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Screening of lupeol, mangiferin and β-carotene contents in pulp of mango (*Mangifera indica* L.) varieties at edible ripe stage

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Mango fruits are an amazing source of numerous bioactive phytonutrients like lupeol (a novel anti-inflammatory and anticancer dietary triterpene), mangiferin (an antidiabetic, anti-HIV, anticancer, immunomodulatory and anti-inflammatory agent) and β-carotene (a significant carotenoid that functions as a precursor to vitamin A, the conscientious reason for our vision). In the present study, ripe pulp of 23 mango varieties was examined for their contents of lupeol, mangiferin and β-carotene using high performance liquid chromatography-photodiode array detector (HPLC-PAD). Mulgoa had the highest lupeol concentration, measuring 42.52 µg/g and Langra stands second with 36.33 µg/g followed by Pairi (33.56 µg/g), Sensation (28.69 µg/g) and Dashehari with 28.22 µg/g. Mangiferin content was highest in variety Arunika (49.58 µg/g) followed by Ambika (34.80 µg/g), Dashehari (33.31 µg/g), Sensation (29.66 µg/g) and Neelum (27.93 µg/g). Sensation's mature pulp has the highest quantity of β-carotene (109.58 µg/g), followed by Kesar (96.87 µg/g), Dashehari (82.13 µg/g), Mulgoa (79.99 µg/g), Arunika (74.26 µg/g), and Amrapali (70.12 µg/g). The pulp of Dashehari, Sensation, Mulgoa, Arunika, Kesar and Amrapali possessed good to moderate amount of these nutraceuticals and are beneficial for consumption at ripe stage. This study has showed the importance of nutraceutical components present in mango; meanwhile it also encourages mango growers to grow these varieties for better profitability.

Keywords: β-Carotene, Edible ripe stage, HPLC-PAD quantification, Lupeol, Mangiferin, Mango pulp

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Mango (*Mangifera indica* L.) is the major significant fruit crop in terms of production, marketing, recognition and utilization. India is the top-producing nation of mangoes with the greatest varietal diversity (about 1,500 varieties of mango are grown in India including 1,000 commercial varieties). Mango is a nutritionally rich fruit due to the presence of many dietary antioxidant phytochemicals like polyphenols, carotenoids, tocopherols, ascorbic acid, mangiferin and lupeol. It is popular both in fresh and processed forms. Lupeol, a dietary triterpene, and mangiferin, a C-glycosyl xanthone, are two bioactive phytochemicals widely distributed in different plant parts of mango like pulp, peel, stem, bark, kernel and leaf. Lupeol (also known as Fagarsterol) is found in many fruits and vegetables like mango, strawberry, grape, guava, bael, mulberry, olive, white cabbage, green pepper, cucumber, tomato, carrot, peas, etc.[1]. The lupeol chemical formula is $C_{30}H_{50}O$ and chemical name is 20(29)-Lupen-3-beta-ol. The lupeol chemical structure is given in Figure 1. It has immense potential to act as an anti-inflammatory, anti-microbial, anti-cancer, analgesic, antipyretic, cardioprotective cholesterol lowering agent, skin protective agent, hepatoprotective and nephroprotective agent as suggested by many *in vitro* and preclinical animal studies[2-9]. Mangiferin (1,3,6,7-tetrahydroxy xanthone-C2-β-D-glucocide) (Fig. 1) is also available in bark, pulp, peel and leaves of mango and shows a broad array of pharmacological actions like anti-diabetic, immunomodulatory, antitumor, HIV preventive, cancer preventive, and inflammatory preventive along with antioxidant activities[10–14]. Ripe mango peel and pulp are an excellent source of β-carotene (Fig. 1) which is the most important carotenoids of mango representing about 80% of the total carotenoids content. β-Carotene provides the highest vitamin A activity, which is essential for vision, immune function, reproduction and antioxidant activities[15].

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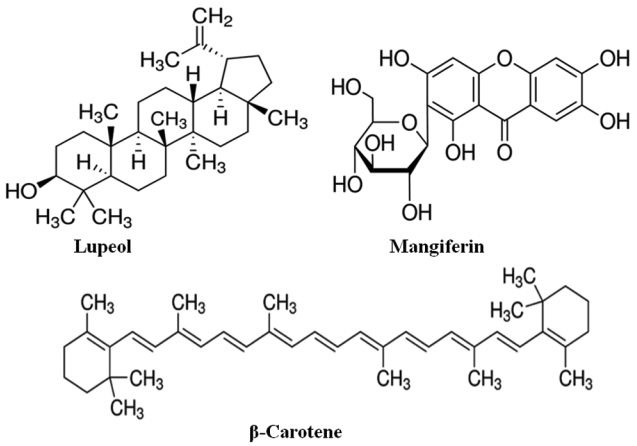

Fig. 1 — Chemical structures of lupeol, mangiferin and β-carotene

Most of the previous studies on isolation and extraction of lupeol and mangiferin have been conducted from mango stem, bark, leaves and peel[16-19]. Some researchers have identified mangiferin content and lupeol concentrations in the pulp and peel of four mango varieties during storage by HPTLC[20]. Total carotenoids or β-carotene has been estimated in the ripe pulp of many mango varieties worldwide[21,22]. However, no literature is available on simultaneous presentation on characterization of these phytochemicals in so many mango varieties. As mango is mostly preferred for consumption in ripe form, characterization of these bioactive phytochemicals in ripe fruit is imperative. Not much work in this aspect has been done in traditional India varieties like Neelum, Totapuri, Dashehari, Banganpalli etc. Keeping these points in view, screening of twenty three popular and commercial mango varieties for lupeol, mangiferin and β-carotene contents in ripe pulp has been done using simple and accurate high performance liquid chromatography (HPLC) techniques.

Methodology

Twenty three popular mango varieties (Dashehari, Langra, Chausa, Totapuri, Banganpalli, Alphonso, Bombay Green, Neelum, Amrapali, Mallika, Arunika, Ambika, Kesar, Lucknow Safeda, Hushn-e-ara, Vanraj, Mulgoa, Saheb Pasand, Janardhan Pasand, Pairi, Kensington, Tommy Atkins and Sensation) were selected for the present investigation. Ten physiologically mature mango fruits were randomly collected for each variety from germplasm block located at Rehmankhera, Lucknow, India. The fruits were kept for ripening at room temperature by keeping them in brown paper bags. The ripe fruits were peeled off, pulp was mixed, homogenized and representative samples (four replications) were collected for extraction of nutraceuticals following quartering method and analysis was done using HPLC technique. The experiment was conducted as completely randomized design with 4 replicates.

Materials and Methods

Extraction of nutraceuticals

For the extraction of lupeol, 10 g homogenized pulp sample was mixed thoroughly in 80% ethanol by keeping them overnight at room temperature with occasional shaking and then extracted thrice with n-hexane. The pooled n-hexane extract was evaporated totally in a rotary vacuum evaporator and residue was diluted in 10 mL of HPLC mobile phase. For mangiferin extraction, 10 g of pulp sample was sonicated with 40% methanol for 30 min in an ultrasonicator and cooled at room temperature[23]. Then it was centrifuged for 20 min at 20,000 x g, supernatant was collected and filtered through 0.45 µm nylon membrane filter paper. β-Carotene was extracted from 15 g pulp samples as per the method available in literature[24] with slight modifications. Glass columns of 30 cm × 1 cm, filled with 5 g silica gel sandwiched between 2 layers of anhydrous sodium sulphate, were used for eluting β-carotene with acetone. After evaporating acetone under nitrogen, the residue was dissolved in mobile phase of HPLC (10 mL) for analysis. The values of all the three nutraceuticals were expressed as µg/g of pulp on fresh weight basis.

Preparation of standard solutions

The technical grade standards of lupeol, mangiferin and β-carotene were obtained from Sigma-Aldrich (St. Louis, USA) with more than 90% purity. Stock solutions of lupeol (940 µg/mL), mangiferin (1000 µg/mL) and β-carotene (1000 µg/mL) were prepared in their respective mobile phases. Working solutions of lower concentrations were also prepared by serial dilution in respective mobile phases. Linearity in standard curve for lupeol was observed between 4.7 to 94 µg/mL, for mangiferin between 0.5 to 50 µg/mL and for β-carotene within 0.2 to 50 µg/mL.

Chromatographic parameters

A Shimardzu make binary HPLC system (model LC 10 ATVP) with photodiode array detector and rheodyne injector (20 µL loop) was used for the analysis of nutraceuticals. Stationary phase was same for mangiferin and β-carotene – reverse phase C-18 column (250 mm × 4.6 mm i.d., 5 µ film thickness) from

Phenomenex® Luna. For the estimation of lupeol, Phenomenex® Luna reverse phase C-8 column (250 mm × 4.6 mm i.d., 5 µ film thickness) was used[25]. Mobile phase, flow rate and maximum detection wavelength for lupeol, mangiferin and β-carotene were acetonitrile: acetic acid (99.99: 0.01), 0.8 mL/min and 210 nm; methanol: 0.1 % aqueous phosphoric acid (35: 65), 1.0 mL/min and 258 nm; and acetonitrile: chloroform: isopropanol: water (78: 16: 3.5: 2.5), 1.0 mL/min and 450 nm, respectively. All the samples were filtered using a sample clarification kit through an Axiva nylon membrane filter (13 mm in diameter and 0.45 mm in thickness) prior to being injected into the HPLC.

Validation of HPLC methods

Single laboratory validation of HPLC techniques was performed by assessing the limits of detection (LOD) and limits of quantification (LOQ) through dilution of standard solutions to a series of concentrations. The LOD and LOQ were determined at signal to noise ratio (S/N) of 3 and 10, respectively. The correctness of the complete method was tested through recovery analysis. Two types of varieties were chosen for recovery study - one with good amount of respective nutraceuticals (Dashehari) and another with least amount (Totapuri). Pulps of samples were fortified with two concentrations each of lupeol (9.4 and 47 µg/mL), mangiferin (5.0 and 20 µg/mL) and β-carotene (1.0 and 10 µg/mL) and extracted and analyzed following the same methods described earlier after keeping them overnight at room temperature for better absorption.

Statistical analysis

The statistical software SPSS version 16.0 was used to perform the analysis of variance (ANOVA). The information was presented as mean standards of replications, and the least significant difference (LSD) at $p \leq 0.05$ was used to distinguish between mean differences.

Results

Method validation and linearity

The LOD and LOQ, based on S/N ratios of 3 and 10, were observed as 0.47 and 0.94 µg/mL for lupeol, 0.5 and 1.0 µg/mL for mangiferin and 0.5 and 1.0 µg/mL for β-carotene. The recovery of lupeol, when fortified with 9.4 and 47 µg/mL to Dashehari pulp, varied between 82.73% to 92.13%, while the same from ripe Totapuri pulp was 100.46% to 105.52% (Table 1). Similarly, the recoveries of mangiferin ranged between 99.21% to 103.44% and 95.73% to 101.63% from Dashehari and Totapuri, respectively, after fortifying with 5.0 and 20 µg/mL concentrations. For β-carotene the recoveries were 89.04% to 95.61% and 83.16% to 90.51%, after fortification at 1.0 and 10 µg/mL concentrations, from the pulp of Dashehari and Totapuri, respectively (Table 1). This proved that the analytical technique is accurate and efficient enough for estimation of these nutraceuticals present in mango pulp. The linearity of HPLC methods was assessed by drawing the standard curve against various concentrations and calculating regression equations and regression coefficient (R^2) value (Fig. 2a-2c). An 'R^2' value of above 0.99 proved the sensitivity and accuracy of the analytical methods.

Estimation of lupeol in mango varieties

The content of lupeol, a dietary triterpene and proven anti-cancer and anti-inflammatory agent, was found maximum in ripe pulp of var. Mulgoa (42.52 µg/g) followed by varieties Langra (36.33 µg/g), Pairi (33.56 µg/g), Sensation (28.69 µg/g) and Dashehari (28.22 µg/g). Four other varieties like Saheb Pasand (20.76 µg/g), Kesar (17.49 µg/g), Neelum (17.21 µg/g) and Lucknow Safeda (16.21 µg/g) also possessed moderate amount of lupeol (Fig. 3A). However, varieties Banganpalli, Janardhan Pasand, Totapuri, Tommy Atkins and Ambika contained

Table 1 — Recoveries of three nutraceuticals from mango pulp

Fortification level (µg/mL)	Recovery from Dashehari pulp (%)				Recovery from Totapuri pulp (%)			
	R1	R2	R3	Mean±SD	R1	R2	R3	Mean±SD
Lupeol								
9.4	80.31	84.95	82.93	82.73±2.326	95.33	101.87	104.19	100.46±4.594
47.0	89.25	94.19	92.13	92.13±2.570	108.55	103.23	104.79	105.52±2.735
Mangiferin								
5.0	100.45	95.67	101.52	99.21±3.115	92.73	97.85	96.61	95.73±2.671
20.0	99.89	107.59	102.85	103.44±3.884	97.48	102.68	104.73	101.63±3.737
β-carotene								
1.0	85.42	92.17	89.54	89.04±3.402	80.27	84.11	85.09	83.16±2.547
10.0	98.27	92.41	96.15	95.61±2.967	88.15	92.23	91.14	90.51±2.112

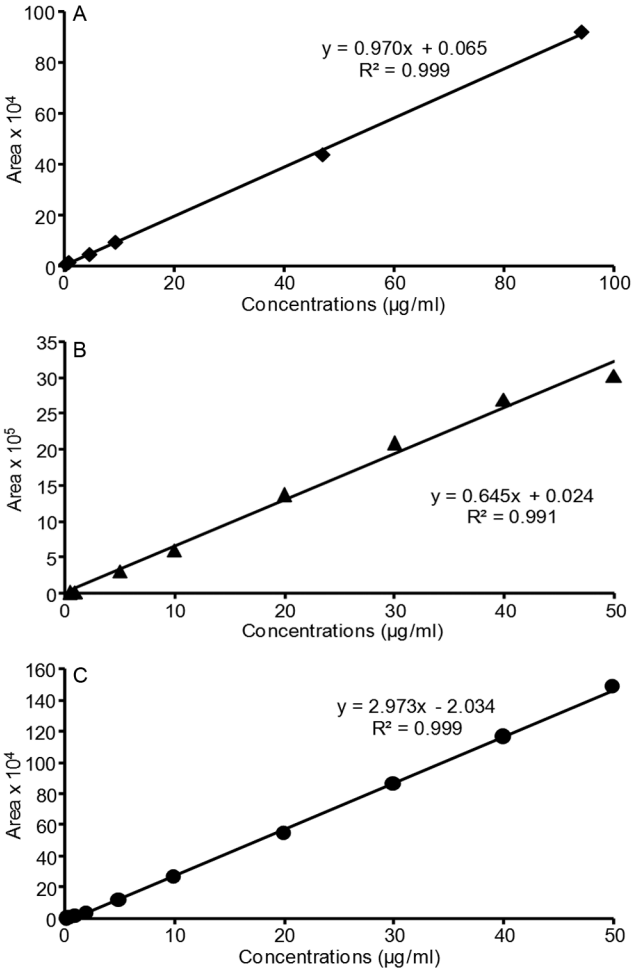

Fig. 2 — Calibration curves for lupeol (A), mangiferin (B) and β-carotene (C) with regression equation and regression coefficient

minimum amounts of lupeol in ripe pulp (1.03, 3.30, 4.07, 4.46 and 4.66 µg/g, respectively). A significantly wide variation in lupeol content was observed among the varieties grown under North Indian conditions at edible ripe stage (LSD at $p \leq 0.05$ = 2.911 and CV = 13.197).

Analysis of mangiferin in ripe mango pulp

Mangiferin, a phenolic compound of xanthone derivative, was analyzed and quantified in ripe pulp of 23 popular mango varieties preserved in the field gene bank of the institute under same environmental conditions. Maximum mangiferin content was noticed in Arunika variety (49.58 µg/g), a coloured variety developed from a cross combination of Amrapali x Vanraj at ICAR-CISH, Lucknow, India, whereas, minimum mangiferin content was recorded in var. Alphonso (2.42 µg/g) which is a commercial variety grown in Lucknow condition, as revealed by HPLC

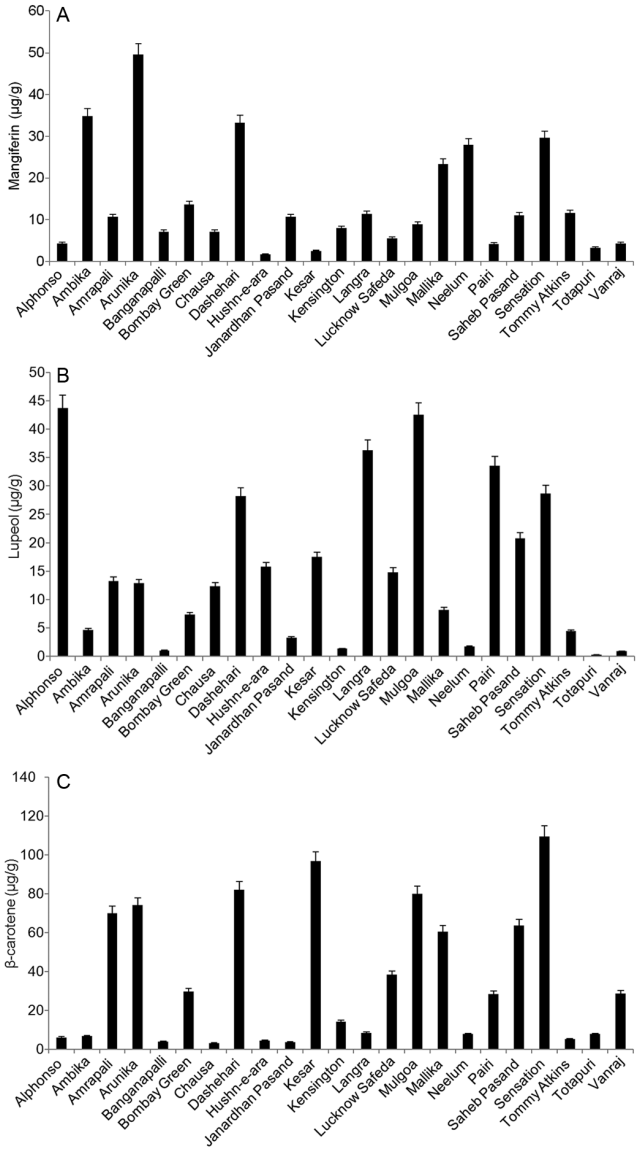

Fig. 3 — Lupeol (A), mangiferin (B) and β-carotene (C) contents in ripe pulp of various mango varieties

data. Several other varieties like Ambika (also a coloured variety developed from a cross combination of Amrapali x Janardhan Pasand at ICAR-CISH, Lucknow), Dashehari, Sensation, Neelum and Mallika also possessed good amount of mangiferin at consumption maturity stage (34.80, 33.31, 29.66, 27.93 and 23.35 µg/g, respectively) (Fig. 3B). However, varieties Kesar, Totapuri, Pairi and Vanraj contained lesser amount of mangiferin at edible ripe stage with 2.50, 3.24, 4.21 and 4.31 µg/g, respectively. Significantly wide variation in mangiferin content (LSD at $p \leq 0.05$ = 2.969 and CV = 14.413) was noticed in ripe pulp of 23 mango varieties grown under North Indian conditions.

Quantification of β-carotene in ripe mango pulp

β-Carotene is a major carotenoid (80%) of total carotenoids noticed in ripe mango pulp and providing highest vitamin A activity. Most of the previous studies revolved around estimation of carotenoids in mango. The present study consisted of quantification of β-carotene, the major carotenoid, in pulp of 23 mango varieties at edible ripe stage. Significantly wide variation was noticed in β-carotene content in pulp of mango varieties at consumption maturity stage (LSD at $p \leq 0.05 = 8.722$ and CV = 17.042). Most of the varieties (12) contained moderate to very good amount of this nutraceutical compound when ready for consumption. Pulp of variety Sensation had the maximum amount (109.58 µg/g) of β-carotene, closely followed by Kesar (96.87 µg/g) which is an export variety of India. Other mango varieties with very good amount of β-carotene were Dashehari (82.13 µg/g), Mulgoa (79.99 µg/g) and Arunika (74.26 µg/g) (Fig. 3C). Varieties Amrapali (70.12 µg/g), Saheb Pasand (63.77 µg/g) and Mallika (60.59 µg/g) also possessed good amount of this nutraceutical. Chausa (3.15 µg/g), Janardhan Pasand (3.71 µg/g), Banganpalli (3.97 µg/g) and Hushn-e-ara (4.43 µg/g) were the varieties possessed significantly lower amount of this vitamin A precursor at edible ripe stage, while other varieties had moderate amount of β-carotene.

Discussion

Bioactive compounds have gained prominence in the food and pharmaceutical industry for their antioxidant activity potential and some beneficial effects on human health. Lupeol, mangiferin and carotenoids compounds in mango are getting more attention due to the health benefits but these compounds will vary from variety to variety. In the present study, the lupeol, mangiferin and carotenoids contents varied significantly from variety to variety. The maximum lupeol content was recorded in pulp of Dashehari and peel of Langra mangoes, while it was minimum in pulp of Bombay Green and peel of Chausa mangoes after HPTLC analysis[20]. HPTLC estimation required derivatization of lupeol with vanillin-sulfuric acid-ethanol combination for quantification, which is a cumbersome and time-consuming process. Meanwhile HPLC is a widely used, more simple and accurate identification and quantification method for lupeol. In Ataulfo mango, more lupeol was estimated in peel than in pulp where it was higher during consumption (at ripe stage) than physiological maturity[17]. Among the three juicy varieties of mango grown in Telangana region of India, lupeol content varied between 67.24 µg/100 g dry powder in variety Chinnarasam and 8.45 µg/100 g dry powder in variety Pandurivari Mamidi[26]. Soujanya *et al*.[27] have also reported that dry powder of coloured mango var. Suvarnarekha possessed higher amount of lupeol (47.26 µg/100g) than Vanraj (28.86 µg/100g) and it increased during storage up to 12 days. Similarly, in dry pulp powder of seven table varieties of mango grown in Telangana, India, highest lupeol content was observed in Baneshan (50.9 µg/100 g) and lowest in Himayath (8.3 µg/100 g)[28]. In a recent study, Tommy Atkins and Keitt pulp contained 2.3 and 4.6 mg/100 g DW of lupeol which is very low even at dry weight basis[29] and similar to our findings. In the present investigation, mango varieties Alphonso, Mulgoa, Langra, Pairi, Sensation, Dashehari, Saheb Pasand, Kesar and Neelum are found to be good sources of lupeol at edible ripe stage.

Significant variation in mangiferin content in pulp of four mango varieties was reported earlier where Bombay Green had maximum amount and Langra had minimum amount[20]. Mango peel contained significantly higher amount of mangiferin compared to mango pulp as tested in 14 mango varieties where mangiferin was detected in peel (dry matter basis) of all 14 varieties but it was found in pulp of only 5 varieties (dry matter basis)[16]. Pulp of Jose mango possessed maximum amount of mangiferin (19.4 mg/kg dry matter) whereas, that of Mini-mango contained minimum amount (3.0 mg/kg dry matter). Mangiferin was not detected in pulp of Tommy Atkins and Keitt mangoes even in dry weight basis in a recent study in Italy[29]. A higher concentration of mangiferin was also observed in mango (Ataulfo) peel at consumption maturity stage than in pulp[17]. Similarly in China, mangiferin content was recorded in pulp of 5 out of 11 mango varieties with Maqiesu had the highest (0.20 mg/g DW) and Zihuamang the lowest (0.002 mg/g DW). However, peel of all 11 Chinese mango varieties contained significantly higher amount of mangiferin than pulp[30]. In the current study, mango varieties Arunika, Ambika, Dashehari, Sensation, Neelum and Mallika were found to be good sources of mangiferin at consumption maturity stage.

Carotenoids impart yellowish / orange colour in mango pulp and peel. The varieties with attractive yellow to orange colour at the time of ripening had higher amount of β-carotene in comparison to

varieties having light yellow coloured pulp. Around 6 µg/g of β-carotene has been recorded in pulp of Tommy Atkins grown in Brazil[22] which is at par with our estimation in the same variety (5.26 µg/g) grown in India. Same authors have mentioned that β-carotene content of Uba and Haden varieties was significantly higher than that of Tommy Atkins and Palmer varieties. In another study, a much higher amount of β-carotene (12.09-14.05 µg/g) was reported for Tommy Atkins mango grown in Brazil[31] compared to the present investigation. Same authors have mentioned that in five commercial mango varieties grown in Brazil, β-carotene content varied between 6.61 µg/g (Haden) to 25.45 µg/g (Extreme) and accordingly shown vitamin A activity. In ripe pulp of 13 different mango samples (varieties not defined) collected from Brazilian market, β-carotene ranged between 8.2 to 28.7 µg/g with significant variation[32]. Significant increase in β-carotene content has been observed during ripening of Tommy Atkins and Keitt mango pulp which was 5.8 and 6.7 µg/g, respectively, in ripe pulp[33] and is in sync with our study. Carotenoids increased significantly during ripening because of carotenogenesis process (conversion of chlorophyll to carotenoids) and ripe fruits contained almost ten times more carotenoids than unripe or partially ripe fruits as evident in one of our earlier studies where ripe pulp of Amrapali, Dashehari, Chausa, Mallika and Langra recorded 63.55, 137.26, 23.99, 164.33 and 45.33 µg/g of total carotenoids, respectively, which is 3 to 300 times higher than mature but unripe fruits[34]. β-Carotene content in ripe pulp of Langra and Mallika varieties was reported to be 19.68 and 34.55 µg/g, respectively[35]. Higher amount of total carotenoids in three mango genotypes namely Saheb Pasand (7.50 mg/100g), Murshidabad (6.93 mg/100 g) and Sensation (6.37 mg/100 g) was reported in one of our earlier research[36]. Mango varieties Sensation, Kesar, Dashehari, Mulgoa, Arunika, Amrapali, Saheb Pasand and Mallika were found as excellent sources of β-carotene in the present investigation.

The difference in these bioactive phyto-nutrients might be attributed to the difference in variety to variety which is mainly depending on genetical and environmental factors. Given that bioactive compounds are synthesized at the secondary metabolism level, a function of gene expression, the genetic factor can be considered as most significant one[17]. Conversely, environmental variables have the ability to alter secondary metabolite production, which in turn affects gene expression[37] and depending on the climatic conditions, maturity, pest infestation, disease infection *etc*, the genes that synthesize bioactive phytochemicals can be activated or deactivated[38]. The theory of environmental influence on bioactive nutraceuticals even in the same mango variety was also supported in literature where Keitt mango collected from Bahia (hot climate) contained more than double β-carotene compared to that collected from Sao Paulo (moderate climate), Brazil[33].

Conclusions

The contents of all three bioactive phyto-nutrients (lupeol, mangiferin and β-carotene) varied significantly from variety to variety at edible ripe stage in mango pulp. Some varieties contained moderate to good amount of all nutraceuticals and can be considered beneficial for human health e.g., Dashehari, Arunika, Sensation, Saheb Pasand, Mulgoa, etc. Some varieties like Kesar, Mallika, Amrapali, Ambika, etc. are rich sources of one or two nutraceuticals but moderate source of other. These varieties can not only be grown by mango orchardists for more profit but can also be used in breeding programme to develop nutrient rich hybrids.

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Conflict of Interest

Authors declare that there is no competing or conflict of interest.

Authors' Contributions

AKB, AD & BMM: Conceptualization, Supervision, estimation and data preparation; SR & VGL: Formal analysis, data calculation and editing; AKB & BMM: Original draft and paper writing.

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