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Varietal Differences in the Bioaccessibility of β-Carotene from Mango (*Mangifera indica*) and Papaya (*Carica papaya*) Fruits

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Mango and papaya, which are rich sources of β-carotene, are widely consumed in India. In this study, β-carotene content and its bioaccessibility were determined in six locally available varieties of mango, namely, Badami, Raspuri, Mallika, Malgoa, Totapuri, and Neelam, and two varieties of papaya, namely, Honey Dew and Surya. Varietal differences were evident in both β-carotene content and its bioaccessibility in the case of mango. β-Carotene content in ripe mango ranged from 0.55 ± 0.03 mg/100 g in the Malgoa variety to 3.21 ± 0.25 mg/100 g in the Badami variety. Similarly, in the Honey Dew and Surya varieties of papaya, β-carotene contents were 0.70 ± 0.10 and 0.74 ± 0.12 mg/100 g, respectively. Bioaccessibility of β-carotene ranged from 24.5% in Badami to 39.1% in Raspuri varieties of mango. Considering both the percent bioaccessibility and the inherent β-carotene content, the amount of bioaccessible β-carotene was highest in the Mallika variety (0.89 mg/100 g), followed by Badami (0.79 mg/100 g). Because mango and papaya are also consumed as a blend with milk, the influence of the presence of milk on the bioaccessibility of β-carotene from these fruits was also examined. Addition of milk generally brought about a significant increase in the bioaccessibility of β-carotene from mango, the increase ranging from 12 to 56%. Bioaccessibility of β-carotene from the two varieties of papaya examined was similar (31.4–34.3%). Addition of milk increased this bioaccessibility by 19 and 38% in these two varieties. Considering the β-carotene content of mango and papaya, the latter has to be consumed in amounts roughly 3 times that of mango to derive the same amount of β-carotene. Thus, this study has indicated that varietal differences exist in the content and bioaccessibility of β-carotene in mango and that the addition of milk is advantageous in deriving this provitamin A from the fruit pulp of mango and papaya.

KEYWORDS: β-Carotene; bioaccessibility; mango fruits; papaya fruits; varietal difference

INTRODUCTION

Micronutrient deficiency is a major public health problem in developing countries, India accounting for nearly half of the world's prevalence (*1*). Among the micronutrient deficiencies, deficiency of vitamin A is recognized as a serious problem leading to blindness. It has been estimated that, globally, 2.8 million preschool children are at risk of blindness (*2*). Animal foods such as eggs, milk, and liver are good sources of preformed vitamin A. Because these foods are expensive, provitamin A carotenoids, the most important of which is β-carotene, are the principal source of vitamin A for a majority of the world's population, including India (*3*). β-Carotene is abundantly found in green leafy and yellow-orange vegetables and fruits (*4*). Several factors affect the bioavailability of carotenoids, including β-carotene, from foods, and these factors have been described in the mnemonic "SLAMENGHI" (*5*). Among these factors, the matrix in which the carotenoids are embedded plays an important role. The carotenoids in dark green leafy vegetables (DGLV) are entrapped as complexes with proteins in chloroplasts within the cell structures. Such entrapment is probably responsible for the poor bioavailability of carotenoids from DGLV. The cell wall structure of fruits is relatively weaker than that in leaves, and carotenoids in fruits are present in oil droplets in chromoplasts and hence may be more easily extracted during digestion (*3, 5*). Fruits such as mango (*Mangifera indica*) and papaya (*Carica papaya*), which are rich sources of β-carotene, are grown abundantly and consumed in India. Whereas mango is a seasonal fruit, papaya is found throughout the year, often grown in kitchen gardens. Several popular varieties of mango are available and consumed during the season. A few studies have reported differences in the β-carotene content of different cultivars of mango (*6–8*). Climatic conditions have been found to influence the β-carotene content of fruits (*7*). Information on the varietal differences, if any, in the concentration of this provitamin in mango and papaya

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native to India is lacking. It would also be interesting to see if varietal differences exist in the bioaccessibility of β-carotene from these fruits in view of the choice of varieties available for consumption. The present investigation was therefore undertaken to examine the β-carotene content as well its bioaccessibility in six popular varieties of mango and two varieties of papaya found in the local market. Mango and papaya are also consumed along with milk in the form of a milkshake. Hence, the influence of milk, if any, on the bioaccessibility of β-carotene from mango and papaya was examined in this investigation.

MATERIALS AND METHODS

Materials. Six popular cultivars of mango, namely, Badami, Raspuri, Mallika, Malgoa, Totapuri, and Neelam, and two cultivars of papaya, Honey Dew (conventional) and Surya (hybrid, seedless), were procured from the local market. These fruits were of a ripeness ideal for consumption and were uniform for all of the varieties. Fresh edible pulp of the fruits was used for the study. Each variety of mango, the annual fruit available during summer, was procured from five different vendors, and mangoes from each vendor were analyzed on different days, in duplicate. The whole pulp of the fruits was homogenized and used for analysis. In the case of papaya which is available more or less throughout the year, the fruits were collected from five different vendors, each at three different seasons.

All chemicals used were of analytical grade. Solvents were distilled before use. Standard β-carotene, porcine pancreatic pepsin, and pancreatin and bile extract (porcine) were procured from Sigma Chemical Co., St. Louis, MO. Double-distilled water was employed throughout the entire study. All glassware used was acid washed.

Determination of Bioaccessibility of β-Carotene in Vitro. The bioaccessibility of β-carotene in vitro was determined according to the method of Garrett et al. (*9*). Briefly, the method involved subjecting the sample (10 g of fresh fruit pulp) to simulated gastric digestion at pH 2.0 in the presence of pepsin at 37 °C (16 g in 100 mL of 0.1 M HCl), followed by simulated intestinal digestion in the presence of a pancreatin–bile extract mixture (4 g of porcine pancreatin) and 25 g of bile extract (porcine) in 1000 mL of 0.1 M $NaHCO_3$), pH 7.5, at 37 °C for 2 h. At the end of simulated intestinal digestion, the micellar fraction was separated by ultracentrifugation at 70000*g* for 120 min using a Beckman L7-65 ultracentrifuge. The β-carotene present in the micellar fraction represents the portion that is bioacessible.

Analysis of β-Carotene. β-Carotene was extracted from the fruit pulp or the micellar portion obtained after simulated gastrointestinal digestion initially with a mixture of acetone/ethanol (1:1) and subsequently with petroleum ether (*10*). The process was repeated several times to ensure complete extraction of β-carotene. The extract was saponified with 30% methanolic potassium hydroxide at room temperature for 3 h. Following saponification, the alkali was removed completely by repeated washing, and the solvent was evaporated to dryness in a rotary evaporator. The residue was redissolved in petroleum ether and stored in the cold pending analysis. Prior to analysis, the petroleum ether was evaporated under nitrogen and the residue was dissolved in the mobile phase.

Determination of β-carotene was carried out by reverse-phase HPLC (Shimadzu LC 10 AVP), equipped with a UV–visible detector (*9*). β-Carotene was separated on a C_{18} column (S.S. Excil). The mobile phase consisted of a mixture of 65% (v/v) acetonitrile, 15 % (v/v) methylene chloride, and 20% (v/v) methanol containing 1.3 mmol/L ammonium acetate. β-Carotene was monitored at a wavelength of 450 nm. The peak identities and λ_{max} values were confirmed by their retention time and characteristic spectra of standard chromatograms.

Table 1. Varietal Differences in the Content and Bioaccessibility of β-Carotene from Mango (*Mangifera indica*)[a]

mango variety	total β-carotene (mg/100 g)	bioaccessible β-carotene (mg/100 g)	% organic acid
Badami	3.21 ± 0.25 a	0.79 ± 0.03 a (24.5)[b]	0.15 ± 0.02 a
Raspuri	1.83 ± 0.08	0.71 ± 0.03 a (39.1)	0.50 ± 0.01 b
Malgoa	0.55 ± 0.028 c	0.18 ± 0.01 b (32.5)	0.42 ± 0.04 b
Mallika	2.77 ± 0.096 a	0.89 ± 0.04 c (31.9)	0.13 ± 0.02 a
Totapuri	1.27 ± 0.10 d	0.48 ± 0.03 d (38.1)	0.47 ± 0.08 b
Neelam	1.45 ± 0.23 d	0.45 ± 0.03 d (30.8)	0.15 ± 0.03 a

[a] Values are mean ± SEM of five independent determinations, each being in duplicate. Within the same column values with different letters are significantly different (ANOVA, $p < 0.05$). [b] Figures in parentheses represent percent bioaccessible β-carotene.

During the steps of simulated gastrointestinal digestion, ultracentrifugation, and extraction of β-carotene, precautions were taken to minimize the exposure of samples to light and air and thus prevent oxidative destruction of β-carotene. Air was replaced by nitrogen before the flask was stoppered at all stages of incubation and storage. The experiments were carried out under yellow lighting, and all of the glassware was covered with black cloth to prevent exposure to light.

Determination of Total Organic Acids in Fruit Pulp. Total organic acid content of the fruit pulp was determined by titrating the boiled aqueous pulp against 0.005 N NaOH, previously standardized using standard oxalic acid (*11*).

Preparation of Mango and Papaya Milkshake. To examine the effect of the presence of milk, 10 g of the fresh fruit pulp was blended with 50 mL of milk and 10 g of sugar, and the bioaccessibility of β-carotene from this blend was determined as above. For this purpose, commercially available pasteurized cow's milk was boiled in the laboratory, as usually practiced in Indian households.

Statistical Analysis. All determinations were made in five experiments using fruits bought from five different vendors, and the average values are reported. Statistical analysis of data was done employing analysis of variance (ANOVA), and the differences between means were determined by Duncan's multiple-range test and were considered to be significant when $p < 0.05$ (*12*).

RESULTS AND DISCUSSION

β-Carotene Content and Bioaccessibility from Different Varieties of Mango. Table 1 presents the β-carotene content and its bioaccessibility from six different varieties of mango. The β-carotene contents (milligrams per 100 g of fresh pulp) varied widely among the different varieties of mango, the highest being present in Badami (3.21), followed by Mallika (2.80), Raspuri (1.83), Neelam (1.45), Totapuri (1.27), and Malgoa (0.55). Thus, there is a 6-fold difference in β-carotene between the variety with the highest (Badami) and that with lowest content (Malgoa). Incidentally the ripe fruits of Malgoa variety are pale yellow in color, whereas the hue of the Badami variety is the most intense. The β-carotene content of mango (variety not specified) as reported by the National Institute of Nutrition (*4*) is 1.99 mg/100 g. This is close to that of the Raspuri variety of mango (1.83 mg/100 g) determined by us.

Varietal differences were also evident in the bioaccessiblity of β-carotene from mango, which were, however, confined to

a range of 25–39%, unlike the wide variation seen in their β-carotene contents. The bioaccessibility values of these mango varieties were also independent of the inherent content of this provitamin. The percent bioaccessible β-carotene was highest in the Raspuri variety (39.0) and lowest in the Badami variety (24.5). Determination of the total organic acids present in the edible portions of these mango varieties (**Table 1**) indicated that bioaccessibility of β-carotene from specific mango varieties roughly corresponded with the organic acid content of the fruits of Raspuri, Totapuri, Malgoa, and Badami varieties; that is, the variety with the highest organic content also showed the highest β-carotene bioaccessiblity and vice versa. Such a correspondence was, however, not seen in Mallika and Neelam varieties. This could be due to the fact that organic acids are not the only modifiers of β-carotene bioaccessibility. Other factors, especially fiber and carotenoids other than β-carotene (which have not been determined here) may also influence the same.

Varietal differences in the concentration of β-carotene in mango grown in Brazil and Thailand have been reported by other workers (*6*–*8*). Mercadante and Rodriguez-Amaya (*6*) found that the Keitt variety of mango contained higher amounts of *all-trans*-β-carotene (0.67 mg/100 g) compared to the Tommy Atkins variety (0.58 mg/100 g). The Keitt vartiety of mango brought from Bahia state of Brazil, however, had a significantly higher content of *all-trans*-β-carotene (1.5 mg/100 g). These authors observed that climatic conditions in which the fruits are grown have an influence on the carotenoids content, with fruits grown in hot regions having a generally higher carotenoid concentration. Pott et al. (*7*) reported differences in the carotenoid content of three cultivars of mangoes. The *all-trans*-β-carotene content in the Kaew variety was 11.6 mg/100 g, whereas the same was 4.60 and 3.70 mg/100 g in the Kent and Tommy Atkins varieties, respectively. Recently, Vasquez-Caicedo and co-workers (*8*) reported differences in the *all-trans*-β-carotene and its *cis*-isomers in nine different cultivars of mango grown in Thailand. Thus, the β-carotene content of the Indian cultivars of mango studied here was higher than that of Keitt and Tommy Atkins varieties grown in Brazil.

Other than the above reports on the total β-carotene content of mango, information on the bioavailability of this provitamin from the same is lacking. Our current study is probably the very first attempt to understand the bioaccessibility of β-carotene from this abundant source and also the existing varietal differences in the same. An increase in the plasma β-carotene content of children supplemented with solar-dried mango (a local variety, Ameli, in Gambia), with and without fat, for a period of 4 months has been recently reported (*13*). There was also an improvement in the plasma retinol concentration in children receiving fat in addition to dried mango, suggesting that fat plays an important role in the absorption of β-carotene and its conversion to vitamin A.

We have earlier reported the beneficial influence of food acidulants on the retention of β-carotene in vegetables (*14*). Inclusion of tamarind and citric acid (0.1 and 0.01%, respectively) along with green leafy vegetables during heat processing brought about a significant improvement in the retention of β-carotene. Results of the present study showed that the higher the organic acids inherent in the fruit, the higher was the bioaccessibility of β-carotene. Thus, organic acids either exogenous or inherent help in the retention of β-carotene in the food matrix during heat processing as well as improve the bioaccessibility of the same.

This study, which has essentially envisaged varietal differences in the content and bioaccessibility values of β-carotene in mango, has indicated that the latter is independent of the former. Considering both the total content and percent bioaccessibilty, the Mallika variety provides the highest amount of β-carotene (0.88 mg/100 g), followed by the Badami (0.79 mg/100 g) and Raspuri (0.71 mg/100 g) varieties for the same amount of edible pulp consumed. Thus, among the average-sized fruit of any of the varieties of mango (around 200 g of edible pulp), the above three varieties would provide 1.4–1.8 mg of β-carotene, which corresponds to 60–75% of the RDA (2.4 mg) of this provitamin for Indians (*15*). The other three varieties (Totapuri, Neelam, and Malgoa) provide <1 mg of β-carotene per fruit of similar size.

Table 2. Varietal Difference in the Content and Bioaccessibility of β-Carotene from Papaya (*Carica papaya*)[a]

papaya variety	total β-carotene (mg/100 g)	bioaccessible β-carotene (mg/100 g)	% organic acid
Honey Dew	0.70 ± 0.10 a	0.24 ± 0.2 a (34.3)[b]	0.14 ± 0.02 a
Surya	0.73 ± 0.12 a	0.23 ± 0.04 a (31.4)	0.20 ± 0.02 b

[a] Values are mean ± SEM of five independent determinations, each being in duplicate. Within the same column values with different letters are significantly different (ANOVA, $p < 0.05$). [b] Figures in parentheses represent percent bioaccessible β-carotene.

***β*-Carotene Content and Bioaccessibility from Two Varieties of Papaya.** There was no significant difference in the content of β-carotene between the two varieties of papaya examined in this study (**Table 2**). Whereas the β-carotene content of the Surya (seedless) variety was 0.73 mg/100 g, the same was 0.69 mg/100 g in the Honey Dew (conventional) variety. Similarly, no varietal differences were evident in the percent bioaccessibility of β-carotene from papaya, the same being 34.3 and 31.4 from the Honey Dew and Surya varieties, respectively. Unlike in the case of mango, the organic acid content of papaya did not seem to influence the bioaccessibility of β-carotene. Despite a higher amount of organic acid in the Surya variety compared to the Honey Dew variety, the percentages of bioaccessibility of β-carotene from both varieties were similar. The absence of a direct relationship between organic acid content and β-carotene bioaccessibility in these two varieties of papaya suggests that factors other than mere organic acids could also be responsible for the observed trend.

The absence of any difference in the β-carotene content of five cultivars of papaya grown in Brazil, values ranging from 0.23 to 0.37 mg/100 g of the ripe fruit pulp, has been reported (*16*). Setiawan et al. (*17*) reported a β-carotene content of 0.44 mg/100 g of fresh edible portion of papaya. The concentration of β-carotene of papaya in our study (0.69 and 0.73 mg/100 g for the Surya and Honey Dew varieties, respectively) is higher than that reported by these two studies. These values agree with those reported by The National Institute of Nutrition, India (0.88 mg/100 g), for papaya grown in India (*4*). This indicates that as in the case of mangoes, geographical location probably influences the β-carotene content of papaya. In the absence of any information on the bioavailability of β-carotene from papaya, ours is the first observation on the same. Although the percentages of bioaccessiblity of β-carotene are similar in both mango and papaya, considering the total content of this provitamin, papaya has to be consumed in amounts nearly 3 times those of mango to derive the same amount of β-carotene. This is feasible in view of the relative abundance and low cost of papaya in this country, where this fruit is especially affordable for the lower economic segments of population, who are at risk of vitamin A deficiency.

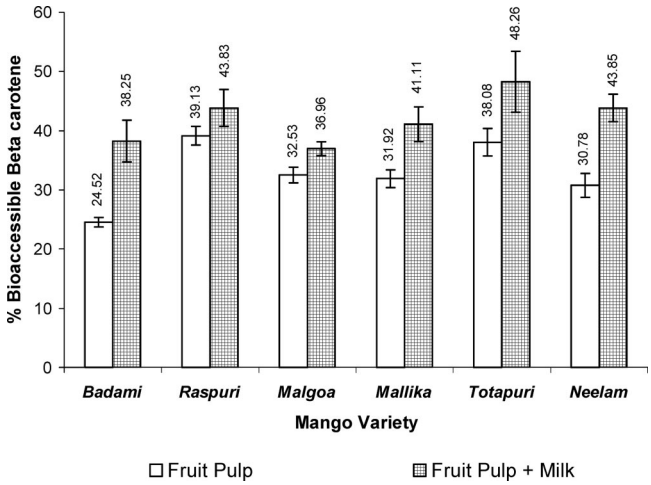

Figure 1. Influence of the presence of milk on the bioaccessibility of β-carotene from mango. The influence of milk was significant in all varieties.

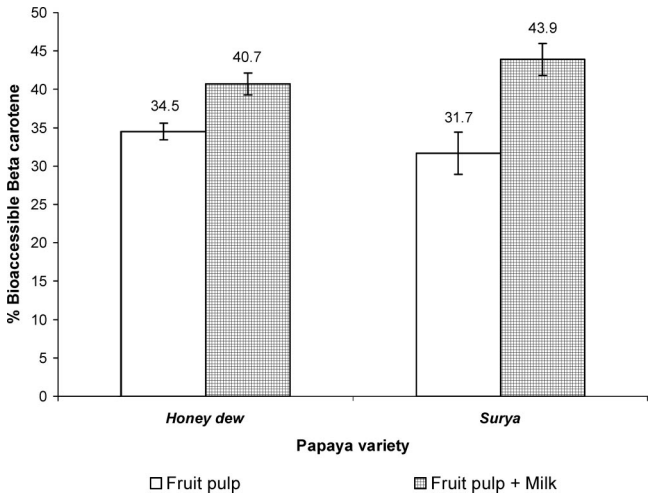

Figure 2. Influence of the presence of milk on the bioaccessibility of β-carotene from papaya. The influence of milk was significant in both varieties of papaya.

The β-carotene content of any of the varieties of mango and papaya examined in this investigation is much less compared to the amounts present in the commonly consumed green leafy vegetables (GLV) (4.8–17.8 mg/100 g) and carrot (6.2 mg/100 g) (*18*). Although the percent bioaccessibility of β-carotene from the fruit pulp of mango and papaya is generally higher than that from pressure-cooked GLV (14.0–22.7%) and carrot (25.3), considering the total amount of this provitamin latent in them, bioaccessible β-carotene per 100 g of pulp of mango and papaya turns out to be less than that from the same amount of pressure-cooked GLV (0.9–2.4 mg/100 g) or carrot (1.3 mg/100 g) (our unpublished data). However, considering the amounts of GLV or carrot consumed in a day's menu and probably the relatively higher amount of either mango or papaya generally consumed, the latter would still be a better provider of bioaccessible β-carotene.

Effect of the Presence of Milk on the Bioaccessibility of β-Carotene from Mango and Papaya. Figures 1 and **2** present the influence of milk on the bioaccessibility of β-carotene from mango and papaya, respectively. Addition of milk to the fruit pulp generally enhanced the bioaccessibility of β-carotene from all varieties of mango as well as the two varieties of papaya. The percent increase in the bioaccessibility of β-carotene in the presence of milk compared to fruit pulp alone ranged from 12 (Raspuri) to 56 (Badami). The enhancement was minimal in Malgoa and Raspuri varieties. Considering the absolute amount of bioaccessible β-carotene, the blend of milk and the Badami variety of mango provides the highest amount (1.2 mg/100 g), followed by those of Mallika (1.1 mg/100 g), Raspuri (0.8 mg/100 g), Neelam (0.63 mg/100 g), Totapuri (0.61 mg/100 g), and Malgoa (0.20 mg/100 g).

The presence of milk had a similar beneficial influence on the bioaccessibility of β-carotene from both varieties of papaya examined. Milk brought about a 40% increase in the bioaccessibility of β-carotene from the Surya variety of papaya, whereas the same was 18% from the Honey Dew variety. Thus, varietal differences existed in papaya only with respect to the influence of exogenous milk on the bioaccessibility of β-carotene. The exogenous milk added to the fruit pulp in this study did not contribute any β-carotene by itself.

The enhancing effect of milk on the bioaccessibility of β-carotene from both mango and papaya could probably be attributed to protein as well as fat present in it. Small amounts of fat are essential for the optimal absorption of carotenoids, which are fat-soluble (*3*). The presence of protein in the small intestine has been found to aid stabilization of fat emulsions and enhance micelle formation (*5*). The addition of fermented milk to a green leafy vegetable local to Tanzania is reported to significantly enhance the bioaccessibility of *all-trans*-β-carotene (*19*). This, as well as our observation of the enhancing effect of milk on the bioaccessibility of β-carotene from mango and papaya, suggests that fat and protein are effective even in vitro, where they probably aid the incorporation of β-carotene into the micellar fraction.

In summary, the present investigation, which examined the bioaccessibility of β-carotene from six varieties of mango and two varieties of papaya, has shown that varietal differences exist in both the content of β-carotene and its bioaccessibility in the case of mango. The bioaccessibility of β-carotene from mango roughly corresponded with the organic acid content of the fruits. Considering the total content and percent bioaccessibility, the Mallika variety of mango provides the highest amount of β-carotene, followed by the Badami variety for the same amount of pulp. There were no significant varietal differences in the β-carotene content or its bioaccessibility from papaya. Consumption of mango and papaya in the form of a milkshake seems to be an ideal approach to improve the bioaccessibility of β-carotene. Thus, if consumed alone, the Mallika variety of mango provides more β-carotene, whereas the amount from the Badami variety can be maximally derived if consumed as a milkshake. Although the percentages of bioaccessiblity of β-carotene are similar in both mango and papaya, considering the total content of this provitamin, papaya has to be consumed in amounts nearly 3 times those of mango to derive the same amount of β-carotene. The present study suggests that differences may also exist in those varieties of mango and papaya not examined here, with respect to both the content and bioaccessibility of β-carotene.

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