Accumulation of All-*trans*-β-carotene and Its 9-*cis* and 13-*cis* Stereoisomers during Postharvest Ripening of Nine Thai Mango Cultivars

ANA LUCÍA VÁSQUEZ-CAICEDO,[†] PITTAYA SRUAMSIRI,[‡] REINHOLD CARLE,[†] AND SYBILLE NEIDHART*,[†]

Institute of Food Technology, Section Plant Foodstuff Technology, Hohenheim University,
August-von-Hartmann-Strasse 3, 70599 Stuttgart, Germany, and Department of Horticulture,
Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand

---

Accumulation of β-carotene during postharvest ripening of nine Thai mango cultivars was assessed after verifying extraction and high-performance liquid chromatographic quantification of the β-carotene stereoisomers for this sample matrix. No relevant *trans*−*cis* isomerization was induced by the analytical procedure. The vitamin A potential of mangoes was evaluated at different ripening stages unequivocally defined by a ripening index ($\mathrm{RPI_{WB}}$). Being rather stable throughout postharvest ripening, the cultivar-specific proportion of *cis*-β-carotene isomers ranged from 14 to 40% of the total β-carotene content. Subjected to the same postharvest ripening conditions, only the cultivars Kaew, Maha Chanok, Chok Anan, and Nam Dokmai #4 developed a bright yellow−orange mesocarp coloration at their fully ripe stage ($\mathrm{RPI_{WB}} = 1.5-1.8$, sugar−acid ratio ~ 50), resulting in total β-carotene contents of 6544−11249 μg/100 g mesocarp dry weight (DW) and vitamin A values of 892−1573 retinol equivalents (RE)/100 g DW. Contrarily, poor-colored cultivars Mon Duen Gao, Rad, Kiew Sawoei, Okrong Kiew, and Okrong Thong reached total β-carotene contents of 1019−2195 μg/100 g DW and vitamin A values of 136−298 RE/100 g DW at comparable sugar−acid ratios. Exponential development of mesocarp color (hue angle, *H*°) and all-*trans*-β-carotene levels, respectively, with $\mathrm{RPI_{WB}}$ was described for each cultivar, allowing good prediction of mesocarp color and vitamin A value at consumption ripeness.

KEYWORDS: ***β*-Carotene stereoisomers; *Mangifera indica* L.; postharvest ripening; vitamin A value; color**

INTRODUCTION

Biosynthesis of carotenoids is one of the major biochemical changes occurring in mango fruits (*Mangifera indica* L.) during postharvest ripening (*1*, *2*). The basic C_5-isoprene unit is subjected to the isoprenoid pathway producing C_{20}-geranylgeranyl diphosphate and finally C_{40}-phytoene. In a subsequent series of dehydrogenation reactions, colored carotenoids are formed (*3*, *4*). All-*trans*-β-carotene results from direct cyclization of all-*trans*-lycopene via the enzyme β-cyclase (*3*, *5*). However, no *cis*-β-carotene isomers are formed by this enzymatical reaction.

Among mango carotenoids, only β-carotene is considered to be of provitamin A relevance, since all-*trans*- and *cis*-β-cryptoxanthin can only be found at very small levels (*6*). Quantifying the proportion of the *cis*-β-carotene isomers is essential in provitamin A estimation, since efficiency of β-carotene conversion into vitamin A is only 53 and 38% for 13-*cis*- and 9-*cis*-β-carotene, respectively (*7*). Although naturally occurring *cis*-β-carotene isomers have been detected in fruits such as mamey (*Mammea americana* L.), buriti (*Mauritia vinifera* Mart.), loquat (*Eriobotrya japonica* Lindl.) (*8*), mango (*M. indica* L.) (*8*−*10*), plums (*Prunus domestica* L.), nectarines [*Prunus persica* (L.) Batsch var. *nucipersica*], and peaches [*Prunus persica* (L.) Batsch] (*8*, *11*), the mechanism of their formation has not been reported. Parallel pathways to all-*trans*-β-carotene and 9-*cis*-β-carotene have been suggested (*3*, *12*), but to date, no biochemical evidence supports this mechanism.

Detection and quantification of *cis*-β-carotene isomers were not achieved in studies on raw mangoes at mature-green, partially ripe, and fully ripe stages (*6*, *13*, *14*) or on frozen and canned mango slices (*15*). Only traces of 13-*cis*-β-carotene were found in some samples of mango cvs. Haden and Tommy Atkins, elucidating its structure by use of nuclear magnetic resonance (NMR) (*8*). The development of high-performance liquid chromatographic (HPLC) techniques based on a polymeric C_{30} stationary phase has improved the separation, identification, and quantification of *cis*-β-carotene isomers and other carotenoids (*10*, *16*). Recently, total *cis*-β-carotene isomers have

---

* To whom correspondence should be addressed. Tel: +49(0)711 459 2317. Fax: +49(0)711 459 4110. E-mail: neidhasy@uni-hohenheim.de.

[†] Hohenheim University.

[‡] Chiang Mai University.

been found to account for 24.4, 25.8, 27.1, and 19.1% of the total *β*-carotene in the cultivars Kent, Tommy Atkins, Nam Dokmai, and Kaew, respectively, when unsaponified fresh fruit extracts were analyzed (*9*). Investigations by LC-(atmospheric pressure chemical ionization)MS confirmed the presence of violaxanthin, neochrom/luteoxanthin, *cis*-violaxanthin, and all-*trans*-*β*-carotene in saponified samples from fully ripe fresh fruits of mango cv. Kent (*17*). In corresponding unsaponified samples, all-*trans*-*β*-carotene, the newly identified violaxanthin dibutyrate, and *cis*-violaxanthin dibutyrate were the major carotenoids (*17*). Additionally, the presence of neoxanthin plus its *cis* isomers, zeaxanthin, and 9- or 9′-*cis*-lutein in saponified extracts have been recently reported for Taiwanese mangoes of an unspecified cultivar, where all-*trans*-*β*-carotene was clearly prevailing (*10*).

Although saponification has been recommended only if strictly necessary (*16*), hydrolysis of carotenol esters simplifies chromatographic separation in mango samples (*18*). No *cis*-*β*-carotene isomers were formed after cold saponification, and minimum losses of all-*trans*-*β*-carotene (1%) and *β*-apo-8′-carotenal (3%) were observed (*19*), although free *β*-cryptoxanthin quantification may be affected (*20*).

Mango fruits are consumed at different ripening stages, depending on eating preferences or adequacy for processing (*21*). However, an objective definition of fruit ripeness was ignored in most of the reviewed studies on the carotenoid composition of mangoes. Classification of fruits on the basis of indistinct maturity grades (*1*, *6*) caused ambiguities. The occurrence of *cis*-*β*-carotene isomers in mangoes at various objective maturity stages still has to be evaluated. Recently, a ripening index combining fruit firmness with the sugar−acid ratio (TSS/TA) has been introduced for postharvest ripeness specification in our parallel studies (*22*) (The definition of the ripening index is described in the Materials and Methods).

Thus, the objective of this study was to evaluate the vitamin A potential together with relevant physicochemical characteristics of various Thai mango cultivars at well-defined fruit ripeness expressed by our ripening index. Adequate extraction and separation procedures were used to assess the occurrence of *cis*-*β*-carotene isomers during postharvest ripening of mangoes. Aiming at improved baseline separation and quantification of *β*-carotene stereoisomers by use of an internal standard (ISTD), cold saponification was applied after its validation prior to routine analysis.

MATERIALS AND METHODS

Samples for Evaluation of *β*-Carotene Extraction. Fresh mango fruits cv. Kent (Peru) at their mature green-ripe stage were obtained from the wholesale market in Stuttgart, Germany. After 4 days of ripening at 25−30 °C, ca. 25 kg of the fruits was manually peeled and destoned. The fruit mesocarp was cut into pieces, which were successively mashed and finished by use of a pulper (type PAP 0533, Bertuzzi, Brugherio, Italy) with sieves of 1.5 mm and 0.4 mm mesh size, to obtain a homogeneous sample. The resulting purée was packed into PE pouches (100−200 g portions), sealed under vacuum, and stored at −80 °C until analysis.

Postharvest Ripening Studies on Thai Mangoes. Mangoes of the cultivars Kaew, Maha Chanok, Chok Anan, Nam Dokmai #4, Mon Duen Gao, Rad, Kiew Sawoei, Okrong Kiew, and Okrong Thong were obtained from Maejo University and a local farm, both in Chiang Mai, Thailand, during the main harvesting season in May 2001. Experiments were repeated in season 2002 (April) for the first five cultivars mentioned above, focusing on the cultivars interesting for industrial processing, due to the high *β*-carotene content and intensive color observed for these varieties in year 2001. Approximately 20 kg of each cultivar was harvested at their mature green-ripe stage and immediately subjected to postharvest ripening for 4−7 days at ambient conditions [season 2001, 29 ± 2 °C and 90−95% relative humidity (RH); season 2002, 35 ± 2 °C and 65−70% RH], imitating typical postharvest ripening practices for mangoes in the area under study. Approximately 5 g calcium carbide/kg fruit was used as a ripening accelerator, packed in paper bags, and distributed in small portions over each of the ripening boxes. Samples of approximately 1 kg were randomly collected for daily physicochemical analyses of fruit ripeness at Chiang Mai University, Chiang Mai, Thailand.

For characterization of ripeness, mango mesocarp was homogenized in a laboratory blender (Moulinex, Groupe SEB, Ecully, France) before determining total soluble solids (TSS), titratable acidity (TA), pH, and color of flesh at Chiang Mai University. TSS was measured refractometrically (*23*). TA was determined by titration with 0.1 M NaOH to pH 8.1 and expressed as g citric acid/100 g (*23*). For associated physical characterization of the fruits, the mesocarp color was evaluated with a colorimeter CR310 and CR300 (both Minolta, Tokyo, Japan) in years 2001 and 2002, respectively, on the basis of the CIELAB color system (L^*, a^*, b^* and L^*, C^*, $H°$, respectively) (*24*). Color data sets were measured 10-fold per sample by use of a freshly obtained purée, which was homogeneously distributed on a Petri dish placed on a white background. Characterizing color intensity, chroma $C^* = [(a^*)^2 + (b^*)^2]^{0.5}$ describes the length of the color vector in the rectangular $a^* \times b^*$ space, where a^* and b^* specify the green−red and the blue−yellow hue axes, respectively, ranging from a^- (green) to a^+ (red) and from b^- (blue) to b^+ (yellow). The hue angle $H° = 180 \cdot \pi^{-1} \cdot \arctan[b^* \cdot (a^*)^{-1}]$, in this study in degrees, marks the position of this vector and hence the nature of the hue with $H° = 0$ and $H° = 90°$ for pure red and yellow, respectively. The lightness L^* completes this coordinate system to the three-dimensional color space. To evaluate mesocarp firmness, the maximum shear force ($F_{\mathrm{WB,i}}$ in N) needed to cut a fruit cube over an area of 1 cm × 1 cm was measured, using an Instron Universal texture analyzer (model 5565; Instron, Canton, MA) equipped with a Warner−Bratzler shear cell. Subsequently, the ripening index $\mathrm{RPI}_{\mathrm{WB}}$, which was broadly explained in a previous study (*22*), was calculated as defined by eq 1, where dimensionless fruit firmness (F_{WB}) $= |F_{\mathrm{WB,i}}|$ is the absolute value of the maximum shear force $F_{\mathrm{WB,i}}$ described above and TSS/TA specifies the sugar−acid ratio of the mesocarp.

$$\mathrm{RPI}_{\mathrm{WB}} = \ln(100 \cdot F_{\mathrm{WB}} \cdot \mathrm{TSS}^{-1} \cdot \mathrm{TA}) \tag{1}$$

Parallel to the physicochemical monitoring of postharvest ripening, mesocarp samples were daily collected for *β*-carotene analyses at Hohenheim University. During the harvest season 2001, mesocarp cubes of 1−2 cm^3 were shock-frozen in liquid nitrogen, vacuum-packed in aluminum pouches, and stored at −22 °C until freeze drying. Shortly before freeze drying, frozen mango pieces were submerged into liquid nitrogen in order to facilitate the pulverization of the sample in a laboratory blender (Moulinex, Groupe SEB, Ecully, France). Pulverized samples were immediately freeze-dried, packed in plastic bottles, and sent deep-frozen (ca. −40 °C) by airfreight to Hohenheim University. Freeze-dried samples were stored at −80 °C until *β*-carotene analyses.

In season 2002, sample collection for *β*-carotene analyses was slightly modified due to limited equipment availability. Mango pieces were shock-frozen in liquid nitrogen, vacuum-packed in aluminum pouches, and stored at −80 °C until their airfreight transport to Hohenheim University at a sample temperature of −40 °C. Frozen mango samples were immediately stored at −80 °C until *β*-carotene analyses. For extraction, the frozen mango pieces were quickly thawed in their pouches in a water bath at 20 °C for 10−15 min and homogenized by use of an ultra-turrax (Janke & Kunkel, Stauffen, Germany).

Chemicals and Solvents. All reagents and solvents were of analytical or HPLC grade (VWR, Darmstadt, Germany). All-*trans*-*β*-carotene (type II, HPLC > 95%, Sigma, St. Louis, MO) and *β*-apo-8′-carotenal (trans) (∼98% UV, Fluka, Buchs, Switzerland) were used for external calibration and as ISTD, respectively.

***β*-Carotene Extraction.** The *β*-carotene extraction procedure previously proposed for mangoes (*9*) was modified to include saponification. Exactly weighed samples of freeze-dried mango mesocarp powder (1−2 g) or mango purée (ca. 10 g) were homogenized for 10−20 s by use of an ultra-turrax after the addition of Celite, calcium carbonate, and cold methanol. The ISTD (ca. 20 mg of β-apo-8′-carotenal/L) was added to the sample prior to extraction. Freeze-dried samples were previously rehydrated with ca. 10 mL of redistilled water. The homogenized extract was filtered through a sintered glass funnel (porosity 2, 40−100 μm) under reduced pressure. The recovered solids were successively extracted 2−3 times with ca. 30 mL of cold methanol each time, until the residue was colorless. Extraction was carried out at subdued light, covering all glassware with aluminum foil to avoid light exposure. The methanolic carotene extract was transferred to a 500 mL amber glass separation funnel with 40 mL of petroleum ether (extra pure; boiling range, 40−60 °C), containing 100 mg of 2,6-di-*tert*-butyl-*p*-cresol (BHT) and 100 mg of 3-*tert*-butyl-4-hydroxyanisol (BHA) per liter, respectively (*25*), and 30 mL of NaCl (10% w/w) to avoid the formation of an emulsion. The petroleum ether fraction was recovered in a 250 mL capped amber glass bottle, while the methanolic aqueous phase was washed 2−4 times successively with 20 mL of petroleum ether until complete extraction of the pigments. After recovery of the combined petroleum ether fractions, an equal volume of methanolic KOH (10% w/w) was added for saponification (*19*), continuously stirring this mixture at 20−25 °C for 3 h under a N_2 atmosphere. The saponified extract was transferred to an amber glass separation funnel and washed with 50 mL of redistilled water. The aqueous phase (yellowish) was washed with 20 mL of petroleum ether for complete recovery of carotenes. The petroleum ether fraction was washed 3−4 times successively with 30−50 mL of redistilled water to remove alkali, until pH 7. The petroleum ether extract was dried with sodium sulfate (2 g) and evaporated in vacuo (<150 mbar, 20−25 °C). The dried residue was dissolved in 2-propanol and adjusted to a volume of 5 mL, membrane-filtered (0.2 μm), and transferred to amber glass vials. All determinations were carried out in duplicate. Aliquots of 20 μL were used for HPLC analysis.

HPLC Analysis. HPLC separation was performed according to Marx et al. (*16*), using the same separation column and HPLC equipment together with a degasser GT 154 (Shimadzu, Kyoto, Japan). 9-*cis*-β-Carotene and 13-*cis*-β-carotene standards were obtained by iodine-catalyzed photoisomerization of all-*trans*-β-carotene and purified as previously described (*26*).

Quantification of all-*trans*-, 9-*cis*-, and 13-*cis*-β-carotene was on the basis of linear calibration curves (eq 2) that resulted from regression analysis between the corresponding isomer concentration (C_{isomer}) of each standard and the respective peak area quotient (Q_{isomer}), obtained from the peak areas of the isomer ($\text{PA}_{\text{isomer}}$) and the ISTD β-apo-8′-carotenal (PA_{ISTD}) in the standard chromatograms (*27*, *28*).

$$C_{\text{isomer}} = a \cdot Q_{\text{isomer}} + b \text{ with } Q_{\text{isomer}} = \text{PA}_{\text{isomer}}/\text{PA}_{\text{ISTD}} \qquad (2)$$

Peak areas were monitored at 453 nm for all-*trans*-β-carotene and β-apo-8′-carotenal and at 445 nm for 9-*cis*- and 13-*cis*-β-carotene, respectively. Concentrations of the standards were measured photometrically using the extinction coefficients $A^{1\%}_{1\text{cm}}$ of 2592 at 450 nm for all-*trans*-β-carotene, 2551 at 445 nm for 9-*cis*-β-carotene, 2094 at 443 nm for 13-*cis*-β-carotene (in hexane), and 2640 at 457 nm for β-apo-8′-carotenal (in petroleum ether) (*29*−*31*). At least five standard concentrations were used for each calibration curve, ranging from 0.5 to 38.8 mg/L, from 0.6 to 6.0 mg/L, and from 0.4 to 3.8 mg/L for all-*trans*-, 9-*cis*-, and 13-*cis*-β-carotene, respectively, on the basis of a quantification limit of approximately 0.30 mg/L for the system used.

Evaluation of the Extraction Procedure. To validate the extraction procedure, the effects of saponification on the stability of the ISTD β-apo-8′-carotenal and the all-*trans*-, 9-*cis*-, and 13-*cis*-β-carotene isomers were studied. Insignificant changes in all-*trans*-, 9-*cis*-, and 13-*cis*-β-carotene contents during saponification with 10% methanolic KOH for 16 h at room temperature had been previously reported (*19*). However, because this saponification time was considered too long, reduction of saponification time to 3 h was examined by use of a mix of standard isomers, which was prepared by iodine-catalyzed photoisomerization of all-*trans*-β-carotene (Sigma, type II) dissolved in 2-propanol (*26*). One milliliter of the ISTD β-apo-8′-carotenal (ca. 20 mg/L) was added to 2 mL of the isomer mix and 30 mL of petroleum ether, prior to the saponification with 30 mL of methanolic KOH (10% w/w) for 3 or 16 h at 20−25 °C under a N_2 atmosphere. A control without saponification, containing the isomer mix and the ISTD, was included. After saponification, the extract was transferred to an amber glass separation funnel and washed 3−4 times with 30−50 mL of redistilled water to remove alkali, until pH 7, following the procedure described above for routine β-carotene extraction.

The stability of β-apo-8′-carotenal during carotene extraction from mango purée was checked by its determination after its addition at the beginning and at the end of the extraction procedure, respectively. Ca. 10 g of mango purée cv. Kent was exactly weighed and extracted as described above for routine β-carotene extraction but varying the order of β-apo-8′-carotenal addition (ca. 40 mg/L): (i) addition of 0.5 mL of ISTD before saponification, (ii) addition of 0.25 mL of ISTD before saponification and 0.25 mL of ISTD before adjusting to the final volume of 5 mL and subsequent membrane filtration into vials, and (iii) addition of 0.5 mL of ISTD at the end of the extraction, before adjusting to the final volume.

To establish the repeatability of the extraction procedure, the purée was successively extracted nine times as described above for routine β-carotene extraction, analyzing each extract twice according to the HPLC method summarized above.

Recovery Studies. To verify the recovery of all-*trans*-β-carotene in a sample, ca. 10 g of mango purée (cv. Kent) was exactly weighed and 0, 0.5, 1, or 1.5 mL of an all-*trans*-β-carotene standard (34.4 mg/L in 2-propanol) was added to the sample at the beginning of the respective extraction, following the routine β-carotene extraction procedure described above. All determinations were carried out in duplicate.

Statistical Analysis. Data were subjected to analysis of variance by application of the general linear model (GLM) of the Statistical Analysis System (SAS, 1999−2001, version 8.02). Correlation analysis between β-carotene content and color was performed by use of the JMP software (SAS Institute Inc., 1989−1997, version 3.2.1). Regression analysis between various quality parameters was calculated with EXCEL 97 (Microsoft).

RESULTS AND DISCUSSION

Evaluation of Extraction Procedure. To exclude the formation of artifacts, chlorinated solvents were avoided for extraction and chromatographic separation as previously suggested (*16*). The suitability of the C_{30}-reversed phase column to separate a broad range of free and esterified carotenoids in unsaponified extracts of paprika (*32*) or mango (*17*) has been recently discussed. However, esterified carotenoids coeluted with the ISTD (β-apo-8′-carotenal) (**Figure 1A** vs **C**), which had to be unavoidably included to achieve an accurate quantification. Moreover, the focus of our study was solely to separate and quantify β-carotene and its stereoisomers. The saponification step produced free carotenols with increased polarity, resulting in a shift of their retention times to the first 12 min (**Figure 1B**). This facilitated the addition of the ISTD, which eluted between 18 and 20 min (**Figure 1C**).

Saponification of fresh mango samples improved separation of all-*trans*-β-carotene and its *cis* isomers when compared to the unsaponified extract, which was obtained as previously described (*9*) (**Figure 1B**,**A**, respectively). As indicated by the low coefficients of variation (CV) in **Table 1** (CV ≤ 5%), *trans*−*cis* isomerization observed after 3 h of saponification was irrelevant. Nevertheless, **Table 1** shows significant time-dependent differences of saponification treatments for all-*trans*-β-carotene and 13-*cis*-β-carotene, respectively. However, when the CVs in **Table 1** were compared with those of the repeatability study, *trans*−*cis* isomerization was obviously negligible. Repeatability was verified by means of nine extractions of a single mango purée. CVs were 6.73, 6.91, and 6.26% for all-*trans*-β-carotene [420.9 ± 28.3 μg/100 g fresh weight (FW)] and the isomers 9-*cis*-β-carotene (57.0 ± 3.9 μg/100 g FW)

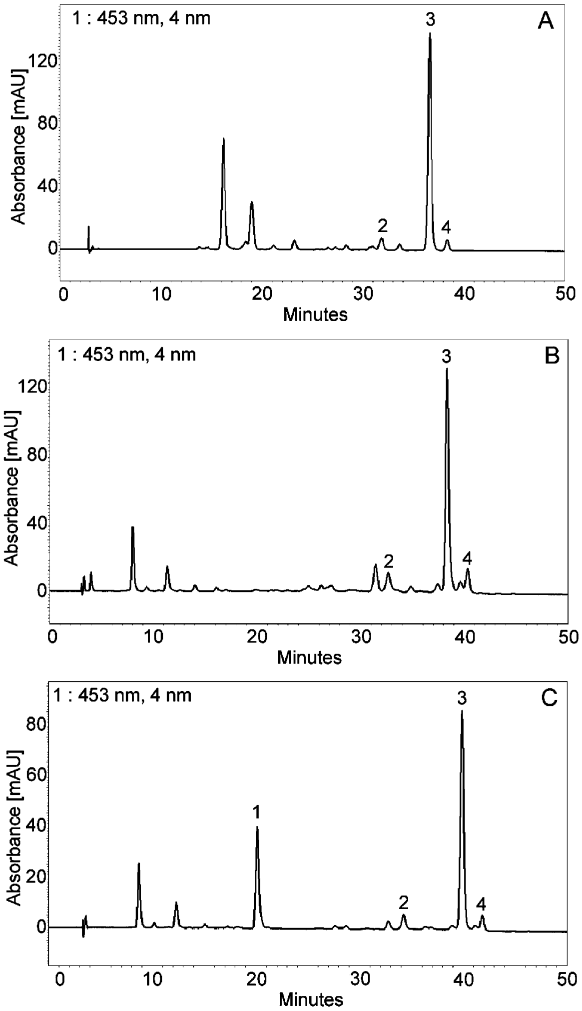

Figure 1. HPLC separation of β-carotene stereoisomers in mango mesocarp cv. Kaew: (**A**) unsaponified sample, (**B**) after saponification without adding ISTD, and (**C**) after saponification with addition of ISTD. Peaks: 1 = β-apo-8′-carotenal (ISTD); 2 = 13-*cis*-β-carotene; 3 = all-*trans*-β-carotene; and 4 = 9-*cis*-β-carotene.

(expressed as all-*trans*-β-carotene) and 13-*cis*-β-carotene (69.4 ± 4.3 μg/100 g FW) (expressed as all-*trans*-β-carotene), respectively. Consistently, the evaluation of an extraction method for the analysis of carotenoids from canned tomato juice resulted in CVs between 4.6 and 10.5% for β-carotene (*28*). Therefore, a saponification time of 3 h was sufficient to achieve satisfactory quantification of the β-carotene stereoisomers. This was in accordance with the extraction procedure suggested for β-carotene from fruit matrices, where the hydrolysis of carotenol esters simplified the separation of the commonly found mixture of carotenoids forming esters with a variety of fatty acids (*18*).

By adding the ISTD to the sample before and after the extraction procedure, respectively, stability of the β-carotene isomers during extraction was examined (**Table 2**). Although significant differences between some treatments could be detected for a stereoisomer, CVs were still below the analytical errors stated above for all-*trans*-β-carotene and the *cis*-β-carotene isomers. Therefore, the ISTD was added at the beginning of the extraction. Losses of 1% of all-*trans*-β-carotene and 3% of β-apo-8′-carotenal were reported after extended saponification of 16 h (*19*), but no loss of all-*trans*-β-carotene occurred after 3 h of saponification in the present study. Moreover, drastically shortening the saponification time from 16 to 3 h should even support carotenoid stability. Potential losses owing to solvent transfer during carotenoid extraction were adjusted by the early addition of the ISTD to the food matrix. In previous studies, the recovery of all-*trans*-β-carotene ranged between 95 and 105% (*9*, *16*). Consistently, the recovery range was between 99.4 and 105.4% for the proposed extraction procedure (**Table 3**).

Postharvest Ripening of Thai Mangoes. The cultivars investigated in this study markedly differed in their total β-carotene contents along their ripening process. Classification in β-carotene rich and poor varieties was possible. Changes in all-*trans*-, 9-*cis*-, and 13-*cis*-β-carotene during postharvest ripening of cultivars rich in β-carotene are shown in **Table 4**. The vitamin A value is reported as retinol equivalents (RE) per 100 g edible part (*7*), expressed on the dry weight (DW) basis to facilitate a direct comparison among cultivars, irrespective of the water content. Mango fruits were classified by both their TSS/TA ratio and the corresponding ripening index ($\mathrm{RPI}_{\mathrm{WB}}$) for an objective comparison of physiological ripening stages. Typical ripeness at fresh consumption was characterized by TSS/TA ratios ranging from 23 to 50. Depending on the cultivar,

Table 1. Effect of Saponification Time on *trans*–*cis* Isomerization of a β-Carotene Isomer Mix Standard

	saponification time (h)				
isomer (mg/L)	0	3	16	CV[a] (%)	Pr > F
all-*trans*-β-carotene	14.28 ± 0.08^{b} b	15.26 ± 0.19 a	13.88 ± 0.19 b	5.0	0.0024
13-*cis*-β-carotene	6.38 ± 0.07 a	6.42 ± 0.05 a	5.94 ± 0.07 b	4.2	0.0016
9-*cis*-β-carotene	7.21 ± 0.05 a	7.44 ± 0.18 a	7.17 ± 0.08 a	3.7	0.3683

[a] CV calculated from all observations ($n = 10$) for the corresponding isomer. [b] ± Standard error. Duncan's means comparison test: Values marked with the same letter (horizontally) are not significantly different ($p > 0.05$); Pr > F, transgression probability.

Table 2. Addition of the ISTD β-Apo-8′-carotenal during Extraction with Saponification of 3 h: Effect of the Timing of ISTD Addition

	isomer content (μg/100 g DW)				
isomer	A	B	C	CV[a] (%)	Pr > F
all-*trans*-β-carotene	594.3 ± 9.4^{b} b	607.4 ± 11.5 b	641.6 ± 4.2 a	4.3	0.0119
13-*cis*-β-carotene	79.4 ± 1.6 b	81.3 ± 2.1 b	86.9 ± 0.6 a	5.3	0.0228
9-*cis*-β-carotene	70.4 ± 1.8 a	76.2 ± 0.7 a	79.6 ± 0.7 a	6.0	0.0014

[a] CV calculated from all observations ($n = 12$) for the corresponding isomer. [b] ± Standard error. A = 0.5 mL of ISTD after extraction; B = 0.25 mL of ISTD before extraction and 0.25 mL of ISTD after extraction; and C = 0.5 mL of ISTD before extraction. Duncan's means comparison test: Values marked with the same letter (horizontally) are not significantly different ($p > 0.05$); Pr > F, transgression probability.

Table 3. Recovery of All-*trans*-β-carotene after Complete Extraction of 10 g of Mango Purée (Cv. Kent) with Addition of All-*trans*-β-carotene Standard

dosage of standard: all-*trans*-β-carotene added (μg/100 g FW)	total content of all-*trans*-β-carotene expected (μg/100 g FW)	total content of all-*trans*-β-carotene detected (μg/100 g FW)	CV (%)	recovery (%)
0		641.6 ± 4.2[a]	1.3	
170.9	812.5	828.7 ± 13.9	3.4	102.0
342.6	984.2	1036.5 ± 47.1	9.1	105.4
513.0	1154.6	1147.6 ± 23.4	4.1	99.4

[a] ± Standard error.

the associated RPI_{WB} ranged from 1.5 to 3.5 in this group of varieties. The use of a ripening index (RPI_{WB}) compensated the limitations of the TSS/TA ratio, since other characteristics, such as adequate texture and color, are equally desired. By definition, RPI_{WB} accounts for mesocarp firmness and sugar−acid ratio (eq 1), combining the most essential physical and chemical basic properties of the ripening fruit regarding sensory ripeness perception (*22*). Because basic color characteristics are a specific property of each fruit cultivar (*33*), color is not included in this ripening index but was rather related to RPI_{WB} in this study in parallel with carotenoid changes as shown below. Although the use of a sensory-based index has been discussed as an alternative to the sugar−acid ratio particularly in the citrus industry (*34*), TSS/TA remains an indicator of harmonic taste (*33*) and is still widely applied by processors as ripening indicator and in fruit product standardization. Hence, it has been included in **Tables 4** and **5** besides RPI_{WB}.

As shown in **Table 5**, cultivars poor in β-carotene are typically consumed at RPI_{WB} levels between 1.5 and 5.0. The wide range reflects the numerous cultivar-specific applications ascribed to this group of mango varieties. Kiew Sawoei and Rad are usually consumed at their mature green-ripe stage (TSS/TA ≤ ∼25), when fruits are still firm. Unlike those varieties, Mon Duen Gao, Okrong Thong, and Okrong Kiew may be consumed after substantial mesocarp softening, irrespective of the poor color development (*33*).

Table 4. Total β-Carotene Content and Isomer Proportion in Cultivars Rich in β-Carotene (Crop Year 2001)

cultivar	TSS/TA	RPI_{WB}	β-carotene total (μg/100 g DW)	β-carotene all-*trans* (%)	β-carotene 13-*cis* (%)	β-carotene 9-*cis* (%)	vitamin A[a] (RE/100 g DW)
Kaew	9.0	5.00	1190 ± 75[c]	86.7	13.3	ND	186 ± 13[c]
	26.9[b]	2.62	5912 ± 231	85.8	8.8	5.4	912 ± 34
	50.2[b]	1.50	8249 ± 14	82.5	9.6	7.8	1246 ± 2
Chok Anan	9.8	5.52	536 ± 6	100.0	ND	ND	89 ± 1
	27.6[b]	3.46	5053 ± 64	65.9	17.3	16.8	686 ± 7
	49.9[b]	1.83	6544 ± 21	66.3	17.6	16.1	892 ± 1
Maha Chanok	10.8	3.47	3060 ± 49	82.8	9.6	7.6	463 ± 8
	23.4[b]	2.56	6700 ± 479	84.1	8.8	7.1	1022 ± 77
	47.7[b]	1.67	8563 ± 305	84.7	9.5	5.9	1312 ± 48
Nam Dokmai #4	8.9	5.28	1658 ± 12	73.4	12.2	14.4	236 ± 1
	26.6[b]	3.14	5513 ± 30	68.8	15.4	15.8	762 ± 2
	47.6[b]	1.61	11249 ± 939	70.5	14.8	14.7	1573 ± 140

[a] Determined as suggested by Zechmeister (*7*): Vitamin A $= (6^{-1} \cdot C_{\text{AT}}) + 0.53(6^{-1} \cdot C_{13-cis}) + 0.38(6^{-1} \cdot C_{9-cis})$. [b] Typical ripeness at fresh consumption. [c] ± Standard error. ND = not detectable.

Table 5. Total β-Carotene Content and Isomer Proportion in Cultivars Poor in β-Carotene (Crop Year 2001)

cultivar[a]	TSS/TA	RPI_{WB}	β-carotene total (μg/100 g DW)	β-carotene all-*trans* (%)	β-carotene 13-*cis* (%)	β-carotene 9-*cis* (%)	vitamin A[b] (RE/100 g DW)
Kiew Sawoei	NA	NA	NA	NA	NA	NA	NA
	24.1[c]	4.69	673 ± 30[d]	100.0	ND	ND	112 ± 5[d]
	52.8	3.13	1544 ± 68	74.2	12.7	13.1	221 ± 10
	111.3	0.62	1831 ± 33	73.5	13.9	12.6	261 ± 5
Rad	11.8[c]	5.00	272 ± 19	100.0	ND	ND	45 ± 3
	24.5[c]	2.95	985 ± 4	62.6	18.5	18.9	131 ± 0
	83.3	1.48	2195 ± 59	65.9	18.2	15.9	298 ± 8
	98.1	NA	2298 ± 86	60.2	21.6	18.2	301 ± 12
Mon Duen Gao	13.7	5.27	613 ± 34	100.0	ND	ND	102 ± 6
	22.7[c]	3.48	675 ± 23	100.0	ND	ND	113 ± 4
	48.5[c]	1.73	1592 ± 33	70.3	14.0	15.7	222 ± 5
	97.0	NA	2035 ± 39	68.2	16.5	15.3	281 ± 6
Okrong Thong	14.5	3.49	289 ± 10	100.0	ND	ND	48 ± 2
	23.4	2.88	329 ± 12	100.0	ND	ND	55 ± 2
	65.1[c]	1.47	1977 ± 39	66.5	17.4	16.1	270 ± 6
	114.1	0.63	2538 ± 62	66.0	18.1	15.8	345 ± 8
Okrong Kiew	14.2	2.80	343 ± 0	100.0	ND	ND	57 ± 0
	28.5	NA	468 ± 17	100.0	ND	ND	78 ± 3
	52.3[c]	1.57	1019 ± 13	62.9	18.3	18.8	136 ± 2
	NA	NA	NA	NA	NA	NA	NA

[a] Cultivars poor in β-carotene may not reach red color (see parameters s_1 and t_1 in **Table 7**). [b] Determined as suggested by Zechmeister (*7*), see footnote a of **Table 4**. [c] Typical ripeness at fresh consumption. [d] ± Standard error. ND = not detectable; NA = sample not available or individual parameter not determined.

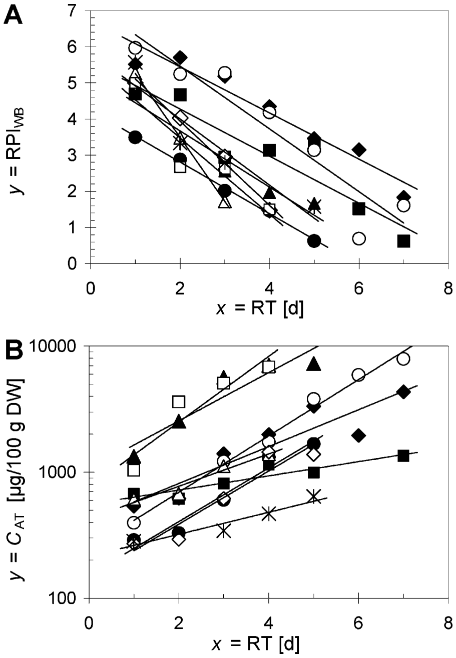

Figure 2. Modeling of postharvest ripening changes as functions of time ($x = $ RT in days): (**A**) cultivar-specific linearity between ripening index ($y = \mathrm{RPI_{WB}}$) and RT and (**B**) exponential accumulation of all-*trans*-β-carotene ($y = C_{AT}$ in μg/100 g DW). R^2 in parentheses for **A** and **B**, respectively: (□), Kaew (0.86, 0.87); (◆), Chok Anan (0.93, 0.85); (▲), Maha Chanok (0.94, 0.88); (○), Nam Dokmai #4 (0.88, 0.99); (■), Kiew Sawoei (0.87, 0.84); (◇), Rad (0.99, 0.90); (△), Mon Duen Gao (1.00, 0.94); (●), Okrong Thong (0.996, 0.95); and (∗), Okrong Kiew (0.87, 0.92).

On the whole, at similar TSS/TA ratios and RPI$_{WB}$, extremely varying β-carotene levels and corresponding vitamin A potentials were noticed. Among all cultivars studied, Nam Dokmai #4 showed by far the highest vitamin A potential representing more than the 11- and 7-fold RE/100 g DW of Okrong Kiew and Kiew Sawoei, respectively. The cultivars Kaew and Maha Chanok were also rich in provitamin A (1246 and 1312 RE/100 g DW, respectively) but mostly because of their markedly higher all-*trans*-β-carotene proportions rather than because of the total β-carotene contents. The former exceeded that of cv. Nam Dokmai #4 by 12 and 14%, respectively, at comparable levels of TSS/TA ratios and RPI$_{WB}$.

At early ripening stages, *cis* isomers were only observed in cultivars rich in β-carotene, possibly because of their occurrence in β-carotene-poor varieties at concentrations below the detection limits. A measurable occurrence of *cis* isomers was associated with total β-carotene contents exceeding ~985 μg/100 g DW (**Tables 4** and **5**). Above this β-carotene level, the proportion of total *cis* isomers only varied slightly with maturity. In all cultivars, total *cis* isomers ranged from 15.3 to 37.1% at TSS/TA ratios around 50. Although recent studies in microalgae revealed that the accumulation of 9-*cis*-β-carotene, among other *cis* isomers, is enhanced by stress factors, such as increased salinity (*35*) and exposure to light and irradiation (*36*), biosynthesis, breakdown, or interconversion pathways of carotenoids greatly vary among plant species (*12*) and even possibly among cultivars, as suggested by the present study (**Tables 4** and **5**). The presence of *cis* isomers could be explained by the site of carotenoid accumulation in the chromoplast structures (plastoglobules) (*12*) and the solubilization of carotenoids in lipid globules (*37*, *38*). However, the rather constant proportions of total *cis* isomers during ripening that were observed for each of the cultivars suggested a natural equilibrium between β-carotene stereoisomers, by analogy with a mixture of synthetic β-carotene stereoisomers (*7*).

To study the development of the β-carotene stereoisomers during postharvest ripening of the mango fruits by application of a kinetic approach, the ripeness index RPI$_{WB}$ was used for unambiguous maturity specification in mathematical modeling of postharvest ripening changes. Its suitability for this purpose is shown by **Figure 2A**, as RPI$_{WB}$ linearly decreased with proceeding ripening time (RT), with coefficients of determination (R^2) ranging from 0.86 to 1.00. All-*trans*-β-carotene (C_{AT}) exponentially rose with RT (**Figure 2B**). Consequently, exponential functions describing the development of all-*trans*-β-carotene with RPI$_{WB}$ could be derived for each cultivar by simple mathematical transformation of the equations underlying **Figure 2A**,**B**. The resulting relationships were confirmed by regression between C_{AT} and RPI$_{WB}$ (eq 3), where R^2 ranged from 0.76 to 0.98 and γ and δ were the estimated cultivar-specific constants of the regression functions (**Table 6**).

$$C_{AT} = \gamma \cdot e^{\delta \cdot \mathrm{RPI_{WB}}} \tag{3a}$$

$$\mathrm{RPI_{WB}} = \delta^{-1} \cdot (\ln C_{AT} - \ln \gamma) \tag{3b}$$

Carotenoid accumulation during postharvest ripening of mangoes is usually accompanied by color changes. According to our previous study (*33*), decreasing mesocarp hue angle ($H°$) and rising TSS/TA were linked by cultivar-specific power law functions. Furthermore, TSS/TA exponentially increased with

Table 6. Postharvest Ripening Formation of All-*trans*-β-carotene (C_{AT} in μg/100 g DW) and Its Interrelations with Mesocarp Color (Crop Year 2001): Functions and Coefficients of Determination (R^2)

		exponential function: C_{AT} vs RPI$_{WB}$ (eq 3a) $y = \gamma \cdot e^{\delta \cdot x}$ with $y = C_{AT}$, $x = \mathrm{RPI_{WB}}$			power law function: $H°$ vs C_{AT} (eq 5) $y = \kappa \cdot x^{\tau}$ with $y = H°$, $x = C_{AT}$			exponential function: C_{AT} vs a^* (eq 9) $y = \upsilon \cdot e^{\omega \cdot x}$ with $y = C_{AT}$, $x = a^*$			comparison of parameters: eq 5 with eqs 3b and 4b $\kappa = \alpha \cdot \gamma^{-\beta/\delta}$, $\tau = \beta/\delta$, $Z_1 \equiv \alpha \cdot \gamma^{-\beta/\delta}$, $Z_2 \equiv \beta/\delta$			
cultivar	N^a	γ	δ	R^2	κ	τ	R^2	υ	ω	R^2	Z_1	κ/Z_1	Z_2	τ/Z_2
Kaew	4	17297	–0.555	0.965	233.9	–0.123	0.955	2308.5	0.082	0.946	233.5	1.00	–0.123	1.000
Chok Anan	7	12660	–0.497	0.810	231.9	–0.122	0.935	2150.7	0.089	0.923	242.1	0.96	–0.128	0.955
Maha Chanok	5	21077	–0.578	0.976	243.4	–0.129	0.954	2229.4	0.073	0.962	247.5	0.98	–0.131	0.985
Nam Dokmai #4	6	13468	–0.508	0.881	200.1	–0.106	0.992	1633.1	0.109	0.974	211.6	0.95	–0.112	0.946
Kiew Sawoei	6	1602	–0.181	0.779	693.7	–0.286	0.908	1249.5	0.044	0.886	974.8	0.71	–0.336	0.850
Rad	5 (4)^b	2800	–0.503	0.948	184.4	–0.105	0.973	878.4	0.101	0.990	183.2	1.01	–0.104	1.013
Mon Duen Gao	4 (3)	1399	–0.170	0.863	315.7	–0.176	0.919	1220.7	0.062	0.923	391.0	0.81	–0.208	0.845
Okrong Thong	5	2761	–0.682	0.946	141.1	–0.063	0.969	1226.6	0.244	0.978	143.1	0.99	–0.065	0.965
Okrong Kiew	4 (3)	724	–0.182	0.757	227.3	–0.139	0.986	972.9	0.137	0.909	225.1	1.01	–0.136	1.021

^{a} N = number of observations. ^{b} The number of observations in parentheses corresponds to eq 3a.

Table 7. Postharvest Ripening Development of Mesocarp Color (Crop Year 2001): Functions and Coefficients of Determination (R^2)

		exponential function: $H°$ vs RPI_{WB} (eq 4a) $y = \alpha \cdot e^{\beta \cdot x}$ with $y = H°$, $x = \mathrm{RPI}_{WB}$			linear function: a^* vs RPI_{WB} (eq 6) $y = s_1 \cdot x + t_1$ with $y = a^*$, $x = \mathrm{RPI}_{WB}$			linear function: b^* vs RPI_{WB} (eq 7) $y = s_2 \cdot x + t_2$ with $y = b^*$, $x = \mathrm{RPI}_{WB}$		
cultivar	N[a]	α	β	R^2	s_1	t_1	R^2	s_2	t_2	R^2
Kaew	4	70.6	0.068	0.920	−6.4	23.4	0.903	−4.9	78.6	0.745
Chok Anan	7	72.3	0.064	0.832	−5.6	20.1	0.891	−6.6	87.3	0.712
Maha Chanok	5	66.9	0.076	0.961	−7.7	30.1	0.963	−1.2	71.8	0.207
Nam Dokmai #4	7	72.8	0.057	0.836	−4.9	19.7	0.878	−4.6	75.7	0.518
Kiew Sawoei	7	81.5	0.061	0.931	−4.6	7.3	0.951	−6.2	71.4	0.823
Rad	4	80.3	0.052	0.991	−4.7	10.3	0.963	−5.3	78.2	0.833
Mon Duen Gao	3	86.6	0.035	0.999	−2.9	2.9	0.999	−3.2	64.6	0.950
Okrong Thong	5	85.3	0.045	0.984	−2.7	3.2	0.920	−10.5	72.6	0.857
Okrong Kiew	4	91.7	0.025	0.745	−1.0	−3.2	0.462	−5.7	61.7	0.859

[a] N = number of observations.

Table 8. Fruit Quality of Selected Cultivars at Similar Ripeness in Different Crop Years

						β-carotene (μg/100 g DW)				
cultivar	year	TSS (°Brix)	TA (g/100 g)	TSS/TA	$H°$	all-*trans*	13-*cis*	9-*cis*	total *cis* isomers (%)	vitamin A[a] (RE/100 g DW)
Kaew	2001	18.2	0.36	50.2	77.6	6809 ± 15	796 ± 3	645 ± 2	17.5	1246 ± 2
	2002	18.9	0.41	45.7	81.0	10717 ± 711	2108 ± 175	1207 ± 151	23.4	2049 ± 143
Chok Anan	2001	16.1	0.32	49.9	83.3	4341 ± 0	1152 ± 2	1051 ± 19	33.7	892 ± 1
	2002	20.4	0.33	61.2	89.5	2213 ± 116	855 ± 57	504 ± 32	38.0	476 ± 26
Maha Chanok	2001	16.7	0.35	47.7	74.9	7253 ± 275	809 ± 27	501 ± 4	15.3	1312 ± 48
	2002	16.9	0.36	47.3	85.9	7048 ± 617	1023 ± 70	376 ± 32	16.7	1289 ± 111
Nam Dokmai #4	2001	15.0	0.32	47.6	77.4	7924 ± 443	1669 ± 67	1656 ± 214	29.5	1573 ± 83
	2002	20.0	0.34	59.3	86.7	4266 ± 267	1078 ± 20	744 ± 59	29.9	853 ± 54
Mon Duen Gao	2001	15.3	0.16	97.0	88.4	1388 ± 32	336 ± 0	311 ± 6	31.8	281 ± 6
	2002	16.0	0.07	227.4	91.5	2081 ± 179	480 ± 45	125 ± 16	22.4	397 ± 35

[a] Determined as suggested by Zechmeister (*7*); see footnote a of **Table 4**.

postharvest RT (data not shown). When both relationships were combined, the kinetics of the mesocarp hue development could be calculated as time-dependent $H° = f(\mathrm{RT})$. By additional consideration of the linear dependency of RPI_{WB} on RT shown in **Figure 2A**, the exponential dependency of the mesocarp hue angle $H°$ on RPI_{WB} could be derived. This transformation confirmed statistical regression between $H°$ and RPI_{WB} (eq 4), with R^2 ranging from 0.75 to 1.00 and α and β as the estimated cultivar-specific constants of the regression functions (**Table 7**).

$$H° = \alpha \cdot e^{\beta \cdot \mathrm{RPI}_{WB}} \tag{4a}$$

$$\mathrm{RPI}_{WB} = \beta^{-1} \cdot (\ln H° - \ln \alpha) \tag{4b}$$

Consequently, changes in mesocarp color ($H°$) followed power law functions with increasing C_{AT} contents (**Figure 3A**). Direct regression analysis between $H°$ and C_{AT} equally resulted in power law equations (eq 5), with R^2 ranging from 0.92 to 0.99 (**Table 6**). The estimated constants of the regression functions, κ and τ, were consistent with those that were calculated after combination of eqs 3b and 4b **(Table 6)**. Therefore, $H°$ of the mesocarp may allow a cultivar-specific estimation of β-carotene contents in mango fruits throughout the postharvest ripening process.

$$H° = \kappa \cdot {C_{AT}}^{\tau} = \alpha \cdot \gamma^{-\beta/\delta} \cdot {C_{AT}}^{\beta/\delta} \tag{5}$$

On the basis of the CIELAB color space, hue angle changes were associated with changes of the hue coordinates a^* and b^* (toward increased redness and yellowness, respectively) by definition. Both hue coordinates linearly increased with declining RPI_{WB} within the considered postharvest period (**Figure 3B**), as described by the regression functions (eqs 6 and 7) presented in **Table 7**. The latter functions could be confirmed by graphical comparison of the measured RPI_{WB} and the calculated ripeness index obtained from modified eq 4b after its conversion into eq 8 (**Figure 3B**).

$$a^* = s_1 \cdot \mathrm{RPI}_{WB} + t_1 \tag{6}$$

$$b^* = s_2 \cdot \mathrm{RPI}_{WB} + t_2 \tag{7}$$

$$\mathrm{RPI}_{WB} = \beta^{-1} \cdot \ln\{180° \cdot \pi^{-1} \cdot \arctan\,[b^* \cdot (a^*)^{-1}]\} - \beta^{-1} \cdot \ln \alpha \tag{8}$$

Linear relationships between RPI_{WB} and the hue coordinates a^* and b^* were characterized by R^2 mostly exceeding 0.87 and 0.71, respectively (**Table 7**). However, the use of $H°$ as a color indicator at any RPI_{WB} level was considered favorable because of improved R^2 ranging from 0.75 to 1.0 for eq 4a (**Table 7**). Moreover, the entire hue changes from greenish−yellow to orange−yellow during ripening directly became evident from this single hue parameter.

Irrespective of the importance of $H°$, the exponential relationship observed between C_{AT} and a^* (**Figure 3C**) should be mentioned. The high coefficients of determination ($R^2 = 0.89-0.99$, **Table 6**) for the underlying regression functions (eq 9) revealed that the green−red coordinate a^* may represent another cultivar-specific indicator of the β-carotene content. Interrela-

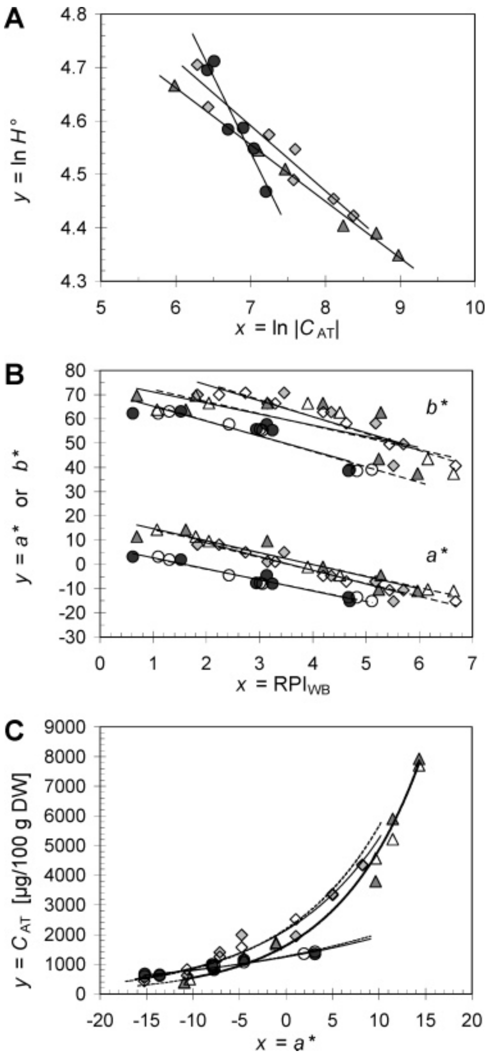

Figure 3. Interrelations between mesocarp color and β-carotene content during postharvest ripening of mangoes (R^2 for measured data sets in parentheses after cultivars). (**A**) Cultivar-specific power law changes in color (y = ln $H°$) with increasing C_{AT} (x = ln |C_{AT}|, calculated from C_{AT} in μg/100 g DW; eq 5). (◆), Chok Anan (0.94); (▲), Nam Dokmai #4 (0.99); and (●), Kiew Sawoei (0.91). (**B**) Development of $a*$ and $b*$ (measured values) with declining RPI$_{WB}$ (filled symbol, measured; open symbol, calculated; eq 8). (◆), Chok Anan (0.89, 0.71); (▲), Nam Dokmai #4 (0.88, 0.52); and (●), Kiew Sawoei (0.95, 0.82) with R^2 for $a*$ and $b*$, respectively. (**C**) Cultivar-specific exponential accumulation of C_{AT} (y; filled symbol, measured; open symbol, calculated; eq 10) with measured $a*$ values (x). (◆), Chok Anan (0.92); (▲), Nam Dokmai #4 (0.97); and (●), Kiew Sawoei (0.89).

tions between eqs 5 and 9 (**Table 6**) could be confirmed by graphical comparison of the measured C_{AT} values and the calculated contents obtained from eq 10, which is a modification of eq 5 (**Figure 3C**).

$$C_{AT} = \upsilon \cdot e^{\omega \cdot a*} \qquad (9)$$

$$C_{AT} = \kappa^{-1/\tau} \cdot \{180° \cdot \pi^{-1} \cdot \arctan [b* \cdot (a*)^{-1}]\}^{1/\tau} \qquad (10)$$

Significance of color as a nutritive indicator also became evident in noncultivar-specific correlation analysis between C_{AT} values and $a*$ or $H°$, respectively, throughout the whole ripening process, resulting in correlation coefficients of 0.89 and −0.83. Cultivars rich in β-carotene were characterized by a marked color change, which was reflected by the high slope of eq 6 ($|s_1| \geq |-4.9|$) associated with high axis intercepts (t_1 at RPI$_{WB}$ = 0) exceeding ∼20 (**Table 7**). In cultivars poor in β-carotene, red coloration was less pronounced, reaching maximal axis intercepts t_1 of ∼10.

As a consequence of the mathematical functions explained above, particularly eq 5, cultivar-specific power law relationships between mesocarp hue angle and vitamin A values can be derived. The natural variability of β-carotene contents and vitamin A potentials of selected cultivars is outlined in **Table 8**. The extents of both β-carotene and color formation considerably varied during postharvest ripening, mainly because of exogenous effects such as climate and physiological maturity (that is, respiratory activity) at harvest (*2*). The total *cis* isomer proportion of the β-carotene content was rather specific for each cultivar.

In conclusion, cultivar-specific relationships between the ripening index RPI$_{WB}$, the mesocarp hue angle, and β-carotene were established to model postharvest ripening changes regarding the nutritive value of the mango fruits on the basis of a kinetic approach for each property studied. Because relative amounts of *cis* isomers were only subjected to slight changes throughout the ripening process within a cultivar, these interrelations allow a rough estimation of vitamin A values based on the simple determination of the ripening index and mesocarp hue ($H°$) to select mangoes with suitable processing ripeness for carotene-rich products. By additional consideration of the underlying kinetics on the basis of a database for the processed raw material, such initial estimations, shortly after harvest, could even facilitate goal-oriented control of postharvest ripening processes.

ABBREVIATIONS USED

C_{AT}, all-*trans*-β-carotene content (μg/100 g DW); C_{13-cis}, 13-*cis*-β-carotene content (μg/100 g DW); C_{9-cis}, 9-*cis*-β-carotene content (μg/100 g DW); CV, coefficient of variation (%); DW, dry weight; FW, fresh weight; F_{WB}, dimensionless fruit firmness (F_{WB} = absolute value of $F_{WB,i}$); $F_{WB,i}$, maximum shear force describing fruit firmness in N at ripening time RT$_i$ (mesocarp analysis with Warner−Bratzler shear cell); $H°$, hue angle in degree; ISTD, internal standard; RE, retinol equivalent; RH, relative humidity; RPI$_{WB}$, ripening index, dimensionless [label “WB” marks the underlying mode of texture analysis with use of the Warner−Bratzler shear cell (F_{WB})]; RT, ripening time in days; R^2, coefficient of determination; TA, titratable acids (= total acidity) in g citric acid/100 g; TSS, total soluble solids in °Brix (that is, g/100 g); TSS/TA, sugar−acid ratio; WB, Warner−Bratzler shear cell.

ACKNOWLEDGMENT

Pairote Wiriyacharee, Faculty of Agro-Industry, Chiang Mai University, is acknowledged for providing laboratory facilities in Thailand. We thank Berit Jöns, Hohenheim University, for her technical assistance in β-carotene analyses.

LITERATURE CITED

(1) John, J.; Subbarayan, C.; Cama, H. R. Carotenoids in 3 stages of ripening of mango. *J. Food Sci.* **1970**, *35*, 262−265.
(2) Schulz, H. Physiologie der lagernden Frucht. In *Physiologische Grundlagen des Obstbaues*; Friedrich, G., Fischer, M., Eds.; Eugen Ulmer Verlag: Stuttgart, Germany, 2000; pp 372−397.
(3) Britton, G. Overview of carotenoid biosynthesis. In *Carotenoids: Biosynthesis and Metabolism*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser Verlag: Basel, Switzerland, 1998; pp 13−147.

(4) Burns, J.; Fraser, P. D.; Bramley, P. M. Identification and quantification of carotenoids, tocopherols and chlorophylls in commonly consumed fruits and vegetables. *Phytochemistry* **2003**, *62*, 939−947.

(5) Delgado-Vargas, F.; Jimenez, A. R.; Paredes-Lopez, O. Natural pigments: Carotenoids, anthocyanins, and betalains—Characteristics, biosynthesis, processing, and stability. *Crit. Rev. Food Sci. Nutr.* **2000**, *40*, 173−289.

(6) Mercadante, A. Z.; Rodriguez-Amaya, D. B. Effects of ripening, cultivar differences, and processing on the carotenoid composition of mango. *J. Agric. Food Chem.* **1998**, *46*, 128−130.

(7) Zechmeister, L. *Cis*−*trans-Isomeric Carotenoids, Vitamin A and Arylpolyenes*; Springer-Verlag: Wien, Austria, 1962.

(8) Godoy, H. T.; Rodriguez-Amaya, D. B. Occurrence of *cis*-isomers of provitamin A in Brazilian fruits. *J. Agric. Food Chem.* **1994**, *42*, 1306−1313.

(9) Pott, I.; Marx, M.; Neidhart, S.; Mühlbauer, W.; Carle, R. Quantitative determination of β-carotene stereoisomers in fresh, dried, and solar-dried mangoes (*Mangifera indica* L.). *J. Agric. Food Chem.* **2003**, *51*, 4527−4531.

(10) Chen, J. P.; Tai, C. Y.; Chen, B. H. Improved liquid chromatographic method for determination of carotenoids in Taiwanese mango (*Mangifera indica* L.). *J. Chromatogr. A* **2004**, *1054*, 261−268.

(11) Chandler, L. A.; Schwartz, S. J. HPLC separation of *cis*−*trans* carotene isomers in fresh and processed fruits and vegetables. *J. Food Sci.* **1987**, *52*, 669−672.

(12) Bonora, A.; Pancaldi, S.; Gualandri, R.; Fasulo, M. P. Carotenoid and ultrastructure variations in plastids of *Arum italicum* Miller fruit during maturation and ripening. *J. Exp. Bot.* **2000**, *51*, 873−884.

(13) Ben Amotz, A.; Fishler, R. Analysis of carotenoids with emphasis on 9-*cis*-β-carotene in vegetables and fruits commonly consumed in Israel. *Food Chem.* **1998**, *62*, 515−520.

(14) Mercadante, A. Z.; Rodriguez-Amaya, D. B.; Britton, G. HPLC and mass spectrometric analysis of carotenoids from mango. *J. Agric. Food Chem.* **1997**, *45*, 120−123.

(15) Cano, M. P.; de Ancos, B. Carotenoid and carotenoid ester composition in mango fruit as influenced by processing method. *J. Agric. Food Chem.* **1994**, *42*, 2737−2742.

(16) Marx, M.; Schieber, A.; Carle, R. Quantitative determination of carotene stereoisomers in carrot juices and vitamin supplemented (ATBC) drinks. *Food Chem.* **2000**, *70*, 403−408.

(17) Pott, I.; Breithaupt, D. E.; Carle, R. Detection of unusual carotenoid esters in fresh mango (*Mangifera indica* L. cv. 'Kent'). *Phytochemistry* **2003**, *64*, 825−829.

(18) Rodriguez-Amaya, D. B. *A Guide to Carotenoid Analysis in Foods*; ILSI Press: Washington DC, 2001.

(19) Kimura, M.; Rodriguez-Amaya, D. B.; Godoy, H. T. Assessment of the saponification step in the quantitative determination of carotenoids and provitamins A. *Food Chem.* **1990**, *35*, 187−195.

(20) Philip, T.; Chen, T. S. Development of a method for the quantitative estimation of provitamin A carotenoids in some fruits. *J. Food Sci.* **1988**, *53*, 1703−1706.

(21) Mahayothee, B.; Mühlbauer, W.; Neidhart, S.; Carle, R. Influence of postharvest ripening processes on appropriate maturity for drying mangoes 'Nam Dokmai' and 'Kaew'. *Acta Hortic.* **2004**, *645*, 241−248.

(22) Mahayothee, B. The influence of raw material on the quality of dried mango slices (*Mangifera indica* L.) with special reference to postharvest ripening. Ph.D. Thesis, Hohenheim University. In *Schriftenreihe des Lehrstuhls Lebensmittel pflanzlicher Herkunft*; Carle, R., Ed.; Shaker Verlag: Aachen, Germany, 2005; Vol. 2.

(23) International Federation of Fruit Juice Producers (IFU). *Methods of Analysis*; Swiss Fruit Association: Zug, Switzerland, 2001.

(24) Hutchings, J. B. *Food Colour and Appearance*; Aspen: Gaithersburg, MD, 1999.

(25) Chen, W. H.; Yan, S. H. An improved analytical method for the determination of carotenes and xantophylls in dried plant materials and mixed feeds. *Food Chem.* **1992**, *44*, 61−66.

(26) Schieber, A.; Marx, M.; Carle, R. Simultaneous determination of carotenes and tocopherols in ATBC drinks by high-performance liquid chromatography. *Food Chem.* **2002**, *76*, 357−362.

(27) Stoll, T.; Schweiggert, U.; Schieber, A.; Carle, R. Process for the recovery of a carotene-rich functional food ingredient from carrot pomace by enzymatic liquefaction. *Innovative Food Sci. Emerging Technol.* **2003**, *4*, 415−423.

(28) Taungbodhitham, A. K.; Jones, G. P.; Wahlqvist, M. L.; Briggs, D. R. Evaluation of extraction method for the analysis of carotenoids in fruits and vegetables. *Food Chem.* **1998**, *63*, 577−584.

(29) Marx, M. Einfluss der Herstellungstechnologie auf die *trans-cis*-Isomerisierung von β-Carotin in Karottensaft. Ph.D. Thesis, Hohenheim University, Stuttgart, Germany, 2003.

(30) Schierle, J.; Härdi, W.; Faccin, N.; Bühler, I.; Schüep, W. Geometrical isomers of β, β-carotene. In *Carotenoids. Vol. 1A: Isolation and Analysis*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser Verlag: Basel, Switzerland, 1995; pp 265−272.

(31) Britton, G. UV/visible spectroscopy. In *Carotenoids. Vol. 1B: Spectroscopy*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser Verlag: Basel, Switzerland, 1995; pp 13−62.

(32) Breithaupt, D. E.; Schwack, W. Determination of free and bound carotenoids in paprika (*Capsicum annuum* L.) by LC/MS. *Eur. Food Res. Technol.* **2000**, *211*, 52−55.

(33) Vásquez-Caicedo, A. L.; Neidhart, S.; Carle, R. Postharvest ripening behavior of nine Thai mango cultivars and their suitability for industrial applications. *Acta Hortic.* **2004**, *645*, 617−625.

(34) Jordan, R. B.; Seelye, R. J.; McGlone, V. A. A sensory-based alternative to Brix/acid ratio. *Food Technol.* **2001**, *55*, 36−38, 40−44.

(35) Gómez, P. I.; Barriga, A.; Cifuentes, A. S.; González, M. A. Effect of salinity on the quantity and quality of carotenoids accumulated by *Dunaliella salina* (strain CONC-007) and *Dunaliella bardawil* (strain ATCC 30861) chlorophyta. *Biol. Res.* **2003**, *36*, 185−192.

(36) Orset, S. C.; Young, A. J. Exposure to low irradiances favors the synthesis of 9-*cis*-β, β-carotene in *Dunaliella salina* (Teod.). *Plant Physiol.* **2000**, *122*, 609−617.

(37) Britton, G.; Gambelli, L.; Dunphy, P.; Pudney, P.; Gidley, M. Physical state of carotenoids in chromoplasts of tomato and carrot: Consequences for bioavailability. In *Proceedings of the 2nd International Congress of Pigments in Foods: Functionalities of Pigments in Foods*; Empis, J. A., Ed.; Lisbon, Portugal, 2002; pp 151−158.

(38) Marx, M.; Stuparić, M.; Schieber, A.; Carle, R. Effects of thermal processing on *trans-cis*-isomerization of β-carotene in carrot juices and carotene-containing preparations. *Food Chem.* **2003**, *83*, 609−617.

Received for review November 3, 2004. Revised manuscript received March 15, 2005. Accepted March 20, 2005. This research is funded by Deutsche Forschungsgemeinschaft (DFG), Bonn, Germany: Project SFB 564-E2. It is part of the Special Research Program "Research for Sustainable Land Use and Rural Development in Mountainous Regions of Southeast Asia". Dedicated to Prof. Dr.-Ing. Karlheinz Gierschner on the occasion of his 75th birthday.

JF048168H