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Stability of carotenoids and vitamin A during storage of carrot juice

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Investigations were conducted on the stability of carotenoids and vitamin A during storage of carrot juice. Carrot juice was acidified, pasteurized, and then subjected to light and dark storage at 4, 25 and 35 °C for 3 months. The isomerization and degradation of carotenoids were monitored by high-performance liquid chromatography with diode-array detection. Results showed that the amounts of lutein, α-carotene, β-carotene and vitamin A in carrot juice decreased with increasing storage temperature. Light can be more destructive to carotenoids than darkness. 9-*cis* carotenoid isomers were the major types formed in carrot juice under light storage, while 13-*cis* types were favoured during storage in the dark. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Carrot (*Daucus carota* L. var. Sativa DC.) is an important crop of Taiwan. In recent years the consumption of carrots in Taiwan has been increasing steadily, mainly because carrot contains an important biological compound—carotenoid. Of the various carotenoids in carrots, β-carotene constitutes a large portion, followed by α-carotene and lutein (Baloch *et al.*, 1977; Seifert & Buttery, 1978; Bushway & Wilson, 1982; Munsch & Simard, 1983; Heinonen, 1990; Chen *et al.*, 1995). The beneficial effect of β-carotene to human health has been well established. For instance, β-carotene has been found to reduce the risk of skin cancer (Mathews-Roth, 1985; Krinsky, 1989; Ziegler, 1989), to increase immune response (Bendich & Shapiro, 1986; Bendich, 1989; Prabhala *et al.*, 1990) and to protect against liver damage (Zamora *et al.*, 1991).

After harvest, some quality defects such as loss of sweetness and carotenoids and formation of bitterness and oxidized flavour can occur in carrots. To reduce quality loss carrots have to be stored at 0 °C with a relative humidity of 93–98% (Salunkhe & Desai, 1984). From a commercial point of view, carrots can be processed into frozen, dried, canned and fermented products, and even baby foods (Niketic-Aleksic *et al.*, 1973; Ramdas & Kulkarni, 1987). In addition, carrots can be used to prepare beverage products such as carrot juice. In recent years carrot juice has become an important food commodity in Taiwan for health reasons. The consumption of fruit and vegetable juice in Taiwan reached 30 000 tons for the year 1992, of which carrot juice constituted 2000 tons (Commission on Fruits and Vegetables in Taiwan, 1993).

Many methods have been used to process carrot juice. One of the drawbacks in processing carrot juice is that the sterilization temperature has to be raised because carrots are low-acid (pH $5.5 \approx 6.5$) foods. However, this treatment can result in great loss of colour (Stephens *et al.*, 1971). To remedy this problem carrot juices are often acidified before processing so that the sterilization temperature can be lowered. It has been reported that heating carrots in acetic acid solution can prevent coagulation of the extracted juice during heat sterilization (Stephens *et al.*, 1971).

The major problem associated with carrot juice quality is colour (Stephens *et al.*, 1971; Bates & Koburger, 1974; Sims *et al.*, 1993; Chen *et al.*, 1995). It has been reported that the colour change of carrot juice during processing correlated well to carotenoid content (Munsch & Simard, 1983; Chen *et al.*, 1995). Saldana *et al.* (1976) studied the effect of storage on quality of carrot juice and found that storage time had no effect on colour or β-carotene when carrot juice was stored at 20 °C for 9 months. Sims *et al.* (1993) determined the effect of heating and acidification on carrot juice and found that heating carrots to 93 °C prior to milling and pressing improved juice colour. These authors also found that acidification of milled carrots to pH 4 or 5 with citric acid could improve juice colour. Chen *et al.* (1995) studied the effect of various processing methods on carotenoid, colour and vitamin A content in carrot juice and found that canning resulted in the highest destruction of carotenoids, followed by HTST heating

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and acidification. The same trend was also observed for colour and vitamin A content change. Very few reports dealt with stability of carotenoids and vitamin A during storage of carrot juice. The purpose of this present study was to determine the isomerization and degradation of various carotenoids, and change in vitamin A content during storage of carrot juice.

MATERIALS AND METHODS

Materials

Fresh carrots (*Daucus carota* L. var. Sativa DC.) were purchased from a local market, and a total of approximately 30 kg of carrots was obtained.

All-*trans*-α-carotene, all-*trans*-β-carotene and all-*trans*-lutein (75% purity) standards were purchased from Sigma (St Louis, MO). Each standard was found to contain a small amount of *cis* isomers by high-performance liquid chromatographic (HPLC) analysis and was used without further purification. All HPLC grade solvents such as methanol and methylene chloride were obtained from Merck (Darmstadt, Germany). Solvents used for extraction of carotenoids, such as hexane, acetone, toluene and absolute ethanol, were of analytical grade and were also from Merck. The HPLC grade solvents were degassed under vacuum and filtered through a 0.2-μm membrane filter prior to use.

Instrumentation

The HPLC instrument consisted of a Jasco PU-980 pump (Tokyo, Japan) with a Schimadzu SPD-M6A photodiode-array detector (Tokyo, Japan) and an SIC chromatocoder 12 integrator (Tokyo, Japan). Data were analysed using an Axxiom 727 and dual-channel chromatography data system (Axxiom Chromatography Inc., Calabasas, CA). A Vydac 201TP54 column (250 mm × 4.6 mm i.d.) packed with 5-μm particles (Hesperia, CA) was used.

Processing of carrots

Thirty kilograms of carrots were washed with 30 litres of water before peeling. The peeled carrots were cut into small pieces and ground into juice with a grinder. A total of approximately 15 litres of carrot juice was obtained, and was acidified to pH 4.0 with citric acid, then heated at 105 °C for 30 s using a laboratory pasteurization system as described by Chen *et al.* (1995). Pasteurized juice was divided into two portions of 10 and 5 litres each. The first portion was used to fill 36 glass bottles (11.2 × 5.5 cm), each containing approximately 250 ml of juice. Similarly, the second juice portion was used to fill 36 aluminium foil bags (190 × 110 × 0.6 mm), each containing 125 ml of juice. The 36 bottles were placed in three incubators (12 bottles in each) and the temperature was maintained at 4, 25 and 35 °C, respectively. A fluorescent tube (General Electric, 20 W) was suspended approximately 20 cm above the bottles, where the light intensity measured 1500 lux. The 36 aluminium foil bags were treated in the same way as the glass bottles with the exception that no light was used. Two bottles from each treatment were randomly selected every 2 weeks for carotenoid analysis.

Extraction of carotenoids

Fresh and processed carrot juice (4-ml aliquots) was extracted for carotenoids using a method described in a previous study (Chen *et al.*, 1995).

HPLC analysis of carotenoids

A polymeric C_{18} column (Vydac 201TP54) and a mobile phase of methanol–methylene chloride (99:1, v/v) were used for separation of carotenoids and their *cis* isomers with a sample solvent of methanol–methylene chloride (45:55, v/v) (Chen *et al.*, 1995). Detection wavelength was set at 450 nm with flow rate at 1.0 ml min^{-1} and sensitivity at 0.16 AUFS. Carotenoids such as lutein, α-carotene and β-carotene in carrot juice were identified by comparing unknown peaks with reference standards and chromatography with added standards. The identification of *cis* carotenoids was based on spectral characteristics and *Q* ratio as described previously (Chen & Chen, 1994; Chen *et al.*, 1994). Quantification of carotenoids was carried out using an absolute calibration curve as described by Chen *et al.* (1995). Duplicate analyses were conducted and mean values determined. Vitamin A was quantified using the following formula:

1 retinal equivalent (RE) = 1 mg of retinal
= 6 mg of β-carotene
= 12 mg of other provitamin A
= 12 mg of other provitamin A carotenoids

RESULTS AND DISCUSSION

In a previous study, we determined the various carotenoids in raw carrot juice and found that β-carotene was present in the largest amount (62.5 μg ml^{-1}), followed by α-carotene (27.6 μg ml^{-1}), lutein (6.0 μg ml^{-1}), 13-*cis*-β-carotene (3.4 μg ml^{-1}), 13,15-di-*cis*-β-carotene (1.3 μg ml^{-1}), 15-*cis*-β-carotene (1.1 μg ml^{-1}), 9-*cis*-β-carotene (1.1 μg ml^{-1}), 13-*cis*-lutein (0.6 μg ml^{-1}), 9-*cis*-lutein (0.4 μg ml^{-1}), 13-*cis*-α-carotene (0.2 μg ml^{-1}) and 9-*cis*-β-carotene (0.2 μg ml^{-1}) (Chen *et al.*, 1995).

Changes of lutein and its *cis* isomers in carrot juice during storage

Figure 1 shows the concentration change of lutein and its *cis* isomers in carrot juice during storage in the dark. After storage for 3 months at 4, 25 and 35 °C, the amount of lutein decreased from 4.36 to 3.96, 3.94 and 3.32 μg ml^{-1}, respectively. This result implied that lutein concentration decreased with increasing storage

temperature. No significant change was observed for 9-*cis*-lutein. For 13-*cis*-lutein, it increased from 0.80 to 1.06, 1.08 and 1.02 μg ml^{-1}, indicating that this isomer was more easily formed than 9-*cis*-lutein during storage in the dark. Figure 2 shows the concentration change of lutein and its *cis* isomers under light storage. Similar to the result in Fig. 1, lutein concentration decreased from 4.63 to 3.77, 3.34 and 2.98 μg ml^{-1}, respectively, after storage for 3 months at 4, 25 and 35 °C. The only difference is that the degradation of lutein during illumination was greater than that in the dark. Only minor change was observed for 9-*cis*-lutein under light storage. This result seems to be contradictory to some reports (Chandler & Schwartz, 1987; Pesek & Warthesen, 1990; Chen *et al.*, 1994), which showed that the 9-*cis* isomer was favoured during illumination of β-carotene. This may be explained as follows: (1) the activation energy required for formation of 9-*cis*-lutein may be greater than that of 13-*cis*-lutein; (2) the presence of suspended particles and large molecules in the juice may offer some protection for 9-*cis*-lutein isomerization; (3) the initial concentration of lutein in the juice is too low, and hence the relative concentration change of 9-*cis*-lutein is small. For 13-*cis*-lutein, it increased from 0.80 to 1.03, 1.09 and 1.15 μg ml^{-1} under light storage at 4, 25 and 35 °C, respectively. This result may imply that 13-*cis*-lutein was formed at a greater rate than 9-*cis*-lutein during illumination. Khachik *et al.* (1986) found that 13-*cis*-lutein was formed in greater amounts than 9-*cis*-lutein after cooking of cabbage. Chen *et al.* (1994) demonstrated that the 13-*cis* isomer was favoured during heating of β-carotene. Apparently, the formation

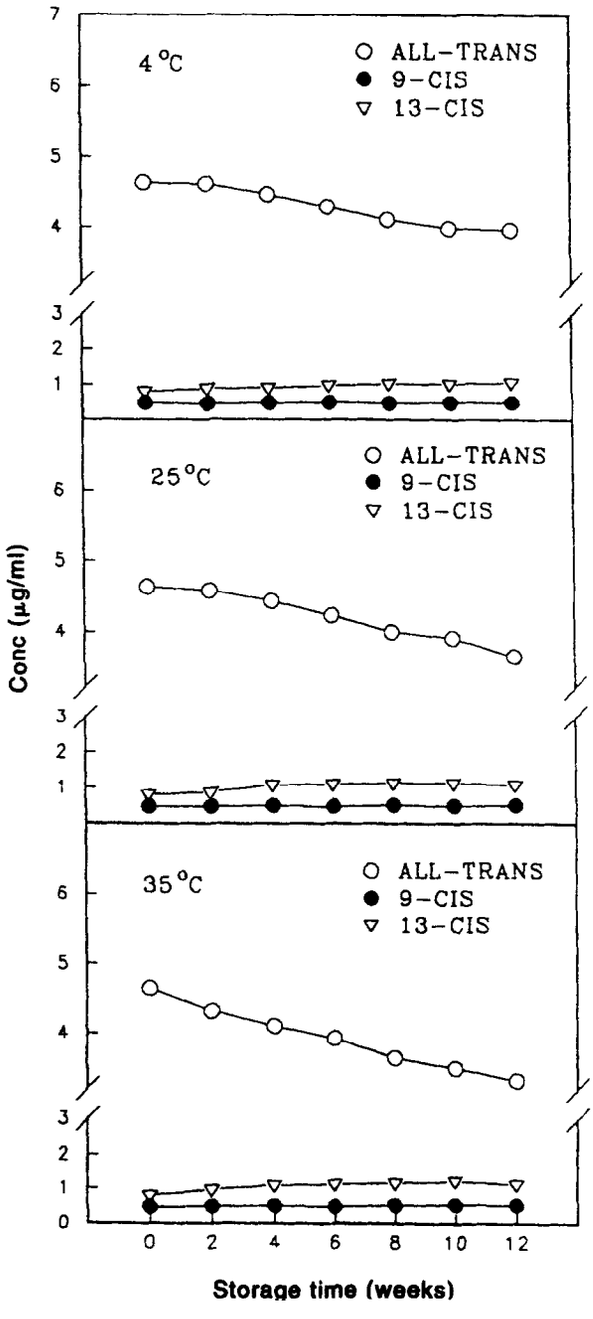

Fig. 1. Concentration changes of all-*trans*-lutein and its *cis* isomers in carrot juice during storage in the dark at 4, 25 and 35 °C.

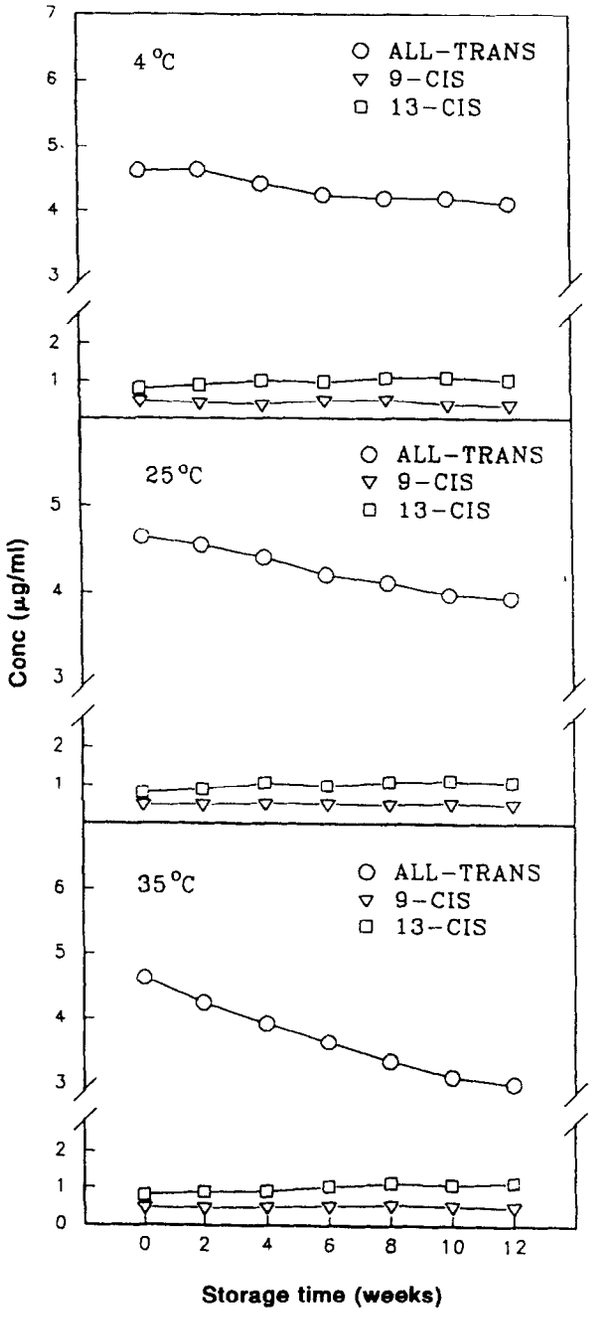

Fig. 2. Concentration changes of all-*trans*-lutein and its *cis* isomers in carrot juice under light storage at 4, 25 and 35 °C.

of the 9-*cis* or 13-*cis* isomer during heating and illumination of lutein can be attributed to differences in activation energy, light intensity and illumination modes. It is also possible that the results observed in carrot juice cannot be identical in the model system because the former contains water and other components.

Changes of α-carotene and its *cis* isomers in carrot juice during storage

Figure 3 shows the concentration change of α-carotene and its *cis* isomers during storage in the dark. Similar to lutein, the amount of α-carotene decreased from 25.4 to 20.7, 19.7, and 19.3 μg ml^{-1} after storage in the dark for 3 months at 4, 25 and 35 °C, respectively. This result implied that the amount of α-carotene decreased with increasing storage temperature. In contrast, 9-*cis*-α-carotene increased from 0.21 to 0.27, 0.30 and 0.30 μg ml^{-1}. 13-*cis*-α-Carotene showed the same trend with an increase from 0.35 to 0.47, 0.51 and 0.52 μg ml^{-1}. Compared to 9-*cis*-α-carotene, 13-*cis*-α-carotene was formed in greater amounts, indicating that the central *cis* isomer of all-*trans*-α-carotene was more easily formed than the other *cis* isomer under dark storage.

Figure 4 shows the concentration change of α-carotene and its *cis* isomers under light storage. Similar to the result in Fig. 3, α-carotene decreased from 25.4 to

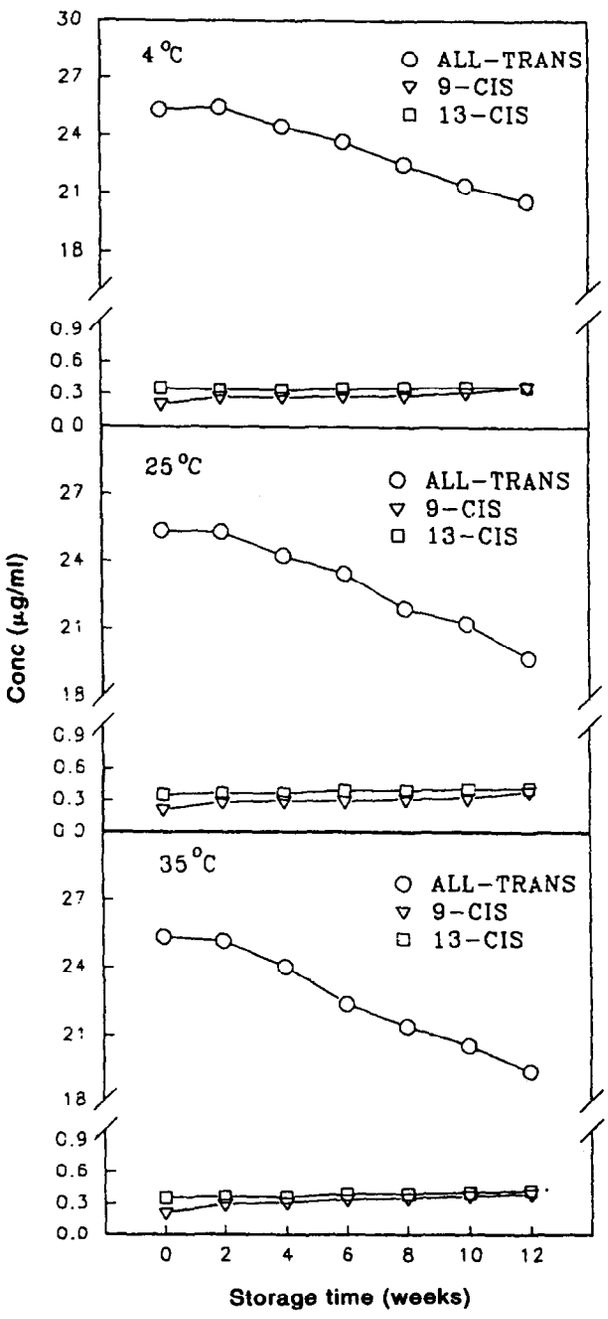

Fig. 3. Concentration changes of all-*trans*-α-carotene and its *cis* isomers in carrot juice during storage in the dark at 4, 25 and 35 °C.

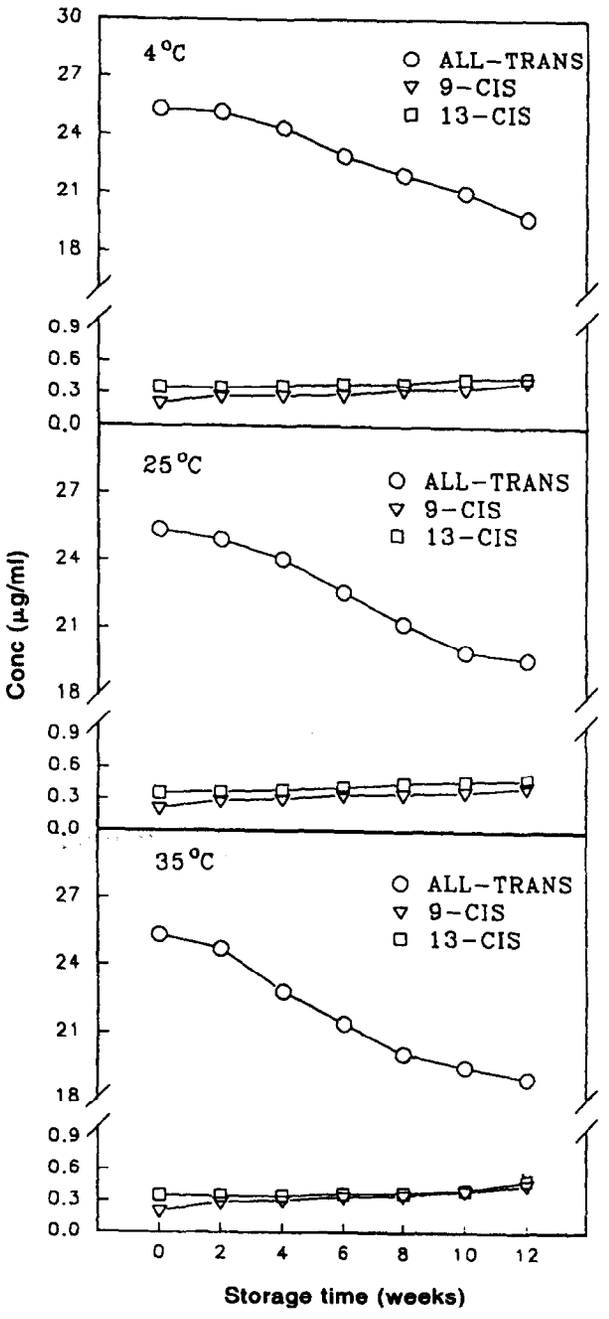

Fig. 4. Concentration changes of all-*trans*-α-carotene and its *cis* isomers in carrot juice under light storage at 4, 25 and 35 °C.

19.7, 19.6 and 18.9 μg ml^{-1}, respectively, after storage for 3 months at 4, 25 and 35 °C. This result implied that the degradation of α-carotene was faster under lighted conditions than under dark storage. The amount of 9-*cis*-α-carotene increased from 0.21 to 0.42, 0.41 and 0.46 μg ml^{-1}, indicating that light can catalyse the formation of 9-*cis*-α-carotene. Chen *et al.* (1994) also found that 9-*cis* was favoured during illumination of α-carotene. The formation of 13-*cis*-α-carotene showed the same trend. However, the amount of 13-*cis*-α-carotene formed during illumination was lower than that in the dark. This is probably because the degradation rate of 13-*cis*-α-carotene was greater than the formation rate when carrot juice was exposed to light. In a recent study Chen *et al.* (1994) demonstrated that the formation of 9-*cis* or 13-*cis* from all-*trans*-α-carotene was dependent upon the extent of heat or iodine/light treatment, and the latter was formed in greater amount under either treatment.

Change of β-carotene and its *cis* isomers in carrot juice during storage

Figure 5 shows the concentration change of β-carotene and its *cis* isomers during storage in the dark. Similar to α-carotene and lutein, the amount of β-carotene decreased with increasing storage temperature. Pesek & Warthesen (1988) also found that β-carotene concentration decreased with increasing storage temperature in an aqueous model system. The amount of β-carotene decreased from 54.7 to 50.9, 49.0 and 46.4 μg ml^{-1} after storage in the dark for 3 months at 4, 25 and 35 °C, respectively. Compared to α-carotene, β-carotene concentration decreased to a greater extent at 35 °C, indicating that the latter was more susceptible to temperature loss than the former. Both 9-*cis*-β-carotene and 13-*cis*-β-carotene showed the same trend as 9-*cis*-α-carotene and 13-*cis*-α-carotene. 13-*cis*-β-carotene was formed at a greater amount than 9-*cis*-β-carotene in the dark. However, only a minor change was observed for 15-*cis*-β-carotene and 13,15-di-*cis*-β-carotene. Chen *et al.* (1995) reported that 13,15-di-*cis*-β-carotene can only be formed under drastic treatment such as canning. In another study, Chen *et al.* (1994) also demonstrated that the amount of 13,15-di-*cis*-β-carotene increased substantially under iodine/light treatment.

Figure 6 shows the concentration change of β-carotene and its *cis* isomers under light storage. Similar to the result in Fig. 5, β-carotene concentration decreased from 59.7 to 48.7, 47.3 and 46.2 μg ml^{-1} after storage for 3 months at 4, 25 and 35 °C, respectively. This result implied that the degradation rate of β-carotene was greater under lighted conditions. Also, the higher the storage temperature, the more destruction of β-carotene. The amount of 9-*cis*-β-carotene increased from 1.21 to 1.54, 1.82 and 1.88 μg ml^{-1}, indicating that this isomer was more easily formed when exposed to light. For 13-*cis*-β-carotene, it increased from 4.46 to 4.62, 4.74 and 4.77 μg ml^{-1}. This result was similar to that for 13-*cis*-α-carotene, which showed that the formation and degradation of the 13-*cis* isomer of α-carotene or β-carotene can proceed simultaneously under light storage, and the degradation rate was found to be greater than the formation rate. Chandler & Schwartz (1987) studied the iodine-catalysed photoisomerization of all-*trans*-β-carotene and found that 9-*cis*-β-carotene was formed in greater amount than 13-*cis*-β-carotene. In another study, Pesek & Warthesen (1990) found that 13-*cis*-β-carotene was formed in a larger amount than 9-*cis*-β-carotene under dark storage. The formation of the 9-*cis* rather than the 13-*cis* isomer under light storage is apparently due to the instability of the former. In this study the photoisomerization rate of all-*trans*-β-carotene was lower than in the model system as reported by Pesek & Warthesen (1990), who demonstrated

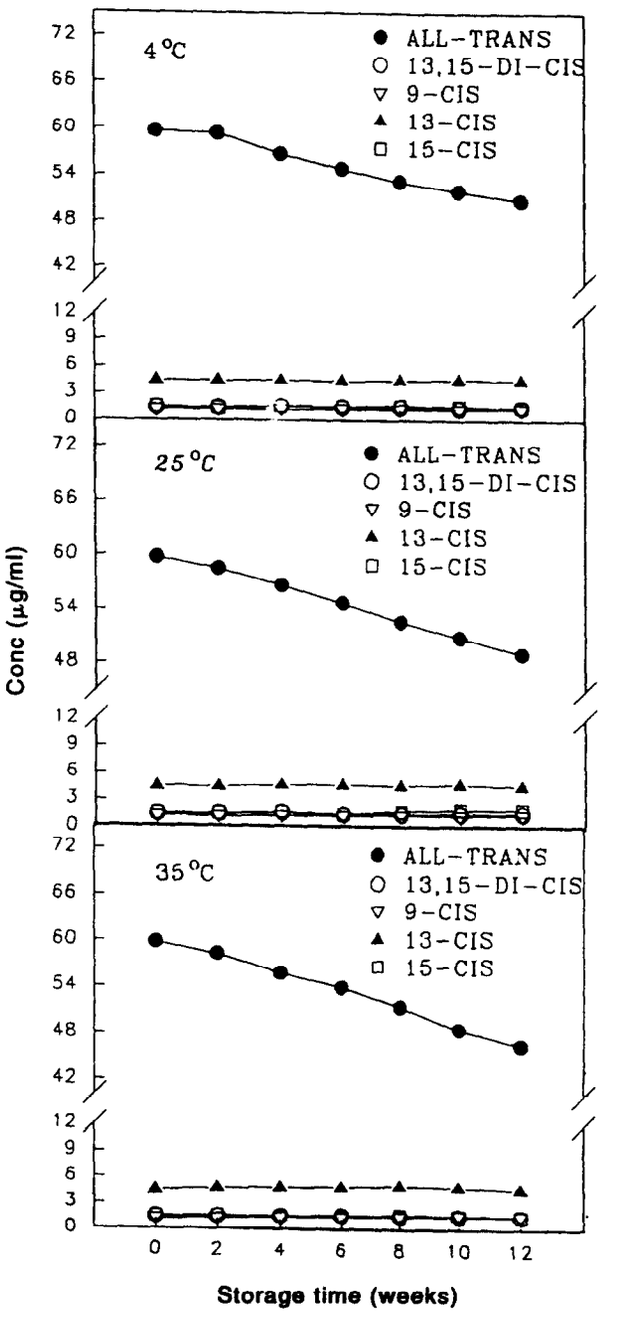

Fig. 5. Concentration changes of all-*trans*-β-carotene and its *cis* isomers in carrot juice during storage in the dark at 4, 25 and 35 °C.

that the activation energy required for isomerization of 13-*cis*-β-carotene was lower than 9-*cis*-β-carotene. This is mainly because carrot juice is a complex system and the presence of large molecules may offer some protection for isomerization of all-*trans*-β-carotene. It is also possible that all-*trans*-β-carotene may be adsorbed to solid particles in the juice, which are shadowed by large particles. Therefore, all-*trans*-β-carotene can be protected from light. Pesek & Warthesen (1988) pointed out that many reactions such as photoisomerization and photodegradation can occur simultaneously and competitively when carotenoids are exposed to light, and the proceeding of either reaction should depend upon light intensity, temperature and presence of catalyst. Bryant *et al.* (1992) further demonstrated that some α-carotene and β-carotene can be complexed with protein in carrots, and the formation of complex may stabilize carotenoids during processing and storage of carrot juice.

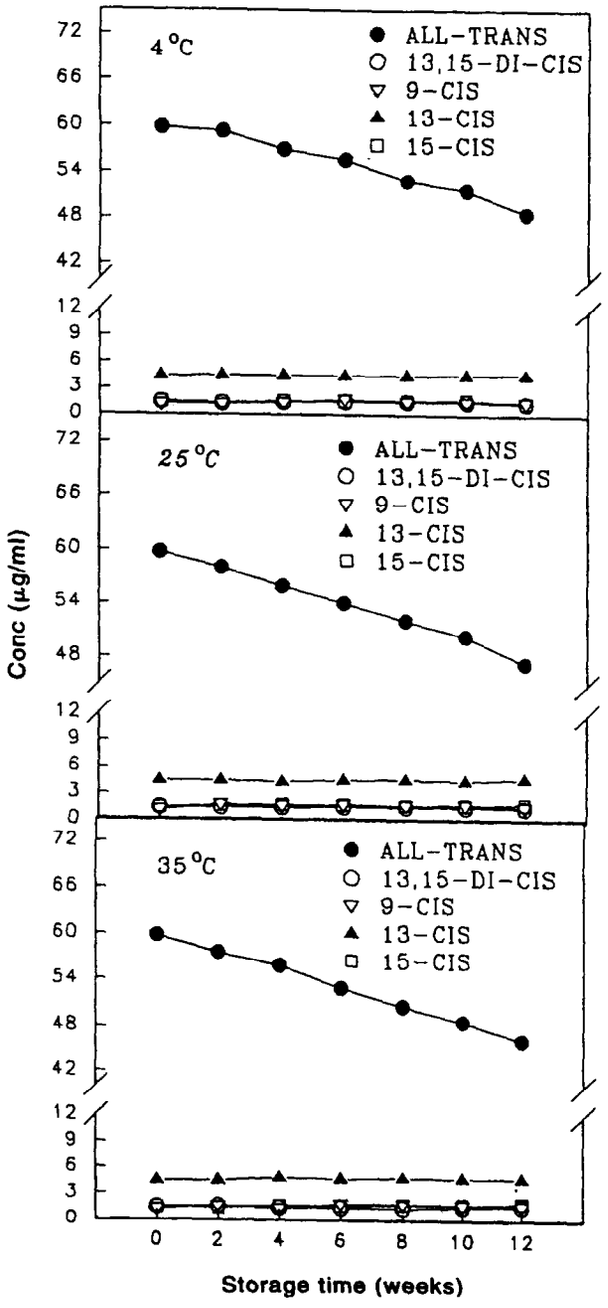

Fig. 6. Concentration changes of all-*trans*-β-carotene and its *cis* isomers in carrot juice under light storage at 4, 25 and 35 °C.

Change of vitamin A content during storage of carrot juice

Figure 7 shows the change in vitamin A content of carrot juice under dark storage. The vitamin A content decreased from 12.1 to 10.2, 9.82 and 9.28 μg ml^{-1} after storage for 3 months at 4, 25 and 35 °C, respectively. This result indicated that vitamin A content decreased with increasing storage temperature. Similar results were observed for vitamin A content change under light storage at 4, 25 and 35 °C for 3 months (Fig. 8). By comparing the results in Figs 7 and 8, it can be found that light can be more destructive to vitamin A than in the dark at the same storage temperature. This result also correlated well with the decrease of both α-carotene

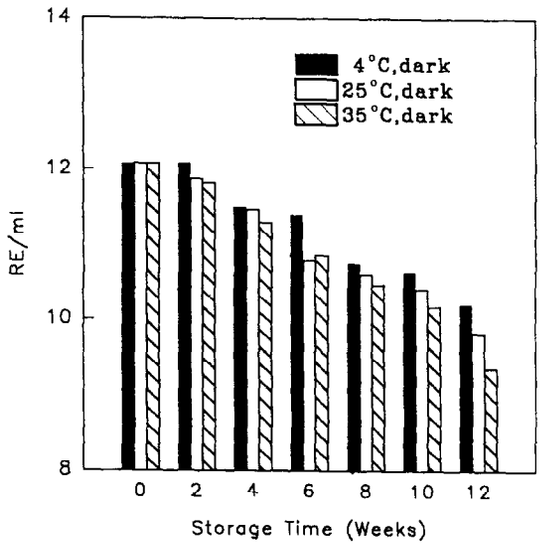

Fig. 7. Concentration changes of vitamin A in carrot juice during storage in the dark at 4, 25 and 35 °C.

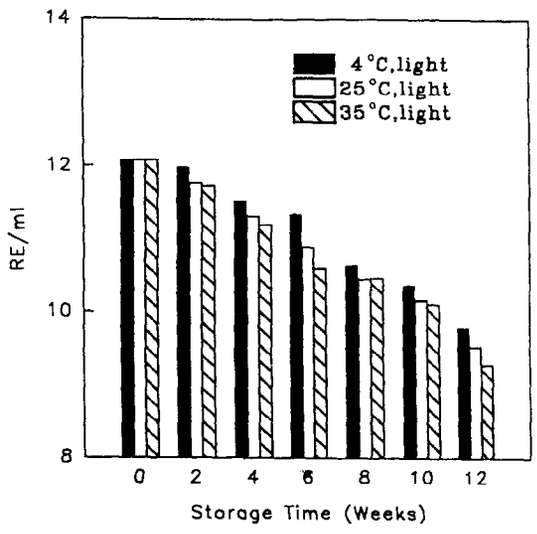

Fig. 8. Concentration changes of vitamin A in carrot juice under light storage at 4, 25 and 35 °C.

and β-carotene contents in the carrot juice under light and dark storage. Deitrich *et al.* (1977) pointed out that the stability of vitamin A during food processing and storage is a very complex phenomenon because of numerous factors such as that food contains metal ions, oxidants, antioxidants and other components. Also, the formation of a protein–carotenoid complex in the carrot juice may not only enhance vitamin A stability but also affect the bioavaibility of vitamin A in the diet (Bryant *et al.*, 1992).

In conclusion, the concentration changes of lutein, α-carotene, β-carotene and vitamin A in the carrot juice decreased with increasing storage temperature. Light storage can be more destructive to each carotenoid and vitamin A than dark storage. The 9-*cis* isomers were the major carotenoid isomers formed in carrot juice under light storage, while 13-*cis* was favoured under dark storage.

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