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ARTICLES

Quantitative Determination of β-Carotene Stereoisomers in Fresh, Dried, and Solar-Dried Mangoes (*Mangifera indica* L.)

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A rapid method for quantitative determination of β-carotene, including *cis*-isomers, in dried mango has been developed. Applicability of available methods to dried products was limited because of formation of artifacts caused by extraction and preparation. The analytical procedure was based on the extraction of carotenoids from dried mango mesocarp using a mixture of methanol and acetone/hexane, allowing the separation of disturbing fibers. No saponification was required. Furthermore, carotenoid determination by HPLC on a C_{30} stationary phase was achieved. This method was applied to determine β-carotene and its stereoisomers in fresh, dried, and solar-dried mango slices of four cultivars. Drying resulted in a complete and partial degradation of xanthophylls and all-*trans*-β-carotene, respectively. Isomerization was shown to depend on the drying process. Whereas conventionally dried mangoes were characterized by elevated amounts of 13-*cis*-β-carotene, solar-dried mango slices contained additional amounts of the 9-*cis*-isomer. Calculation of vitamin A values was based on the real amount of the β-carotene stereoisomers and ranged from 113 to 420 and from 425 to 1010 RE/100 g for fresh and dried mango slices, respectively.

KEYWORDS: **β-Carotene; *trans*−*cis*-isomerization; vitamin A value; mango; *Mangifera indica* L.; drying**

INTRODUCTION

Among carotenoid pigments, which are widely distributed in plant tissues, β-carotene provides the highest vitamin A activity. Vitamin A deficiencies are widespread in developing countries, influencing the growth of young children severely (*1*−*3*). UNICEF and WHO consider that improving the vitamin A status of young children with marginal deficiency may reduce the mortality by 23% on average. Dietary approaches are needed to replace supplementation programs, ensuring sustainability and an adequate coverage of children in need (*4*). Although fruits and vegetables containing carotenoids are available in developing countries, deficiencies are often found during the off season (*5*). Thus, small scale processing, such as drying of fresh mangoes, can significantly contribute to improve the vitamin A intake during the off season. Because drying and solar drying may result in partial degradation of vitamins, especially of carotenoids (*6*), a validated quantitative and rapid analytical method for dried fruit tissue is required to investigate process-related losses of carotenoids and vitamin A potential of the products.

Mango is regarded as a rich source of carotenoids. For ripe mangoes cv. Tommy Atkins, average amounts of 1920 μg of carotenoids per 100 g of mango pulp, comprising 68% β-carotene, were reported (*7*). Even higher contents were found for cv. Keitt containing 5500 μg of total carotenoids per 100 g of mango with 27% all-*trans*-β-carotene (*8*). Also, mango pulp of cv. Gedong, having 3300 μg of all-*trans*-β-carotene per 100 g, is considered as a very good source of carotenoids and provitamin A (*9*). Retinol equivalents (RE) calculated according to the NRC (*10*) were 224, 251, and 550 per 100 g of fresh weight. According to the FAO (*11*), the daily requirements for vitamin A are about 800 and 500 RE for healthy adults and children, respectively. In addition to the vitamin A relevant characteristics, carotenoids are known to protect humans against different types of cancer (*12*) and cardiovascular diseases (*13*).

Postharvest treatments and processing affect carotenoid content of fruits and vegetables. Enzymatic and/or thermally induced oxidative degradation commonly cause considerable

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losses of carotenoids (*14*). Medium-temperature drying processes are usually characterized by extended exposure to air temperatures of 40−80 °C and permanent exposure to atmospheric oxygen. Solar- and sun-drying techniques imply solar radiation as an additional factor enhancing vitamin degradation. While solar-dried samples of fruits and leafy vegetables showed only slightly lower β-carotene retention than oven-dried samples, sun drying of fruit pieces may result in considerable losses of carotenoid and provitamin A content (*6*).

Especially, isomerization of all-*trans*-carotenoids is known to occur during thermal processing, including drying (*15*). Additionally, Marx et al. (*16*) associated the tendency of all-*trans*-isomers toward isomerization in carrot tissue with exposure time and the physiological state of carotenes. In this study, the solubilization of carotene by cellular lipids was identified as a crucial factor of *trans*-*cis*-isomerization. Exposure of all-*trans*-β-carotene to light predominantly leads to the formation of 9-*cis*-isomers, whereas 13-*cis*-β-carotene is mainly formed by thermal treatment (*17*). The nutritional consequences of both degradation types are the reduction of vitamin A activity (*6*, *18*), as well as alterations regarding their bioavailability and their antioxidative properties (*19*, *20*).

Therefore, it is necessary to quantify vitamin A active carotenoids by separating the *cis*- and *trans*-isomers for correct calculation of their provitamin impact (*18*). Sample preparation and analytical methods are mainly based on time-consuming extraction and saponification steps or are not applicable for dried and fibrous plant material (*8*, *21*). Marx et al. (*22*) described a validated rapid extraction method for juices and an HPLC method allowing the separation and quantification of β-carotene stereoisomers.

With special emphasis on dried plant tissue, the objective of this study was to develop a rapid and reliable method for the quantification of all-*trans*-β-carotene, including its stereoisomers, in fresh and differently dried mango products enabling process-dependent estimation of vitamin A activity.

MATERIALS AND METHODS

Raw Material and Drying Processes. Fresh monoembryonic mangoes, cvs. Kent and Tommy Atkins, were purchased from the wholesale market in Stuttgart, Germany. The polyembryonic Thai cultivars Nam Dokmai and Kaew were obtained from the central fruit market of Chiang Mai, Thailand. Green mature fruits were allowed to reach approximately 75−80% of full ripeness, according to Vásquez-Caicedo et al. (*23*). Fruits were cut into slices of 8 mm thickness. Dehydrated products of monoembryonic cultivars were produced at Hohenheim University in a laboratory over-flow dryer described by Guarte et al. (*24*), with drying air temperature of 75 °C, air velocity of 1.0 m/s, and a fixed relative humidity of 4.5% in the dark. After 3−3.5 h, the final water activity was 0.6, corresponding to a water content of 14−16%. The Thai polyembryonic mangoes were dried in a solar-tunnel-dryer, type Hohenheim, described by Esper and Mühlbauer (*25*), with average drying air temperatures between 60 and 62 °C at the Faculty of Agro-Industry, Chiang Mai University, Thailand. Drying took place in April at the best position of the sun, according to the drying temperature, between 9 a.m. and 5 p.m., with solar radiation rates ranging between 400 and 830 W/m^2. Drying time varied between 7 and 8 h.

Chemicals. All chemicals used (Merck, Darmstadt, Germany) were of reagent grade. The internal standard β-apo-8′-carotenal was from Fluka (Basel, Switzerland). For determining the dry weight (DW), the water content was quantified by Karl Fischer titration in a one-component system at 50 °C (*26*).

HPLC Analysis. The analytical method described by Marx et al. (*22*) has been adopted to the extraction of carotenoids from fresh and dried mango flesh. An HPLC system Shimadzu (Kyoto, Japan) with a diode array detector SPD-M10Avp. was used. For chromatographic analysis a C_{30} reversed-phase column of analytic scale (250 mm × 4.6 mm i.d.) with a particle size of 5 μm (YMC, Wilmington, USA) was applied. HPLC conditions were as follows: eluent A consisted of methanol/*tert*-butyl methyl ether (MTBE)/water (81:15:4, v/v). Eluent B was prepared by mixing MTBE, methanol, and water (90:6:4, v/v). Baseline separation of carotenoids was achieved by using a linear gradient from 100% A to 56% B within 50 min at a flow rate of 1 mL/min. Carotenoid isomers were identified by their retention time and their spectral characteristics (UV−Vis spectrum). Except for 13-*cis*-β-carotene, individual carotenoid peaks were monitored at their spectral maximum (all-*trans*-β-carotene at 452 nm, 9-*cis*-β-carotene and 13-*cis*-β-carotene at 445 nm).

Quantification was carried out by an external all-*trans*-β-carotene standard. Recovery rate was calculated by using β-apo-8′-carotenal as internal standard. Purity of the standards was checked prior to use. Concentrations of the solutions were determined spectrophotometrically (*22*). All standards were characterized by their characteristic absorption spectra and were eluted as individual peaks. Calculations of concentrations were based on linear calibration graphs, using the extinction coefficient $A^{1\%}_{1cm} = 2592$ at 450 nm for all-*trans*-β-carotene and $A^{1\%}_{1cm} = 2640$ at 457 nm for β-apo-8′-carotenal (*27*, *28*). Isomer concentrations were calculated using all-*trans*-β-carotene standard curves at $\lambda_{max} = 452$ nm.

Preparation of Samples. Fresh and dried mango flesh (5−10 g) was homogenized using an ultra-turrax (Janke & Kunkel, Stauffen, Germany) after adding Celite, calcium carbonate and methanol for complete extraction of the carotenoids from the mango mesocarp. Finely cut dried fruits were rehydrated for 5 to 10 min with cold distilled water (10 mL) in the dark before extraction. Separation of fiber was achieved by washing several times with methanol and filtering the mixture through a glass suction filter funnel until the filter cake was colorless. The clear fluid was extracted in an amber glass separatory funnel with a mixture of acetone and hexane (1:1, v/v), because the suitability of the used solvents has been shown by Marx et al. (*22*). In case of a remaining yellow colored aqueous epilayer, the extraction step was repeated. Sodium chloride solution (10%, w/v) was added to facilitate layer separation and to prevent the formation of emulsions. Acetone was removed by washing twice with distilled water (20 mL each). The extract was dried with sodium sulfate (2 g) and butylated hydroxytoluene (BHT) was added as an antioxidant to a final concentration of 0.1%. Hexane was evaporated in vacuo (25 °C, 150 bar), the residue was dissolved in 2-propanol and adjusted to a volume of 10 mL. Aliquots of 20 μL were used for HPLC analysis.

RESULTS AND DISCUSSION

Qualitative and Quantitative Results. The method presented in this study allowed a fast extraction of carotenoids, including stereoisomers, and a quantitative determination of vitamin A relevant carotenes from fresh and dried mango flesh. In contrast to Kimura et al. (*29*), suggested saponification was not required because of a negligible amount of lipids, and additional *cis*-isomerization by sample preparation was avoided. The simple extraction meets the requirements of a standard method for routine determination of provitamin A in dried plant tissues, provided that β-cryptoxanthin esters are not present in appreciable amounts.

Depending on cultivar and ripening, mangoes show a wide range of different carotenoids. In addition to β-carotene, considerable amounts of different xanthophylls, their stereoisomers, and esterified compounds were reported (*30*). Furthermore, the occurrence of esterified or free cryptoxanthin in fresh mango flesh has already been described (*8*, *31*−*33*). The earlier reports showed that their contents differed widely, depending on cultivar, ripening status, and sample preparation method. However, in the present study, cryptoxanthin and related esters were not detected in dried mangoes of any cultivar investigated. As demonstrated by the HPLC chromatogram (**Figure 1**), carotene pattern of unprocessed mango cv. Kent is characterized

Table 1. β-Carotene Content of Fresh and Dried Mango Flesh of Different Cultivars

samples	all-*trans*-β-carotene μg/100 g DW[c]	9-*cis*-β-carotene μg/100 g DW	13-*cis*-β-carotene μg/100 g DW	relative amount of *cis*-isomers[a] %	vitamin A value[b] RE/100 g
Kent					
fresh	4580	tr[d]	1120	24.4	142
dried[e]	4270	180	1390	36.8	752
Tommy Atkins					
fresh	3650	nd[f]	940	25.8	114
dried[e]	2510	tr[d]	930	37.1	431
Namdok Mai					
fresh	3650	tr[d]	990	27.1	121
solar-dried[g]	2400	810	730	64.2	425
Kaew					
fresh	11 680	1010	1220	19.1	423
solar-dried[g]	6820	2050	1430	51.0	1011

[a] Calculated as percentage of all-*trans*-β-carotene. [b] Retinol equivalent (RE) according to Zechmeister (*41*). [c] Dry weight. [d] In traces. [e] Standard drying process (ϑ_a = 75 °C, a_w = 0.6, t_D = 3–3.5 h). [f] Not detected. [g] Solar-drying process (a_W = 0.6, t_D = 7–8 h).

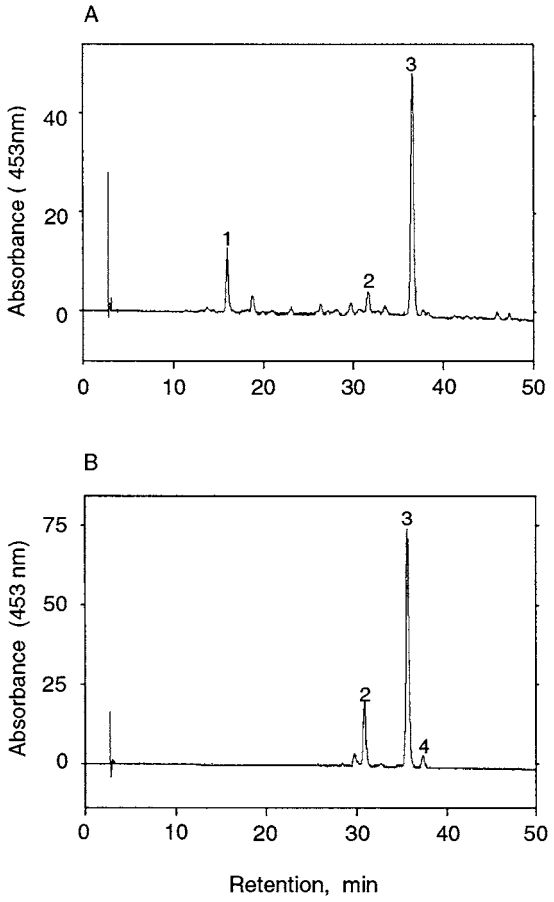

Figure 1. HPLC chromatograms of the carotenoids of ripe mango, cv. Kent: fresh (**A**) and dried in a laboratory over-flow dryer at 75 °C (**B**). Peak numbers: **1**, violaxanthin ester; **2**, 13-*cis*-β-carotene; **3**, all-*trans*-β-carotene; **4**, 9-*cis*-β-carotene.

by all-*trans*-β-carotene with the accompanying 13-*cis*-isomer and xanthophylls as minor compounds, with the latter displaying a retention time between 12 and 25 min. The predominant xanthophyll was tentatively identified as violaxanthin ester, according to the method of Breithaupt and Schwack (*34*). The occurrence of violaxanthin as a major carotenoid of fresh Brazilian mangoes, cvs. Keitt and Tommy Atkins, was earlier described by Mercadante and Rodriguez-Amaya (*30*). Consistent with their finding, xanthophylls were partly degraded by processing. The unstable character of violaxanthin derivatives as di-epoxides may explain their complete degradation during the drying process. Within the spectrum of carotenoids identified so far in fresh, ripe mango flesh, we found that β-carotene was the only carotenoid displaying significant vitamin A activity in dried mangoes.

Although photosensitizing properties of acetone were described (*35*), no light-induced isomerization was monitored when using amber glass for all steps of sample preparation, and extraction time for fresh and dried samples was about 1 h.

Recovery rate of the internal standard β-apo-8′-carotenal added for quantification of carotenoids in the dried samples was 95−105%. Comparable to other fruits such as peach, nectarine, and plum (*21*), a natural portion of mainly 13-*cis* stereoisomers in fresh mango flesh was found. The amount of *cis*-isomers in a sample with added all-*trans*-β-carotene was the same as that in the respective control sample. Thus, chromatographic analysis after identical sample extraction showed that no additional formation of isomers occurred during sample preparation.

***Trans*−*Cis*-Isomerization.** Since Marx et al. (*16*) did not find *trans*-*cis*-isomerization of carotene in fresh carrots, known to contain crystal-like chromoplasts (*36*), an interrelation between isomerization and alteration of crystalline carotenes by thermal processing steps was established. In particular, dissolution of carotene in oil enhanced isomerization rates significantly. Analogous effects were described for lycopene by Nguyen et al. (*37*), during tomato processing. Whereas the chromoplasts of red-fruited tomato were shown to be strikingly similar to those described for carrots, high-beta tomato mutants showed little similarity to those of the carrot, though both were high in β-carotene (*38*−*40*). Although ultrastructural studies of carotenoid-containing structures in mango mesocarp are lacking, due to its natural 13-*cis*-isomer content, globulose mango chromoplasts are assumed to contain carotenoid-carrying lipid droplets. Apart from that, globular chromoplasts are the most common type, and widely found in orchard fruits (*36*).

Table 1 presents the effect of drying processes on the loss of β-carotene and increased isomerization rate of mango. The relative amount of *cis*-isomers in fresh mango flesh was approximately 19−27% of all-*trans*-β-carotene. Provitamin A activity of *cis*-β-carotene is lower compared to the all-*trans*-isomer. For 9-*cis*-β-carotene and 13-*cis*-β-carotene, provitamin A activity was estimated to be 38 and 53% of that of all-*trans*-β-carotene, respectively (*41*). Because calculation of the provitamin A activity based on the total amount of β-carotene leads to overestimation, a quantitative separation of stereoisomers was recommended by several authors (*18*, *42*). Calculation of vitamin

A activity of dried fruits will be more accurate and convincing with the knowledge of isomerization rate and amount of different stereoisomers.

Influence of Drying Processes. Lower provitamin A contents of the investigated fresh mango cultivars compared to those given by several authors for fully ripe fruits (*7*−*9*) may partly be explained by the shorter period of carotenogenesis in ripening fruits used for processing and drying. Mercadante and Rodriguez-Amaya (*30*) distinguished three maturity stages (mature-green, partially ripe, and ripe) for fresh Brazilian mangoes, cvs. Keitt and Tommy Atkins, and found a 30−40% increase of the final vitamin A value of ripe fruits within the last ripening period.

When drying of mango slices, cvs. Kent and Tommy Atkins, was performed in the dark, using an over-flow tray dryer, the formation of 9-*cis*-isomers was negligible. However, the relative amount of *cis*-isomers significantly increased because of considerable formation of 13-*cis*-isomers. Therefore, the raised isomerization rate had to be mainly ascribed to elevated temperature. Exposure to light predominantly leads to the formation of the 9-*cis*-isomer (*17*). As expected, solar drying of the Thai mangoes, cvs. Nam Dokmai and Kaew, in a sunlit tunnel dryer with maximum drying temperatures of 62 °C and maximum solar radiation rate of 830 W/m^2 resulted in an increased formation of the 9-*cis*-isomer and, consequently, in higher relative amounts of total *cis*-isomers (64.2 and 51% for Nam Dokmai and Kaew, respectively) compared to the conventional drying process in the dark.

Due to the short drying time of 3.5 h, max, all-*trans*-β-carotene degradation was less (7 and 30%) for tray-dried mango slices. During drying, fruit temperatures rose within 2 h from ambient air conditions to more than 50 °C. Disintegration of mango tissue was minimized by cutting the fruit into slices of 8 mm. Therefore, cell integrity of the major part of the mesocarp was maintained, and enzymatic degradation of carotenoids within the tissue was limited. As described by several authors, minimal inactivation rate for lipoxygenase (carotene oxidase), being responsible for main enzymatic carotene degradation of different plant materials (*43*), was found at temperatures between 40 and 50 °C (*44*−*47*). Even increasing lipoxygenase activity was reported within the range of 25 to 40 °C (*48*).

Solar-dried mango slices were exposed to sunlight for about 7.5 h. During the solar-drying process, fruit temperature was below 50 °C for a period of 5.5 h. Prolongation of drying time was reflected by a lower retention of all-*trans*-β-carotene (65 and 58%) and an increased isomerization rate.

Vitamin A Value. Nevertheless, both drying processes allowed the production of dried mango slices with significant amounts of provitamin A reaching up to 1010 RE per 100 g edible portion, depending on cultivar. Thus, 80−190 g and 50−120 g of the given dried mango slices would be sufficient to meet daily requirements of healthy adults and children, respectively. Due to their high β-carotene content, dried mangoes are regarded as a very good source of provitamin A, although more detailed knowledge of the bioavailability of carotenoids in dried fruits is still needed.

The described method was already successfully applied in a preliminary study of postharvest ripening behavior of nine Thai mango cultivars (*23*). The application of the method for the determination of carotenes in solid or fluid mango products such as slices in syrup, puree, or juices of more commercially used cultivars is the subject of an ongoing study. Because unidentified carotenoids in fresh, canned, or otherwise processed mangoes of other cultivars might coelute with β-carotene stereoisomers, the aim of the current study is the adoption of extraction method and HPLC separation to other provitamin A-containing fruits and vegetables, irrespective of cultivar or processing mode.

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