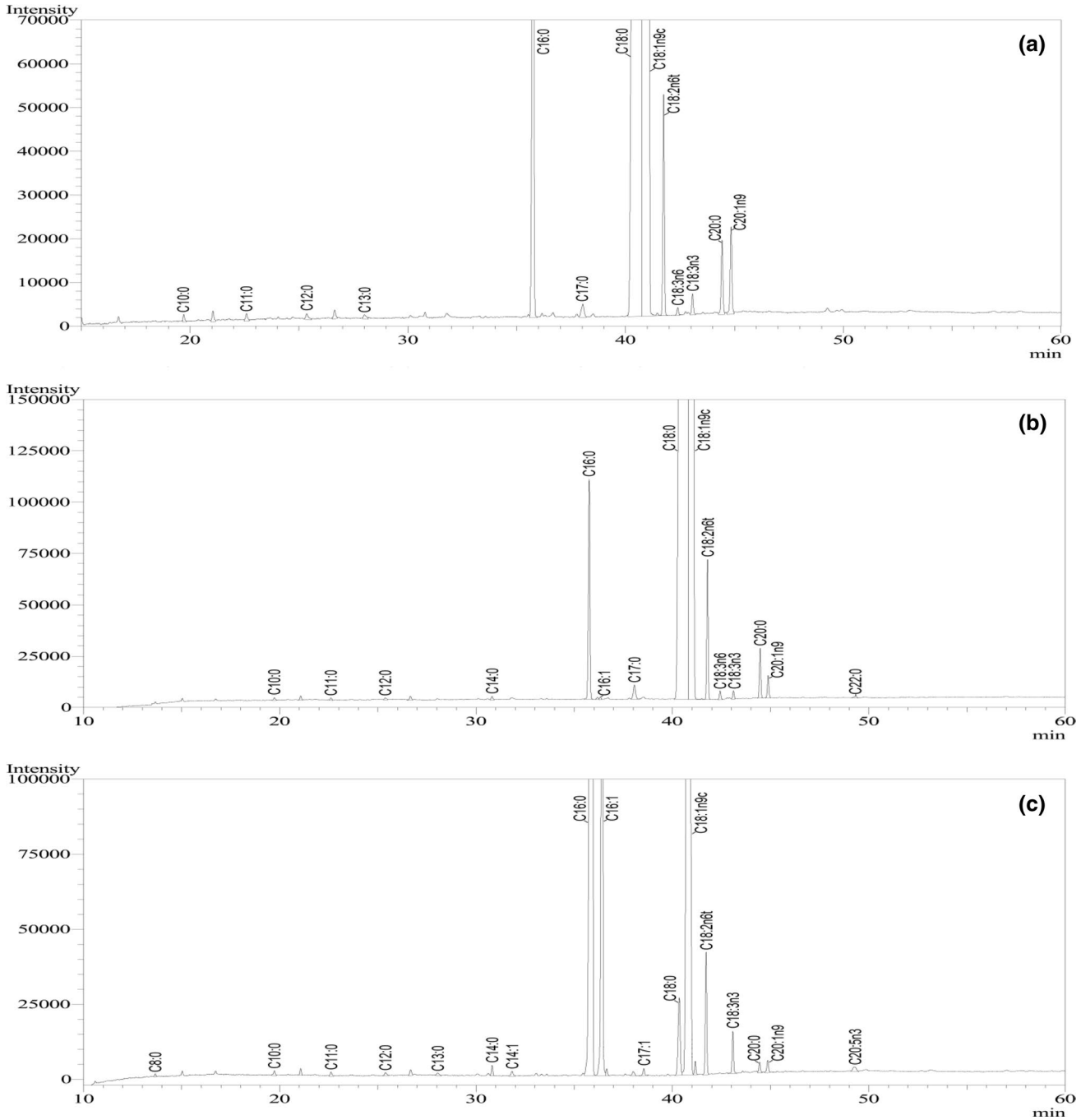

Fig. 2 Representative chromatograms showing FAME of *Garcinia* pericarp determined by GC: **a** *G. gummi-gutta*, **b** *G. indica*, and **c** *G. xanthochymus*

1 mL/min, and the split ratio was 20:1. Initially, the column temperature was maintained at 50 °C for 2 min, which was gradually increased to 240 °C at the rate of 5 °C/min. The column temperature was maintained at 240 °C for 10 min. The constituent identity of the FAME was confirmed by comparing their mass spectral fragmentation pattern with inbuilt library provided along with the instrument and also with the NIST library.

Table 1 Proximate composition of *Garcinia* fruits (g/100 g sample)

Parameter	*G. gummi-gutta*		*G. indica*		*G. xanthochymus*	
	Pericarp	Seed	Pericarp	Seed	Pericarp	Seed
Fruit	93.57 ± 0.76^{a}	06.43 ± 0.43^{d}	88.35 ± 0.79^{b}	11.24 ± 0.32^{c}	86.61 ± 1.15^{b}	13.38 ± 0.32^{c}
Moisture	87.21 ± 0.04^{a}	40.70 ± 0.08^{d}	81.63 ± 0.04^{b}	42.58 ± 0.03^{c}	81.41 ± 0.06^{b}	35.45 ± 1.28^{e}
Ash*	1.95 ± 0.01^{d}	1.78 ± 0.01^{d}	2.24 ± 0.05^{c}	2.62 ± 0.01^{b}	3.0 ± 0.1^{a}	1.04 ± 0.17^{e}
Oil*	4.43 ± 0.26^{c}	11.21 ± 0.43^{b}	1.53 ± 0.12^{d}	16.80 ± 1.63^{a}	1.38 ± 0.07^{d}	16.90 ± 0.82^{a}
Protein*	4.04 ± 0.04^{e}	7.72 ± 0.06^{b}	4.83 ± 0.06^{c}	10.79 ± 0.10^{a}	4.87 ± 0.09^{c}	4.38 ± 0.07^{d}
Wax*	1.1 ± 0.14^{a}	ND	0.3 ± 0.08^{b}	ND	0.2 ± 0.08^{b}	ND

Values are expressed as means ± SD ($n = 3$)

ND not detected

Values in the same row followed by the same superscript letter are not significantly different at $p < 0.05$

* Dry weight (w/w %)

Analysis of the Sample and Residues by Scanning Electron Micrograph (SEM)

Garcinia powder before and after solvent extraction was subjected to SEM [32, 33]. Dry powder passed through 120-micron metal sieve to obtain uniform residues. The shape and surface characters of sample residues were recorded on SEM (LEO 435 VP, LEO Electron Microscopy Ltd., Cambridge, UK).

Determination of Fatty Acid Desaturation Ratios

Oleic desaturation ratio (ODR) and linoleic desaturation ratio (LDR) were calculated. ODR determine the efficiency of the desaturation from oleic acid to linoleic acid and LDR determine the efficiency of the desaturation from linoleic acid to α-linolenic acid. Within the desaturation pathway, ODR and LDR were calculated as follows:

$$\text{ODR} = \frac{\%\text{C18:2} + \%\text{C18:3}}{\%\text{C18:1} + \%\text{C18:2} + \%\text{C18:3}}$$

$$\text{LDR} = \frac{\%\text{C18:3}}{\%\text{C18:2} + \%\text{C18:3}}$$

Statistical Analysis

All estimations were carried out in triplicate making three determinations ($n = 3$). Statistical analysis was carried out to calculate mean ± standard deviation (SD) and summarized the characteristics of the subjects. One-way analysis of variance (ANOVA) was performed to determine the significance of the result using Tukey–Kramer comparison test and Dunnett's test. Minitab 17 (Minitab Ltd., UK) was used for correlation analysis. A difference of $p < 0.05$ was considered statistically significant.

Results and Discussion

Fruit Composition

The pericarp/seed ratio was found to be high in *G. gummi-gutta* fruits (Table 1). As expected, the fresh seeds had significantly ($p < 0.05$) lower moisture content (35.45–42.58 %) than those of fresh pericarps (81.41–87.21 %). Moisture content of seeds analyzed was more than those reported by Naveen and Krishnakumar for the same fruits [34]. Ash content was found to be significantly high ($p < 0.05$) in *G. indica* fruits. The ash content of seeds ranged between 1.04 and 2.62 g/100 g was greater than the values determined for seeds such as coconut but less than those of castor and groundnut oil seeds [35]. Previous studies showed that the free fatty acid concentration was more in *G. xanthochymus* seed [34]. Similar results were observed in the present study. *Garcinia* seed contains a considerable amount of protein, and it appears to be the nutritious part of the fruit. The protein content, *G. gummi-gutta* and *G. indica* seeds (7.72 and 10.79 %, respectively) was greater than the value determined for *G. mangostana* (6.57 %) by Ajayi *et al*. [36]. *G. indica* seeds had significantly ($p < 0.05$) more protein content than other samples.

G. indica pericarp that is used by natives for different culinary and therapeutic purposes contains 18–19 % dry matter. *G. gummi-gutta* pericarp had a high amount of oil compared to *G. indica* and *G. xanthochymus* pericarp. Similar results were reported by Mazi *et al*. [37] in *Garcinia kola*. Since the pericarp was sticky to handle, wax content was estimated which indicated that the pericarp contains 0.2–1.1 % of wax. The wax content was not determined in *Garcinia* seeds.

Total Amino Acid Profile of Fruits

The aim of this study was to contribute to the knowledge about the total amino acids of *Garcinia* fruits. This is

Table 2 Total amino acid composition (g/100 g protein) of *Garcinia* fruits

Amino acid	*G. gummi-gutta*		*G. indica*		*G. xanthochymus*	
	Pericarp	Seed	Pericarp	Seed	Pericarp	Seed
Essential amino acids						
Histidine (HIS)	–	1.36 ± 0.01^{b}	–	1.88 ± 0.04^{a}	–	1.71 ± 0.04^{a}
Isoleucine (ILE)	6.15 ± 0.07^{a}	5.67 ± 0.32^{abc}	4.79 ± 0.08^{d}	5.32 ± 0.11^{cd}	5.86 ± 0.16^{ab}	5.21 ± 0.18^{bcd}
Leucine (LEU)	15.25 ± 0.12^{b}	18.71 ± 0.11^{a}	12.48 ± 0.30^{c}	18.56 ± 0.36^{a}	14.07 ± 0.52^{bc}	13.20 ± 0.54^{bc}
Lysine (LYS)	2.17 ± 0.03^{c}	2.11 ± 0.07^{c}	4.89 ± 0.08^{a}	1.13 ± 0.03^{d}	1.91 ± 0.01^{c}	3.69 ± 0.11^{b}
Methionine (MET)	2.78 ± 0.03^{a}	ND	2.60 ± 0.05^{a}	ND	2.01 ± 0.06^{b}	2.77 ± 0.07^{a}
Phenylalanine (PHE)	12.02 ± 0.21^{bc}	14.60 ± 0.29^{a}	10.89 ± 0.13^{c}	14.73 ± 0.32^{a}	12.59 ± 0.21^{ab}	11.01 ± 0.35^{bc}
Threonine (THR)	ND	1.19 ± 0.02^{c}	1.65 ± 0.03^{b}	1.16 ± 0.06^{c}	–	2.31 ± 0.06^{a}
Valine (VAL)	8.36 ± 0.08^{b}	9.77 ± 0.03^{a}	7.05 ± 0.11^{c}	5.51 ± 0.08^{d}	9.09 ± 0.11^{ab}	4.69 ± 0.04^{d}
Non-essential amino acids						
Alanine (ALA)	12.10 ± 0.14^{a}	7.49 ± 0.04^{c}	11.18 ± 0.18^{a}	7.86 ± 0.05^{c}	9.51 ± 0.35^{b}	8.39 ± 0.17^{bc}
Aspartic acid (ASP)	6.13 ± 0.07^{d}	7.50 ± 0.09^{c}	7.81 ± 0.12^{bc}	11.72 ± 0.23^{a}	8.72 ± 0.32^{b}	6.99 ± 0.22^{cd}
Glycine (GLY)	8.54 ± 0.10^{a}	7.05 ± 0.08^{b}	8.21 ± 0.21^{a}	8.05 ± 0.15^{ab}	6.95 ± 0.12^{b}	8.19 ± 0.33^{a}
Glutamic acid (GLU)	1.78 ± 0.02^{c}	4.58 ± 0.07^{b}	4.47 ± 0.07^{b}	6.93 ± 0.18^{a}	3.96 ± 0.09^{b}	6.48 ± 0.10^{a}
Glutamine (GLN)	9.16 ± 0.15^{a}	3.45 ± 0.05^{c}	2.05 ± 0.03^{e}	1.36 ± 0.01^{f}	2.74 ± 0.05^{d}	3.95 ± 0.09^{b}
Hydroxyproline (HYP)	ND	ND	2.79 ± 0.12^{b}	ND	4.33 ± 0.13^{a}	2.15 ± 0.04^{b}
Proline (PRO)	11.87 ± 0.12^{bc}	11.35 ± 0.12^{bc}	9.39 ± 0.15^{d}	10.64 ± 0.07^{cd}	13.70 ± 0.08^{a}	11.82 ± 0.25^{b}
Serine (SER)	1.75 ± 0.02^{d}	2.07 ± 0.01^{cd}	3.77 ± 0.10^{a}	2.45 ± 0.04^{c}	2.01 ± 0.07^{cd}	3.09 ± 0.12^{b}
Tyrosine (TYR)	1.94 ± 0.02^{d}	3.10 ± 0.03^{c}	5.98 ± 0.15^{a}	2.69 ± 0.09^{c}	2.55 ± 0.03^{c}	4.35 ± 0.17^{b}

ND not detected

Values in the same row followed by the same superscript letter are not significantly different at $p < 0.05$

– Concentration is less than 0.1 %

* Essential amino acids

with a view to the information obtained from this analysis being used to understand and explain its impact on human health. To the best of our knowledge this is the first report and explain the amino acid composition of these fruits (Table 2). There was a remarkably predominance of hydrophobic amino acids such as alanine, leucine, proline and phenylalanine. Several authors have reported that these amino acids are major components of antioxidant peptides and exhibit good antioxidant activity [38, 39]. Leucine and phenylalanine, which are essential amino acids, were found significantly ($p < 0.05$) high in the pericarp of *G. gummi-gutta* and *G. indica* when compared to the seed. Leucine showed significantly ($p < 0.01$) strong positive correlation with phenylalanine (data not shown). Sulfur-containing amino acids like methionine were a minority in some samples while asparagine and tryptophan were not detected. Overall, we conclude that these varieties of *Garcinia* are a good source of hydrophobic amino acids and may exhibit antioxidant properties.

SEM Analysis of *Garcinia*

In order to explain the efficiency of solvent (chloroform:methanol) extraction of fatty acids, the microstructures of residue before and after solvent extraction were examined by SEM. The microstructures of extracted material changed significantly with large pores and aggregated into a larger mass compared with that of material before solvent extraction, which had more compact structure (Fig. 3). It indicated that the extraction changed the inner structure of the materials, due to the higher extraction efficiency of fatty acids.

Fatty Acid Profile of Fruits

The present study demonstrates that *G. gummi-gutta*, *G. indica* and *G. xanthochymus* seeds are good sources of fatty acids. Further characterization of individual fatty acids was carried out by GC (Fig. 2) and GC–MS analysis.

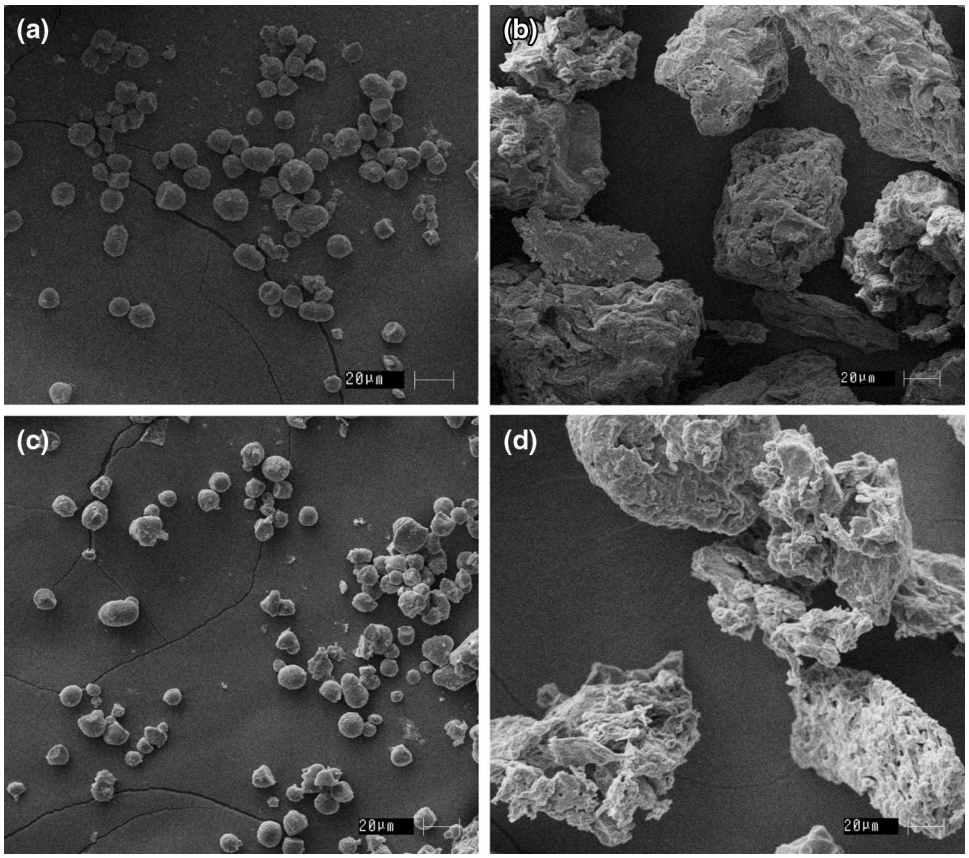

Fig. 3 Scanning electron micrographs of *Garcinia* powder, **a** raw pericarp, **b** pericarp residues obtained after solvent extraction, **c** raw seed, and **d** seed residues obtained after solvent extraction

Comprehensive fatty acids (37 fatty acids) were screened from three commercially important *Garcinia* varieties (Table 3). Earlier Daniel *et al*. [40] had indicated that seed fatty acid percentage increased with seed maturation in *G. indica*. We observed that oleic acid (OA) was one of the primary fatty acids found in all samples of *Garcinia*. Ajayi *et al*. [36] also reported that oleic acid was the most prevalent fatty acid in *G. mangostana* seed oil. The presence of OA in the diet is good for health. It is reported that oleic acid is effective in lowering low-density lipoprotein (LDL) cholesterol level [41]. The relative percentage of OA was significantly higher ($p < 0.05$) in seeds than pericarp. After OA, the other major fatty acid identified was stearic acid (SA) however, it was found to be in a minor quantity in *G. xanthochymus*. The earlier report also indicates that the SA and OA are the primary fatty acids present in *G. indica* seed [40, 42, 43]. Such combinations lead to the solidification of the fats with a rough surface [18]. Whereas palmitic acid (PA) content was found to be high in *G. xanthochymus* fruits compared to the other *Garcinia* fruits.

The fatty acid compositions of palm oil and *G. xanthochymus* seed oil were found to be similar [44]. Among the main edible oils of India, palm oil holds the highest market share of up to 38 % [45]; hence, *G. xanthochymus* seed oil can be the next option for palm oil. In *Garcinia*, it has been observed that many fatty acids were present in very less quantity (<0.1 %) and most of the fatty acids vary from species to species for their presence. The common fatty acids observed were C10:0 (capric acid), C11:0 (undecanoic acid), C12:0 (lauric acid), C16:0 (PA), C18:0 (SA), C18:1n9c (OA) and C18:2n6t (linolelaidic acid). Studies on *Garcinia kola* also revealed that oleic, linoleic and palmitic acid were the dominant fatty acids in the seed, while, PA and linoleic acid (LA, C18:2n6c) were predominant in the hull [46]. Among all fatty acids C13:0 (tridecanoic acid), C18:3n6 (γ-linolenic acid) and C22:0 (behenic acid) were either not determined or were present in very less quantity (<0.1 %) in all fruits.

On screening of fat type, we observed omega-3 and omega-6 fatty acids in *Garcinia* fruits. The mammalian body cannot synthesize essential fatty acids, and these fatty acids are obtained from food sources [47]. Earlier, there were reports that the ratio of omega-6 and omega-3 fatty acids in human's diet has to be 1:1 because, mammalian cells cannot convert omega-6 and omega-3 fatty acids [48]. Our findings suggest that *G. xanthochymus* fruits are close to this ratio while other fruits contained significantly high ($p < 0.05$) amount of omega-6 fatty acids (Table 4). Eicosapentaenoic acid (C20:5n3) is a n-3 long chain fatty acid detected in *G. xanthochymus* seed. n-3 long chain fatty

Table 3 Fatty acid composition (relative %) of *Garcinia* oil

Fatty acid	*G. gummi-gutta*		*G. indica*		*G. xanthochymus*	
	Pericarp	Seed	Pericarp	Seed	Pericarp	Seed
C8:0	1.61 ± 0.004	ND	ND	ND	ND	–
C10:0	2.98 ± 0.002^{a}	–	2.66 ± 0.474^{a}	–	2.58 ± 0.269^{a}	–
C11:0	2.61 ± 0.002^{a}	–	2.57 ± 0.403^{a}	–	2.38 ± 0.141^{a}	–
C12:0	2.99 ± 0.001^{a}	–	2.57 ± 0.304^{a}	–	3.56 ± 0.134^{a}	–
C13:0	ND	–	ND	ND	ND	–
C14:0	ND	ND	ND	–	5.24 ± 0.106	–
C16:0	14.44 ± 0.001^{c}	3.45 ± 0.007^{d}	16.61 ± 2.298^{bc}	1.91 ± 0.092^{d}	20.17 ± 0.601^{b}	38.53 ± 0.134^{a}
C17:0	ND	–	ND	0.14 ± 0.064	ND	ND
C18:0	11.16 ± 0.001^{cd}	36.52 ± 1.492^{b}	13.73 ± 0.863^{c}	58.79 ± 1.280^{a}	8.99 ± 0.460^{d}	1.41 ± 0.007^{e}
C20:0	ND	0.36 ± 0.085^{a}	ND	0.35 ± 0.085^{a}	ND	0.15 ± 0.007^{b}
C22:0	ND	–	ND	–	ND	ND
Σ Saturated fatty acid (SFA)	35.79 ± 0.011^{c}	40.36 ± 1.584^{bc}	38.14 ± 4.342^{bc}	61.20 ± 1.521^{a}	42.92 ± 1.711^{b}	40.12 ± 0.148^{bc}
C14:1	3.56 ± 0.002^{b}	ND	4.04 ± 0.163^{ab}	ND	4.73 ± 0.240^{a}	–
C16:1	2.29 ± 0.001^{c}	ND	ND	–	6.66 ± 0.021^{b}	11.94 ± 0.042^{a}
C17:1	ND	ND	ND	ND	ND	0.14 ± 0.007
C18:1n9c	31.65 ± 0.003^{d}	58.60 ± 1.754^{a}	31.46 ± 1.450^{d}	37.47 ± 0.728^{c}	29.24 ± 0.007^{d}	45.31 ± 0.163^{b}
C18:1n9t	7.90 ± 0.003	ND	ND	ND	ND	ND
C20:1n9	ND	0.18 ± 0.028^{b}	3.54 ± 0.233^{a}	0.18 ± 0.028^{b}	ND	0.19 ± 0.007^{b}
Σ Mono-unsaturated fatty acid (MUFA)	45.40 ± 0.009^{b}	58.78 ± 1.782^{a}	39.04 ± 1.846^{c}	37.65 ± 0.756^{c}	40.63 ± 0.268^{c}	57.60 ± 0.219^{a}
C18:2n6t	12.45 ± 0.004^{a}	0.86 ± 0.007^{c}	13.90 ± 0.156^{a}	1.15 ± 0.078^{c}	6.43 ± 0.106^{b}	1.56 ± 0.007^{c}
C18:2n6c	6.36 ± 0.002^{b}	ND	8.92 ± 0.339^{a}	ND	ND	ND
C18:3n6	ND	–	ND	–	ND	ND
C18:3n3	ND	–	ND	–	10.02 ± 0.042^{a}	0.57 ± 0.007^{b}
C20:5n3	ND	ND	ND	ND	ND	0.15 ± 0.007
Σ Poly- unsaturated fatty acid (PUFA)	18.81 ± 0.006^{b}	0.86 ± 0.007^{c}	22.82 ± 0.495^{a}	1.15 ± 0.078^{c}	16.45 ± 0.148^{b}	2.28 ± 0.021^{c}

Values are expressed as means ± SD ($n = 3$)

ND not detected

Values in the same row followed by the same superscript letter are not significantly different at $p < 0.05$

– Concentration is less than 0.1 %

acids have been recommended for the prevention of cardiovascular disease [49]. α-Linolenic acid (ALA, C18:3n3) was another predominant omega-3 fatty acid mainly, identified in *G. xanthochymus* fruit pericarp and seed. ALA lowers the risk of fatal ischemic heart disease and myocardial infarction. A lower amount of γ-linolenic acid (GLA, C18:3n6) was present in seeds of *G. gummi-gutta* and *G. indica,* which has selective antitumor properties with negligible systemic toxicity [50]. *Garcinia gummi-gutta* and *G. indica* pericarp intake may protect against the development of cancer as these are a good source of LA. Linoleic acid has been reported to be an anticancer molecule [51].

The comparative study of saturated and unsaturated fatty acid indicates that the percentage of total unsaturated fatty acids was significantly high ($p < 0.05$) in all fruits (Table 4). Our findings suggest that *Garcinia* fruits are rich in monounsaturated fatty acids (MUFA). It has been reported that MUFA rich diet reduces cardiovascular disease (CVD) risk by favorably modulating blood lipids [52]. MUFA also contributes to reducing coronary heart disease (CHD) in both healthy adults and those with established chronic disease. Comparatively polyunsaturated fatty acids (PUFA) were found to be significantly high ($p < 0.05$) in pericarp than the seeds.

Lipid Classes and Their Fatty Acid Composition

Garcinia oil was further studied for lipid sub-classes, i.e. neutral lipids, glycolipids and phospholipids with fatty acid composition. Lipid sub-classes of *Garcinia* oil are

Table 4 Comparison of saturated and unsaturated fatty acids composition of *Garcinia* fruits

Fatty acids	*G. gummi-gutta*			*G. indica*			*G. xanthochymus*		
	Pericarp	Seed	Fruit	Pericarp	Seed	Fruit	Pericarp	Seed	Fruit
Total saturated fatty acids (%)*	35.79 ± 0.011^{x}	40.36 ± 1.584^{x}	$38.07 \pm 0.798^{b,x}$	38.14 ± 4.342^{x}	61.20 ± 1.521^{w}	$49.67 \pm 2.931^{a,w}$	42.92 ± 1.711^{x}	40.12 ± 0.148^{x}	$41.52 \pm 0.930^{b,x}$
Total unsaturated fatty acids (%)*	64.21 ± 0.015^{w}	59.64 ± 0.895^{w}	$61.93 \pm 0.455^{a,w}$	61.86 ± 2.341^{w}	38.8 ± 0.834^{x}	$50.33 \pm 1.588^{b,w}$	57.08 ± 0.416^{w}	59.88 ± 0.240^{w}	$58.48 \pm 0.328^{a,w}$
Ratio of saturated/unsaturated fatty acids	0.56	0.68	0.61	0.62	1.58	0.99	0.75	0.67	0.71
Total *n-6**	18.81 ± 0.006^{y}	0.86 ± 0.007^{y}	$9.84 \pm 0.006^{a,y}$	22.82 ± 0.495^{y}	1.15 ± 0.078^{y}	$11.99 \pm 0.287^{a,x}$	6.43 ± 0.106^{z}	1.56 ± 0.007^{y}	$3.40 \pm 0.057^{b,z}$
Total *n-3**	ND	–	–	ND	–	–	10.02 ± 0.042^{y}	0.72 ± 0.014^{z}	5.37 ± 0.028^{y}
Ratio *n-6/n-3*	ND	0.86 ± 0.007^{y}	$9.84 \pm 0.006^{a,y}$	ND	1.15 ± 0.078^{y}	$11.99 \pm 0.287^{a,x}$	0.64	2.17	0.63

Values are expressed as means ± SD ($n = 3$)

ND not detected

Values in the same row followed by the same superscript letter (a, b) are not significantly different at $p < 0.05$. Values in the same column followed by the same superscript letter (w, x, y, z) are not significantly different at $p < 0.05$

* The values are relative % of fatty acids in sample

– Concentration is less than 0.1 %

Table 5 Lipid composition (relative %) of the *Garcinia* oil

Sample	Neutral lipids	Glycolipids	Phospholipids
G. gummi-gutta pericarp	37.6 ± 0.12^{d}	55.6 ± 0.13^{a}	6.8 ± 0.05^{a}
G. gummi-gutta seed	95.8 ± 0.25^{a}	3.2 ± 0.10^{d}	1.0 ± 0.03^{d}
G. indica pericarp	77.0 ± 0.09^{c}	20.6 ± 0.07^{b}	2.4 ± 0.01^{c}
G. indica seed	79.6 ± 0.18^{bc}	19.6 ± 0.13^{b}	0.8 ± 0.02^{d}
G. xanthochymus pericarp	39.5 ± 0.02^{d}	55.9 ± 0.07^{a}	4.6 ± 0.01^{b}
G. xanthochymus seed	82.0 ± 0.12^{b}	16.1 ± 0.10^{c}	1.9 ± 0.03^{c}

Values are expressed as means ± SD ($n = 3$)

Values in the same column followed by the same superscript letter are not significantly different at $p < 0.05$

summarized in Table 5. Neutral lipids were predominant (37.6–95.8 %) in most of the samples followed by glycolipids (3.2–55.9 %) and phospholipids (1.0–6.8 %). Lipid subclasses pattern of *G. indica* seed oils agreed well with the literature [40, 53]. The neutral lipids of *Garcinia* oils showed MUFA oleic (32.9–63.74 %) as the major fatty acid followed by saturated stearic (1.58–67.06 %), palmitic (0.02–44.21 %) and omega-6 (0.01–14.85 %), omega-3 (0.08–5.41 %) fatty acids (Table 6). The fatty acid composition of neutral lipids agreed well with the *Garcinia* oil fatty acid composition provided in Table 3. The results concurred with the findings of Thippeswamy and Raina [53] who have reported similar observations. The glycolipids of *Garcinia* oils showed oleic (38.21–62.1 %), stearic (14.92–55.25 %), palmitic (2.32–45.44 %), omega-6 (0.81–19.71 %) and omega-3 (0.14–17.64 %) fatty acids. The phospholipids of *Garcinia* oil showed oleic (43–71.54 %) as predominant fatty acid against palmitic (3.57-33.01 %), stearic (1.88-48.85 %), omega-6 (4.02–32.5 %) and omega-3 (2.23–14.38 %) fatty acids. Palmitic was found to be the major fractions ($p < 0.05$) of *G. xanthochymus* seed glycolipids

Table 6 Fatty acid composition (relative %) of neutral, glyco and phospho-lipid fractions of *Garcinia* fruit

Fractions	Palmitic acid [C16:0]	Stearic acid [C18:0]	Oleic acid [C18:1n9c]	Omega-6 (ω-6)	Omega-3 (ω-3)
Neutral lipids					
Pericarp					
G. gummi-gutta	21.58 ± 0.137^{e}	16.28 ± 0.372^{g}	47.29 ± 0.286^{ef}	14.85 ± 0.096^{d}	ND
G. indica	16.25 ± 0.122^{f}	27.63 ± 0.284^{e}	44.61 ± 0.334^{fg}	11.51 ± 0.16^{e}	ND
G. xanthochymus	28.40 ± 0.414^{d}	19.39 ± 0.449^{f}	40.79 ± 0.946^{hi}	6.01 ± 0.139^{f}	5.41 ± 0.849^{c}
Seed					
G. gummi-gutta	ND	35.32 ± 0.912^{d}	63.74 ± 0.919^{b}	0.86 ± 0.014^{h}	0.08 ± 0.017^{d}
G. indica	0.02 ± 0.001^{j}	67.06 ± 0.923^{a}	32.91 ± 0.903^{j}	0.01 ± 0.001^{h}	ND
G. xanthochymus	44.41 ± 0.141^{a}	1.58 ± 0.013^{j}	51.68 ± 0.424^{cd}	1.58 ± 0.013^{h}	0.75 ± 0.083^{d}
Glycolipids					
Pericarp					
G. gummi-gutta	33.94 ± 0.202^{b}	46.35 ± 0.505^{c}	ND	19.71 ± 0.303^{c}	ND
G. indica	31.33 ± 0.082^{c}	14.92 ± 0.125^{g}	39.10 ± 0.181^{i}	14.65 ± 0.138^{d}	ND
G. xanthochymus	26.93 ± 0.178^{d}	16.41 ± 0.388^{g}	38.21 ± 0.253^{i}	0.81 ± 0.005^{h}	17.64 ± 0.132^{a}
Seed					
G. gummi-gutta	6.44 ± 0.038^{h}	28.91 ± 0.175^{e}	62.10 ± 0.259^{b}	ND	2.55 ± 0.053^{d}
G. indica	2.32 ± 0.376^{i}	55.25 ± 0.449^{b}	40.61 ± 0.147^{hi}	1.68 ± 0.185^{h}	0.14 ± 0.001^{d}
G. xanthochymus	45.44 ± 0.318^{a}	54.56 ± 0.450^{b}	ND	ND	ND
Phospholipids					
Pericarp					
G. gummi-gutta	16.26 ± 0.180^{f}	8.24 ± 0.156^{h}	43.00 ± 0.400^{gh}	32.50 ± 0.503^{a}	ND
G. indica	16.43 ± 0.354^{f}	4.97 ± 0.100^{i}	48.54 ± 0.977^{de}	30.06 ± 0.863^{b}	ND
G. xanthochymus	28.96 ± 0.640^{d}	1.88 ± 0.062^{j}	54.78 ± 0.676^{c}	ND	14.38 ± 0.884^{b}
Seed					
G. gummi-gutta	12.31 ± 0.477^{g}	6.22 ± 0.083^{hi}	71.54 ± 0.911^{a}	9.93 ± 0.361^{e}	ND
G. indica	3.57 ± 0.051^{i}	48.85 ± 0.822^{c}	43.56 ± 0.662^{fgh}	4.02 ± 0.057^{g}	ND
G. xanthochymus	33.01 ± 0.633^{bc}	1.95 ± 0.037^{j}	62.81 ± 1.243^{b}	ND	2.23 ± 0.104^{d}

Values are expressed as means ± SD ($n = 3$)

ND not detected

Values in the same column followed by the same superscript letter are not significantly different at $p < 0.05$

Table 7 Range of variation in *Garcinia* oil desaturation ratios

Fatty acid	*G. gummi-gutta*		*G. indica*		*G. xanthochymus*	
	Pericarp	Seed	Pericarp	Seed	Pericarp	Seed
ODR	0.322	0.016	0.420	0.032	0.360	0.045
LDR	0	0.104	0	0.080	0.609	0.268

The ODR and LDR values were calculated by the average value of % fatty acids composition

ODR oleic desaturation ratio, *LDR* linoleic desaturation ratio

Table 8 Relationship among fatty acid compositions (% in oil) in *Garcinia*

Pearson correlation matrix	Palmitic acid	Stearic acid	Oleic acid	Linolelaidic acid
Palmitic acid	1			
Stearic acid	−0.838[a]	1		
Oleic acid	−0.153	0.298	1	
Linolelaidic acid	0.071	−0.470	−0.697[a]	1

[a] Correlation is significant at $p < 0.05$

(45.44 %), stearic in *G. indica* seed neutral lipids (67.06 %) and oleic acid in *G. xanthochymus* seed phospholipids (71.54 %). These results agreed well with the *Garcinia* oil fatty acid composition of neutral lipids and glycolipids.

Desaturation Ratios of Unsaturated Fatty Acids and Association of Individual Fatty Acids

Evaluation of potential of cultivars by comparing individual fatty acid values with beneficial health attributes is difficult. The biosynthetic pathway of fatty acids is complex and highly regulated by a desaturation pathway [54]. For this reason, the oleic desaturation ratio (ODR) and the linoleic desaturation ratio (LDR) were additionally analyzed. ODR and LDR values were calculated to estimate the efficiency of the fatty acid desaturation pathway (Table 7). Among the varieties studied, highest LDR values were found in *G. xanthochymus*. LDR value was zero in the pericarp of *G. gummi-gutta* and *G. indica* indicating no α-linolenic acid formation. The high LDR values in seed samples imply that the biosynthetic pathway is efficient in the conversion of linoleic acid to α-linolenic acid. Relatively low ODR values in seeds indicating less conversion of oleic acid to linoleic acid. Based on ODR and LDR values, *G. xanthochymus* could be a potential target cultivar to develop a more suitable and health beneficial *Garcinia* variety with enhanced α-linolenic acid content.

The results of correlation analyses of major *Garcinia* fatty acids are shown in Table 8. PA showed significantly ($p < 0.05$) negative correlation with SA. PA and SA are undesirable for human health as they are saturated fatty acids. On the other hand, significant ($p < 0.05$) negative correlation was observed between OA and linolelaidic acid (LNA, C18:2n6t). OA is MUFA and hence beneficial for human consumption, while, LNA is *trans* PUFA and studies reported that it may have an atherogenic effect [55].

Conclusion

The purpose of this study was to compare the fatty acid composition of the major Indian *Garcinia* fruits. The results have shown these varieties to be an important oil seed cultivar in the Indian subcontinent. The present study concludes that the fatty acid profile of *Garcinia* fruits varied from species to species not only in their quantities but also in the type of fatty acids. Oleic acid is the predominant MUFA identified in *Garcinia* fruits and present data also reveals that *Garcinia* fruits are a rich source of unsaturated fatty acids, which are essential in the diet. A detailed analysis of *Garcinia* oil, lipid sub-classes and their fatty acid composition has provided us with the preliminary idea about the fatty acid interconnection. As *Garcinia* fruits were collected for three consecutive seasons from different regions, the data have provided a holistic overview about the nutritional and medicinal benefit of these fruits. The study of these fruits indicates that the fats can be used for blending with different edible oils and their physicochemical properties make them valuable. Our findings may have important implications for the commercial production of these fats.

Acknowledgments The authors thank Director, CSIR-Central Food Technological Research Institute (CFTRI) for providing access to the resources necessary for the completion of this study. The first author acknowledges the fellowship provided by the Department of Science and Technology (DST), Government of India under the INSPIRE fellowship program.

References

1. Ito N, Fukushima S, Tamano S, Hirose M, Hagiwara A (1986) Dose response in butylated hydroxyanisole induction of forestomach carcinogenesis in F344 rats. J Natl Cancer Inst 77:1261–1265
2. Han QB, Qiao CF, Song JZ, Yang NY, Cao XW, Peng Y, Yang DJ, Chen SL, Xu HX (2007) Cytotoxic prenylated phenolic compounds from the twig bark of *Garcinia xanthochymus*. Chem Biodivers 4:940–946
3. Hemshekhar M, Sunitha K, Sebastin SM, Devaraja S, Kemparaju K, Vishwanath BS, Niranjana SR, Girish KS (2011) An overview on genus *Garcinia*: phytochemical and therapeutical aspects. Phytochem Rev 10:325–351
4. Patil MM, Anu-Appaiah KA (2013) In: Martirosyan DM (ed) Introduction to functional food science, 1st edn. Food Science Publisher, USA
5. Oraphin C, Ayse B, Warinthorn C, Emmanuel AT (2010) Chemistry and biology of the caged *Garcinia* xanthones. Chem Eur J 16:9944–9962
6. Sergio W (1988) A natural food, the malabar tamarind, may be effective in the treatment of obesity. Med Hypotheses 27:39–40
7. Lim TK (2012), In Lim TK (ed) Edible Medicinal and Non-Medicinal Plants vol. 2, Fruits, 1st edn. Springer, Heidelberg
8. Padhye S, Ahmad A, Oswal N, Sarkar FH (2009) Emerging role of garcinol, the antioxidant chalcone from *Garcinia indica* Choisy and its synthetic analogs. J Hematol Oncol. doi:10.1186/1756-8722-2-38
9. Baliga MS, Bhat HP, Pai RJ, Boloor R, Palatty PL (2011) The chemistry and medicinal uses of the underutilized Indian fruit tree *Garcinia indica* Choisy (kokum): a review. Food Res Int 44:1790–1799
10. Koshy AS, Vijayalakshmi NR (2001) Impact of certain flavonoids on lipid profiles-potential action of *Garcinia cambogia* flavonoids. Phytother Res 15:395–400
11. Rai AK, Prakash M, Anu-Appaiah KA (2010) Production of *Garcinia* wine: changes in biochemical parameters, organic acids and free sugars during fermentation of *Garcinia* must. Int J Food Sci Tech 45:1330–1336
12. Kapadia GJ, Rao GS (2011) In: Rai M, Chikindas M (eds) Natural antimicrobials in food safety and quality, 1st edn. CAB International, UK
13. Mathew GE, Mathew B, Shaneeb MM, Nyanthara B (2011) Diuretic activity of leaves of *Garcinia cambogia* in rats. Indian J Pharm Sci 73:228–230
14. Jayaprakasha GK, Sakariah KK (2002) Determination of organic acids in leaves and fruit rinds of *Garcinia indica* (Desr.) by LC. J Pharm Biomed Anal 28:379–384
15. Chanmahasathien W, Li Y, Satake M, Oshima Y, Ruangrungsi N, Ohizumi Y (2003) Prenylated xanthones with NGF-potentiating activity from *Garcinia xanthochymus*. Phytochemistry 64:981–986
16. Asghar M, Monjok E, Kouamou G, Ohia SE, Bagchi D, Lokhandwala MF (2007) Super CitriMax (HCA-SX) attenuates increases in oxidative stress, inflammation, insulin resistance, and body weight in developing obese Zucker rats. Mol Cell Biochem 304:93–99
17. Kureel RS, Ram K, Pandey A, Dutt D (2009) Kokum a potential tree borne oilseed. National Oilseeds and Vegetable Oils Development Board, India
18. Reddy YS, Prabhakar JV (1994) Cocoa butter extenders from Kokum (*Garcinia indica*) and Phulwara (*Madhuca butyracea*) butter. J Am Oil Chem Soc 71:217–219
19. Jeyarani T, Reddy YS (1999) Heat-resistant cocoa butter extenders from Mahua (*Madhuca latifolia*) and Kokum (*Garcinia indica*) fats. J Am Oil Chem Soc 76:1431–1436
20. Maheshwari B, Reddy YS (2005) Application of Kokum (*Garcinia indica*) fat as cocoa butter improver in chocolate. J Sci Food Agric 85:135–140
21. Firestone D (1998) Official methods and recommended practices of the American Oil Chemists Society, AOCS method No. Ai 2-75, AOCS method No. Ca 5a-40, AOCS method No. Cd 8-53, AOCS method No. Cd 3c-91, AOCS method No. Cd 1-25, AOCS method No. Ca 6a-40, AOCS method No. Ce 1-62, AOCS method No. Ce 5b-89, AOCS method No. Ce 8-89, 5th edn. AOCS press, Illinois
22. Sharma PB, Handique PJ, Devi HS (2015) Antioxidant properties, physico-chemical characteristics and proximate composition of five wild fruits of Manipur, India. J Food Sci Technol 52:894–902
23. Baudilio C, Carmen M, José LP, Juan JE, María DR (2008) A new method for the determination of wax precipitation from non-diluted crude oils by fractional precipitation. Fuel 87:2090–2094
24. Thiex NJ, Manson H, Andersson S, Persson JA (2002) Determination of crude protein in animal feed, forage, grain, and oilseeds by using block digestion with a copper catalyst and steam distillation into boric acid: collaborative study. J AOAC Int 85:309–317
25. Ibekwe HA, Orok EE (2010) Proximate composition of *Aframomum melegueta* seeds, *Garcinia kola* seeds and growth performance of broiler chicks treated with powders from these seeds. Int J Poult Sci 9:1152–1155
26. Thiex N, Novotny L, Crawford A (2012) Determination of ash in animal feed: AOAC official method 942.05 revisited. J AOAC Int 95:1392–1397
27. Bhatnagar AS, Gopala Krishna AG (2014) Lipid classes and subclasses of cold-pressed and solvent-extracted oils from commercial Indian niger (*Guizotia abyssinica* (L.f.) Cass.) seed. J Am Oil Chem Soc 91:1205–1216
28. Ramadan MF, Moersel JT (2003) Determination of the lipid classes and fatty acid profile of niger (*Guizotia abyssinica* Cass.) seed oil. Phytochem Anal 14:366–370
29. Ichihara K, Shibahara A, Yamamoto K, Nakayama T (1996) An improved method for rapid analysis of the fatty acids of glycerolipids. Lipids 31:535–539
30. Tufan B, Koral S, Kose S (2011) Changes during fishing season in the fat content and fatty acid profile of edible muscle, liver and gonads of anchovy (*Engraulis encrasicolus*) caught in the Turkish Black Sea. Int J Food Sci Tech 46:800–810
31. Swapna HC, Rai AK, Bhaskar N, Sachindra NM (2010) Lipid classes and fatty acid profile of selected Indian fresh water fishes. J Food Sci Technol 47:394–400
32. Li D, Wang LJ, Wang DC, Chen XD, Mao ZH (2007) Microstructure analysis of rice kernel. Int J Food Prop 10:85–91
33. Zhang W-G, Jin G-M (2011) Microwave puffing-pretreated extraction of oil from *Camellia oleifera seed* and evaluation of its physicochemical characteristics. Int J Food Sci Tech 46:2544–2549
34. Naveen GPNA, Krishnakumar G (2012) Biochemical analysis and seed oil characterizations of *Garcinia indica*, *G. xanthochymus* and *G. gummi-gutta* for nutritional qualities. Indian J Sci 1:71–73
35. Onyeike EN, Acheru GN (2002) Chemical composition of selected Nigerian oil seeds and physiochemical properties of oil extracts. Food Chem 77:431–437
36. Ajayi IA, Oderinde RA, Ogunkoya BO, Egunyomi A, Taiwo VO (2007) Chemical analysis and preliminary toxicological evaluation of *Garcinia mangostana* seeds and seed oil. Food Chem 101:999–1004
37. Mazi EA, Okoronkwo KA, Ibe UK (2013) Physico-chemical and nutritive properties of bitter kola (*Garcinia kola*). J Nutr Food Sci. doi:10.4172/2155-9600.1000218

38. Guo H, Kouzuma Y, Yonekura M (2009) Structures and properties of antioxidative peptides derived from royal jelly protein. Food Chem 113:238–245
39. Ajibola CF, Fashakin JB, Fagbemi TN, Aluko RE (2011) Effect of peptide size on antioxidant properties of African yam bean seed (*Sphenostylis stenocarpa*) protein hydrolysate fractions. Int J Mol Sci 12:6685–6702
40. Daniel J, Abraham L, Balaji K, Rajasekharan R (2003) Biosynthesis of stearate-rich triacylglycerol in developing embryos and microsomal membranes from immature seeds of *Garcinia indica* Chois. Curr Sci India 85:363–370
41. Grundy SM (1989) Monounsaturated fatty acids and cholesterol metabolism: implication for dietary recommendations. J Nutr 119:529–533
42. Hilditch TP (1941) The chemical constitution of natural fats. Wiley, New York
43. Coleman MH (1965) The pancreatic hydrolysis of natural fats. IV. Some exotic seed fats. J Am Oil Chem Soc 42:751–754
44. Che Man YB, Haryati T, Ghazali HM, Asbi BA (1999) Composition and thermal profile of crude palm oil and its products. J Am Oil Chem Soc 76:237–242
45. Johnson S, Saikia N (2009) Fatty acids profile of edible oils and fats in India. Centre for Science and Environment, New Delhi
46. Eleyinmi AF, Bressler DC, Amoo IA, Sporns P, Oshodi AA (2006) Chemical composition of bitter cola (*Garcinia kola*) seed and hulls. Pol J Food Nutr Sci 56:395–400
47. Simopoulos AP (1991) Omega-3 fatty acids in health and disease and in growth and development. Am J Clin Nutr 54:438–463
48. Simopoulos AP (1999) Evolutionary aspects of omega-3 fatty acids in the food supply. Prostag Leukotr Ess 60:421–429
49. Kris-Etherton PM, Harris WS, Appel LJ (2002) Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation 106:2747–2757
50. Kenny FS, Pinder SE, Ellis IO, Gee JM, Nicholson RI, Bryce RP, Robertson JF (2000) Gamma linolenic acid with tamoxifen as primary therapy in breast cancer. Int J Cancer 85:643–648
51. Horrobin DF, Ziboh VA (1997) The importance of linoleic acid metabolites in cancer metastasis and in the synthesis and actions of 13-HODE. Adv Exp Med Biol 433:291–294
52. Gillingham LG, Harris-Janz S, Jones PJ (2011) Dietary monounsaturated fatty acids are protective against metabolic syndrome and cardiovascular disease risk factors. Lipids 46:209–228
53. Thippeswamy HT, Raina PL (1989) Lipid composition of Kokum (*Garcinia indica*) and Dhupa (*Veteria indica*). J Food Sci Technol 26:322–326
54. Velasco L, Goffman FD, Becker HC (1998) Variability for the fatty acid composition of the seed oil in a germplasm collection of the genus *Brassica*. Genet Resour Crop Evol 45:371–382
55. Dashti N, Feng Q, Freeman MR, Gandhi M, Franklin FA (2002) Trans polyunsaturated fatty acids have more adverse effects than saturated fatty acids on the concentration and composition of lipoproteins secreted by human hepatoma HepG2 cells. J Nutr 132:2651–2659