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Effects of thermal processing and fruit matrix on β-carotene stability and enzyme inactivation during transformation of mangoes into purée and nectar

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Abstract

Effects of thermal mango processing on β-carotene stability and inactivation of peroxidase (POD) and polyphenol oxidase (PPO) were systematically evaluated on a laboratory scale, mimicking typical operations in continuous and small-size batch production of mango purée. Treatments comprised pasteurisation between 85 and 93 °C up to 16 min of holding time, with pasteurisation values ($P^{z=8.9\ °C}_{T_{ref}=93.3\ °C}$) of 0.34–14.91 min, and thermal routines usually applied in enzymatic maceration for pectin degradation to standardise mango pulp viscosity. Although significant *trans–cis*-isomerisation of β-carotene ($P < 0.05$) was shown by the formation of 13-*cis*-β-carotene, maximum vitamin A loss during pasteurisation of mango purée did not exceed 15.4%, owing to a total β-carotene retention of 93%. PPO was readily inactivated after 1 min, whereas residual POD activities of 4.0–6.3% were detected, even after 16 min, at all pasteurisation temperatures. To study the influence of the fruit matrix during pasteurisation, β-carotene stability was additionally evaluated at modified physical states in aqueous and in oil-in-water (o/w) emulsified model preparations. After disintegration of the mango matrix, *trans–cis*-isomerisation was higher than in model preparations, indicating a non-crystalline state of β-carotene in the mango mesocarp. In semi-continuous production of mango nectar on a pilot-plant scale, evaluated by stepwise process control, four heating treatments used for steam peeling, thermal inactivation of endogenous enzymes prior to enzymatic pulp liquefaction, and pasteurisation of purée and nectar, respectively, consistently resulted in a final retention of 83% of the vitamin A value of the fruit dry matter.

Keywords: *Mangifera indica* L.; β-Carotene stereoisomers; Maceration; Pasteurisation; Vitamin A value

1. Introduction

Products, derived from mango fruit (*Mangifera indica* L.), are increasingly used in beverage, dairy, and confectionery industries, where fruit purées and concentrate are the major intermediates. Generally, modern industrial year-round production of mango juice, that is, nectar, is mostly from such purée intermediates, produced during peak harvest seasons. Accordingly, the world's total export volume of mango juice and mango pulp increased between 1999 and 2003 by 179% and 49% to 11,848 t and 9510 t, respectively, while the world production has risen by 13% and 17%, since 1999 to 151,998 t of mango juice and 925,263 t of mango pulp in 2004 (FAOSTAT, 2005). Enzymatic purée liquefaction may be applied to standardise viscosity by controlled pectin degradation for technological and sensory reasons, since otherwise, the highly viscous mango pulp will often display inappropriate flow properties (Janser, 1997). Therefore, the fruit component in the final nectar may have been subjected to up to four heating treatments in the form of steam-peeling, thermal inactivation of endogenous enzymes prior to enzymatic

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pulp liquefaction, and pasteurisation of purée and nectar (Dube et al., 2004). Apart from final pasteurisation, obligatory to achieve microbial product stability, early thermal inactivation of oxidative enzymes after tissue disintegration is a prerequisite to prevent product browning in processing of fully ripe mango fruits, since peroxidase (POD) and polyphenol oxidase (PPO) activities displayed cultivar-specific logarithmic increases during postharvest ripening (Vásquez-Caicedo, Neidhart, & Carle, 2004). Furthermore, heat-stable peroxidases eventually contribute to the formation of oxidation products that may oxidise a wide range of hydrogen-donor molecules (Khan & Robinson, 1993), thus causing deleterious discolouration of mango purées and beverages.

Although unavoidable for achieving desired shelf-life, heat treatments may affect the nutritional properties of purées and concentrates, by reducing carotenoid contents that exert provitamin A activity, particularly all-*trans*-β-carotene.[2] β-Carotene (β,β-carotene) is oxidised in the presence of free radicals, producing epoxy, hydroxy and carbonyl compounds (Yanishlieva, Aitzetmüller, & Raneva, 1998) and low molecular fragments, such as β-ionone, 5,6-epoxy-β-ionone and dihydroactinidiolide (DHA), often associated with fruity, floral and woody notes (Waché, Bosser-DeRatuld, Lhuguenot, & Belin, 2003). Although carotenoids are naturally stabilised by the plant matrix, cutting or disrupting of fruit and vegetable tissues favours their exposure to oxygen and endogenous oxidative enzymes, thus provoking their oxidation (Rodriguez-Amaya, 1999). Accordingly, when fresh mango slices were compared with canned (Godoy & Rodriguez-Amaya, 1987) and frozen ones (Cano & de Ancos, 1994), the minor β-carotene losses found were attributed to the still limited disruption of the fruit ultrastructure. The protective role of the fruit matrix was conversely demonstrated by a 13% decrease of β-carotene in mango purée heated at 80 °C for 10 min (Godoy & Rodriguez-Amaya, 1987).

Despite associated carotenoid degradation, enzymatic tissue disintegration was proven to enhance β-carotene bioavailability from whole leaf and minced spinach (Castenmiller, West, Linssen, van het Hof, & Voragen, 1999). Similarly, bioavailability of lycopene and β-carotene from tomato was improved in samples homogenised under high pressure, confirming that the insufficient release of carotenoids from intact cells is a limiting factor for carotenoid uptake (van het Hof et al., 2000). β-Carotene ingestion from heated carrot and spinach purées resulted in a 3-fold higher plasma response than from the unheated vegetables (Rock et al., 1998). However, possibly due to thermally induced *trans–cis*-isomerisation (van het Hof et al., 2000), a similar increase was not obtained with homogenised tomato samples subjected to an additional heat treatment, since both absorption in the gastrointestinal tract (Ben-Amotz & Levy, 1996; Gaziano et al., 1995) and conversion into vitamin A (Castenmiller & West, 1998; Zechmeister, 1962) are known to be considerably reduced for the *cis*-isomers of β-carotene.

cis–β-Carotene isomers are naturally occurring in mango fruits (Godoy & Rodriguez-Amaya, 1994; Pott, Marx, Neidhart, Mühlbauer, & Carle, 2003a; Vásquez-Caicedo, Sruamsiri, Carle, & Neidhart, 2005), suggesting that their exposure to heat and light may propagate *trans–cis*-isomerisation. Formation of 13-*cis*- and 9-*cis*–β-carotene[3] in vegetable preparations due to heat and illumination, respectively, has been well established (Chen, Chen, & Chien, 1994; Chen, Peng, & Chen, 1996; Marx, Stuparić, Schieber, & Carle, 2003). Differences between fruit and vegetable species, such as localisation of the β-carotene in the tissue and its physical state, may be crucial factors for the susceptibility to *trans–cis*-isomerisation and β-carotene stability. Accordingly, in carrot juice production, 13-*cis*–β-carotene formation was enhanced in the presence of lipids as a result of extensive blanching at temperatures of 80–100 °C for 30–60 min, and by the addition of grape seed oil to the coarse mash prior to thermal preservation (Marx et al., 2003). In solar-dried mango fruits, 9-*cis*–β-carotene was mostly formed via photo-induced isomerisation at mild temperatures of 60–62 °C (Pott et al., 2003a). However, also extensive heating (130 °C) and prolonged sterilisation times, with *F*-values of 20–40 min, facilitated the formation of 9-*cis*–β-carotene in carrot juices (Marx et al., 2003).

On the whole, retention of nutritive quality and colour through limitation of carotenoid degradation and prevention of enzymatic browning are major, strongly coupled production aims in mango processing. Therefore, this study focussed on the effects of various heating conditions on total loss and *trans–cis*-isomerisation of β-carotene, and the simultaneous inactivation of PPO and POD in mango purée, systematically investigated in batch heating processes on a laboratory scale. Additionally, the contribution of the mango purée matrix to β-carotene stability was assessed by comparison of purées with model preparations at various time–temperature regimes. Finally, the aim of this work was to verify the contributions of the individual processing steps previously evaluated under laboratory conditions for the semi-continuous production of mango purée and nectar on an industry-like pilot-plant scale, determining β-carotene degradation and enzyme inactivation by stepwise process control. Aside from their sensory relevance as regards enzymatic browning, PPO and POD may be used as technological indicators, due to their extraordinary thermostability (Askar & Treptow, 2001; Khan & Robinson, 1993), since their thermal inactivation during processing would also indicate that of other quality-related, but less thermostable enzymes.

[2] Semi-systematic IUPAC-IUB nomenclature: all-*E*-β,β-carotene.

[3] Semi-systematic IUPAC-IUB nomenclature: 13Z- and 9Z-β,β-carotene, respectively.

2. Materials and methods

2.1. Materials and reagents

Mature-green mango fruits, cv. 'Tommy Atkins', were obtained from the wholesale market in Stuttgart, Germany. All reagents and solvents used were of analytical or HPLC grade (VWR, Darmstadt, Germany). All-*trans*-β-carotene (type II, HPLC purity >95%; Sigma, St. Louis, MO, USA) and *trans*-β-apo-8′-carotenal (purity ~98% UV; Fluka, Buchs, Switzerland) were used for external calibration and as internal standard, respectively. Water-dispersible β-carotene beadlets (β-carotene 1% CWS) were obtained from DSM Nutritional Products (Basel, Switzerland). A carotenoid-free product of medium-chain triglycerides of vegetable origin (MCT oil type S), mainly consisting of caprylic and capric acids, was provided by Gustav Heess Oleochemische Erzeugnisse GmbH (Stuttgart, Germany). Apple pectin (Classic AU-L 003/01), with a degree of esterification of 77%, was donated by Herbstreith & Fox KG (Neuenbürg, Germany). Enzyme preparations with pectolytic activities for pulp maceration, namely a polygalacturonase (Rohament® PL) and a pectin lyase (Rohapect® PTE), were supplied by AB Enzymes GmbH (Darmstadt, Germany).

2.2. Sample preparation

2.2.1. Mango purées subjected to thermal batch processing on a laboratory scale

Two lots of mature-green mangoes were consecutively ripened at 25–30 °C for 4 days. Afterwards, defective fruits were removed from each lot. The fruits of both lots were divided into four and two batches of approximately 8 kg, respectively, according to the six temperatures of the process variants studied (Fig. 1), to investigate β-carotene stability and inactivation of endogenous enzymes in mango pulp under the well-defined thermal conditions of mimicked pasteurisation and maceration processes. The fruits of each batch were washed, manually peeled and pitted. The fruit mesocarp was successively cut into pieces, mashed and finished in a pulper (PAP 0533, Bertuzzi, Brugherio, Italy) with sieves of 10 mm, 1.5 mm, and 0.4 mm mesh size, respectively, in order to obtain a homogeneous intermediate. By use of a reaction vessel (EL 10, Esco-Labor AG, Riehen, Switzerland), ca. 2 kg of unheated purée per process variant were immediately pasteurised at 85 °C, 88 °C, 90.5 °C and 93 °C, for 0, 1, 4, and 16 min, or tempered at 30 °C and 45 °C for 30 and 60 min, respectively (Fig. 1), under continuous stirring with an anchor stirrer. The vessel was equipped with a reflux cooling system to avoid evaporation. The pulp temperature inside the reaction vessel was regularly recorded using a PT100 thermocouple. At each nominal process temperature, pulp aliquots of approximately 200 g were transferred from the reaction vessel into a round bottom flask after the holding times mentioned above, according to the process variants shown in Fig. 1. The flask was immediately capped with an inserted thermocouple, placed in an ice bath, and continuously stirred for cooling of the pulp to 15 °C. The effects of the respective heating regimes were expressed by the pasteurisation value ($P^{z=8.9\ ^\circ C}_{T_{ref}=93.3\ ^\circ C}$) (Hoppe et al., 2006), which is the duration of an analogous treatment at the reference temperature ($T_{ref} = 93.3$ °C) needed to achieve the same lethal effect on a population of microorganisms characteristic of the product under study. Calculated from the purée temperature (T) recorded every minute throughout the whole thermal treatment (Eq. (1); t, process time), the $P^{z=8.9\ ^\circ C}_{T_{ref}=93.3\ ^\circ C}$ value comprised the contributions of heating, holding and cooling times of the considered thermal processes to their total lethal effects. The z-value ($z = 8.9$ °C) was characteristic of microorganisms in products within the pH range of mango purée (Brown, 1991).

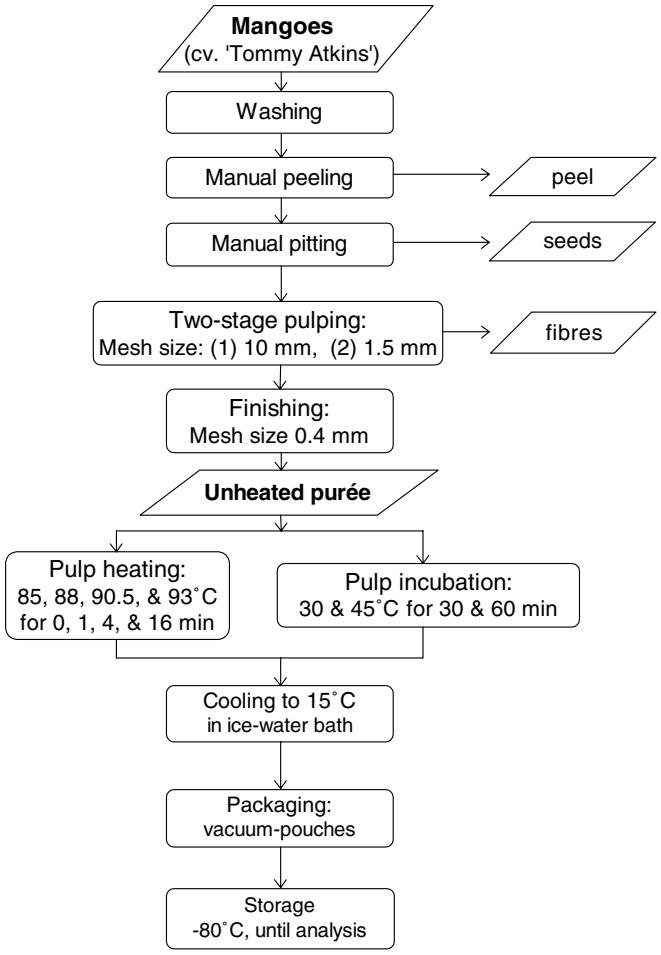

Fig. 1. Mango purée production with standardised thermal treatments to study different variants of pasteurisation and maceration routines (batch processing on laboratory scale). Maceration was mimicked regarding the thermal conditions by incubation treatments performed without the addition of enzymes.

$$P^{z=8.9\ ^\circ C}_{T_{ref}=93.3\ ^\circ C} = \int_0^t 10^{(T(t)-T_{ref})/z} dt \quad (1)$$

Finally, the cooled pulp was divided into sample aliquots for subsequent parallel physicochemical analyses [colour, pH, total soluble solids (TSS), titratable acidity

(TA), and viscosity]. Further sample aliquots were packed into aluminium pouches, sealed under vacuum and stored at −80 °C until analyses of β-carotene content and enzyme activities.

2.2.2. Model preparations subjected to thermal batch processing on a laboratory scale

To study the matrix effects of a typical mango purée under the well-defined thermal conditions of the processes described in Section 2.2.1, model preparations with varying lipid contents were formulated, mimicking mango pulp composition. A sugar syrup of 68° Brix was prepared from crystalline sucrose (Südzucker, Mannheim, Germany) and distilled water. Pectin was dispersed in distilled water at 40 °C and homogenised with an Ultra-turrax homogeniser (Janke & Kunkel, IKA-Werk, Staufen, Germany) to prepare a pectin solution of 2.5% (w/w). An aqueous dispersion of β-carotene 1% CWS beadlets (10% w/w) was prepared under dark conditions by the use of a magnetic stirrer, and kept at 4 °C until the final dosage to the model preparations produced on the same day. The preparations of pectin, sugar, and β-carotene were mixed at the necessary doses with an Ultra-turrax homogeniser to achieve a model formulation of 14° Brix, containing 0.648 g of pectin/100 g of fresh weight (FW) and 1694 μg of β-carotene/100 g of FW. The β-carotene content was analogous to that found in the fully ripe mesocarp of the Thai cultivar 'Kaew' (Vásquez-Caicedo et al., 2002). The pH value was adjusted to pH 4.4 with 2 M potassium hydroxide, by analogy with the pH measured in the mango purées mentioned in Section 2.2.1. To modify the physical state of the β-carotene in the model preparations, oil was added to the aqueous system in amounts corresponding to mango pulp (Herrmann, 1994). An aqueous system was used as control (0% of oil), while the oil-in-water (o/w) emulsions contained 0.2% and 0.4% of MCT oil. Each of the three model preparations was homogenised in a high-pressure homogeniser (LAB 60-10 TBSX, APV Gaulin, Lübeck, Germany) at 300 bar before subjecting it to one of the thermal process variants under study. Pasteurisation of each model preparation was performed at 88 °C and 93 °C for 0, 1, 4, and 16 min, respectively, by analogy with Section 2.2.1.

2.2.3. Mango nectar production on a pilot-plant scale with stepwise process control

β-Carotene stability and enzyme activities (PPO, POD) were evaluated at each processing step during mango nectar production (Fig. 2). By analogy with Section 2.2.1, mature-green mangoes cv. 'Tommy Atkins' were ripened at 25–30 °C for 4 days, reaching a sugar-acid ratio (TSS/TA) of 24 ± 0.22 at a content of total soluble solids (TSS) of 13.9 ± 0.02° Brix and pH 3.94 until processing. Approximately 18 kg of mango fruits were manually washed, steamed for 7 min and immediately cooled with tap water to facilitate manual peeling. After manual pitting, the fruit mesocarp was successively cut into pieces, mashed and finished in a pulper (PAP 0533, Bertuzzi, Brugherio, Italy) with sieves of 10 mm, 1.5 mm and 0.4 mm mesh size, respectively. To inactivate endogenous enzymes, this purée was continuously heated at a flow rate of 95 l/h, using a tubular heater (Ruland Engineering & Consulting, Neustadt, Germany). A product temperature of approximately 70 °C was achieved by heat-exchange against hot water, followed by final heating up to 91.3 ± 1.3 °C for 25 s using the integrated tubular Actijoule® unit, and by subsequent cooling to approximately 30 °C. The Actijoule® unit consisted of a series of stainless steel tubes subjected to low voltage, creating an electrical resistance. This system allows a direct heat transfer from the tube walls to the product, providing a constant temperature throughout the tube length in contrast to conventional tubular heating systems on the basis of water or steam. Therefore, this equipment is considered particularly suitable for thermal degradation and inactivation studies of active ingredients

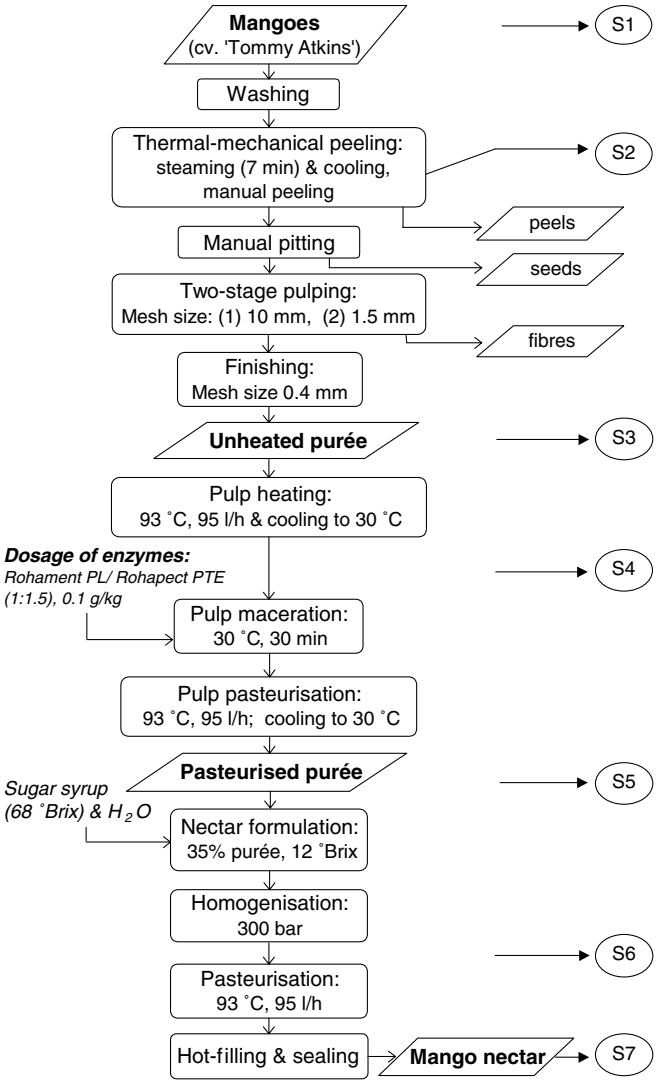

Fig. 2. Mango processing into purée and nectar on a pilot-plant scale with stepwise quality control (sampling at processing steps S1–S7). Thermal treatments are characterised by their nominal product temperatures and holding times. The real thermal conditions achieved are described in the text.

and enzymes, respectively. After inactivation of endogenous enzymes, the re-cooled purée was transferred into a reaction vessel type EL 10 (Esco-Labor AG, Riehen, Switzerland) for enzymatic pectin degradation to lower pulp viscosity for improved sensory properties. A mix of the pectolytic enzymes Rohament PL and Rohapect PTE (1:1.5) was added at a concentration of 0.1 g/kg of purée (Dube et al., 2004). The pulp was incubated at 30 ± 1 °C for 30 min under constant stirring. Immediately after incubation, the liquefied purée was pasteurised at a flow rate of 95 l/h by use of the tubular heat-exchanger with the Actijoule® system, holding at 86.8 ± 0.5 °C for 25 s with subsequent cooling to 30 °C. Subsequently, a mango nectar of 12° Brix and 35% of pulp was produced by diluting the mango purée with a sugar syrup of 66.7° Brix and drinking water. The nectar was then homogenised in a high-pressure homogeniser (LAB 60-10 TBSX, APV Gaulin, Lübeck, Germany) at 300 bar. Finally, the nectar was continuously pasteurised at 95 l/h and 94.6 ± 1.6 °C for 25 s, as previously described for the pulp. The pasteurised nectar was hot-filled into 0.5 l glass bottles that were sealed under steam-injection and cooled to room temperature in a water bath. An overall minimum value for $P^{z=8.9\ °C}_{T_{ref}=93.3\ °C}$, representing the cumulative effect of all three continuous heating operations involved, was estimated, based on Eq. (1) from the product temperatures [$T(t)$] recorded at 11 positions of the heating and cooling sections of the tubular heat-exchanger and the flow times t up to each of those positions. Flow times were calculated from flow rates and tube dimensions. The contributions provided by recooling of the hot-filled nectar in the bottles and by steaming of the fruits for peeling were disregarded, due to metrological constraints. Samples for β-carotene analysis, residual activities of PPO and POD, and physicochemical characterisation were collected throughout the process (S1–S7), as indicated in Fig. 2. To determine the dry matter at each processing step (S1–S7), ca. 3 g of sample were exactly weighed and evenly distributed on an aluminium plate for subsequent drying at 85 °C to constant weight, using an infrared-drying scale type MA 40 (Sartorius, Göttingen, Germany).

2.3. Physicochemical analyses

Total soluble solids (TSS), titratable acidity (TA), pH, colour and the flow properties, namely, the dependence of the viscosity on the shear-rate, were determined, to control the quality of the raw and heated samples and hence the processes. TSS was measured refractometrically [International Federation of Fruit Juice Producers (IFU), 2001] by use of a digital refractometer type RX 5000 (Atago, Tokio, Japan). TA was determined by titration with 0.1 N NaOH to a pH of 8.1 and expressed as g citric acid/100 g [International Federation of Fruit Juice Producers (IFU), 2001], using an automatic titration system Titrino 718 STAT (Deutsche Metrohm, Filderstadt, Germany). For physical characterisation of the samples, colour was evaluated on the basis of the CIELAB colour space (L^*, a^*, b^* and L^*, C^*, $H°$, respectively) (Hoppe et al., 2006; Hutchings, 1999). For the colour measurements with a colorimeter CR300 (Minolta, Langenhagen, Germany), the purées, model preparations and nectar samples, were homogeneously distributed on a Petri dish placed on a white background and analyzed 7-fold at different test points per sample surface. Additionally, colour spectra from non-heated and heated purée samples were compared by scanning the respective ethanolic pigment extracts from 280 to 780 nm, as reported by Bonora, Pancaldi, Gualandri, and Fasulo (2000), using an UV/Vis spectrophotometer Lambda 20 (Perkin–Elmer, Überlingen, Germany). Flow curves were recorded at 25 °C, measuring sample viscosity within a shear rate range from 0.06 to 500 s^{-1} with a rheometer CVO 120 HR that was equipped with a coaxial cylinder device C25 (Bohlin Instruments, Pforzheim, Germany).

2.4. β-Carotene analysis

Samples of frozen mango purée and nectar or model preparations, respectively, were quickly thawed in their pouches at 20 °C in a water bath. An aliquot of approximately 10 g of sample was exactly weighed before β-carotene extraction that was performed as described previously (Vásquez-Caicedo et al., 2005).

HPLC analysis was performed as detailed by Marx, Schieber, and Carle (2000). Quantification of all-*trans*-β-carotene at 453 nm was based on a linear calibration curve, as previously reported (Vásquez-Caicedo et al., 2005). By analogy, 9-*cis*- and 13-*cis*-β-carotene isomers were monitored at 445 nm, calculating their concentrations from the respective all-*trans*-β-carotene calibration curve. A minimum of five standard concentrations was used for each calibration curve, ranging from 0.97 to 19.4 mg/l ($R^2 = 0.9992$) and from 0.49 to 9.7 mg/l ($R^2 = 0.9989$) for quantification of all-*trans*-β-carotene and the *cis*-β-carotene isomers, respectively. The concentrations of the injected sample solutions were at least 1.1, 1.8, and 2.7 times larger than that of the lowest standard used for calibration of 9-*cis*-, 13-*cis*-, and all-*trans*-β-carotene levels, respectively. In the calibrated ranges relevant for the sample peaks, recoveries of the β-carotene standards were 88.6–111% for the concentration range of the *cis*-isomers and 93.9–102% for that of all-*trans*-β-carotene. The quantification limit was 0.25 mg/l (Vásquez-Caicedo, Schilling, Carle, & Neidhart, 2006). Samples were extracted and analyzed in duplicate.

2.5. Determination of enzymatic activities

2.5.1. Enzyme extraction

For the extraction of POD and PPO, frozen purée samples were thawed until approximately 4 °C was reached. The extraction procedure described by Baur, Klaiber, Koblo, and Carle (2004) was slightly modified.

Approximately 3 g of sample and 600 mg of polyvinylpolypyrrolidone (PVPP), equivalent to one part of dry matter to one part of PVPP, were exactly weighed and mixed with 10 ml of chilled McIlvaine buffer (pH 6.5), consisting of 30% 0.1 M citric acid and 70% 0.2 M disodium phosphate, under continuous stirring for 1 h at 4 °C. Subsequently, the homogenate was centrifuged twice to separate solid residues from the extract. The first centrifugation was carried out in capped 10 ml glass tubes for 10 min at 3000 rpm in a centrifuge type Z 320 (Hermle Labortechnik, Wehingen, Germany). The supernatant was transferred into 2 ml Eppendorf safe-lock tubes and centrifuged for 10 min at 10,000 rpm in a microlitre-centrifuge type 1394 (Andreas Hettlich GmbH & Co. KG, Tuttlingen, Germany). The clear supernatant was kept in an ice bath until POD and PPO activity assays were performed, shortly after. All samples were extracted in triplicate. The protein contents of the enzyme extracts were determined according to Bradford (1976), using bovine serum albumin (BSA) as protein standard.

2.5.2. PPO assay

PPO activity was determined by use of a modification of a previously described assay (Baur et al., 2004). Aliquots of 1.4 ml of reaction buffer [0.5 mM sodium dodecyl sulfate (SDS) in McIlvaine buffer (pH 6.5), consisting of 30% 0.1 M citric acid and 70% 0.2 M disodium phosphate], 0.2 ml of a 0.5 M L-proline reagent in reaction buffer, and 0.2 ml of the enzyme extract (Section 2.5.1) were mixed in a cuvette at ambient temperature. The reaction was started by adding 0.2 ml of 25 mM 4-methylcatechol in reaction buffer. The formation of a pink proline-catechol product was measured at 525 nm ($\varepsilon_{25\,^\circ C} = 1550\ l\ mol^{-1}\ cm^{-1}$) every 15 s for 3 min, using a Cary 100 spectrophotometer (Varian, Mulgrave, Victoria, Australia) adjusted at 25 °C. Each extract was analyzed in triplicate. The respective heat-denatured protein extract that had been boiled up four times in a test tube was always used as a control. PPO activity was calculated from the highest slope of the linear segment of the absorption–time curve measured for a period of 60 s. The enzyme activity was expressed in nKat/mg of protein and in either nKat/100 g of purée fresh weight or in nKat/g of pulp dry weight.

2.5.3. POD assay

POD activity was assayed in triplicate per enzyme extract as described by Baur et al. (2004). The reaction was started by adding 0.2 ml of the enzyme extract (Section 2.5.1) to 1.3 ml of McIlvaine buffer (pH 6.5) that consisted of 30% 0.1 M citric acid and 70% 0.2 M disodium phosphate and contained 12 mM tropolone (2-hydroxy-2,4,6-cycloheptatrien-1-one) and 3.3 mM H_2O_2 as substrates. After 40 s of preincubation, the accumulation of a yellow product was measured at 418 nm ($\varepsilon_{25\,^\circ C} = 2075\ l\ mol^{-1}\ cm^{-1}$) every 15 s for 3 min at 25 °C. Equipment, controls and calculations of POD activity from the absorption–time curves were by analogy with Section 2.5.2.

2.6. Statistical analyses

The laboratory-scale experiments on mango purée pasteurisation were based on a 4×4 matrix, comprising four temperature levels and four different holding times. A 2×2 full factorial was the basis for the experiments that were directed towards thermal maceration conditions, including two temperature levels and two holding times. Experiments with the aqueous and oil/water emulsified model preparations were planned as a $2 \times 3 \times 4$ matrix covering two pasteurisation temperatures, three oil concentrations, and four holding times. Analyses of variance (ANOVA) and Pearson correlation coefficients were calculated using JMP IN software, version 3.2.1 (1997), obtained from SAS Institute, Cary, NC, USA.

3. Results

3.1. The influence of the thermal conditions of pasteurisation routines on mango purée studied in batch processes on the laboratory scale

According to the four pasteurisation temperatures to be studied in respective batch processes (Fig. 1), the mango fruits (cv. 'Tommy Atkins') had to be divided into four lots after postharvest ripening. Minimum variations in TSS/TA ratios, pH values, colour intensity (C^*) and hue ($H°$) among the four purée batches prior to heating confirmed uniform ripeness of the lots, thus minimizing any errors due to heterogeneity of the raw material (Table 1). Similarly, all unheated purées presented identical non-Newtonian flow curves with viscosities of 1.2–1.3 Pas at a shear rate of 8.0 s^{-1} and the typical shear-thinning characteristics of mango purée, as previously shown (Dube et al., 2004). Under the applied postharvest ripening conditions, the fruits only reached approximately 50% of the TSS/TA ratio levels recommended for purée production from selected Thai cultivars (Vásquez-Caicedo et al., 2004). Also, instead of the desired bright yellow-orange colour and soft mesocarp texture, the four purée lots presented a yellow colour with a slight greenish hue ($H° \approx 92$), altogether indicating restrained ripening. This may be attributed to an early harvesting of the fruits at their mature-green preclimacteric stages and long transportation under cool conditions before reaching the German market. Postharvest ripening of fruits harvested too early may be incomplete, resulting in higher acidity, lower total reducing sugar contents, and poorer peel colour (lower a^* values) than for fruits harvested at springy-mature green, half-ripe or ripe stages (Lalel, Singh, & Tan, 2003). Insufficient mesocarp redness, that is, low a^* values and hue angles of $H° > 90°$, may be closely related to low β-carotene contents, since a strong linear correlation ($r = 0.96$) between both properties has previously been reported (Vásquez-Caicedo et al., 2002) and postharvest β-carotene formation is closely associated with the cultivar-specific colour development of the mesocarp (Vásquez-Caicedo et al., 2005). Nevertheless, pH

Table 1
Physicochemical characteristics of the mango pulp prior to heating in the batch pasteurisation experiments

Variant		pH	TSS/TA	C^*	$H°$ (°)
Temperature[a] (°C)	Holding time[b] (min)				
85.0	0	3.89 ± 0.02	21.6 ± 0.03	47.38 ± 0.03	92.41 ± 0.01
88.0	0	3.82 ± 0.01	20.8 ± 0.03	49.50 ± 0.03	92.06 ± 0.01
90.5	0	3.85 ± 0.00	22.6 ± 0.04	49.83 ± 0.02	92.06 ± 0.01
93.0	0	3.73 ± 0.05	22.3 ± 0.42	49.39 ± 0.03	91.71 ± 0.01

TSS/TA, sugar-acid ratio; C^*, chroma (dimensionless length of the CIE colour vector); $H°$, hue angle (angle of the CIE colour vector in degrees).
[a] Pasteurisation temperature subsequently studied by use of the respective batch of mango purée.
[b] As in Table 2, a holding time of 0 min represents the still unheated purée used as control in the subsequent pasteurisation experiment which the respective batch of mango purée was subjected to.

values (3.73–3.89) and titratable acidity (0.57–0.63 g/100 g) were within the ranges recommended for the production of mango purée (Askar & Treptow, 2001; Dukel, 1988).

β-Carotene stability, under various pasteurisation regimes that were characterised by their $P^{z=8.9\,°C}_{T_{ref}=93.3\,°C}$ values, is shown in Table 2. Ranging from 85 to 93 °C, the nominal process temperatures were typical for pasteurisation of fruit products. Nominal lethal effects, reflecting the thermal stress in each process variant, covered a broad range from $P^{z=8.9\,°C}_{T_{ref}=93.3\,°C} = 0.34$ min to 14.91 min, where holding times of 1 min and 16 min were used in this experiment to mimic the thermal conditions of continuous pasteurisation ($P^{z=8.9\,°C}_{T_{ref}=93.3\,°C} = 0.34 - 2.09$ min) and bottle pasteurisation after cold-filling ($P^{z=8.9\,°C}_{T_{ref}=93.3\,°C} = 2.16 - 14.91$ min), respectively. Regarding their lethal effects, the treatments with 16 min at 85 °C, 4 min at 90.5 °C, and 1 min at 93 °C were nearly equivalent ($P^{z=8.9\,°C}_{T_{ref}=93.3\,°C} = 2.16, 2.59$, and 2.09 min), by analogy with those of 16 min at 88 °C and 4 min at 93 °C ($P^{z=8.9\,°C}_{T_{ref}=93.3\,°C} = 4.71$ and 4.41 min).

In the unheated control samples of all four temperature variants, both the total β-carotene content and the relative proportions of 9-*cis*- and 13-*cis*-isomers were similar with average values of 363 μg/100 g of purée fresh weight (FW), 8.3%, and 12.9%, respectively (Table 2), being consistent with the amount of naturally occurring *cis*-isomers in cv. 'Tommy Atkins' previously reported (Pott et al., 2003a). The maximum total β-carotene loss that could be ascribed to pasteurisation of mango purée was only 7.2%. This total loss was observed after 16 min at 90.5 °C ($P^{z=8.9\,°C}_{T_{ref}=93.3\,°C} = 6.76$ min). Irrespective of the differences in the lethal effects, total β-carotene losses caused by any pasteurisation treatment studied were rather similar and very

Table 2
Influence of pasteurisation conditions applied to mango purée on the stability of β-carotene and the vitamin A value

Temperature (°C)	Holding time (min)	$P^{z=8.9\,°C}_{T_{ref}=93.3\,°C}$ (min)	β-carotene				Vitamin A (RE/100 g FW)[c]
			Total[a] (μg/100 g FW)	all-*trans* (%)[b]	13-*cis* (%)	9-*cis* (%)	
85	0[d]	–	$361^{a,v} \pm 4.1$	78.0	13.4	8.7	$53.1^{a,v} \pm 0.6$
	1	0.34	$354^{a,v} \pm 4.4$	75.5	15.8	8.7	$51.5^{ab,v} \pm 0.6$
	4	0.76	$353^{a,v} \pm 3.4$	71.9	18.8	9.3	$50.3^{b,v} \pm 0.6$
	16	2.16	$336^{b,v} \pm 1.4$	66.4	22.3	11.4	$46.1^{c,v} \pm 0.2$
88	0	–	$359^{a,v} \pm 4.4$	78.4	13.2	8.4	$53.0^{a,v} \pm 0.8$
	1	0.88	$332^{a,w} \pm 3.5$	75.0	16.5	8.6	$48.1^{a,w} \pm 0.5$
	4	1.76	$324^{a,w} \pm 13.0$	72.1	18.8	9.1	$47.7^{b,w} \pm 0.7$
	16	4.71	$351^{a,v} \pm 16.6$	68.5	20.8	10.7	$48.9^{a,v} \pm 2.5$
90.5	0	–	$383^{a,v} \pm 0.7$	79.9	12.0	8.1	$57.0^{a,v} \pm 0.1$
	1	1.47	$362^{b,v} \pm 6.6$	74.1	17.2	8.7	$52.2^{b,v} \pm 1.0$
	4	2.59	$363^{b,v} \pm 0.2$	71.5	19.6	9.0	$51.5^{b,v} \pm 0.0$
	16	6.76	$356^{b,v} \pm 0.0$	64.1	23.4	12.4	$48.2^{c,v} \pm 0.1$
93	0	–	$349^{a,v} \pm 34.2$	78.8	13.1	8.1	$53.1^{a,v} \pm 4.0$
	1	2.09	$341^{a,v} \pm 2.6$	74.0	17.6	8.4	$49.1^{a,vw} \pm 0.5$
	4	4.41	$338^{a,vw} \pm 3.2$	68.8	21.7	9.5	$47.3^{a,w} \pm 0.4$
	16	14.91	$346^{a,v} \pm 0.2$	65.2	23.5	11.4	$47.2^{a,v} \pm 0.0$

[a,b,c,v,w] On the basis of the least significant difference test (LSD), same letters indicate non-significant differences of the means obtained at different holding times within a temperature level (a, b, c; vertically) and at different temperatures within a holding time level (v, w; vertically), respectively, at a level of significance of $\alpha = 0.05$.
[a] Mean ± standard error (analysis in duplicate).
[b] Relative isomer proportions calculated in g of isomer/100 g of total β-carotene.
[c] Calculated on the basis of the bioconversion rate concluded by FAO/WHO Joint Expert Consultation (1988) for β-carotene from a mixed meal and the relative bioconversion of the *cis*-isomers described by Zechmeister (1962); vitamin $A = C_{AT}/6 + 0.53 \cdot (C_{13\text{-}cis}/6) + 0.38 \cdot (C_{9\text{-}cis}/6)$ with C_{AT}, $C_{13\text{-}cis}$, and $C_{9\text{-}cis}$ being the contents of all-*trans*-, 13-*cis*-, and 9-*cis*-β-carotene (in μg/100 g of FW), respectively (Castenmiller & West, 1998; Godoy & Rodriguez-Amaya, 1994).
[d] Holding times of 0 min represent the respective unheated control samples for each level of pasteurisation temperature.

limited. Therefore, the β-carotene results neither allowed the calculation of the degradation kinetics of all-*trans*-β-carotene nor the evaluation of the data matrix by regression analysis, as originally intended.

As a result of a two-way crossed ANOVA performed instead, pasteurisation temperature and holding time significantly affected the all-*trans*-β-carotene content and the vitamin A value of the purées ($P < 0.05$). However, both factors acted independently, because the respective two-factor interactions between them were not significant ($P > 0.05$). Contrarily, the effects of the temperature level on the contents of 13-*cis*- and 9-*cis*-β-carotene, respectively, significantly depended on the holding time applied ($P < 0.01$). Decreases of all-*trans*-β-carotene and vitamin A average levels mostly depended on the heating duration. 13-*cis*- and 9-*cis*-β-carotene markedly accumulated with extended holding times, but their rates of formation depended on the temperature level (Fig. 3a). Formation of 13-*cis*-β-carotene was particularly induced above 90 °C, while the initially high rates of formation declined with rising holding time. Although linear regression between $P^{z=8.9\ °C}_{T_{ref}=93.3\ °C}$ and the relative contents of the β-carotene isomers was always poor ($R^2 = 0.50$–0.69, Fig. 3b),

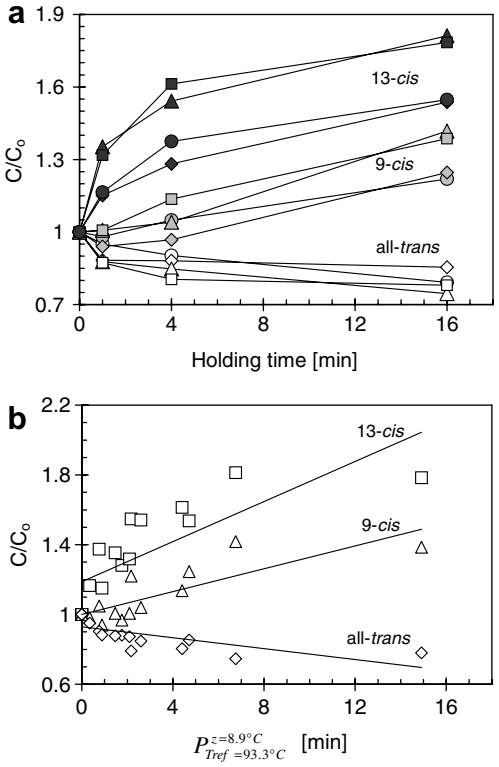

Fig. 3. β-Carotene stability in mango purée with various pasteurisation routines: (a) Relative contents (C/C_0) of β-carotene isomers with rising holding times at various pasteurisation temperatures. Pasteurisation temperatures: 85 °C (●), 88 °C (◆), 90.5 °C (▲), and 93 °C (■). (b) Relative contents of all-*trans*- (◇), 13-*cis*- (□), and 9-*cis*-β-carotene (△) as a function of the lethal effect ($P^{z=8.9\ °C}_{T_{ref}=93.3\ °C}$) achieved by the pasteurisation routines ($R^2 = 0.50$, 0.62 and 0.69, respectively). C = isomer content of the pasteurised sample (μg/100 g of purée fresh weight (FW)); C_0 = isomer content of the unheated control (μg/100 g of purée FW).

due to the rather weak alterations of the latter, Pearson correlation coefficients between $P^{z=8.9\ °C}_{T_{ref}=93.3\ °C}$ and all-*trans*-, 13-*cis*-, and 9-*cis*-β-carotene contents (−0.66, 0.76 and 0.69, respectively) were significant ($P < 0.05$). Consistent with a previous study (Marx et al., 2003), the $P^{z=8.9\ °C}_{T_{ref}=93.3\ °C}$ value had a marked effect on the formation of 13-*cis*-β-carotene. After all, 13-*cis*-β-carotene almost doubled its percentage of the total β-carotene content after 16 min above 90 °C ($P^{z=8.9\ °C}_{T_{ref}=93.3\ °C} \geqslant 6.76$ min), as compared to the unheated purée. Hence, *trans*–*cis*-isomerisation, and especially the formation of 13-*cis*-β-carotene, was closely related to the intensity of the heat treatment.

On the whole, total β-carotene degradation and *trans*–*cis*-isomerisation resulted in a drop of the vitamin A value. Its loss, caused by pasteurisation, ranged from 7.7% to 15.4% (Table 2) after the maximum holding time (16 min) at any of the temperature levels studied.

β-Carotene degradation was accompanied by detrimental colour changes. A marked loss of colour intensity (C^*), which significantly correlated with holding time ($r = -0.82$, $P < 0.05$), but only a slight hue ($H°$) increase were observed (Fig. 4). Colour changes induced by pasteurisation were better reflected by the absorption spectra of the pigment extracts between 340 and 520 nm (Fig. 5). Peak shifts were detected by overlaying the spectra derived from samples obtained after different holding times at 88 °C. The time-dependent formation of a peak between 360 and 380 nm indicated the formation of the corresponding *cis*-isomers, which usually show an additional absorption band at 320–360 nm (van den Berg et al., 2000). Consistent with the loss in colour intensity, formation of mono- and di-*cis*-isomers produced a small hypsochromic shift and a hypochromic effect (Chen et al., 1994; Rodriguez-Amaya, 2001).

Investigations of peroxidase (POD) and polyphenol oxidase (PPO) activities in the same purées revealed that PPO was readily inactivated after 1 min of holding time at all temperature levels applied (data not shown). On the contrary, Askar and Treptow (2001) considered PPO the most thermostable enzyme in mangoes, requiring 3 min at 85 °C

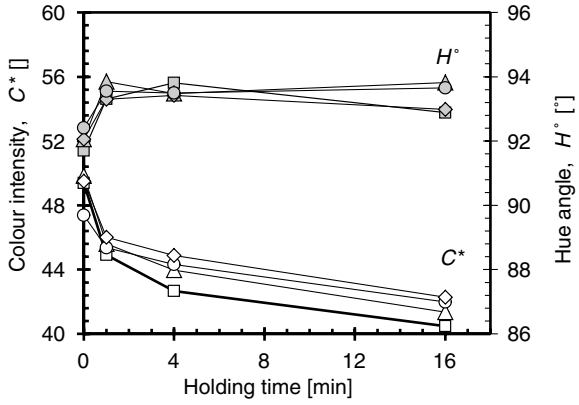

Fig. 4. Changing colour intensity (chroma, C^*) and hue (hue angle, $H°$) of mango purée as a result of rising pasteurisation temperatures and holding times. Pasteurisation temperatures: 85 °C (○), 88 °C (◇), 90.5 °C (△), and 93 °C (□). $P^{z=8.9\ °C}_{T_{ref}=93.3\ °C}$ values for all variants as described in Table 2.

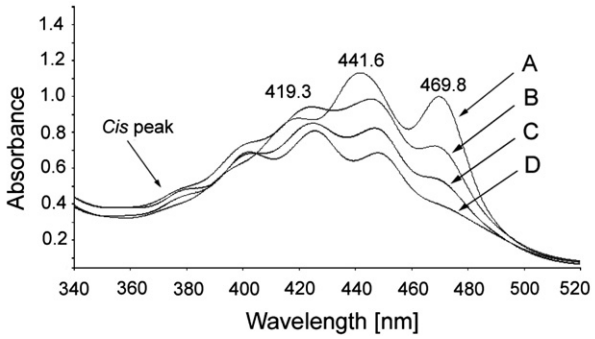

Fig. 5. UV/vis spectra of ethanolic carotenoid extracts obtained from mango purée: (a) unheated control; (b) heated at 88 °C for 1 min; (c) heated at 88 °C for 4 min and (d) heated at 88 °C for 16 min. $P^{z=8.9\,^{\circ}C}_{T_{ref}=93.3\,^{\circ}C}$ values for all variants as described in Table 2.

to reach 50% of its initial activity. In contrast, POD residual activities, ranging from 16.8% to 7.8% and 8.5% to 4.0%, respectively, were found after 1, 4, and 16 min at 85–93 °C (Table 3), when the residual activity was related to the protein content of the enzyme extract ($A_P/A_{P,0}$) and to the fresh weight of the purée ($A_M/A_{M,0}$), respectively. Compared to the unheated purées, less protein was extractable after pasteurisation (0.20–0.23 g/l and 0.10–0.13 g/l of enzyme extract, respectively), irrespective of the thermal conditions. Therefore, the residual activities $A_P/A_{P,0}$, based on the activity of the extracted protein, were excessive. Nevertheless, residual POD activity was detectable in the pasteurised purées by the applied quantitative method but did not significantly vary among the process variants.

Table 3
Influence of pasteurisation conditions applied to mango purée on the inactivation of endogenous peroxidase (POD)

Temperature (°C)	Holding time (min)	$P^{z=8.9\,^{\circ}C}_{T_{ref}=93.3\,^{\circ}C}$ (min)	Residual activity of POD (%)	
			$A_P/A_{P,0}$[a]	$A_M/A_{M,0}$[b]
85	0[c]	–	100	100
	1	0.34	14.7	8.1
	4	0.76	16.8	8.5
	16	2.16	10.3	6.1
88	0	–	100	100
	1	0.88	12.2	6.9
	4	1.76	13.6	7.6
	16	4.71	10.2	6.3
90.5	0	–	100	100
	1	1.47	11.7	6.3
	4	2.59	8.8	4.7
	16	6.76	12.5	6.3
93	0	–	100	100
	1	2.09	10.7	4.8
	4	4.41	8.7	4.4
	16	14.91	7.8	4.0

[a] Residual activity of POD in the purée based on the activity A_P of the extracted protein in nKat/mg of protein of the enzyme extract, with $A_{P,0}$ being the reference value of the respective unheated purée.

[b] Residual activity of POD in the purée based on the activity A_M of the mango pulp in nKat/100 g of pulp fresh weight, with $A_{M,0}$ being the reference value of the respective unheated control.

[c] Holding times of 0 min represent the respective unheated control samples for each level of pasteurisation temperature.

3.2. The influence of the thermal conditions of pasteurisation routines on model preparations studied in batch processes on the laboratory scale

To evaluate the influence of the tissue matrix on the degradation and *trans–cis*-isomerisation of β-carotene observed in the purées under pasteurisation conditions in Section 3.1, the respective experiments described in Fig. 1 were repeated with tissue-free model preparations at two temperature levels (88 °C and 93 °C), while modifying the physical state of β-carotene by the addition of oil.

The various contents of MCT oil added [0%, 0.2%, and 0.4% (w/w)] resulted in slight colour differences among the model preparations prior to heating. The water-dispersible β-carotene formulation caused a deep-orange colour of the two model preparations produced without oil ($H° = 74.0°$ and 78.2°) that increasingly turned to a yellow tonality as the oil content rose, owing to the lipophilic affinity of the originally crystal-like β-carotene beadlets released from their protective hydrophilic capsules. Consequently, the hue angle ($H°$) of the oil-containing model preparations increased from 83.4°–85.6° at 0.2% of oil added to 88.6°–88.8° at 0.4% of oil. Therefore, the relative proportions of the isomers were evaluated, to assess the contributions of both pasteurisation and partial lipophilic β-carotene solubilisation in o/w-emulsions to *trans–cis*-isomerisation (Table 4). Measured total β-carotene contents of the model preparations prior to heating ranged from 1716 to 1824, 1277–1343 and 1586–1682 μg/100 g of sample FW for the products with 0, 0.2, and 0.4% (w/w) of MCT oil.

As shown by a three-way ANOVA, the ratio between all-*trans*-β-carotene and its *cis*-isomers was affected by the interaction of holding time, temperature, and oil content ($P < 0.05$). While the relative proportion of 9-*cis*-β-carotene remained rather constant (Table 4), a major impact of increasing holding times on the formation of 13-*cis*-β-carotene, together with overall effects of the heating temperature and oil concentration, could be detected. By heating of the formulations containing 0%, 0.2%, and 0.4% of oil at 93 °C for 16 min ($P^{z=8.9\,^{\circ}C}_{T_{ref}=93.3\,^{\circ}C}$ = 15.9–16.2 min), that is, under the maximum thermal stress applied, the relative proportions of the 13-*cis*-isomers rose from 21.4%, 23.8%, and 24.2% to maximum levels of 27.0%, 27.9%, and 28.6%, respectively, corresponding to relative increases by 26, 17, and 18%. However, as *trans–cis*-isomerisation was very limited and the total β-carotene contents of the pasteurised model preparations mostly varied around those of the respective unheated controls within the analytical error range, the stability of β-carotene in the model preparations (Table 4) during pasteurisation was superior to that in mango purées (Table 2), possibly because the protective capsule that coated the β-carotene in the beadlets stabilized the solid state of the β-carotene.

Table 4
Ratio of the β-carotene isomers in model preparations of β-carotene 1% CWS beadlets, at different oil contents, depending on the pasteurisation conditions

Oil (g/100 g)	Holding time (min)	$P^{z=8.9\,^{\circ}C}_{T_{ref}=93.3\,^{\circ}C}$ (min)		β-carotene							
				Total[a] (μg/100 g FW)		all-*trans* (%)[b]		13-*cis* (%)		9-*cis* (%)	
		88 °C	93 °C	88 °C	93 °C	88 °C	93 °C	88 °C	93 °C	88 °C	93 °C
0	0[c]	–	–	1716 ± 18	1824 ± 130	57.1	57.9	22.3	21.4	20.6	20.7
0	1	0.61	2.19	1786 ± 17	2107 ± 44	57.5	55.4	22.4	23.3	20.1	21.3
0	4	1.36	4.74	1680 ± 38	1801 ± 161	56.0	54.6	23.2	24.1	20.8	21.3
0	16	4.30	15.92	1698 ± 4	1669 ± 86	54.3	51.3	25.2	27.0	20.5	21.6
0.2	0	–	–	1277 ± 66	1343 ± 8	56.7	55.8	22.6	23.8	20.7	20.4
0.2	1	0.40	2.07	1384 ± 13	1388 ± 10	57.0	55.4	22.0	24.3	20.9	20.3
0.2	4	1.10	5.06	1358 ± 34	1420 ± 61	56.4	53.5	22.4	24.3	21.2	22.1
0.2	16	4.05	16.24	1347 ± 30	1549 ± 6	54.4	52.3	24.7	27.9	20.9	19.8
0.4	0	–	–	1586 ± 22	1682 ± 24	57.3	55.8	23.0	24.2	19.7	20.0
0.4	1	0.68	2.35	1565 ± 48	1795 ± 8	56.0	55.1	23.7	25.1	20.3	19.8
0.4	4	1.42	5.00	1451 ± 21	1727 ± 24	54.5	54.0	25.2	26.2	20.3	19.8
0.4	16	4.48	16.15	1574 ± 74	1600 ± 30	53.0	51.9	27.0	28.6	20.0	19.5

[a] Mean ± standard error (analysis in duplicate).
[b] Relative isomer proportions calculated in g of isomer/100 g of total β-carotene.
[c] Holding times of 0 min refer to the respective unheated control samples for each level of pasteurisation temperature and oil content.

Furthermore, an equilibrium ratio of the stereoisomers according to their relative thermodynamic stabilities may have already been reached in the unheated model preparations or even in the beadlets (Weedon & Moss, 1995).

Consistent with the minor β-carotene changes induced by pasteurisation, colour changes were primarily provoked by the addition of the oil to the formulation but hardly by the heat treatments. The longest heating time (16 min) resulted in insignificant maximum variations of $\leqslant$2.5% and $\leqslant$1.3% around the values of the respective control for C^* and H° of the oil-containing model preparations, respectively.

3.3. The influence of the thermal conditions of maceration routines on mango purée and model preparations studied in batch processes on the laboratory scale

To study the influence of the thermal conditions of maceration treatments on β-carotene stability and purée colour at two temperature levels, two lots of mango fruits were processed, corresponding to the four process variants of purée incubation in Fig. 1. With TSS/TA and pH values ranging from 33.7 to 36.4 and 4.08 to 4.09, respectively, similar initial physicochemical characteristics of the two purée lots, prior to the thermal treatments studied, documented uniform ripeness of the fruits used for this experiment.

Total β-carotene contents and vitamin A values did not show any significant changes ($P > 0.05$) induced by purée incubation for 30 or 60 min at 30 °C or 45 °C (Table 5). A minor apparent decrease in total β-carotene of 8.3% during 60 min at 45 °C, on the one hand, and a similar increase at 30 °C during the same time, on the other hand, particularly illustrated the insignificance of these changes that could be attributed to analytical error, with the coefficients of variation (CV) ranging from 1.4% to 14.7% for the respective samples. Reproducibility of the analytical method applied was previously documented by a CV of

Table 5
Influence of the thermal conditions of pulp maceration applied to mango purée on the stability of β-carotene, the vitamin A value and product colour

Temperature (°C)	Holding time (min)	β-carotene			Vitamin A (RE/100 g FW)[c]	L^*	C^*
		Total[a] (μg/100 g FW)	13-*cis* (%)[b]	9-*cis* (%)			
30	0[d]	$951^{a,v} \pm 9.5$	13.3	6.8	$142^{a,v} \pm 1.3$	56.4 ± 0.6	58.0 ± 0.8
	30	$938^{a,v} \pm 37.4$	13.8	6.6	$140^{a,v} \pm 5.5$	54.1 ± 0.4	55.0 ± 0.7
	60	$1034^{a,v} \pm 107.5$	13.7	6.5	$154^{a,v} \pm 16.2$	53.5 ± 0.9	53.8 ± 1.3
45	0	$977^{a,v} \pm 20.6$	13.3	8.0	$145^{a,v} \pm 2.9$	58.6 ± 1.0	58.4 ± 0.7
	30	$936^{a,v} \pm 39.9$	13.3	8.4	$138^{a,v} \pm 5.8$	53.0 ± 0.4	50.9 ± 0.6
	60	$896^{a,v} \pm 14.6$	14.2	8.0	$134^{a,v} \pm 2.1$	51.5 ± 0.5	46.4 ± 0.7

[a,v] On the basis of the least significant difference test (LSD), same letters indicate non-significant differences of the means obtained at different holding times within a temperature level (a; vertically) and at different temperatures within a holding time level (v; vertically), respectively, at a level of significance of $\alpha = 0.05$.
[a] Mean ± standard error (analysis in duplicate).
[b] Relative isomer proportions calculated in g of isomer/100 g of total β-carotene.
[c] Calculated as described in footnote a of Table 2.
[d] Holding times of 0 min represent the respective unheated control samples for each level of maceration temperature.

6.73%, 6.26% and 6.91% obtained for all-*trans*-, 13-*cis*- and 9-*cis*-β-carotene, respectively, after nine extractions of a single mango purée with subsequent HPLC analyses in duplicate (Vásquez-Caicedo et al., 2005). Consistently, Taungbodhitham, Jones, Wahlqvist, and Briggs (1998) reported a CV ranging from 4.6% to 10.5% for the determination of β-carotene, validated for the analysis of canned tomato juice. Concordantly, the relative proportions of 13-*cis*- and 9-*cis*-β-carotene were not significantly affected by the thermal conditions of maceration treatments ($P > 0.05$).

Colour changes during purée incubation were most clearly reflected by a reduction of lightness (L^*) and colour intensity (C^*) (Table 5). Although PPO and POD activities of the samples were not investigated in this experiment, the colour loss may be related to enzymatic browning of the mango purées, since no inactivation of endogenous enzymes was performed in the purées prior to their incubation at 30 or 45 °C (Fig. 1). Both L^* and C^* values were significantly affected by increasing incubation temperatures ($P < 0.05$), with relative losses of 5.1% and 12.1% for L^* and of 7.2% and 20.5% for C^* at 30 °C and 45 °C, respectively, when incubation times were extended to 60 min. Documented β-carotene stability and constant colour hue ($H° = 87.8°–89.0°$), under the thermal conditions of purée maceration, clearly indicated other causes of the observed colour changes than β-carotene degradation.

As opposed to the mango purées, incubation of the two aqueous model preparations studied at 30 °C and 45 °C resulted in slight colour intensification, from $C^* = 55.4$ and 61.7 in the unheated control samples to $C^* = 73.2$ and 72.6 in the formulations incubated for 30 and 60 min, respectively. This was accompanied by a weak red shift of the hue angle that declined from 86° by 1.95°–1.65°. These colour changes may be due to the improved dispersion of the β-carotene formulation (1% CWS) with increasing incubation time, favoured by constant stirring. Unlike the colour, all-*trans*-, 13-*cis*- and 9-*cis*-β-carotene contents of the two aqueous model preparations varied very little (<1%) around 1214, 413 and 396 μg/100 g, respectively, under the set incubation conditions, not revealing any degradation or *trans–cis*-isomerisation of β-carotene.

3.4. β-Carotene stability during mango nectar processing on a pilot-plant scale

After the evaluation of the thermal effects of single processing steps (Sections 3.1 and 3.3), the whole process of mango nectar production was considered to assess the combined effect of all thermal processes involved beside their individual impacts on product quality. β-Carotene stability throughout nectar production was studied by means of stepwise process analysis, based on the samples taken at the processing steps S1–S7 (Fig. 2). While effects of heating could be expected for the samples S2, S4, S5, and S7, effects of oxidation, after severe tissue disintegration, were particularly possible for the samples S3 and S6. As opposed to S4, sample S5 had been subjected to maceration besides continuous heating of the pulp at pasteurisation temperatures.

Total β-carotene, the respective isomers and the vitamin A value were documented over the nectar production, relating the respective contents to the dry matter (DW) of the fruit pulp for each sample (Fig. 6a). For the samples S1 and S2, the mesocarp of two randomly selected fruits of the raw material and of the blanched and recooled fruits prior to manual peeling, respectively, was ground in a laboratory mixer. Hence, the samples S1 and S2 may be less representative than those of the other stages that could be taken out of the fluid bulk products. After steaming of the intact fruits (S2), a decrease of all-*trans*-β-carotene by 9% and a formation of 9-*cis*-β-carotene by 24% were

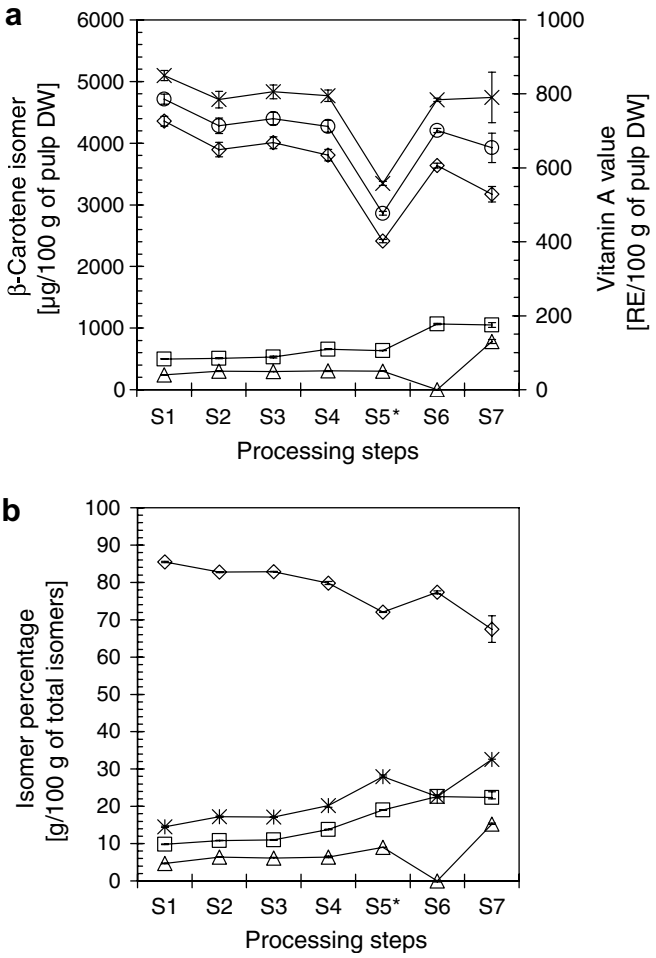

Fig. 6. β-Carotene changes during the production of mango nectar on pilot-plant scale: (a) β-carotene degradation as monitored by the contents of total β-carotene and its detectable stereoisomers and by the vitamin A value. All data presented were related to pulp dry weight (DW). (b) *trans–cis*-isomerisation identified by the stereoisomer percentages of the total β-carotene content. Processing steps as displayed in Fig. 2. The asterisk (*) marks an outlier as discussed in the text. Legend: vitamin A value (○) in retinol equivalents (RE) calculated as described in footnote *a* of Table 2, total β-carotene (✳), all-*trans*-isomer (◇), 13-*cis*-isomer (□), 9-*cis*-isomer (△), and relative proportion of all *cis*-isomers identified (∗).

observed, resulting in an initial loss of total β-carotene and vitamin A by 6% and 8%, respectively. However, despite the intensive tissue disintegration during the two-stage pulping process and subsequent finishing (S3) contributing considerably to the process time, with exposure to light and oxygen, no significant degradation or isomerisation of β-carotene was detectable at that stage by comparison of S2 and S3 (Fig. 6a, b). Hence, the differences recorded between S1 and S2 may only partly result from thermal degradation induced by steaming but may also partly be attributed to the natural variability among individual fruits. Maximum degradation effects induced by pulping and finishing (S3) may thus rather be estimated on the basis of the changes from S1 to S2 described above.

The first continuous heating of the pulp, with immediate recooling (S4) prior to its maceration, only caused an increase in 13-*cis*-β-carotene by approximately 30% that, however, did not significantly affect the vitamin A value of the pulp (Fig. 6a). After maceration and subsequent continuous pasteurisation of the purée (S5), a further decrease of all-*trans*-β-carotene by 38% at constant levels of both *cis*-isomers was found (Fig. 6a), apparently reflecting marked *trans–cis*-isomerisation induced at this stage (Fig. 6b). However, the content of all-*trans*-β-carotene in the pasteurised nectar was finally only reduced by a total of 27%, while a cumulative increase to the 2-fold and 3-fold amounts of 13-*cis*- and 9-*cis*-β-carotene, respectively, were detected for the whole process. Since the nectar was formulated with 35% of purée, all comparisons between purée and nectar rested upon the β-carotene contents of the pulp dry matter included in the samples. In the light of the findings for S6 and S7 and the trends observed in the batch pasteurisation experiments (Section 3.1), the all-*trans*-β-carotene content determined after pulp pasteurisation (S5) should be considered an outlier. A total thermal stress ($P^{z=8.9\ ^\circ C}_{T_{ref}=93.3\ ^\circ C}$) of at least 1.72 min was estimated for the whole process by summation of the individual lethal effects assessed for thermal inactivation of endogenous enzymes (S4), pulp pasteurisation (S5) and nectar pasteurisation (S7). It was approximately equivalent to the lethal effect of batch pasteurisation between 90.5 and 93 °C for 1 min ($P^{z=8.9\ ^\circ C}_{T_{ref}=93.3\ ^\circ C} = 1.47$ and 2.09, respectively) or 88 °C for 4 min ($P^{z=8.9\ ^\circ C}_{T_{ref}=93.3\ ^\circ C} = 1.76$) that always caused a decline of all-*trans*-β-carotene by only 12–13% and an increase in the relative proportion of the 13-*cis*-isomers from 12–13.2% to 17.2–18.8% (Section 3.1, Table 2).

The progression of *trans–cis*-isomerisation during nectar production is summarised in Fig. 6b. The relative proportions of the β-carotene stereoisomers altered rather steadily during processing from 85.5%, 9.8%, and 4.7% for all-*trans*-, 13-*cis*-, and 9-*cis*-β-carotene, respectively, in the raw mesocarp (S1) to 67.5%, 22.4%, and 15.2% in the pasteurised nectar (S7). On the whole, the vitamin A value dropped from 786 to 655 RE/100 g of pulp DW, representing a vitamin A loss of 17% over the whole process up to the pasteurised mango nectar with a pulp content of 35% (Fig. 6a). In contrast, total vitamin A losses ranging from 7.5% to 10% were detected at analogous $P^{z=8.9\ ^\circ C}_{T_{ref}=93.3\ ^\circ C}$ values in the small-scale batch pasteurisation processes (Section 3.1). Therefore, a synergistic effect of light, oxygen, heat, and loss of cell compartmentalisation on β-carotene stability could be assumed for the whole process of mango nectar production, with the total thermal stress accounting for approximately one half of the all-*trans*-β-carotene loss.

By analogy with the pasteurisation experiments on a laboratory scale (Section 3.1), the thermal inactivation of endogenous enzymes (PPO, POD) was monitored for the pulp, investigating the samples S3–S5. PPO, only quantifiable in the unheated purée (S3), was readily inactivated after the first continuous heating of the pulp (S4), whereas POD was very thermostable. From a decline of POD activity from 158 ± 0.27 nKat/g of pulp DW in S3 to 3.6 ± 0.27 nKat/g in S4 and 5.2 ± 0.31 nKat/g in S5, which were significantly different from the respective control samples, residual activities of 2.2% and 3.2% resulted after the first heating of the pulp (S4) prior to maceration and after the final pasteurisation of the purée (S5), respectively.

4. Discussion

Unlike previous observations made on carrot juices subjected to pasteurisation temperatures (Marx et al., 2003), the present study clearly proved a time- and temperature-dependent *trans–cis*-isomerisation of the β-carotene in the mango matrix for all pasteurisation routines evaluated. The observed proneness to *trans–cis*-isomerisation supported the idea that mango carotenoids may be naturally dissolved in lipid droplets usually found in globular chromoplast structures, which are the most widely distributed type of chromoplasts in fruits (Sitte, Falk, & Liedvogel, 1980). Consistently, heating of a dissolved β-carotene model solution resulted in a temperature-dependent *trans–cis*-isomerisation, whereas water-dispersible crystalline β-carotene formulations showed neither time- nor temperature-dependent *trans–cis*-isomerisation (Marx et al., 2003).

According to Chen et al. (1994), all-*trans*-β-carotene degradation followed a first-order kinetics veritably only when crystalline β-carotene was heated at 150 °C for 30 min, but during heating at 50 or 100 °C for 30 min, relative amounts of all-*trans*-β-carotene and the *cis*-isomers were only slightly affected. Similarly, first-order kinetics of β-carotene degradation in carrot juice resulted only after long-term exposure to elevated temperatures (Hojilla, García, & Raymundo, 1985). Consistently, Marx et al. (2003) observed an increase of 7.8% and 12.3% in the relative proportions of 13-*cis*-β-carotene only after prolonged sterilisation times in carrot juices heated at 130 °C with $F = 20$ min and 40 min, respectively. High β-carotene stability in carrot juices could be explained by the pigment crystals found in carrot roots (Sitte et al., 1980). This implied that crystalline β-carotene is very stable at pasteurisation temperatures, thus supporting the assumption that the physical state of the β-carotene is the crucial factor for the proneness to

trans–cis-isomerisation. This is also in accordance with the behaviour of β-carotene at pasteurisation temperatures in the model preparations produced in the present study with or without addition of MCT-oil (Section 3.2). Changes were only minor compared to the mango purée matrix, even in the presence of oil, an observation that was ascribed to the high initial relative proportions of the *cis*-isomers found in the model preparations, irrespective of the oil content and the protective coating of the β-carotene.

In view of the changing physical state of all-*trans*-β-carotene in the mango purée matrix during processing, with loss of cell compartmentalisation, the present study aimed at the evaluation of its degradation kinetics at usual pasteurisation temperatures. However, since the extent of all-*trans*-β-carotene degradation was very limited, the kinetic models did not fit the data. β-Carotene degradation, during air-drying of different food systems (butternut squash, sweet potato and yellow corn) at temperatures ranging from 60 to 80 °C for 50 h, fitted first-order kinetics, characterised by varying activation energies and rate constants (Stefanovich & Karel, 1982). However, such long-term treatments are applicable to drying processes that are not comparable to the conditions of mango purée/nectar production investigated in the present study.

Despite suppression of enzymatic browning and limited degradation of all-*trans*-β-carotene, changes in colour intensity occurred that were both time- and temperature-dependent. However, the hypsochromic shift and the hypochromic effect observed, along with *trans–cis*-isomerisation of β-carotene (Rodriguez-Amaya, 2001), cannot solely be assumed to cause the described colour changes. Pott, Breithaupt, and Carle (2003b) characterised free and esterified carotenoids in the ripe mesocarp of mango cv. 'Kent' and identified violaxanthin dibutyrate in its *trans*- and *cis*-forms, presenting maximum UV absorption at 416/440/470 and 414/436/466 nm, respectively. When the carotenoid patterns in mango cv. 'Kent' were compared before and after processing (Pott et al., 2003a), violaxanthin esters and derivatives were completely degraded after drying in an overflow-dryer at 75 °C for 3–3.5 h. Similarly, Mercadante and Rodriguez-Amaya (1998) concluded, from the carotenoid patterns of commercial mango juices offered in Brazilian markets, that violaxanthin had been degraded to undetectable levels, while β-carotene, followed by auroxanthin, was the major carotenoid of those products. Hence, due to the different thermostabilities of carotenoids, the thermal sensitivity of the mesocarp colour during processing may differ among mango varieties, depending on the carotenoid pattern of the fresh fruit. For example, a ratio of all-*trans*-violaxanthin to all-*trans*-β-carotene of 2.7 by weight characterised the major carotenoids of ripe mesocarp of cv. 'Keitt' (Mercadante & Rodriguez-Amaya, 1998), while an elevated ratio of 3.9 was reported for fruits of cv. 'Tommy Atkins'. In our study, also performed using the latter variety, pasteurisation of mango purée did not completely decompose the xanthophylls not quantified but present in the sample. Nevertheless, they were degraded to a certain extent, which may be related to a decrease in absorbance of the peaks at 441.6 and 469.8 nm observed in Fig. 5. Moreover, the decrease in absorption was directly related to the increasing holding time. Due to our focus on provitamin A retention, the loss of violaxanthin esters could not be quantified and directly correlated with the loss of colour intensity, because the chromatograms obtained from the β-carotene extraction procedure included a saponification step that partly destroyed xanthophylls (Rodriguez-Amaya, 2001). Moreover, an increasing solubilisation of the carotenoids present in the mango matrix upon heating may also partly explain an absorption shift to yellow hues. However, to fully understand the interrelation between colour and changing carotenoid pattern during processing of carotenoid-rich fruits or vegetables, further detailed studies are required.

The present work shows how cell disruption and enzymatic maceration of the mango matrix enhances degradation processes, including the activation of native oxidative enzymes and chemical decomposition of total β-carotene. The findings were consistent with a minor β-carotene degradation in canned mango pieces (Godoy & Rodriguez-Amaya, 1987). The reduction of the total β-carotene content from 742 μg/100 g of mesocarp fresh weight to 201 μg/100 g of nectar fresh weight, by 73%, for a product with 35% of pulp content, proved that the enormous loss of total β-carotene of 70–80%, reported by Rodríguez-Arce and Díaz (1992) for mango nectar production, resulted rather from the dilution of the pulp than from degradation. The latter only caused a cumulative loss of total β-carotene in the nectar of 7% on the basis of the pulp dry weight and a 2.4-fold increase of the *cis*-isomers. As previously suggested for vegetable juices (Pesek & Warthesen, 1987) and shown by the formation of the 9-*cis*-isomers during processing steps with some exposure to light, such as peeling, pitting, pulping (S2, S3), bottling and recooling (S7), photodegradation of all-*trans*-β-carotene may partly account for the total β-carotene loss during mango nectar production. However, maceration in a closed vessel did not affect β-carotene stability (Section 3.3), while heating at pasteurisation temperatures primarily favoured the formation of the 13-*cis*-isomers (S4). As deduced above (Section 3.4), a total loss of vitamin A of 17%, contrasting with only 7.5% to 10% of vitamin A reduction in the thermally comparable standard processes of mango purée pasteurisation described in Section 3.1, was the result of the combined effects of light, oxygen, heat, and loss of cell compartmentalisation during mango nectar production.

On the whole, good vitamin A retention was achieved by continuous mango nectar production, even though the processes necessarily implied three heating operations (S4, S5 and S7) in addition to steaming of the fruits in the course of peeling. Improvements might even be expected for closed industrial processing lines, where processing times and exposure to light can be shortened by replacing some manual operations still applied on a pilot-plant scale and by limiting standby times between batchwise or semi-con-

tinuous operations. Although the carotene pigments may be partly dissolved in lipid droplets, a relatively high stability of the β-carotene revealed a protective matrix effect, which may partly be associated with the high pectin and fibre contents of mango fruits. By analogy with purées of tomato (van het Hof et al., 2000), carrot and spinach (Rock et al., 1998), both an improved release of carotenoids from the plant tissue and increased bioavailability of the carotenoids from mango purée may occur due to matrix degradation, thus compensating for β-carotene losses caused by processing. Finally, to support the present assumptions, investigations on the nature of mango chromoplasts are currently under way.

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