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Impact of the Stage of Ripening and Dietary Fat on *in Vitro* Bioaccessibility of β-Carotene in 'Ataulfo' Mango

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Pulp from "slightly ripe", "moderately ripe", or "fully ripe" mangoes was digested *in vitro* in the absence and presence of processed chicken as a source of exogenous fat and protein to examine the impact of stage of ripening of mango on micellarization during digestion and intestinal cell uptake (i.e., bioaccessibility) of β-carotene. The quantity of β-carotene transferred to the micelle fraction during simulated digestion significantly increased as the fruit ripened and when chicken was mixed with mango before digestion. Qualitative and quantitative changes that occur in pectin from mango pulp during the ripening process influenced the efficiency of micellarization of β-carotene. Finally, the uptake of β-carotene in micelles generated during simulated digestion by Caco-2 human intestinal cells confirmed the bioaccessibility of the provitamin A carotenoid in mango.

KEYWORDS: ***Mangifera indica*** **L.; β-carotene isomers; bioavailability; depolymerized pectin; carotenoid micellarization; Caco-2 cells**

INTRODUCTION

Mango (*Mangifera indica* L.) is a popular fruit in many countries, commonly consumed at different ripening stages either alone or combined with other foods (*1*). The typical yellow-orange color of ripened mango fruit is due to the presence of carotenoids (*2*), with all-*trans*-β-carotene being one the most abundant (*3, 4*). The relatively high content of all-*trans*-β-carotene supports the likelihood that mango contributes to the health of populations routinely ingesting the fruit. This carotenoid possesses the highest provitamin A activity (*5*) and seems to prevent some types of cancer (*6*) and the oxidation of LDL (*7*), a process implicated in the development of atherosclerosis. Several studies have demonstrated that the beneficial effects of carotenoids depend upon the amount that is actually absorbed and/or metabolized by the organism (*5*). The absorption of carotenoids is a complex process that requires the transfer of the carotenoids from the food to mixed micelles for delivery to small intestinal epithelial cells and incorporation of the intact carotenoid or retinyl ester products into chylomicrons secreted into lymph (*5, 8*). Numerous factors affect these processes, including carotenoid speciation, food matrix, type and intensity of food matrix processing, and some components such as the amount of fat and fiber in the food and meal (*9, 10*). Food matrix refers to the combined effects of all factors from a food that simultaneously promotes or reduces the bioavailability of endogenous carotenoids. The matrix certainly contributes to the variable bioavailability of carotenoids in different foods and meals (*10, 11*), as well as that from the same food processed according to different styles (*12*). The different matrices in a plant food at different stages of ripening are also likely to affect carotenoid bioavailability. Marked qualitative and quantitative changes in carotenoids, organic acids, lipids, phenolics, volatile compounds, and nonstructural and structural carbohydrates have been reported during the short duration of the ripening process of mango fruit (*13–16*). The effects of such compositional changes might modify the bioavailability of mango carotenoids at different stages of ripening. Existing data on the bioavailability of mango carotenoids are limited and contradictory. The accumulation of vitamin A in rat liver fed with a diet containing mango suggests that all-*trans*-β-carotene in this fruit is readily bioavailable (*17*). However, supplementation with retinyl palmitate and mango was insufficient to correct vitamin A deficiency in Senegalese children (*18*).

The primary objective of this investigation was to examine the influence of stage of ripening on the bioaccessibility of β-carotene from mango using the coupled *in vitro* digestion and Caco-2 human cell model. 'Ataulfo' mango fruit was selected because it is one of the most important mango cultivars in Mexico (*1*). We also investigated the influence of dietary fat and mango pectin, promoter and probable inhibitor, respectively,

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of carotenoid bioavailability (*9*), on micellarization of β-carotene during simulated digestion.

MATERIALS AND METHODS

Foods, Chemicals, and Standards. Processed chicken (Del Monte stage 2 chicken baby food) and 'Ataulfo' mangoes were purchased from a local grocery. Glycodeoxycholate and taurodeoxycholate were from Calbiochem (San Diego, CA). Pepsin (porcine), pancreatin (porcine), bile extract (porcine), taurocholate, and all-*trans*-β-carotene were purchased from Sigma Chemical (St. Louis, MO) and all-*trans*-violaxanthin was from CaroteNature GmbH (Lupsingen, Switzerland). Other reagents and materials were from Sigma Chemical or Fisher Scientific Co. (Norcross, GA), unless stated otherwise.

Selection of Fruits and Preparation for *in Vitro* Digestion. Fresh 'Ataulfo' mangoes from Mexico were uniform in size and free from blemishes. "Slightly ripe" (SR), "moderately ripe" (MR), and "fully ripe" (FR) fruit were included in the experiment. Fruit classification according to ripening stage was based on subjective evaluation of fruit firmness and intensity of the yellow-orange pigmentation of the skin and pulp and on analysis of carotenoid content. Two longitudinal slices of similar weight (10 g) were obtained from opposite cheeks of each fruit and homogenized to puree, which was immediately subjected to the simulated *in vitro* digestion.

Simulated *in Vitro* Digestion. Digestion reactions (50 mL total volume) contained puree (1.5 g) from SR, MR, or FR mangoes without or with 1 g of processed chicken containing 5.6% fat, 8.5% protein, and 0% fiber by weight. The gastric phase consisted in subjecting the test food in saline to the activity of porcine pepsin at pH 3 for 1 h at 37 °C under reciprocal shaking (95 rpm). The small intestinal phase included the digestive action of porcine pancreatin and bile extract at pH 7 for 2 h under the same conditions of temperature and shaking. Other details of the procedure for simulating the gastric and small intestinal phases of digestion have been described by Garrett et al. (*19*). The mixture after completion of the small intestinal phase of digestion was referred to as digesta. A portion of digesta was centrifuged (167000*g* at 4 °C for 20 min) to separate the aqueous fraction containing micellarized carotenoids from residual undigested materials. The aqueous fraction was passed through a Millex GP syringe filter unit with 0.22 μm pores (Millipore Corp., Bedford, MA) to obtain carotenoids that partitioned into micelles. Puree, digesta, and filtered aqueous fraction were immediately blanketed with nitrogen and stored at −20 °C for 3 days until analysis.

The effect of pectin isolated from SR and FR mangoes on the micellarization of β-carotene was examined using simulated small intestinal digestion. Capsular contents of a β-carotene supplement (15 000 μg of β-carotene and soybean oil) were extracted into and successively diluted with petroleum ether to a concentration of 76.6 μg of β-carotene/mL. Aliquots (200 μL) were transferred to a reaction tube, and solvent was evaporated under a stream of nitrogen. The starting amount of β-carotene in these reactions (15.4 ± 0.01 μg) was less than that present in samples of mango subjected to simulated gastric and small intestinal digestion (i.e., 16.8–63.5 μg), because the extent of micellarization of β-carotene from the supplement was expected to exceed that in the more complex food matrix (*9*). The digestion reactions contained pectin from either SR or FR mangoes, providing a final concentration of 0, 108, or 225 μg/mL. These quantities of pectin were similar to those for digestion of SR and FR mangoes in the initial series of experiments. The final volume of the reaction was decreased to 20 mL, and the porcine bile extract used in the standard digestion reaction including gastric and intestinal phases was replaced by a mixture of pure bile salts (0.80 mmol/L glycodeoxycholate, 0.45 mmol/L taurodeoxycholate, and 0.75 mmol/L taurocholate), according to Chitchumroonchokchai et al. (*20*).

Uptake of β-Carotene from Micelles by Caco-2 Human Intestinal Cells. Test cultures of Caco-2 cells (HTB37, American Type Culture Collection; passage 29) were maintained (in 6-well plates) as previously described (*19, 20*) and used at 12 days postconfluency. Filtered aqueous fraction from digested FR mango plus chicken were pooled, diluted 1:3 (v/v) with Dulbecco's minimum essential medium, sterilized by filtration (0.22 μm pores), and added to washed monolayers of Caco-2 cells (2 mL/well). Plates were incubated for 4 h in a humidified atmosphere of 95% air/5% CO_2. Integrity of cell layers was microscopically assessed during incubation by comparing monolayers exposed and not exposed to test medium. Medium with micellar β-carotene was also added to a set of wells without cells to monitor the stability of the carotenoid during incubation. After incubation, test medium was removed and the monolayers were washed twice with ice cold phosphate-buffered saline (PBS) containing albumin from bovine serum (2 mg/mL). Monolayers were washed 2 times again with 2 mL of pure PBS, collected in 1 mL PBS, and centrifuged at 100*g* at 4 °C for 5 min. Cell pellets were stored under nitrogen at –20 °C and analyzed 3 days later. Medium with micellar β-carotene was also added to a set of wells without cells to monitor the stability of the carotenoid during incubation.

Isolation of Pectin from Mango. Pectin was isolated according to the method of Mesbahi et al. (*21*) with several modifications. Puree (230 g) from either SR or FR mango was mixed with 1.5 L of distilled water and heated to 100 °C. After the mixture was cooled at room temperature, the pH of the mixture was adjusted to 1.5 with 25% sulfuric acid. The mixture was reheated at 90 °C for 2.5 h, cooled, and filtered through a Whatman paper no. 1. The solid residue was discarded while the liquid extract was neutralized with 1 N NaOH and mixed with 3 L absolute ethanol to precipitate the pectin. The suspension was filtered through Whatman paper no. 1 of known weight, and retained pectin was sequentially washed with absolute ethanol (200 mL) and acetone (200 mL). Finally, the pectin was dried at 60 °C for 8 h, ground in a mortar, and stored at −20 °C. The yield of pectin was determined gravimetrically.

Carotenoid Extraction and High-Performance Liquid Chromatography (HPLC) Analysis. Carotenoids from digesta (3 mL) and filtered aqueous fraction (3 mL) were extracted by washing with 4 mL of a mixture of petroleum ether/acetone (2:1, v/v) 3 times. Cell pellets and mango puree (0.3 g) were mixed with 1 mL of PBS, sonicated for 15 s, and extracted as described for digesta and filtered aqueous fraction. The petroleum ether fractions were combined and dried under a stream of nitrogen. The residue was reconstituted in 250–500 μL of isopropyl alcohol and filtered for injection (25–50 μL). The HPLC system consisted of a separations module (Alliance 2695) coupled to a temperature-controlled (10 °C) autosampler and a photodiode array detector (Waters, Milford, MA). The chromatographic system used a high-resolution YMC carotenoid C_{30} (4.6 × 150 mm, 3 μm) analytical column (YMC, Inc., Wilmington, NC) at 25 °C. The solvent system was composed of methanol (A) and *tert*-butyl methyl ether (MTBE, B). A linear gradient from 72% A and 28% B to 64.4% A and 35.6% B in 12 min was used at a flow rate of 1 mL/min.

All-*trans*-β-carotene from analytical samples was identified by comparing its retention time and absorption spectra with those obtained with a reference standard. Other carotenoids were identified by comparing their spectral characteristics with those generated with reference standards or reported in the literature. The spectral fine structure (% III/II), which describes the percentage of the ratio of the peak height of the longest wavelength absorption band ($\lambda_{max\ III}$) to that of the middle absorption band ($\lambda_{max\ II}$), taking the minimum between two peaks as baseline, was also used for identification. Quantification of *cis*- and all-*trans*-carotenoids was carried out by calibration curves constructed with all-*trans*-carotenoids. β-Apo-8′-carotenal (1.1 μg) was used as an internal standard.

Miscellaneous Assays. The protein content of cell pellets was determined by the bicinchoninic acid method (Pierce, Rockford, IL) at 562 nm using a Synergy HT microplate reader (Biotek Instruments, Inc., Winooski, VT) and bovine serum albumin as a standard. 9-*cis*-Violaxanthin was generated by I_2-catalyzed photoisomerization of all-*trans*-violaxanthin according to Molnár et al. (*22*).

Statistical Analysis. All measurements were made in triplicate ($N = 3$). Results are expressed as means ± standard errors of the mean. Tukey−Kramer honestly significant difference test was used to determine statistically significant differences. All analyses were performed using the JMP statistical software (SAS Institute, Inc., Cary, NC).

RESULTS

Carotenoid Profile of Mango at Different Stages of Ripening. All analyzed fruit presented an excellent external and

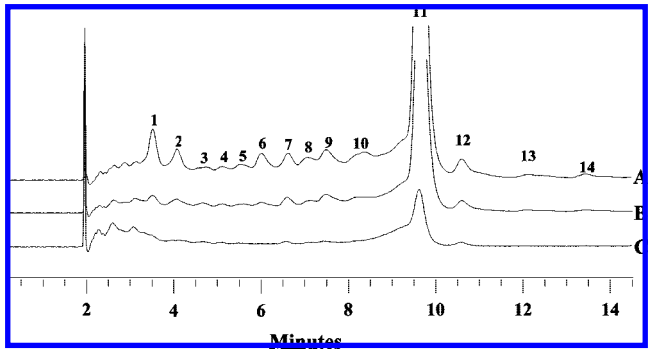

Figure 1. Representative chromatograms at 452 nm obtained with crude extracts of pulp from "moderately ripe" mango (MR, **A**), digesta (**B**), and filtered aqueous fraction (**C**). Peaks 1, 4, 5, 6, 7, 8, 9, 10, and 13 were identified as all-*trans*-violaxanthin and likely represent various ester derivatives; peaks 2 and 14 were assigned as 9-*cis*-violaxanthin; peaks 3, 11, and 12 were identified as *cis*-β-cryptoxanthin, all-*trans*-β-carotene, and 9-*cis*-β-carotene, respectively.

Table 1. Carotenoid Content in Pulp of 'Ataulfo' Mango at Different Stages of Ripening

		concentration (μg/g)[a]		
carotenoid	$\lambda_{max\ I}/\lambda_{max\ II}/\lambda_{max\ III}$	SR	MR	FR
all-*trans*-violaxanthin	416/439/469	7.9 ± 0.12	8.4 ± 0.01	24.2 ± 0.19
9-*cis*-violaxanthin	413/436/465	2.1 ± 0.00	3.2 ± 0.03	8.0 ± 0.06
cis-β-cryptoxanthin	419/445/474	0.10 ± 0.00	0.1 ± 0.01	0.3 ± 0.00
all-*trans*-β-carotene	427/452/478	10.7 ± 0.88	18.3 ± 1.19	39.4 ± 2.60
9-*cis*-β-carotene	426/446/472	0.45 ± 0.04	1.6 ± 0.09	2.9 ± 0.14

[a] Data represent the mean ± the standard error of triplicate samples of pulp from "slightly ripe" (SR), "moderately ripe" (MR), and "fully ripe" (FR) 'Ataulfo' mangoes.

internal appearance and their physiological stage fell in one of three increasing stages of ripening (SR, MR, and FR), as judged by color, firmness, and carotenoid content. No qualitative changes were detected in carotenoid composition of 'Ataulfo' mango at the three stages of ripening. The typical carotenoid profile of mango fruit included 14 peaks (**Figure 1A**). Several peaks (peaks 1, 4, 5, 6, 7, 8, 9, 10, 13, 2, and 14) were tentatively identified as all-*trans*-violaxanthin and 9-*cis*-violaxanthin containing compounds by comparing their absorption spectra (**Table 1**) with those obtained from standard compounds of all-*trans*-violaxanthin and 9-*cis*-violaxanthin (generated by photoisomerization of all-*trans*-violaxanthin). The absorption spectra of all-*trans*-violaxanthin and 9-*cis*-violaxanthin (λ_{max} at 416, 440, and 469 and 414, 436, and 466 nm, respectively) were similar to those reported by Pott et al. (*23*) for these xanthophylls in water–methanol−MTBE. The 3 nm hypochromic shift that characterizes these violaxanthin isomers (*3, 23*) was detected (**Table 1**). Peak 3 (**Figure 1A**) was identified as *cis*-β-cryptoxanthin by comparing its spectral maximum (445 nm) with that previously reported (444 nm) for this carotenoid in water–methanol−MTBE (*24*). Peaks 11 and 12 (**Figure 1A**) were identified as isomers of β-carotene based on their elution sequence from the C_{30} stationary phase and spectral maxima, i.e., 452 and 446 nm for all-*trans*-β-carotene and 9-*cis*-β-carotene in water–methanol−MTBE (*25*). Because the focus of the present study was provitamin A carotenoids and β-carotene is the sole contributor to vitamin A value of mango (*4*), a detailed study of the spectra from the detected β-carotene isomers was performed. The absorption spectra (λ_{max} at 427, 452, and 478 nm) and spectral fine structure (III/II = 22%) of peak 11 were virtually identical to those obtained using a

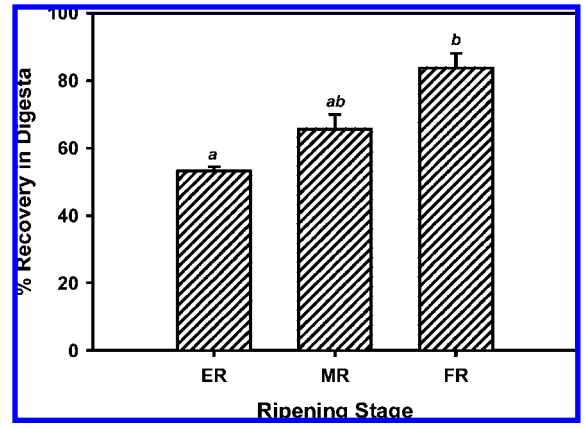

Figure 2. Recovery of BC after simulated *in vitro* digestion of "slightly ripe" (SR), "moderately ripe" (MR), and "fully ripe" (FR) 'Ataulfo' mangoes. Data represent the mean of three replicated samples ± the standard error of the mean (vertical bars). The presence of different letters above the error bar indicates significant ($p < 0.05$) differences.

standard compound of all-*trans*-β-carotene (λ_{max} at 427, 452, 478 nm; III/II = 24%). The spectral fine structure of other carotenoids, including 9-*cis*-β-carotene (peak 12, III/II = 33%), were similar to those obtained with standard compounds or previously reported by Lee et al. (*26*).

The total carotenoid content (sum of free and esterified carotenoids, see **Figure 1**) in pulp from SR, MR and FR 'Ataulfo' mangoes was 21.3 ± 0.7, 31.6 ± 1.3, and 74.8 ± 3.0 μg/g, respectively. All-*trans*-violaxanthin, 9-*cis*-violaxanthin, and all-*trans*-β-carotene were the most abundant carotenoids in 'Ataulfo' mango, comprising 26.7–37.0, 9.9–10.7, and 50.5–57.9% of the total carotenoid content, respectively, at the three stages of ripening. The content of the most abundant carotenoids during ripening is shown in **Table 1** and seems to follow an exponential behavior in relation to the stage of ripening.

Digestive Stability and Micellarization of β-Carotene from Mango. Digestive stability represents the percentage of β-carotene in the test food recovered in the digesta, whereas micellarization reflects the percentage of β-carotene in the test food present in the filtered aqueous fraction. All-*trans*-β-carotene and 9-*cis*-β-carotene were detected in mango puree, digesta, and filtered aqueous fraction (**Figure 1**). The relative amounts of 9-*cis*-β-carotene in mango puree, digesta, and filtered aqueous fraction were only 6.2 ± 0.5, 2.5 ± 0.2, and 4.7 ± 0.3%, respectively, of the total β-carotene content. Therefore, subsequent data are presented as content of β-carotene (BC) and represents the sum of all-*trans*-β-carotene and 9-*cis*-β-carotene. The violaxanthin (all-*trans* and 9-*cis*) from 'Ataulfo' mango predominantly exists as several mono- and diesters (*27*). Only free forms of xanthophylls are taken up by intestinal cells and absorbed (*9*), and because porcine pancreatin generally lacks carboxyl ester lipase activity (*28, 29*), micellarization and cellular uptake of violaxanthin isomers were not monitored.

The starting quantities of BC in samples of SR, MR, and FR 'Ataulfo' mangoes to be digested were 16.8 ± 1.3, 29.8 ± 1.9, and 63.5 ± 4.1 μg, respectively. Recovery of BC after simulated gastric and small intestinal digestion was dependent upon the ripening stage of the fruit and increased from 53.2 ± 1.4 to 83.7 ± 4.4% in simulated *in vitro* digestion of SR and FR mangoes, respectively (**Figure 2**). Recoveries of BC after digestion of unripe mangoes was considerably lower (30.7 ± 1.3%). The addition of chicken to mango puree did not affect recovery of BC after simulated gastric and small intestinal (data

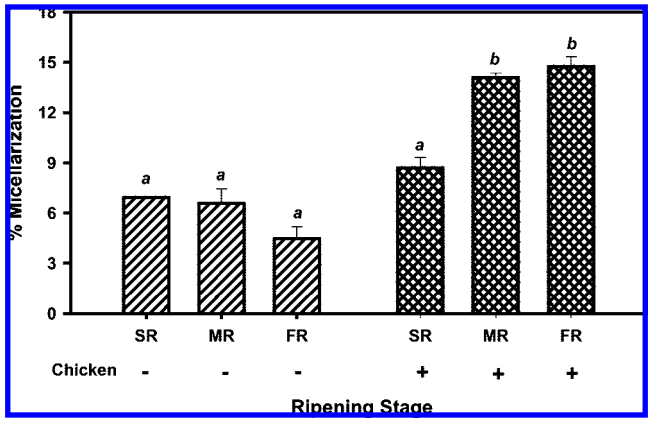

Figure 3. Effect of processed chicken on the micellarization of BC during digestion of "slightly ripe" (SR), "moderately ripe" (MR), and "fully ripe" (FR) 'Ataulfo' mangoes. Data represent the mean of three replicated samples ± the standard error of the mean (vertical bars). Means within the same set of bars with different letters above the error bar differ significantly ($p < 0.05$).

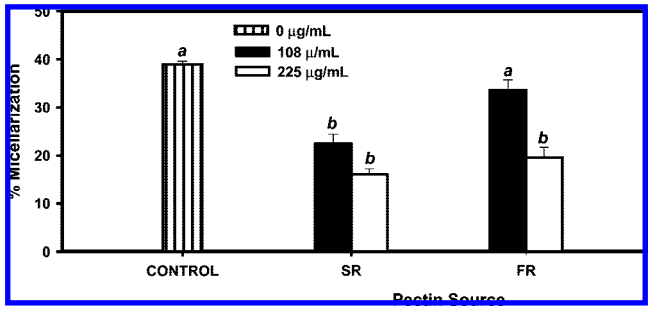

Figure 4. Impact of pectin isolated from "slightly ripe" (SR) or "fully ripe" (FR) 'Ataulfo' mangoes on micellarization of BC during simulated small intestinal digestion. BC in soybean oil supplement was diluted and mixed with a solution with indicated quantities of pectins and subjected to simulated small intestinal digestion. The aqueous fraction was isolated to determine the quantity of the carotenoid transferred to micelles for comparison with that transferred from control samples without pectin. Data are the mean of three replicated samples ± the standard error of the mean (vertical bars). The presence of different letters above the error bar indicates significant differences ($p > 0.05$).

not shown). Efficiency of micellarization of BC during small intestinal digestion declined from 6.9 ± 0.1 to 4.5 ± 0.7% when SR, MR, and FR mangoes without chicken were digested (**Figure 3**). The addition of chicken to mango increased ($p < 0.05$) micellarization of BC from SR, MR, and FR mangoes by 25.7, 114.4, and 231.1%, respectively, compared to digestion of mangoes alone (**Figure 3**).

Uptake of Micellarized β-Carotene from Mango by Caco-2 Cells. The cellular content of BC after 4 h of incubation with medium containing micelles generated during small intestinal digestion of FR mango with chicken represented 16.9 ± 0.5% (13.8 ± 0.3 pmol/mg cell protein) of that in the medium. Recovery of BC added to medium after 4.5 h of incubation in cell-free wells was 101.3 ± 0.6%. The relative amounts of 9-*cis*-β-carotene in the test medium before and after incubation period were 2.3 ± 0.1 and 4.8 ± 0.2% of the total β-carotene content, respectively ($p < 0.05$), indicating limited isomerization.

Effect of Pectin from SR and FR Mangoes on the Micellarization of β-Carotene from an Oil-Based Supplement. Transfer of BC from oil to filtered aqueous fraction was decreased by pectin (**Figure 4**). The addition of both concentrations of pectin from SR significantly decreased ($p < 0.05$) transfer of BC to filtered aqueous fraction during simulated small intestinal digestion but was not concentration-dependent. In contrast, transfer of BC to micelles during simulated small intestinal digestion was not significantly decreased ($p > 0.05$) by the presence of 108 μg/mL pectin from FR mango. However, addition of higher concentration (225 μg/mL) of pectin isolated from FR mango inhibited micellarization of BC to similar extent as pectin from SR mango.

DISCUSSION

This study has demonstrated that the ripening stage of mango affects the content of BC in pulp and the bioaccessibility of the carotenoid as determined by its efficiency of transfer to micelles during simulated digestion. The results also suggest that ingestion of mango with dietary fat will increase the micellarization of BC from mango during digestion, thereby increasing the potential for absorption of the carotenoid itself or its cleavage products. We also observed that the micellarization of BC was affected by the type and amount of pectin present in mango pulp at different stages of ripening.

Although the HPLC method employed for carotenoid analysis was exclusively designed for the rapid separation and quantification of all-*trans*-β-carotene, additional carotenoids including 11 violaxanthin-containing peaks were identified in mango pulp extracts. The presence of all-*trans*-β-carotene and 10 violaxanthin-containing peaks (free and esterified) have been also demonstrated in crude extracts from 'Alphonso' mango (*30*). The carotenoids present in 'Ataulfo' mango (*cis*/all-*trans* isomers of violaxanthin, β-cryptoxanthin, and β-carotene) have also been previously reported in other mango cultivars (*2, 3*).

The total carotenoid content of 'Ataulfo' mango was mainly represented by the content of all-*trans*-violaxanthin, 9-*cis*-violaxanthin, and all-*trans*-β-carotene, with all-*trans*-β-carotene predominant at all stages of ripening. Similar findings have been reported in 'Keitt', 'Tommy Atkins', and 'Kent' mangoes (*4, 23, 31*). The accumulation of such carotenoids followed an exponential behavior with ripening of the fruit as recently reported for all-*trans*-β-carotene in nine Thai cultivars of mango (*2*). The change of mango flesh color from white to yellow-orange is caused by the accumulation of carotenoids (*2, 16*), correlates with the ripening stage (*2*), and has been suggested as a reliable maturity index (*2, 13, 14*). Therefore, the markers used to assess maturation in the present work (carotenoid content, subjective color, and firmness) were appropriate.

Both the quantity and recovery of BC after digestion were affected by the stage of mango ripening. Recovery of BC improved as the fruit ripened. The high content of organic acids in unripe mango fruit decrease during the ripening process (*13*), and organic acids can induce losses of carotenoids in the juice and pulp of fruit and vegetables (*31, 33*). Thus, the stability of BC during digestion of mangoes at different stages of ripening may reflect acid-induced degradation that occurred during the preparation of the puree alone or in combination with simulated gastric digestion.

The amount of BC in mango transferred to the micelle fraction during simulated digestion of mango alone was influenced by the ripening stage of digested fruit. Softening of mango might increase the accessibility of carotenoids by facilitating the mechanical and enzymatic disruption of the pulp during digestion with release of carotenoids to oil droplets. Thus, ripening likely has a similar effect as homogenization and thermal processing that disrupt cell walls to provide digestive enzymes with access to macromolecules to facilitate transfer

of carotenoids to micelles and enhance the potential for absorption (*12, 34*).

Chicken was included in the digestion reactions to test the effect of a complex food on the micellarization of BC during digestion of mango. Mango fruit is consumed as a dessert, often after a typical meal that generally contains meat, or in meals containing both meat and mango (*1*). Chicken increased the efficiency of micellarization of BC from mango. The fat in the chicken probably promoted micellarization of BC from mango (*35*), although protein also may have stabilized the fat emulsification (*9*). *In vivo*, ingested fat increases secretion of pancreatic lipases and bile (*36*), thereby enhancing the formation of micelles and bioaccessibility of carotenoids. However, pectin isolated from mango partially inhibited the transfer of BC from soybean oil to micelles during simulated small intestinal digestion. This suggests that pectin has the potential to offset the promotional influence of dietary fat on the bioavailability of BC. Several investigators have reported that ingestion of pectin and other soluble fibers decreases carotenoid bioavailability in human subjects (*37, 38*). The negative effects of mango pectin can be attributed to its ability to alter the process of mixed micelle formation. Pasquier et al. (*39*) demonstrated that several dietary fibers including pectins increased the viscosity of reconstituted duodenal medium and affected emulsification and lipolysis of fat, indispensable steps for carotenoid micellarization (*40*). Pectin can also decrease pancreatic lipase activity (*41*) and bind bile acids (*42, 43*), thus reducing the formation of micelles and micellarization of carotenoids (*8*). We observed that the magnitude of the adverse impact of mango pectin on micellarization of BC depends upon the concentration and stage of ripening of fruit from which it was isolated. The effect of the increasing concentration of mango pectin on the bioaccessibility of BC can be explained in terms of an increased disruption of micelle formation (*39, 40*). The basis for the effect of the pectin source on the bioaccessibility of BC appears to be more complex. Depolymerization of pectin during mango ripening is associated with softening of the fruit (*15*). The mean molecular weight for the most abundant fraction of pectin in 'Alphonso' mango decreased from 1300 to 21 kDa in unripened and ripened fruit, respectively. This ripening-induced depolymerization of mango pectin may also be accompanied by a decrease in its degree of esterification as has been shown for other fruits (*44*). The viscosity of solutions containing high-molecular-weight pectin is greater than that with low-molecular-weight pectin (*45*). Interactions between pectin and bile salts decrease as the degree of esterification of pectin is reduced (*46*). Therefore, the inhibitory effect of pectin (at both 108 and 225 μg/mL) from SR mangoes (high molecular weight and degree of esterification) on micellarization of BC during simulated small intestinal digestion may have been due to increased viscosity and interaction with bile salts. Pectin from FR mangoes (low molecular weight and decreased esterification) was expected to have lower viscosity and interaction with bile salts. Depolymerized pectin from citrus and sugar beet exhibit emulsion stabilizing properties (*47*). This facilitates the small droplet size of the emulsion (a fine emulsion) and favors lipolysis of emulsified fat and subsequent micelle formation under physiological conditions (*39, 40*). Therefore, the more efficient micellarization of BC during simulated digestion with the lower concentration of FR mango pectin may have been due to greater lipolysis than in reactions with SR mango pectin. Inhibition of micellarization of BC in the reaction containing the higher concentration of FR mango pectin suggests that the outcome is dependent upon the relative amounts of pectin. Therefore, qualitative and quantitative changes of mango pectin during ripening are expected to affect the bioavailability of BC.

The accessibility of BC transferred to micelles during digestion of mango was confirmed by the ability of Caco-2 cells to accumulate the carotenoid from a medium containing diluted aqueous fraction. The extent of uptake during incubation fell in the range previously reported for other plant foods (*19, 48*).

We speculate that the bioavailability of BC in ripe mango (FR) alone is relatively low but can be increased when coconsumed with fat containing foods. Our results also suggest that the proposed provitamin A content for mango fruit that is solely based on BC content (*31, 49*) may be overestimated.

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