Effect of heating on solid β-carotene

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ABSTRACT

The effect of heating on isomerisation and stability of solid β-carotene was investigated, and the products generated by heating were analysed by a number of analytical techniques, including high-performance liquid chromatography (HPLC), UV/VIS-spectroscopy (UV) and gel permeation chromatography (GPC). For the first time, isomerisation of *cis*- to all-*trans*-isomer was demonstrated in partly melted solid β-carotene when β-carotene was heated at 90 and 140 °C. Only a few high molecular weight components were detected by GPC when β-carotene was heated in a nitrogen environment. In contrast, more high molecular weight polymers, as well as low molecular fragments, were produced when β-carotene was heated and exposed to air, suggesting that polymerisation was one of the dominant side-reactions of β-carotene change, in addition to degradation.

1. Introduction

β-Carotene is one of the most important food additives and is widely used as a colourant in foods and beverages (Faure, Galabert, Le Moel, & Nabet, 1999). β-Carotene is also a most effective vitamin A precursor, and has been reported to protect humans against certain types of cancer (Steinmetz & Potter, 1996) and cardiovascular diseases (Gaziano, Manson, Buring, & Hennekens, 1992).

The geometry of β-carotene might be changed from *trans*- to *cis*-forms. All-*trans*-β-carotene mainly converts to 9-*cis*- and 13-*cis*-β-carotene by thermal treatment and exposure to light. Some other *cis*-forms, such as 15-*cis*-β-carotene and 13,15-di-*cis*-β-carotene, were also produced. The increase of the amount of *cis*-isomers decreases the activity of provitamin A and colour intensity (Chen & Chen, 1993; Chen, Peng, & Chen, 1995).

Various studies have been reported on the thermo-isomerisation of β-carotene (Aman, Schieber, & Carle, 2005; Liu & Chen, 1998; Marx, Stuparic, Schieber, & Carle, 2003; Vásquez-Caicedo, Schilling, Carle, & Neidhart, 2007). The results of isomerisation in solution indicate that the isomeric composition depends upon the solvent system. In general, 9-*cis*- and 13-*cis*-β-carotene could not convert to each other (Pesek, Warthesen, & Taoukis, 1990). The isomerisation of crystallized all-*trans*-β-carotene showed that the concentration of all-*trans*-β-carotene decreased significantly, but *cis*-isomers were not produced until the temperature was over 125 °C (Chen, Chen, & Chien, 1994; Chen & Huang, 1998).

The studies of oxidation of β-carotene showed that the kinetics of β-carotene loss were of zero-order or first-order, under heating and illumination, respectively (Chen & Huang, 1998; Pesek & Warthesen, 1990). Furthermore, the investigations on the thermal oxidation of β-carotene in a 1-butanol (El Oualja, Perrin, & Martin, 1995), aqueous model system (Henry et al., 2000) and lipid (Kasaikina et al., 2002; Liu & Chen, 1998) have suggested that isomerisation is not the only reaction during different treatments. The study of mechanisms during auto-, photo-catalyzed and photosensitized oxidations of β-carotene has also shown that the stability of β-carotene is influenced by the heating temperature and the concentration of oxygen during thermal processing (Bonnie & Choo, 1999).

In addition to solution and lipid systems, solid β-carotene occurs widely in storage and food applications with different particle sizes, and is usually heated to melting and milled during manufacture. Considerable work has been devoted to preparing β-carotene nanoparticles by nanotechnology (Tan & Nakajima, 2005). However, the changes of β-carotene in solid form are not well understood. The objective of this study was to examine solid β-carotene, during heating, exposed to air or under the protection of nitrogen in the dark. The isomerisation and stability of β-carotene at different temperatures were examined. Furthermore, the byproducts of β-carotene after each treatment were characterised by various analytical techniques, including high-performance liquid chromatography (HPLC), UV/VIS-spectroscopy (UV) and gel permeation chromatography (GPC). The results would help to understand the reaction mechanism of β-carotene during thermal processing, and promote the application of β-carotene in the food industry.

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2. Materials and methods

2.1. Chemicals and materials

The crystallized all-*trans*-β-carotene (total content >98%) was supplied by NHU Co. Ltd. (XinChang, PR China). Analytical grade dichloromethane, chloroform and tetrahydrofurane (THF) were obtained from Shuanglin Chemical Reagent Factory (HangZhou, PR China). HPLC grade methanol and cyclohexane were purchased from Shield Company (TianJin, PR China). HPLC grade methyl *tert*-butyl ether (MTBE) was from the Tedia Company, Inc. (Fairfield, OH, USA), and water was from Wahaha Group Co. Ltd. (HangZhou, PR China).

2.2. Preparation of trans–cis-β-carotene sample

Crystallized all-*trans*-β-carotene (20 g) was placed in a 100 ml flask and melted at 180 °C *in vacuo* for 15 min. The flask containing melted β-carotene was rapidly placed in ice-water for 3 min. After the β-carotene was solidified, the sample was taken out and milled in a mortar box under nitrogen atmosphere. Thus 10 g of *trans–cis*-β-carotene were prepared.

2.3. Heating experiments

Three groups of samples were prepared at 40, 90 and 140 °C, respectively. Each group consisted of four samples. Sample 1 (approximately 5.0 g of crystallized all-*trans*-β-carotene) was placed in 50 ml flasks and heated and exposed to air. Sample 2 (approximately 0.5 g of crystallized all-*trans*-β-carotene) was placed in 6 small glass tubes, and each tube was flushed with nitrogen and then sealed by fusing immediately. Sample 3 (approximately 5.0 g of *trans–cis*-β-carotene) was placed in 50 ml flasks and heated and exposed to air). Sample 4 (approximately 0.5 g of *trans–cis*-β-carotene) was placed in 6 small glass tubes, and each tube was flushed with nitrogen and then sealed by fusing immediately. These samples were heated in an oil-bath for 14 days and were analysed every day. All operations were conducted in the dark, and experiments were carried out in triplicate.

2.4. HPLC analysis

Agilent 1100 Series HPLC system (Agilent, Waldbronn, Germany) consisted of a G1311A pump, an automatic injector with a 20 μl loop, and a G1315B diode array detector (DAD) equipped with work station computer. A Waters YMC carotenoid C_{30} column (5 μm, 250 × 4.6 mm; YMC, Wilmington, NC, USA) was employed to analyse β-carotene samples at 30 °C. A solvent system of methanol–MTBE–water–dichloromethane (50:45:5:1, v/v/v/v) was used as mobile phase. The flow rate was 1.0 ml/min and the detection wavelength was set at 455 nm. All samples (8.60 mg each) were weighed and dissolved in 10 ml of chloroform, and 20 μl of solution was collected and diluted with 2 ml of mobile phase before injection. Isomers of β-carotene were identified as described by several authors (Chen, Tai, & Chen, 2004; Lacker, Strohschein, & Albert, 1999; Strohschein, Pursch, & Albert, 1999), and the concentration of the isomers was determined from the peak areas.

2.5. UV/VIS-spectroscopy

Approximately 8.60 mg samples were dissolved in 10 ml chloroform, and 30 μl of solution were collected and diluted with 12 ml of cyclohexane. Using cyclohexane as a reference, the absorbance of cyclohexane solutions at 455 nm were measured on a Varian Cary 100 UV/VIS-spectrophotometer (Varian Australia Pty. Ltd., Victoria, Australia). The total content of samples could be calculated by the following formula:

$$\text{Total content } \% = A \times 4000 \Big/ \left(M \times A_{1\,\text{cm}}^{1\%} \times 100 \right)$$

where A was the absorbance of sample at 455 nm, $A_{1\,\text{cm}}^{1\%}$ was the extinction coefficient of all-*trans*-β-carotene standard (the value was 2500) at 455 nm in cyclohexane, 4000 was the dilution factor and M represented the weight of the sample (g).

2.6. GPC analysis

GPC analysis was performed on a Waters 1525 HPLC pump equipped with Waters 2414 RI detector (Milford, MA), and Waters styragel HR columns (7.8 × 300 mm, HR3-HR2-HR1) were used. The system was calibrated using polystyrene standards (Polysciences, Warrington, PA). The eluent was THF, and the flow rate was 1.0 ml/min. Samples (15 mg) were dissolved in THF (10 ml) to prepare a 1.5 mg/ml solution and 20 μl of solution was injected for the test of GPC at 30 °C.

3. Results and discussion

3.1. Identification of β-carotene isomers by HPLC

Fig. 1 shows the HPLC chromatograms and spectral overlays of starting materials (crystallized all-*trans*-β-carotene and *trans–cis*-β-carotene). Separation was completed within 20 min. Isomers could be identified by Q ratio, which was defined as the absorbance ratio of the *cis* peak to the middle main absorption peak (Chen & Chen, 1993; Vecchi, Englert, Maurer, & Meduna, 1981; Zechmeister, 1962). By comparing the Q ratios we obtained with the reported values (Chen et al., 1994, 2004; Tsukida, Saiki, Takii, & Koyama, 1982), peaks 1-3 were identified as 13-*cis*-, all-*trans*- and 9-*cis*-β-carotene, whereas peaks 4-8 were other *cis*-isomers of β-carotene. The identification data are shown in Table 1. Some positions are vacant in Table 1, when the values could not be identified. The identifications of peaks 7 and 8 were difficult because of their weak response in HPLC chromatograms.

Our HPLC results are in agreement with the report by Lacker et al. (1999), who separated and identified isomers of β-carotene by on-line HPLC-UV-APCI. In their work, a self-synthesized polymeric C_{30} phase was used, and five isomers were determined: 13-*cis*-, 9,13-di-*cis*-, all-*trans*- and 9-*cis*-β-carotene. Strohschein et al. (1999) separated isomers by a 3 μm C_{30} stationary phase, and the structures of seven *cis*-isomers were determined by a stopped-flow HPLC-^{1}H NMR mode. The identifications of 13-*cis*-, 9,13-di-*cis*-, all-*trans*- and 9-*cis*-β-carotene were similar to our results.

Based on the area of each peak and the sum of the total areas in Fig. 1, the percentage of each component were calculated. Crystallized all-*trans*-β-carotene consisted of 93.29% all-*trans* (22.09 mAU[*] min), 1.81% 9-*cis* (0.43 mAU[*] min), 3.72% 13-*cis* (0.88 mAU[*] min) and 1.18% other-*cis*-β-carotene (0.28 mAU[*] min). In contrast, 42.9% all-*trans* (7.69 mAU[*] min), 23.7% 9-*cis* (4.25 mAU[*] min), 13.7% 13-*cis* (2.45 mAU[*] min) and 19.8% other-*cis*-β-carotene (3.55 mAU[*] min) were found in *trans–cis*-β-carotene. The species and concentrations of *cis*-isomers increased considerably in *trans–cis*-β-carotene.

3.2. Isomerisation of β-carotene

When sample 1 was heated at 40, 90 and 140 °C, the concentrations of all isomers decreased with time. Total peak areas became lower than 0.1 mAU[*] min and could not be detected by HPLC after 9, 2 and 1 day at 40, 90, and 140 °C, respectively (Fig. 2a). Similar
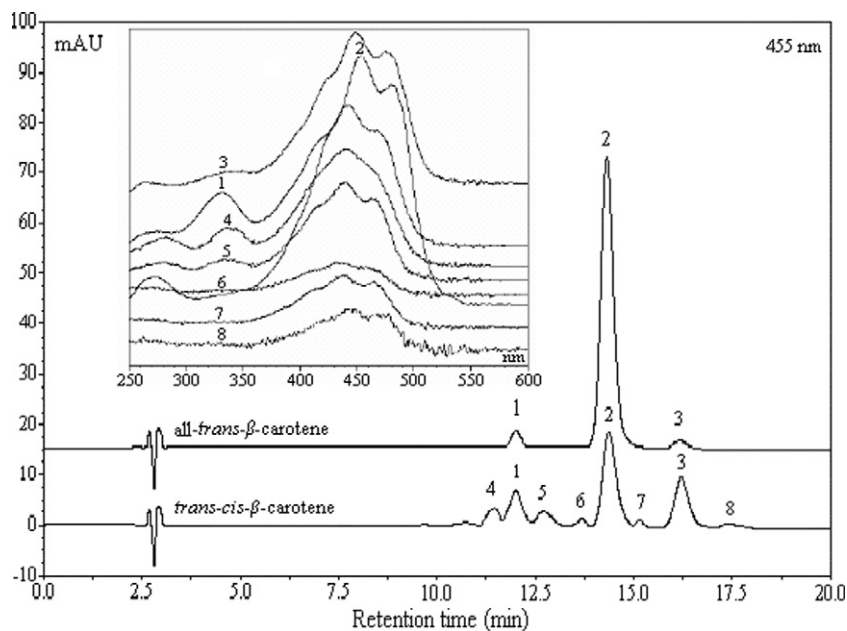
Fig. 1. HPLC chromatograms and spectral overlays of peaks 1–8 of crystallized all-*trans*-β-carotene and *trans*–*cis*-β-carotene.

Table 1
Identification data for β-carotene isomers by HPLC[a]

Peak no.	β-Carotene isomer	Retention time (min)	λ (nm, in-line)[a]			λ (nm, reported)				Q ratio	
										Found	Reported
1	13-*cis*	12.08	337	446	471	333		447	471	0.36	0.35[b]
2	All-*trans*	14.50		454	478			453	477	0.04	0.04[b]
3	9-*cis*	16.26	340	445	472			444	471	0.11	0.11[d]
4	15-*cis*	11.46	334	441		327	417	441	464	0.39	0.41[b]
5	9,15-di-*cis*	12.71	333	440	466	334		441		0.21	0.20[c]
6	9,13-di-*cis*	13.72	411	438	462	335		439		0.16	0.16[c]
7	13,15-di-*cis*	15.16		440	467	339		436		0.14	0.14[c]
8	9,13'-di-*cis*	17.50		440	472	334		438		0.18	0.18[c]

[a] HPLC mobile phase of methanol–MTBE–water–dichloromethane (50:45:5:1, v/v/v/v) was used.
[b] A gradient mobile phase of methanol–isopropanol (99:1, v/v) and methylene chloride (from 100:0 to 70:30, v/v) was used by Chen et al. (2004).
[c] *n*-Hexane was used by Tsukida et al. (1982).
[d] Solvent of acetonitrile–methanol (80:20, v/v) was used by Chen et al. (1994).

results, on heating all-*trans*-β-carotene crystals at 50, 100, 125 and 150 °C, were reported by other researchers (Marx et al., 2003; Vásquez-Caicedo et al., 2007), and they all found no significant isomerisation after heating below 125 °C for 30 min. The concentrations of *cis*-β-carotene changed a little when heating at over 125 °C for 30 min, whereas the degradation of all-*trans*-β-carotene was significant after heating at 50, 100, 125 or 150 °C for several minutes.

It seems that when all-*trans*-β-carotene crystals were exposed to air, isomerisation among isomers of β-carotene was not significant, but the concentrations of isomers all decreased (Fig. 2a). For example, the concentrations of all-*trans*-β-carotene were decreased from 22.09 mAU* min to 0 mAU* min within 2 days at 90 °C.

When sample 2 was heated at 40, 90 and 140 °C for two weeks, no significant concentration change was found for any isomer (Fig. 2b). These results suggest that, if all-*trans*-β-carotene crystals were protected by nitrogen, no isomerisation would occur.

During heating of sample 3 at 40, 90 and 140 °C, concentrations of isomers changed rather markedly (Fig. 3a). The concentrations of *cis*-isomers decreased rapidly, but the concentration of all-*trans*-β-carotene increased at 90 °C from 2 to 7 days, and decreased slowly thereafter. The initial increase in all-*trans*-β-carotene was primarily due to the isomerisation of *cis*- to all-*trans*-isomer; after 7 days, other side-reactions probably took place. When sample 3 was

heated at 140 °C and 40 °C, the concentrations of isomers decreased faster than those of the sample heated at 90 °C.

However, the concentrations of all-*trans*-β-carotene of sample 3 were larger than that of sample 1 (Fig. 2a) when heated at the same temperature, and the differences were more evident at 90 and 140 °C. These results suggest that the isomerisation became more noticeable at higher temperatures when the slick surface of the sample was partly melted.

The results above suggest that, when solid β-carotene was exposed to air, isomerisation of *trans*–*cis*-β-carotene was more significant than that of crystallized all-*trans*-β-carotene, especially at higher temperatures.

From Fig. 3b, it can be seen that, during heating of sample 4 at 40 °C, the concentrations and proportions of isomers changed slightly after 14 days. However, when sample 4 was heated at 90 and 140 °C, the changes of concentration and proportions became more obvious, and the surface of sample also became slick like sample 3. Fig. 3b shows the concentration changes of isomers during heating of sample 4 at 90 and 140 °C. A conversion of *cis*-isomers into all-*trans*-isomer was clearly observed, and the rate of formation of all-*trans*-β-carotene at 140 °C was faster than that at 90 °C. This phenomenon was quite different from the previous report on isomerisation of β-carotene, while the sharp differentia-
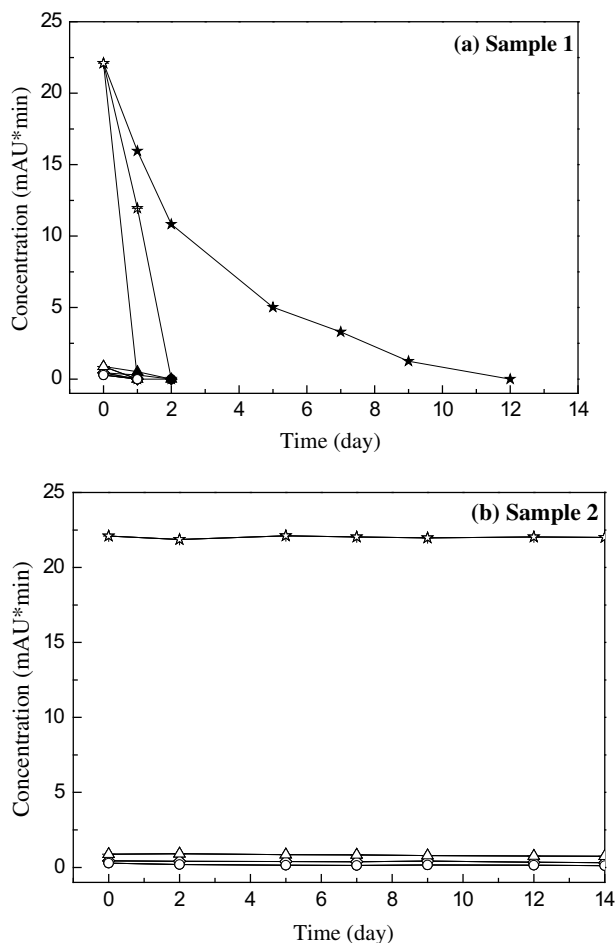
Fig. 2. Concentration changes of isomers during heating of sample 1 (air-exposed all-*trans*-β-carotene) and sample 2 (nitrogen-protected all-*trans*-β-carotene) at 40, 90 and 140 °C. (──★── all-*trans* of 40 °C, ──◆── 9-*cis* of 40 °C, ──▲── 13-*cis* of 40 °C, ──●── other-*cis* of 40 °C, ──✳── all-*trans* of 90 °C, ──⊕── 9-*cis* of 90 °C, ──△── 13-*cis* of 90 °C, ──▢── other-*cis* of 90 °C; ──☆── all-*trans* of 140 °C, ──◇── 9-*cis* of 140 °C, ──△── 13-*cis* of 140 °C, ──○── other-*cis* of 140 °C).

Fig. 3. Concentration changes of isomers during heating of sample 3 (air-exposed *trans*–*cis*-β-carotene) and sample 4 (nitrogen-protected *trans*–*cis*-β-carotene) at 40, 90 and 140 °C. (──★── all-*trans* of 40 °C, ──◆── 9-*cis* of 40 °C, ──▲── 13-*cis* of 40 °C, ──●── other-*cis* of 40 °C, ──✳── all-*trans* of 90 °C, ──⊕── 9-*cis* of 90 °C, ──△── 13-*cis* of 90 °C, ──▢── other-*cis* of 90 °C, ──☆── all-*trans* of 140 °C, ──◇── 9-*cis* of 140 °C, ──△── 13-*cis* of 140 °C, ──○── other-*cis* of 140 °C).

tion could be attributed to the difference in methods of sample handing and components of β-carotene.

Isomerisation of β-carotene has been studied for many years. The isomerisation reaction in solvent was considered a reversible reaction with first-order reaction kinetics (Chen et al., 1994; Pesek et al., 1990), but the isomerisation of solid β-carotene reported in this paper was different from that model. This is presumably due to the different state of the β-carotene molecules. Furthermore, we found that solid *cis*-β-carotene was isomerized into the all-*trans*-molecule only when *cis*-β-carotene was partly melted. The presence of melted component enhanced the probability of reversal of β-carotene molecules, which is comparable to that when β-carotene is completely melted in solvent.

3.3. Stability of β-carotene

During the study of isomerisation of β-carotene, all-*trans*-β-carotene was more stable than was *cis*-β-carotene (Figs. 2 and 3). Fig. 4 shows the change in total content of β-carotene (%), during heating at 40, 90 and 140 °C, of samples 1–4. The total content of the *trans*–*cis*-β-carotene sample (64.5%) was lower than that of the all-*trans*-β-carotene sample (98.03%), mainly because of the lower extinction coefficients of *cis*-β-carotene. The nitrogen-protected samples (samples 2 and 4) were more stable than the air-exposed samples (sample 1 and 3). Moreover, the increase of all-*trans*-β-
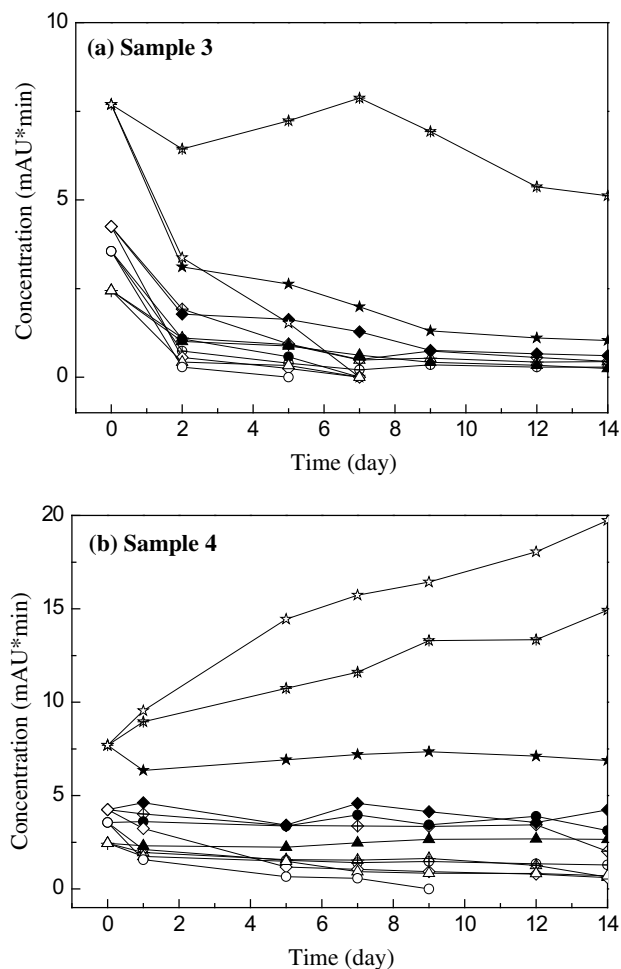
carotene resulted in the augmentation of content of sample 4 during heating. When sample 1 and 3 were both heated and exposed to air, sample 3, which contained more *cis*-isomers, was more stable than was sample 1. Isomerisation took place in sample 3, and that enhanced β-carotene stability.

Based on the total β-carotene contents and HPLC concentrations of samples 1–4 at different temperatures (Figs. 2–4), four conclusions could be drawn.

1. β-Carotene sample were more stable at lower temperatures.
2. When β-carotene sample was exposed to air, it was adequately affected by oxidation, and the content decreased much faster than that under the protection of nitrogen at the same temperature.
3. Isomerisation of *cis*- to all-*trans*-β-carotene occurred in *trans*–*cis*-β-carotene at higher temperatures.
4. The *trans*–*cis*-β-carotene sample was more stable than was the all-*trans*-β-carotene sample when they were exposed to air at the same temperature. We hypothesize that, as the surface of the *trans*–*cis*-β-carotene sample became slick by partly melting, its specific surface area would decrease, which resulted in smaller influence of oxygen. The occurrences of isomerisation in *trans*–*cis*-β-carotene samples, however, resulted in the increase of concentrations of all-*trans*-β-carotene.

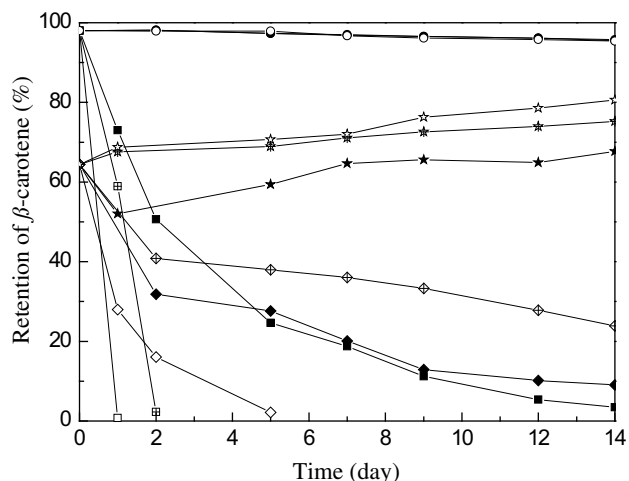**Fig. 4.** Retention of β-carotene (%) during heating at 40, 90 and 140 °C of samples 1–4. (Sample 1: air-exposed all-*trans*-β-carotene, sample 2: nitrogen-protected all-*trans*-β-carotene, sample 3: air-exposed *trans*–*cis*-β-carotene, sample 4: nitrogen-protecting *trans*–*cis*-β-carotene; ■ sample 1 of 40 °C, ⊟ sample 1 of 90 °C, ⊡ sample 1 of 140 °C, ● sample 2 of 40 °C, ⊕ sample 2 of 90 °C, ○ sample 2 of 140 °C, ◆ sample 3 of 40 °C, ⊕ sample 3 of 90 °C, ◇ sample 3 of 140 °C, ★ sample 4 of 40 °C, ✳ sample 4 of 90 °C, ☆ sample 4 of 140 °C).

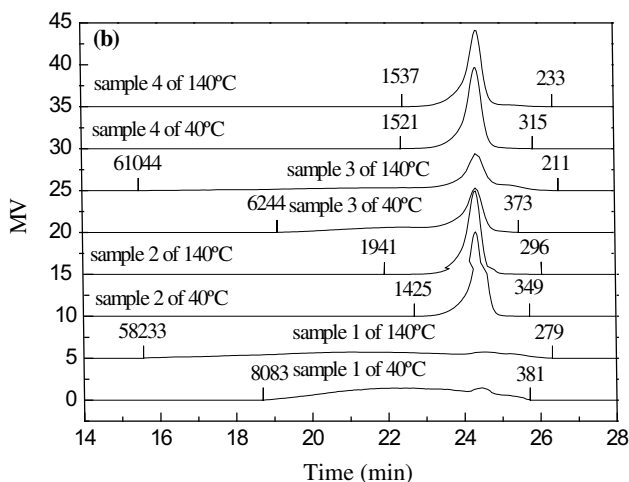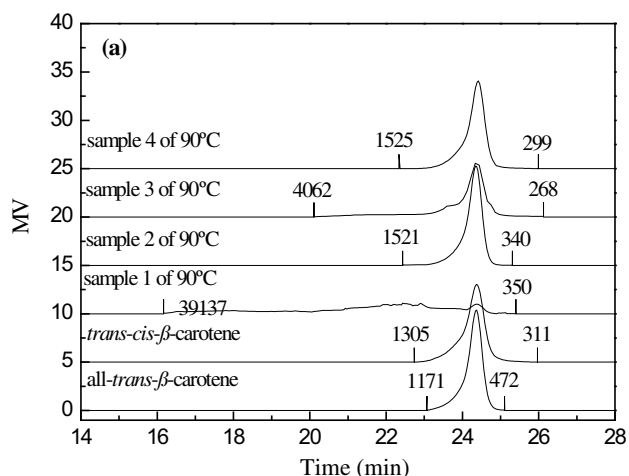**Fig. 5.** GPC traces of crystallized all-*trans*-β-carotene, *trans*–*cis*-β-carotene and samples 1–4 after heating at 40, 90 and 140 °C for 14 days. (Sample 1: air-exposed all-*trans*-β-carotene, sample 2: nitrogen-protected all-*trans*-β-carotene, sample 3: air-exposed *trans*–*cis*-β-carotene, sample 4: nitrogen-protected *trans*–*cis*-β-carotene).

Fig. 5a shows the molecular weight and molecular weight (MW) distribution of starting materials and samples 1–4 after heating at 90 °C for 14 days. Using polystyrenes as the standards, the weight-average molecular weight (M_w) of all-*trans*-β-carotene was approximately 710, while the real MW of β-carotene was 536. It is reported that the use of polystyrene as a standard may overestimate the molecular weight (Jayakannan, van Dongen, & Janssen, 2001). The all-*trans*-β-carotene sample was highly pure (98.03%) and could be used as the standard of β-carotene. The components below MW of 472 amu or above MW of 1171 amu could be considered as byproducts of β-carotene after heating. GPC curves of samples 2 and 4 were similar to those of all-*trans*-β-carotene except for a few high MW byproducts, indicating that no other side-reactions but slight polymerisation had occurred.

When β-carotene was heated and exposed to air at 90 °C for two weeks, a lot of high MW byproducts and low molecular fragments were found by the GPC analysis of samples 1 and 3. These results suggest that polymerisation, as well as degradation, occurred. The all-*trans*-β-carotene sample polymerised more readily than did the *trans*–*cis*-β-carotene sample.

The molecular weight and molecular weight distribution of samples 1–4 after heating at 40 and 140 °C for 14 days were also investigated by GPC (Fig. 5b). These results had the same trend as that at 90 °C. The characters of nitrogen-protected samples at different temperature were similar to that of all-*trans*-β-carotene, and the GPC traces of air-exposed samples suggested that the contents of byproducts increased at a higher temperature.

In summary, we have demonstrated, for the first time, that isomerisation of *cis*- to all-*trans*-isomer occurred in partly melted solid β-carotene when β-carotene was heated at 90 and 140 °C. High stabilities were found during heating of nitrogen-protected β-carotene. Only a few high molecular weight components were detected in nitrogen-protected β-carotene. In contrast to samples under the protection of nitrogen, solid β-carotene was easily oxidised when exposed to air. More high molecular weight compounds, as well as low molecular weight compounds, were found in air-exposed β-carotene, suggesting that the concentration of oxygen had more influence on the stability of β-carotene than had the heating temperature. When β-carotene was heated and exposed to air, polymerisation took place in addition to degradation.

Acknowledgment

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foodchem.2008.05.071.

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