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Effects of thermal processing on *trans–cis*-isomerization of β-carotene in carrot juices and carotene-containing preparations

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Abstract

The effects of thermal processing (blanching, pasteurization, sterilization) on *trans–cis*-isomerization of β-carotene in carrot juice produced on pilot plant scale and in β-carotene-containing preparations (bovine serum albumine and lecithine suspensions) were studied. While pasteurization and sterilization at 121 °C caused only minor isomerization, sterilization at 130 °C and blanching resulted in increased levels of *cis*-isomers. Dissolution of crystalline carotenes by cellular lipids during blanching of carrots was identified as the prerequisite for isomerization. Also addition of grape seed oil to the coarse mash enhanced isomerization both in unheated and heat-preserved juices. Model preparations containing crystalline β-carotene showed pronounced stability during heating, whereas thermal treatment of β-carotene dissolved in toluene resulted in temperature-dependent isomerization. UV/vis spectroscopy of carotene-containing particles obtained by isopycnic density-gradient centrifugation of carrot juice indicated the presence of crystalline carotene. The carotene-stabilizing principles present in carrot juice are assumed to consist of chromoplast fragments as the core coated by water-insoluble juice constituents such as pectin, cellulose and protein which prevent carotenes from being dissolved in neutral lipids upon moderate heating.

Keywords: Carrot juice; β-Carotene; *trans–cis*-Isomerization; Process technology; Physical state

1. Introduction

Carrot juice is one of the most popular vegetable juices and represents a rich source of natural β- and α-carotene (Marx, Schieber, & Carle, 2000). Its production involves various technological steps such as blanching, acidification, pasteurization or canning which have been shown to cause partial conversion of the all-*trans*-carotenes to their *cis*-isomers (Chen, Peng, & Chen, 1995; Jonsson, 1991). Since carotene stereoisomers display different chemical and biological properties (Bauernfeind, 1972; Britton, 1995; Krinsky, 1994), and differ in their antioxidant capacity (Boehm, Puspitasari-Nienhaber, Ferruzzi, & Schwartz, 2002) and bioavailability (Deming, Teixeira, & Erdman, 2002), the knowledge of the various factors affecting the formation of *cis*-isomers in food is of fundamental interest.

Our recent studies have shown that commercial carrot juices and vitaminized drinks, the latter hereinafter referred to as ATBC drinks, contained considerable relative amounts of *cis*-isomers of β-carotene which ranged from 4 to 17 and 30 to 44%, respectively, calculated as percentage of all-*trans*-β-carotene (Marx et al., 2000). Only few studies have dealt with the kinetics and mechanism of thermally induced isomerization of β-carotene. According to von Doering, Sotiriou-Leventis, and Roth (1995), thermal treatment of all-*trans*-β-carotene at temperatures below 100 ° C predominantly results in the formation of 13- and 15-*cis*-β-carotene, whereas the 9-*cis*-isomer is mainly formed above 100 °C. These authors also stated that during heating of all-*trans*-β-carotene at 100 °C, (pseudo-)equilibrium concentrations of β-carotene-isomers are reached after 11 min. Kuki, Koyama, and Nagae (1991) reported the formation of *cis*-isomers, namely of 13-*cis*- and 15-*cis*-β-carotene, at a level of 30% when all-*trans*-β-carotene was heated at 80 °C for 30 min in *n*-hexane. In contrast to these findings, β-carotene in carrot juices showed a higher thermal stability, resulting in no or only minor

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trans–cis-isomerization during pasteurization of raw carrot juices at 100 °C (Chen et al., 1995; Jonsson, 1991).

Canning of carrot juice at 121 °C led to the formation of β-carotene *cis*-isomers at rates of 9% (Jonsson, 1991) and 30% (Chen et al., 1995), 13-*cis*-β-carotene being the predominant isomer in both studies. Blanching of carrots at 95 °C resulted in a significantly higher degradation of β- and α-carotene of 40% each (Dietz, Sacchi, & Erdman, 1988). The combined effects of blanching and thermal preservation have not yet been assessed.

Our previous investigations on ATBC-drinks clearly showed improved chemical and physical stability for beverages containing carrot juice as a natural source of carotenes compared to those based on synthetic β-carotene preparations (Carle, 1999; Marx et al., 2000). The production of the latter usually involves hot dissolution of β-carotene in a suitable lipophilic phase, e.g. citrus oils. Since isomerization does not occur in crystalline carotene (Gaier, Angerhofer, & Wolf, 1991), dissolution is a prerequisite for the formation of *cis*-isomers. Therefore, hot dissolution is considered the critical step leading to comparatively high amounts of *cis*-isomers in ATBC drinks. However, carrot juice represents a more complex matrix, with a variety of parameters affecting the chemical stability of carotenoids. Especially studies on the physical state of carotenes in carrot juices have been neglected so far. According to Kasha, Rawls and Ashraf El-Barjoumi (1965), UV/vis spectra provide useful information on the physical state of the carotenoids. While Wloch and Wieckowski (1986) observed a crystalline structure for carrot chromoplasts using UV/vis spectroscopy, the physical state of carotene in carrot juice particles remains unclear.

The main objective of the present study was to investigate the effects of thermal treatment (blanching, pasteurization, sterilization) and the addition of lipids on *trans–cis*-isomerization of β-carotene during semi-industrial production of carrot juice. Due to its relevance for stability and bioavailability (Britton, Gambelli, Dunphy, Pudney, & Gidley, 2002), particular attention was also paid to effects on the physical state of carotenes. Furthermore, *trans–cis*-isomerization was studied in model preparations containing crystalline and dissolved β-carotene together with proteins and lecithine in order to draw conclusions as to possible stabilizing principles present in carrot juice.

2. Materials and methods

2.1. Materials and reagents

Standards of all-*trans*-α-carotene and 15-*cis*-β-carotene were a gift of Hoffmann-La Roche (Basel, Switzerland); all-*trans*-β-carotene was purchased from Sigma Chemical Co. (St. Louis, USA). The 9-*cis*- and 13-*cis*-isomers of β-carotene were prepared by iodine-catalyzed photoisomerization of all-*trans*-β-carotene according to Zechmeister (1962), purified by semi-preparative HPLC, and identified by their retention times, UV/vis and ^{1}H NMR spectra as described previously (Schieber, Marx, & Carle, 2002). All solvents used for extraction and HPLC were purchased from Merck (Darmstadt, Germany) and were of analytical and HPLC grade, respectively. For juice production, carrots (*Daucus carota* L. ssp. *sativa* cv. 'Karotan') were provided by Hohenheim University Research Station.

2.2. Carrot juices

Carrots juices were produced in autumn/winter 1999/2000 and 2000/2001 according to a standard process previously described by Reiter, Stuparic, Neidhart, and Carle (2003). Carrots were washed, blanched at 80 °C for 10 min, successively comminuted using a grinding mill and a colloid mill, and finally dejuiced with a decanter (CA 150-01-33, Westfalia, Oelde, Germany) on pilot-plant scale. The juices obtained were either filled in glass jars (370 ml) and sterilized at T_{max} = 121 °C (F = 5 min) in a rotary retort (Rotopilot 5, Stock, Neumuenster, Germany) or acidified to pH 4.4 with citric acid, pasteurized (95 °C, 30 s, $P \approx 3$) in a plate heat exchanger (Schmid, Bretten, Germany) and aseptically filled in glass jars. Samples of unheated juices were collected after the decanter and stored at −20 °C until analysis of carotenes.

2.2.1. Effects of blanching

Water blanching was carried out at 80, 90 and 100 °C for 10, 30, and 60 min, each. The core temperatures of the carrots during blanching ranged from 60 (80 °C, 10 min) to 96 °C (100 °C, 60 min). Since blanching at 80 °C for 10 min has shown to be a prerequisite for organoleptically acceptable juices (Reiter et al., 2003), less intensive blanching was not considered. Juices were produced according to the standard process as described above. For each blanching regime sterilized and pasteurized juices were produced.

2.2.2. Effects of sterilization

Juices produced according to the standard process were subjected to varying sterilization conditions (F = 5–40 min at 121 °C and F = 20–40 min at 130 °C).

2.2.3. Effects of lipid addition

Grape seed oil (1%, w/w) which was tested to be devoid of carotenoids was added to the coarse mash under stirring to ensure homogenous distribution. The resulting juices were pasteurized or sterilized as described above. A control sample was produced without addition of grape seed oil.

2.3. Characterization of juices

2.3.1. HPLC analysis of carotenes

The juices were extracted with a mixture of acetone/*n-hexane* (1:1, v/v) using amber glassware to avoid photoisomerization and -oxidation of carotenes. The chromatographic separation of carotene stereoisomers was performed on a YMC (Wilmington, USA) C_{30} column (250×4.6 mm i.d.; particle size 5 μm) as described previously (Marx et al., 2000).

2.3.2. Light microscopy

Samples of raw and blanched carrot tissues were examined microscopically using an Axioskop 2 (Zeiss, Oberkochen, Germany) equipped with a CCD-camera (JVC, Osaka, Japan).

2.3.3. Separation of carotene-containing juice particles

Carotene-containing juice particles of carrot juices were fractionated by density centrifugation using a sucrose gradient of 5–55 °Bx (Reiter et al., 2003). Carotene content and volume of each fraction were used for calculation of relative carotene distribution.

2.3.4. UV/vis spectroscopy of carotene-containing particles

For investigations on the physical state of the carotenes, particle fractions of juices produced according to the standard process and by addition of grape seed oil were studied by UV/vis spectroscopy. Spectra were recorded from 400 to 600 nm using a Lambda 2 spectrometer (Perkin-Elmer, Ueberlingen, Germany) equipped with an integration sphere to compensate for Mie-scattering and compared to those of crystalline α- and β-carotene. Crystal suspensions were prepared by adsorption of carotenes to bovine serum albumine (BSA, Roth, Karlsruhe, Germany) as previously described by Purcell, Walter, and Thompkins (1969) and Wloch, Wiekowski, and Turek (1987).

2.4. Model preparations

Studies on carotene stability and possible stability principles were carried out using three model preparations. Raw carrot juice obtained by the standard process was used as a control. Aqueous dispersions of crystalline carotene were obtained by unspecific adsorption to proteins using BSA and β-carotene crystals (Fluka, Buchs, Switzerland) as described above (Purcell et al., 1969; Wloch et al., 1987). The formation of micelles by carotenes and carrot chromoplast membranes was modelled by aggregation of crystalline β-carotene and lecithine (Fluka, Buchs, Switzerland) according to Yamamoto and Bangham (1978). BSA and lecithine suspensions were checked for crystallinity of β-carotene by UV/vis spectroscopy (data not shown). A solution of β-carotene (25 mg/l) in toluene containing 0.1% *tert.*-butylhydroxytoluene as an antioxidant was used as a model for completely dissolved carotene.

Carrot juice and model preparations were subjected to varying time-temperature regimes. To study the effect of heat load on *trans–cis*-isomerization, the model preparations and control were heated at 100, 110, 120, 130 and 140 °C for 10 min. Control and β-carotene BSA suspensions were heated in lacquered tinplate cans (430 ml, Schmalbach-Lubeca, Ratingen, Germany) using a rotary retort (Rotopilot 5, Stock, Neumuenster, Germany), while heating of β-carotene–toluene solutions and of β-carotene–lecithine suspensions were performed in Pyrex glass tubes (Pyrex, Marne-La-Vallée, France) using a tube heater (Stuart Scientific, Stone, UK). To avoid glass burst, β-carotene–lecithine suspensions and β-carotene–toluene solutions were only heated up to 120 and 130 °C, respectively. The effects of heating time on *trans–cis*-isomerization were investigated at 100 and 120 °C for 10, 20 and 30 min using Pyrex glass tubes. All samples were immediately analyzed for their *cis*-isomer contents by HPLC as described above.

3. Results

3.1. Trans–cis-*isomerization of β-carotene in carrot juices*

3.1.1. Standard process

Whereas unheated juices produced from carrots blanched at 80 °C for 10 min were devoid of *cis*-isomers, heat-preservation resulted in minor *trans–cis*-isomerization of β-carotene. In pasteurized and sterilized samples, 13-*cis*-β-carotene was the only isomer detectable at approximately 2 and 5%, respectively (Tables 1 and 2).

3.1.2. Effects of blanching

Depending on the time-temperature regime, blanching of whole carrots strongly affected *trans–cis*-isomerization of β-carotene in the corresponding unheated, pasteurized and sterilized juices (Table 1). In all samples, 13-*cis*-β-carotene was the predominant *cis*-isomer, whereas 9-*cis*-β-carotene could only be detected in sterilized juices produced from extensively blanched carrots. Since 15-cis-β-carotene could not be completely separated from an unknown *cis*-isomer of α-carotene, quantitative data could not be obtained. In pasteurized juices, elevated heat load during blanching (80, 90, and 100 °C for 30 and 60 min, respectively) led to the formation of 13-*cis*-β-carotene at levels between 1.8 and 10.0% (Table 1). Consistent with Bielig and Wolf (1971) who reported that mash texture affected carotene contents of decanter juices, a decrease of total carotenes was observed for juices produced from excessively blanched carrots.

Table 1
Effects of blanching conditions on contents and isomerization of carotenes in raw and heat-preserved carrot juices

Blanching conditions	Total β-carotene [mg/l]	All-*trans*-β-carotene [mg/l]	13-*cis*-β-carotene [%]	9-*cis*-β-carotene [%]
Unheated juices				
80 °C, 10 min[a]	105.6±0.4	105.6±0.4	n.d.	n.d.
90 °C, 10 min	123.6±1.4	122.4±1.4	1.0±0.0	n.d.
100 °C, 10 min	118.2±4.1	116.5±4.0	1.7±0.1	n.d.
100 °C, 60 min	72.3±2.1	64.4±2.0	11.0±0.2	n.d.
Pasteurized[b] *juices*				
80 °C, 10 min[a]	104.8±1.4	102.9±1.0	1.8±0.2	n.d.
80 °C, 30 min	123.4±1.8	119.6±1.8	3.1±0.0	n.d.
80 °C, 60 min	120.9±3.3	116.8±3.5	3.4±0.1	n.d.
90 °C, 10 min	104.7±0.4	100.6±1.3	3.9±0.1	n.d.
90 °C, 30 min	101.0±2.0	95.8±1.0	5.2±0.1	n.d.
90 °C, 60 min	99.0±2.1	92.7±1.8	6.5±0.4	n.d.
100 °C, 10 min	117.7±0.4	114.8±2.8	2.5±0.1	n.d.
100 °C, 30 min	105.1±0.4	96.6±2.0	8.1±0.1	n.d.
100 °C, 60 min	77.4±1.9	69.7±1.0	10.0±0.9	n.d.
Sterilized[c] *juices*				
80 °C, 10 min[a]	99.4±1.9	94.6±2.0	4.9±0.4	n.d.
80 °C, 30 min	119.5±1.1	112.7±1.0	5.7±0.1	n.d.
80 °C, 60 min	115.0±3.5	104.9±5.5	8.8±0.2	n.d.
90 °C, 10 min	113.1±5.4	105.2±2.6	7.0±0.3	n.d.
90 °C, 30 min	103.1±0.8	94.0±1.0	8.7±0.3	n.d.
90 °C, 60 min	97.2±1.8	81.8±2.6	13.1±0.5	2.8±0.4
100 °C, 10 min	113.1±2.0	104.7±2.0	7.4±0.1	n.d.
100 °C, 30 min	96.9±1.7	83.9±1.5	11.3±0.3	2.1±0.1
100 °C, 60 min	71.1±4.1	59.3±2.0	13.6±0.4	3.1±0.2

n.d., not detectable; *cis*-isomers calculated as percentage of total β-carotene; amounts given are mean values±standard deviations of two replicate determinations

[a] Standard process conditions.
[b] Pasteurization conditions: $T_{max}=95$ °C, $P\approx3$.
[c] Sterilization conditions: $T_{max}=121$ °C, $F=5$.

Table 2
Effects of sterilization conditions on contents and isomerization of carotenes in carrot juices

F-value [min]	Total β-carotene [mg/l]	all-*trans*-β-carotene [mg/l]	13-*cis*-β-carotene [%]	9-*cis*-β-carotene [%]
Sterilization at T_{max} *121 °C*				
5[a]	72.6±1.9	69.1±1.6	4.9±0.3	n.d.
8	82.1±2.2	77.8±3.1	5.3±0.5	n.d.
12	83.2±2.0	78.5±2.7	5.6±0.5	n.d.
15	75.1±5.0	71.1±4.7	5.3±0.3	n.d.
20	72.6±1.0	68.7±1.0	5.4±0.1	n.d.
40	78.1±0.7	72.5±0.7	5.2±0.1	2.0±0.1
Sterilization at T_{max} *130 °C*				
20	77.4±3.0	69.6±1.0	7.8±0.1	2.3±0.1
40	80.7±3.6	68.6±3.2	12.3±0.5	2.7±0.1

n.d., not detectable; *cis*-isomers calculated as percentage of total β-carotene; amounts given are mean values±standard deviations of two replicate determinations.

[a] Standard process.

While tissues of raw and moderately blanched (80 °C, 10 min) carrots contained helical and ribbon-like crystalline chromoplasts, extensive blanching (100 °C, 60 min) resulted in the formation of yellow-coloured lipid droplets containing solubilized carotene.

3.1.3. Effects of pasteurization

Compared with unheated juices which were blanched under identical conditions, the respective juices pasteurized at $T_{max}=95$ °C ($P\approx3$) did not show significant reduction of total β-carotene (Table 1). However, a slightly higher level of 13-*cis*-β-carotene was generally found for the pasteurized juices.

3.1.4. Effects of sterilization

Total β-carotene content of sterilized samples ($T_{max}=121$ °C, $F=5$) did not greatly differ from the corresponding unheated juices (Table 1). In general, heat preservation had an additive effect on isomerization, with an average increase of 13-*cis*-β-carotene of approximately 5% for sterilization. Variation of sterilization conditions did not affect the total β-carotene content (Table 2). Even extended sterilization at $T_{max}=121$ °C (*F*-values 5–40) did not result in increased levels of 13-*cis*-β-carotene which ranged from 4.9 to 5.6%. In contrast, enhanced *trans–cis*-isomerization including the formation of 9-*cis*-β-carotene was observed when sterilization was carried out at $T_{max}=130$ °C. Prolonged sterilization time (*F*-values 20–40) had a strong impact on isomerization as shown for the 13-*cis*-β-carotene content.

3.1.5. Effects of lipids

Compared with the control, addition of grape seed oil to the coarse mash enhanced *trans–cis*-isomerization both in unheated and heat-preserved samples (Table 3). Even unheated juices contained 3.6% 13-*cis*-β-carotene, while 9-*cis*-β-carotene could not be detected. Pasteurization and sterilization resulted in the formation of 13-*cis*-β-carotene at levels of 6.0 and 18.8%, respectively. After sterilization, small amounts of 9-*cis*-β-carotene were also detected.

3.1.6. Separation of carotene-containing cloud particles

Isopycnic density-gradient centrifugation of juices generally yielded four carotene-containing fractions with densities of 1.02 (#1), 1.07 (#2), 1.13 (#3) and 1.18 g/ml (#4) (Table 4). Fraction #1 was a clear, yellow solution, devoid of visible particles. Minimal amounts of carotenes were usually found in fractions #1 (7–11%) and #4 (6–8%), whereas fraction #2 contained 60–70% of total carotene. In contrast, juice produced by adding grape seed oil to the mash showed a different carotene distribution. Surprisingly, a particle fraction (#4) was missing. Since more than one third of total carotene was shifted to fraction #1, partial solubilization of carotene

Table 3
Carotene contents of unheated and heat-preserved carrot juices produced by addition of grape seed oil to the coarse mash

Sample	Total β-carotene [mg/l]	all-*trans*-β-carotene [mg/l]	13-*cis*-β-carotene [%]	9-*cis*-β-carotene [%]
Control				
Sterilized[a] juice	47.8±2.0	44.9±2.1	6.0±0.3	n.d.
Addition of fatty oil to the mash				
Unheated juice	43.1±2.0	41.5±2.1	3.6±0.2	n.d.
Pasteurized[b] juice	41.6±2.1	39.1±2.0	6.0±0.3	n.d.
Sterilized[a] juice	43.6±1.9	33.6±1.0	18.8±0.4	4.1±0.2

n.d., not detectable; *cis*-isomers calculated as percentage of total β-carotene; amounts given are mean values±standard deviations of two replicate determinations

[a] Sterilization conditions: $T_{max} = 121$ °C, $F = 5$.

[b] Pasteurization conditions: $T_{max} = 95$ °C, $P \approx 3$.

is assumed. Also harsh blanching produced higher carotene levels in fraction #1.

3.1.7. UV/vis spectroscopy of carotene-containing particles

Investigations on the physical state of carotenes in the cloud particles were carried out spectroscopically with density fractions of juices produced according to the standard process and after addition of grape seed oil. Without lipids, spectra could only be recorded for fractions #2–4. Due to its poor carotene content, fraction #1 displayed very weak absorption.

Particle fractions #2–4 of carrot juices produced with and without grape seed oil provided spectra with main absorption bands at 434, 457, 488, and 533 nm (Fig. 1a), corresponding well to the spectrum of a mixture of crystalline α- and β-carotene in the ratio of 1:2 (n/n) typical of carrots (Fig. 1b). Spectra were in good agreement with those reported by Wloch and Wiekowski (1986) for carotene crystals isolated from carrot chromoplasts. According to these authors, the appearance of an absorption band at 535 nm, indicating J-aggregates (head-to-tail aggregation) of β-carotene, is characteristic of crystalline β-carotene (Fig. 1b). It is therefore concluded that particles of fractions #2–4 contained crystalline carotene. The spectrum of fraction #1 of a juice produced with lipid addition showed main absorption bands at 490, 458, and 429 nm (Fig. 1c) and a shoulder extending to higher wavelengths. Since dissolved β-caroene does not show absorption at 535 nm, the shoulder may indicate the presence of partially crystalline and dissolved β-carotene in fractions #1 and #2.

4. *Trans–cis*-isomerization of β-carotene in model preparations

When heated at 100–140 °C for 10 min (Fig. 2a), no increase of *cis*-isomers was observed in model preparations containing crystalline β-carotene, i.e. β-carotene/BSA and β-carotene/lecithine suspensions. It should be noted that the β-carotene/BSA preparation initially contained about 3–8% *cis*-isomers as an artefact. Thermal treatment of carrot juices at temperatures between 100 and 120 °C did not cause significant isomerization. However, when the temperature was raised to 130 and 140 °C, 15 and 30% of *cis*-isomers, respectively, were found. Heating of β-carotene dissolved in toluene resulted in a linear increase of *cis*-isomers, indicating temperature-dependent isomerization.

Investigations on time-dependent isomerization revealed constant *cis*-isomer content in preparations containing crystalline β-carotene when heated at 100 and 120 °C (Fig. 2b and c). Stability of β-carotene was also observed for carrot juices heated at 100 °C. However, when heating at 120 °C for 10–30 min, *cis*-isomers amounted to approximately 5%. Dissolved β-carotene suffered significant *trans–cis*-isomerization (Fig. 2b) within 10 min. Prolongation of heating time up to 30 min did not increase *trans–cis*-isomerization. Extended heating at 120 °C caused a linear decrease of mono-*cis*-isomers in favor of di- and poly-*cis*-isomers which were identified by their PDA spectra. Due to the lack of reference compounds, their quantification was not possible.

5. Discussion

Significant differences concerning the extent of β-carotene *trans–cis*-isomerization were observed upon heating of carrots, carrot juice and β-carotene-containing

Table 4
Distribution of carotenes (%) in carotene-containing fractions obtained by isopycnic density-gradient centrifugation of sterilized carrot juices

Process variation	Density fraction	% of total carotene			
		#1 [1.02 g/ml]	#2 [1.07 g/ml]	#3 [1.13 g/ml]	#4 [1.18 g/ml]
Standard process		7.0	70.9	16.1	6.0
Carrots blanched at 90 °C for 10 min		7.6	70.8	15.9	5.7
Carrots blanched at 100 °C for 60 min		10.7	61.0	20.2	8.2
Addition of lipids to coarse mash		37.4	49.8	12.8	–

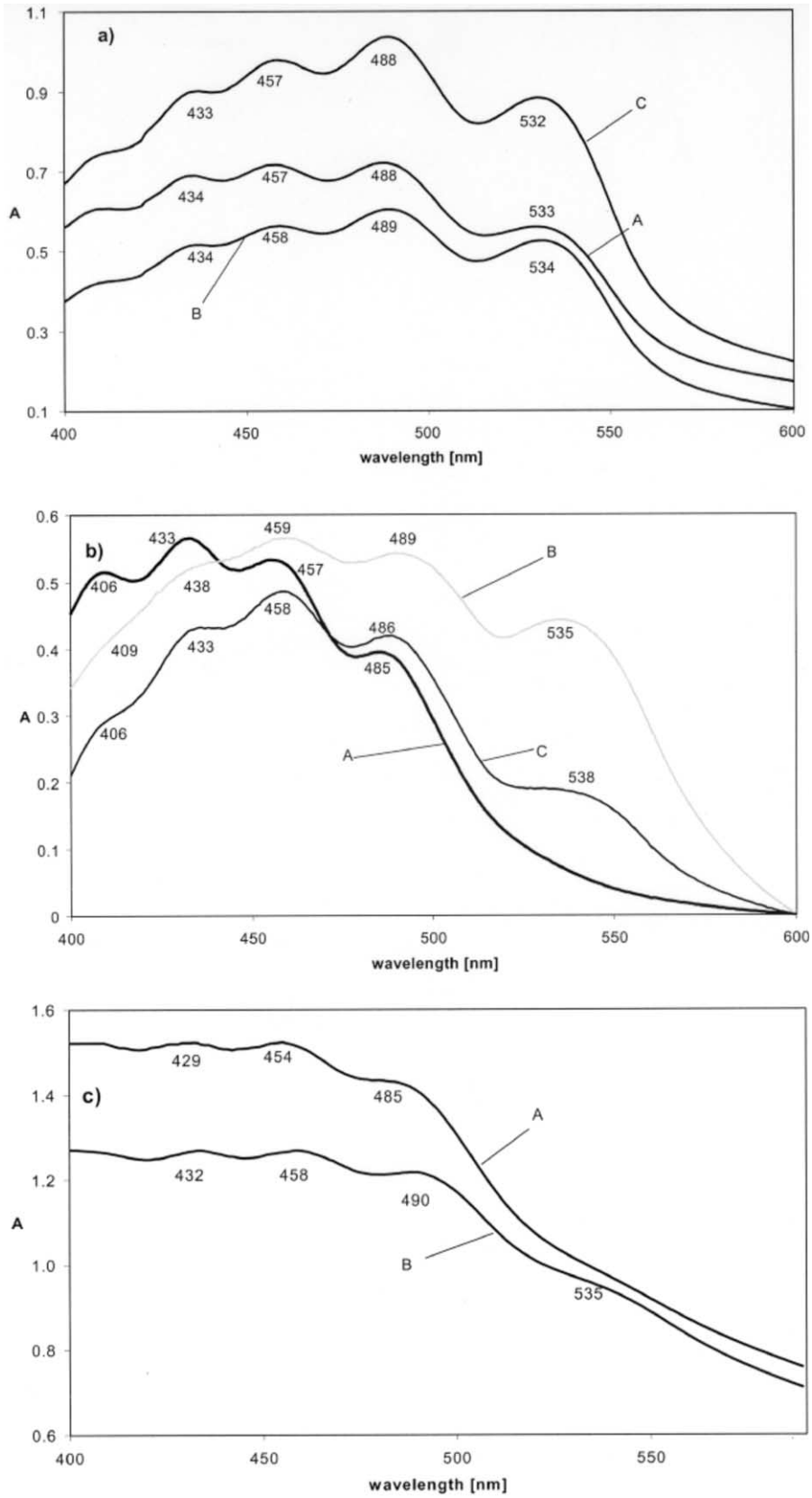

Fig. 1. UV/vis spectra of carotenes and carotene-containing fractions obtained by isopycnic density-gradient centrifugation. (a) fractions isolated from the juices produced by the standard process: A fraction #2 (1.07 g/ml), B fraction #3 (1.13 g/ml), C fraction #4 (1.18 g/ml). (b) A crystalline α-carotene, B crystalline β-carotene, C mixture of crystalline α- and β-carotene in ratio 1:2 (w/w). (c) fractions isolated from juices produced by addition of lipids to the mash: A fraction #1 (1.02 g/ml), B fraction #2 (1.07 g/ml).

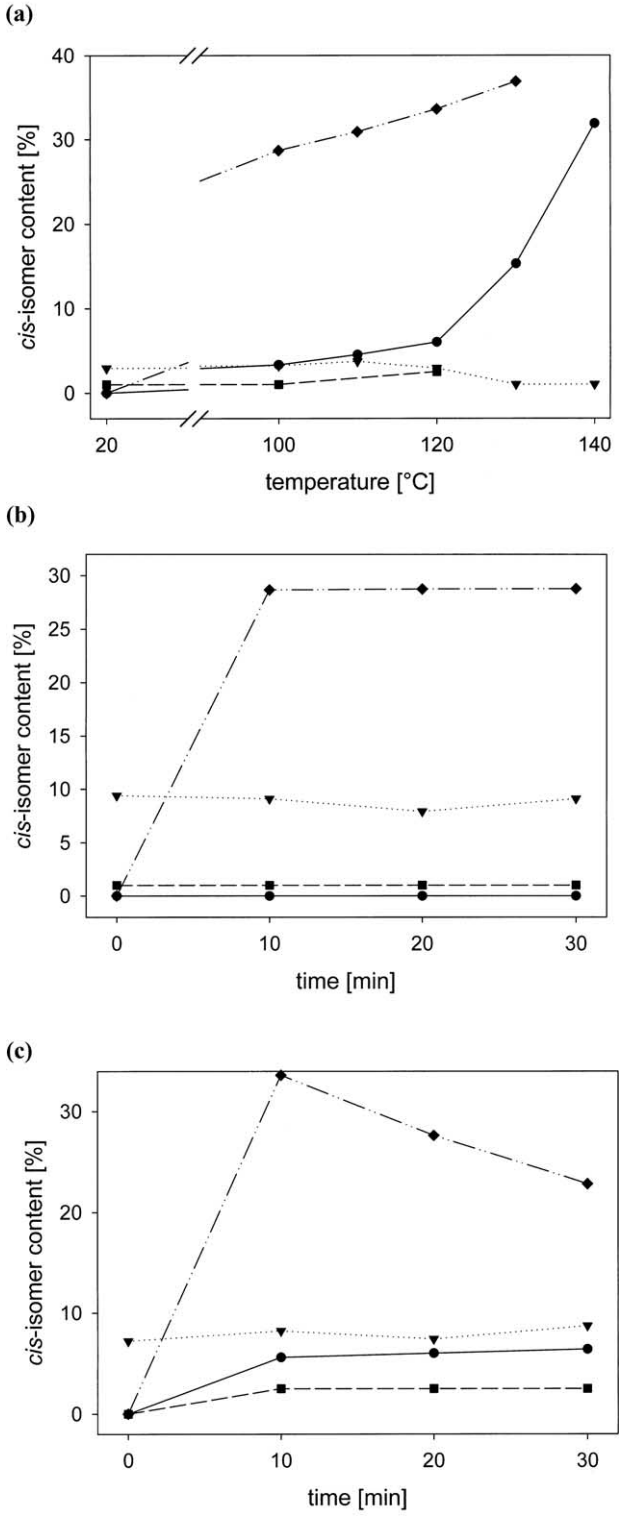

Fig. 2. Effects of heating on *trans–cis*-isomerization of β-carotene in model preparations and carrot juice (control). (a) temperature-dependent isomerization; (b) time-dependent isomerization at 100 °C; (c) time-dependent isomerization at 120 °C. • carrot juice; ▼ β-carotene-BSA-suspension; ■ β-carotene-lecithine-suspension; ◆ β-carotene-toluene-solution.

model preparations. Juices prepared from carrots subjected to gentle blanching conditions (80 °C, 10 min) were either devoid of or contained only low levels of *cis*-isomers. In contrast, elevated heat load during blanching (80–100 °C for 30 and 60 min) gave rise to enhanced isomerization. However, in industrial practice blanching time usually does not exceed 10 min, except for process interruption.

Depending on the time–temperature regime applied, heat preservation was shown to have an additive effect to blanching with respect to the formation of *cis*-isomers. While considerable *trans–cis*-isomerization was observed for juices of excessively blanched carrot roots, this effect was less marked for standard carrot juice subjected to harsh sterilization conditions (121 °C, $F = 5$–40 min). These findings are consistent with the results of previous investigations by Dietz et al. (1988) and Jonsson (1991), reporting that degradation of α- and β-carotene was more pronounced for carrots boiled for 30 min than for carrot juice heated at 100 °C for 60 min.

Useful information on changes of the physical state of carotenes was gained from microscopic studies of raw and excessively heated carrots. While exclusively helical and ribbon-like crystalline chromoplasts were found in tissues of raw and gently blanched carrots, extensive blanching (100 °C, 60 min) resulted in the formation of yellow-coloured lipid droplets containing dissolved carotene. This phenomenon has earlier been described by Purcell et al. (1969) and, very recently, by Britton et al. (2002). The latter authors also pointed out that only severe cooking leads to dissolution of carotene crystals in lipid globules. Purcell et al. (1969) hypothesized that upon heating carotenes are solubilized by cellular lipids which are released after thermal breakdown of cell structure. Lipid content of carrots is about 0.2–0.5 mg/kg, thus exceeding 14-fold the average carotene content (Soimajärvi & Linko, 1973). Therefore, the amount of lipids may suffice at least for partial dissolution of carotenes. Since *trans–cis*-isomerization does not appear with crystalline carotene (Gaier et al., 1991), solubilization of carotenes during blanching is a prerequesite for the formation of *cis*-isomers, and can therefore be considered the crucial step of carrot juice production. This assumption is supported by the fact that the addition of grape seed oil during juice processing resulted in significantly higher levels of *cis*-isomers compared with the control sample. Furthermore, heating of an all-*trans*-β-carotene solution at 100 and 120 °C also produced high amounts of 13-*cis*-β-carotene. In a recent study, no evidence of enhanced isomerization was obtained when tomato segments were heated in water–oil mixtures (Nguyen, Francis, & Schwartz, 2001). However, since only a minor part of total carotenes localized on the surface of the segments is supposed to be solubilized by added lipids, these findings are not considered contradictory to our results. Since model preparations consisting

of crystalline β-carotene with BSA and lecithine proved to be absolutely stable towards isomerization irrespective of the time–temperature regime, carrot juice is assumed to contain mainly crystalline carotene as well as more or less dissolved carotene, depending on the process conditions. In the spectra of the carotene-containing particles of juices obtained from the standard process solely crystalline carotene was detected, although the *cis*-isomer content of the juices was approximately 5%. This observation may be ascribed to recrystallization of dissolved carotene when cooling after sterilization.

Heating of the model preparations showed temperature-dependent *trans–cis*-isomerization for dissolved β-carotene (Fig. 2a). After prolonged heating of dissolved β-carotene at 100 °C, a (pseudo-)equilibrium was found, which is in accordance with von Doering et al. (1995). Due to the conversion of mono-*cis*-isomers into di- and poly-*cis*-isomers, isomerization was time-dependent at 120 °C (Chen, Chen, & Chien, 1994). Carrot juices also showed temperature-dependent behavior. While *trans–cis*-isomerization at 100 and 120 °C was independent of time (Fig. 2b), sterilization at 130 °C resulted in a time-dependent *trans–cis*-isomerization for short heating times ($F < 40$ min, corresponding to approx. 4 min at 130 °C, Table 2). Furthermore, temperature rise from 120 to 130 °C led to a stronger increase in the *cis*-isomer content than the elevation of temperature from 100 to 120 °C (Fig. 2a). In contrast, water-soluble crystalline β-carotene formulations, showed neither time- nor temperature-dependent *trans–cis*-isomerization. Therefore, it is assumed that the carrot juice matrix prevents carotenes from dissolution in lipids during heating. However, this stabilizing matrix effect is affected at temperatures above 130°C, thus indicating thermolability of underlying chemical components. These results also indicate that HTST-heating may cause stronger *trans–cis*-isomerization than conventional heat preservation at 120 °C, since isomerization in carrot juices was shown to be temperature-dependent.

These observations pose questions as to the chemical nature of the carotene stabilizing matrix. Due to its pronounced hydrophobicity, carotene is not dissolved in the aqueous phase of carrot juice. Isopycnic gradient centrifugation revealed that carotene is distributed over cloud particles of different densities. Their composition, morphology and carotene stabilizing mechanisms are not fully understood. Spectroscopic data of density fractions #2–4 provided evidence of crystalline carotene in cloud particles of carrot juices obtained from the standard process. Since complete elucidation of cloud particles is almost impossible, basic principles of stabilizing crystalline carotene had to be taken into consideration.

Wloch and Wiekowski (1986) gave evidence of unspecific adsorption of crystalline carotene to proteins with the formation of water-soluble aggregates. This mechanism was considered for carrot juices by Klaui and Bauernfeind (1981). However, carotene in carrots was found to be located in crystalline chromoplasts with membranes rich in polar lipids (Schmid & Ohlrogge, 1994). Since juice production requires crushing of the carrot tissue, the chromoplasts are comminuted during milling. Therefore, the core structure of the cloud particles is assumed to consist of chromoplast fragments.

Preparations containing crystalline β-carotene stabilized by either protein adsorption or micelle formation with polar lipids were taken as models to mimic the core structure of carotene-containing cloud particles. In consideration of their UV/vis spectra and their extreme stability towards *trans–cis*-isomerization, both model preparations may act as the stabilizing principles in carrot juices. The four density fractions observed are assumed to consist of chromoplast fragments as the core coated by water-insoluble juice constituents such as pectin, cellulose and protein, which prevent carotenes from dissolution in neutral lipids upon moderate heating of juices.

6. Conclusion

Consequences of thermal treatment of carrots and heat preservation of carrot juice were shown to be closely associated with the alteration of the physical state of carotenes. In particular, excessive blanching allowed the solubilization of carotenes by cellular lipids, a process which has been identified as the crucial factor of *trans–cis*-isomerization. In juices, crystalline carotene is suspended and presumably covered by polar lipids forming the core structure of cloud particles. Hydrocolloids such as proteins or polysaccharides supposedly protect carotenes from dissolution in neutral lipids during thermal preservation. However, at temperatures above 120 °C significant destruction of the protective matrix occured. Therefore, deeper studies into the effects of HTST-preservation on the stability of those complexes and *trans–cis*-isomerization are needed.

Minimizing *trans–cis*-isomerization requires prevention of carotenes from being solubilized during juice production. However, it should be noted that bioavailability of carotenoids is affected by a number of factors such as the food matrix, intracellular location, physicochemical characteristics, interactions between carotenoids, and presence of dietary fat, and that processing, especially mechanical disruption and heat treatment, may enhance carotenoid bioavailability (van het Hof, West, Weststrate, & Hautvast, 2000). Considering the low rates of *trans–cis*-isomerization demonstrated in the present study, it is concluded that moderate heat treatment is not deleterious with respect to bioavailability of carotenoids.

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