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Effect of Storage and Cooking on β-Carotene Isomers in Carrots (*Daucus carota* L. cv. 'Stefano')

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Carrots are one of the highest dietary sources of β-carotene and are naturally high in the (all-*E*)-β-carotene isomer, which has higher bioavailability, provitamin A activity, and antioxidant capacity compared to *Z* (*cis*) isomers. The objectives of the present study were to investigate the effects of storage temperature, time, and cooking (boiling for 15 min) on the levels of carotene isomers in 'Stefano' carrots. Storing carrots at either 4 °C to simulate long-term storage or 20 °C to simulate marketing practices resulted in increases in (all-*E*)-β-carotene of 20.3% after 3 days at 4 °C and 34.4% after 14 days at 20 °C, respectively. The levels of *Z* isomers in raw carrots were low with (13*Z*)-β-carotene and (9*Z*)-β-carotene accounting for less that 1.8% of the total β-carotene present. Levels of (9*Z*)-β-carotene decreased during storage at either temperature, whereas storage at 4 °C resulted in a 109% increase in (13*Z*)-β-carotene after 56 days. Cooking significantly increased the levels of (13*Z*)-β-carotene and (9*Z*)-β-carotene and resulted in the production of (15*Z*)-β-carotene, which was absent in raw carrots. Storage at 4 °C for 15 days or more prior to cooking reduced the susceptibility of (all-*E*)-β-carotene to thermal isomerization during cooking, resulting in lower levels of all three *Z*-β-carotene isomers being generated, while storage at 20 °C for up to 21 days resulted in significantly higher levels of (all-*E*)-β-carotene before and after cooking but had no effect on *Z*-isomer production during cooking. Consequently, we conclude that, for the greatest health benefit, fresh carrots can be stored for up to 21 days at 20 °C or at 4 °C for up to 56 days without significant reduction in (all-*E*)-β-carotene and should be consumed raw or boiled for less than 15 min to limit *Z*-β-carotene isomer formation.

KEYWORDS: (all-*E*)-β-Carotene; (9*Z*)-β-carotene; (13*Z*)-β-carotene; (15*Z*)- β-carotene; HPLC; storage; cooking

INTRODUCTION

Since the pioneering work of Zechmeister (*1*), carotenoids and their stereoisomers have been intensively studied. Carotenoids are a large group (> 600) of isoprenoid compounds found in a wide range of fruits and vegetables consumed by humans (*2*); details on structure can be found in Britton (*3*). Diets rich in carotenoids have been implicated in the protection against serious diseases, such as cancer (*4*), heart disease (*5*, *6*), and macular degeneration (*7*, *8*). Vitamin A is essential for human health and development, and certain plant-based carotenoids, principally β-carotene, are converted to vitamin A *in vivo* (*9*). β-Carotene possesses the largest provitamin A activity of any carotenoid, while the other principal precursors, α-carotene, α- and β-cryptoxanthin, γ-carotene, and β-zeacarotene have approximately 50% of the provitamin A activity of β-carotene (*2*, *10*). Orange carrots contain relatively high levels of β-carotene and are commonly consumed either raw or cooked (*11*).

β-Carotene in raw fruits and vegetables occurs only as the all-*E* (all-*trans*) isomer (*11*, *9*); however, processed forms can contain significant amounts of *Z* (*cis*) isomers, which are generated during cooking, juicing, or canning (*10*, *12*). Processing carrots can also result in a significant reduction in β-carotene content and resultant provitamin A activity, but the extent of degradation of all-*E* and concomitant *Z* isomerization varied depending upon the temperature (*12*, *13*). Isomerization occurs when temperatures are high enough to induce dissolution of the crystalline structure of β-carotene, i.e., > 100 °C (*13*, *14*).

The *Z* isomers of β-carotene (9*Z*, 13*Z*, and 15*Z*) possess lower provitamin A activity and bioavailability compared to all-*E* (*9*) and lower antioxidant capacity (*15*). Deming et al. (*16*) reported that (all-*E*)-β-carotene was more bioavailable than (9*Z*)-β-carotene and (13*Z*)-β-carotene in gerbils, while During et al. (*17*) reported that Caco-2 cells preferentially accumulated (all-*E*)- over (9*Z*)-β-carotene. Böhm et al. (*18*) found that (all-*E*)-β-carotene was a more potent antioxidant than (13*Z*)-β-carotene. Clearly, there are marked differences in the biological properties of the various β-carotene isomers and their subsequent impact on human health, and there is an increasing need to quantify their levels in foods and understand any effect common storage conditions and cooking may have. In this study, we investigate the effect of storage at 4 or 20 °C for 21 and 56 days, respectively, and cooking (15 min boil after storage) on the carotene isomer content of cv. 'Stefano' carrots.

MATERIALS AND METHODS

Reagents. (all-*E*)-β-Carotene was purchased from Sigma (Sydney, Australia). All other chemicals were reagent-grade and purchased from Merck (Victoria, Australia).

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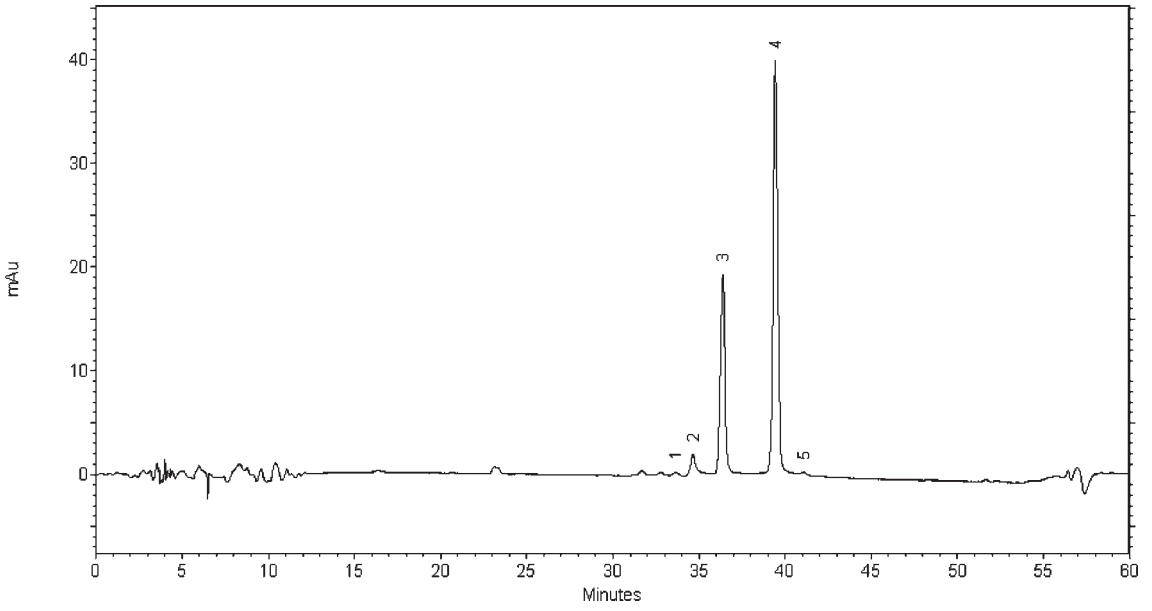

Figure 1. HPLC chromatogram showing carotene isomers in cooked carrots. Peak assignments: 1, (15*Z*)-β-carotene; 2, (13*Z*)-β-carotene; 3, (all-*E*)-α-carotene; 4, (all-*E*)-β-carotene; and 5, (9*Z*)-β-carotene.

Carrots. Freshly harvested carrots cv. 'Stefano' were collected from the wholesale markets and transferred to the laboratory at the Department of Primary Industries, Knoxfield, Victoria, Australia, and stored at 1 °C for 3 days prior to the commencement of storage and cooking. Carrots were harvested 5 days prior to collection and cooled at 4 °C.

Storage. Carrots ($n = 4$) were placed into zip-lock bags (255 × 205 mm) with one hole (6 mm) on each side to maintain a RH of approximately 90%. The bags were then placed in the dark at either 4 or 20 °C for 21 or 56 days, respectively. Upon removal, carrots were cut into 8 pieces (longitudinally in half and then each half into quarters) and frozen at −20 °C prior to analysis.

Cooking. Carrots were cut into 8 pieces (longitudinally in half and then each half into quarters), boiled for 15 min in 1 L of water, cooled in ice water, dried with a paper towel, cut further into 1 cm segments, and frozen at −20 °C prior to analysis.

Extraction and Analysis of Carotenoids. Carotenoid extraction was based on Lessin et al. (*11*). Carrots from each sample were chopped into pieces (as described above) and then mixed, and a representative subsample was used for carotenoid analysis. A total of 100 g of carrot was combined with 1 g of $CaCO_3$, 1 g of celite, and 100 mL of ultrafiltered water in a Warring blender and blended for 90 s. The homogenate was extracted with 100 mL of methanol and vacuum-filtered. The residue was scraped from the filter paper, re-extracted in acetone/hexane (1:1, v/v), and refiltered using the same filter paper. The process was repeated until no visible color remained in the retentate, and all carotenoids were then assumed to be extracted. Filtrates were combined in a separating funnel, and the aqueous layer was discarded. The hexane layer, containing the carotenoids, was washed several times with water and then passed through a column of anhydrous sodium sulfate to remove water. Volumes were adjusted to 250 mL with hexane, and samples were filtered into autosampler vials and flushed with a stream of nitrogen before being capped and analyzed by high-performance liquid chromatography (HPLC).

Chromatography. Carotenoid analysis was performed on a YMC C30 carotenoid column (5 μm, 250 × 4.6 mm, Waters Corporation) maintained at 30 °C, using a tertiary gradient of methanol, *tert*-butylmethylether (MTBE), and water, from ref *19*. The mobile-phase composition changed from 95% methanol, 5% MTBE, and 5% water at the time of injection to 95:5:0 at 12 min, 86:14:0 at 20 min, 75:25:0 at 30 min, and 50:50:0 at 50 min. The UV−vis spectra, from 200 to 650 nm, were collected, and carotenoids were quantified as (all-*E*)-β-carotene equivalents at 450 nm. *Z* isomers were identified by spectral characteristics, relative retention times, and *Q* ratios. Because *Z* isomer standards were not sourced commercially, these were quantified as all-*trans* equivalents.

Statistical Analysis. Each trial was set up as a randomized complete block design, with each block replicated 3 times at 20 °C and 4 times at 4 °C. Analysis of variance was used to determine the least significant difference (LSD, at $p = 0.05$) between mean values using GenStat 11.1.

RESULTS AND DISCUSSION

A typical chromatogram detailing separation of carotene isomers is shown in **Figure 1**. Clear baseline separation was achieved for all isomers. Retention times and spectral data are also presented in **Table 1** in a comparison to data published by Bohm et al. (*18*). Both the elution profile and retention times are similar to Marx et al. (*20*) and Bohm et al. (*18*), giving some degree of confidence in peak identification.

The effects of storing raw carrots at 4 °C for 56 days on the levels of (all-*E*)-α- and β-carotene are shown in **Figure 2**. Significant increases were found in both (all-*E*)-β-carotene (25%) and (all-*E*)-α-carotene (35%) during the first 3 days of storage. Subsequently, there was a significant decline in each carotenoid between 3 and 10 days, with levels being maintained thereafter for up to 56 days of storage. There are several accounts in the literature of increased carotenoid levels in cool-stored carrots. An early study by Brown (*21*) investigated the effects of cold storage (< 4 °C) on the carotene content of nine varieties of carrots and observed increases in β-carotene in all varieties over a 5−30 week period, while Lee (*22*) also observed a small increase in α- and β-carotene in carrots stored at 2 °C, reaching a maximum after 100 days. Similarly, Howard et al. (*23*) found a 10% increase in (all-*E*)-β-carotene in carrots over the first 2 weeks of a 3 week storage period at 4 °C, followed by similar declines to those observed in this study. Berger et al. (*24*) also reported increases in (all-*E*)-β-carotene in two varieties of carrots, when cooked after 7 days of storage at 4 °C, but both varieties showed substantial declines over the next 7 days of storage. Conversely, Kopas-Lane (*25*) found no significant increases in (all-*E*)-β-carotene in carrots stored under similar conditions for up to 12 days, and Koca and Karadeniz (*26*) did not observe any significant differences in α- or β-carotene in carrots stored for up to 6 months at 0 °C.

Retail display of carrots at the point of sale often occurs at temperatures between 18 and 22 °C. Carrots may be exposed to these temperatures for many days, and there are few reports on

Table 1. Retention Times, Spectral Data, and *Q* Ratio Data for Carotene Isomers from Cooked Carrots

peak	compound	retention time (min)	λ (nm, observed[a])				λ (nm, Bohm et al.[b])				*Q* ratio[c] (observed[a])	*Q* ratio[c] (Bohm et al.[b])
1	(15-*Z*)-β-carotene	33.6	338	423	451	473	338	424	449	474	0.44	0.497
2	(13-*Z*)-β-carotene	34.7	337	419	444	469	339	420	445	470	0.35	0.371
3	(all-*E*)-α-carotene	36.4	334	421	445	473	336	424	446	473	0.04	0.052
4	(all-*E*)-β-carotene	39.4	338	423	451	477		426	452	478	0.05	0.000
5	(9-*Z*)-β-carotene	41.1	339	421	446	473	340	422	447	473	0.10	0.094

[a] A gradient mobile phase of methanol/MTBE/water (from 95:5:5 to 50:50:0, v/v/v) was used. [b] Data were from Bohm et al.; gradient mobile phase of methanol/MTBE. [c] Ratio of absorbance intensity of the near-UV maximum (*Z* peak) to the absorption intensity at the main absorption maximum.

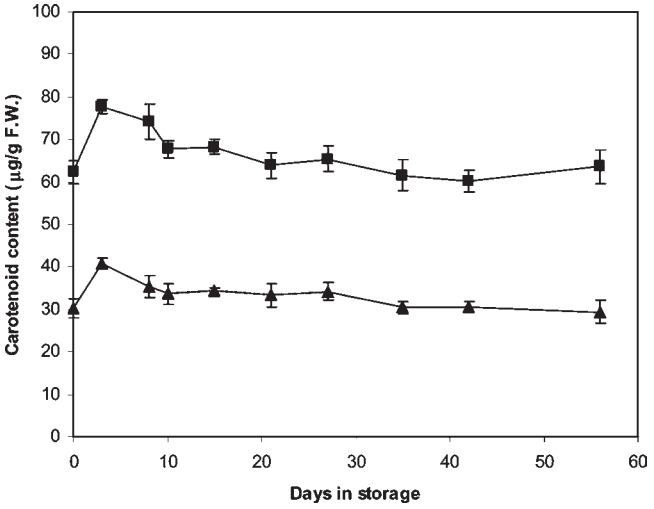

Figure 2. Effect of storing raw carrots in the dark at 4 °C for 56 days on (■) (all-*E*)-β-carotene and (▲) (all-*E*)-α-carotene. Error bars = standard error (SE).

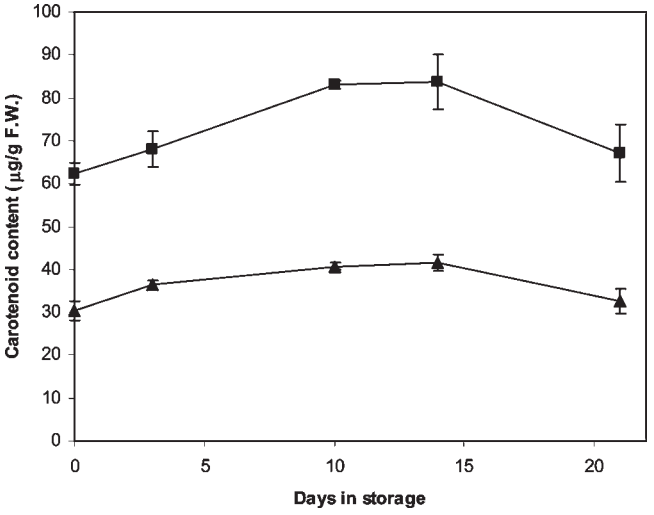

Figure 3. Effect of storing raw carrots in the dark at 20 °C for 21 days on (■) (all-*E*)-β-carotene and (▲) (all-*E*)-α-carotene. Error bars = SE.

the effects of storing carrots above 4 °C. **Figure 3** presents the effects of storage at 20 °C on (all-*E*)- α- and β-carotene levels over 21 days. Storage at 20 °C resulted in an increase in (all-*E*)-β-carotene of 34% and (all-*E*)-α-carotene of 42% during the first 10 days of storage. After 21 days of storage at 20 °C, however, the levels of (all-*E*)-α- and β-carotene had declined and were not significantly different to levels prior to the commencement of storage. Our results agree with the report by Berger et al. (*24*), who found a significant increase in (all-*E*)-β-carotene in both 'Nevis' and 'Kingston' carrot varieties stored at 20 °C for 7 days. From our results (**Figures 2** and **3**) and reports in the literature, either carotenoid synthesis appears to be occurring in carrots during storage or carotene extraction became more efficient after storage because of cell wall softening and membrane destabilization. Post-harvest carotogenesis has been demonstrated in many climacteric fruits (*27*), and Lee (*22*) reported increases in β-zeacarotene and γ-carotene and the appearance of phytoene and phytofluene in stored carrots, which coincided with storage induced increases in β-carotene. Alternatively, increases in carotene content during storage at 4 or 20 °C may be due to improved extractability of the carotenes after enzymatic degradation of macromolecular matrix compounds, as concluded by Berger et al. (*24*) and Marx et al. (*13*).

In raw carrot tissues, α- and β-carotene are predominately present in the all-*E* form (*9*). Quackenbush (*28*), however, found that raw carrots also contained 1.2% 13*Z*- and 0.4% (9*Z*)-β-carotene, while Kopas-Lane and Warthesen (*25*) reported 1% of total β-carotene was in the form of *Z* isomers. Lessin et al. (*11*), however, did not detect any *Z* isomers in fresh carrots or sweet potato. In our investigation, (9*Z*)-β-carotene and (13*Z*)-β-carotene were detected in raw unstored carrots and accounted for approximately 1% of the β-carotene present (**Figure 3**), in agreement with Kopas-Lane and Warthesen (*25*). It is possible, however, that the small quantities of *Z* isomers detected in raw carrots could be due to isomerization during extraction (*2*).

To investigate the effect of typical domestic handling and cooking conditions, carrots were stored at either 4 °C for 56 days or at 20 °C for 21 days and then boiled for 15 min (**Table 2**). In raw carrots, there was a transient increase in (9*Z*)-β-carotene during storage at 4 °C, with significantly higher levels observed on day 3. However, levels of (9*Z*)-β-carotene did not change significantly in carrots during storage at 20 °C. A significant increase was also observed in the level of (13*Z*)-β-carotene after 27, 42, and 56 days of storage at 4 °C, whereas levels declined significantly after 10 days at 20 °C.

Boiling carrots for 15 min resulted in a decrease in all-*E*-β-carotene, significant increases in both (13*Z*)- and (9*Z*)-β-carotene, and the appearance of (15*Z*)-β-carotene, which was not detected in raw carrots (**Table 2**). (13*Z*)-β-Carotene was the predominant *Z* isomer after cooking followed by (15*Z*)-β-carotene, with (9*Z*)-β-carotene being the least abundant isomer. In unstored carrots, cooking resulted in a > 10-fold increase in (13*Z*)- and an almost 2-fold increase in (9*Z*)-β-carotene. After storage at 20 °C, cooking resulted in approximately 9% of (all-*E*)-β-carotene being converted to (13*Z*)-β-carotene, approximately 2% to (15*Z*)-β-carotene, and less than 0.5% to (9*Z*)-β-carotene, regardless of the duration of storage (data not shown). Storage time at 20 °C had no significant effect on *Z* isomerization from all-*E*-β-carotene, and the decline in 13*Z* seen in raw carrots stored at 20 °C was not reflected after cooking. Storage time at 4 °C, however, had a significant effect on the proportion of (all-*E*)-β-carotene converted to *Z* isomers (**Figure 4**). Cooking of unstored carrots resulted in 7.7% of (all-*E*)-β-carotene being converted to (13*Z*)-β-carotene, 1.7% to (15*Z*)-β-carotene, and 0.4% to (9*Z*)-β-carotene. These proportions declined significantly with increasing storage time at 4 °C, with significant decline in 13*Z* and 15*Z* production during cooking after 15 days at 4 °C and

Table 2. Carotene Isomer Content (μg/g FW) in Raw or Cooked Carrots Stored in the Dark at Either 4 or 20 °C[a]

	(15*Z*)-β-carotene		(9*Z*)-β-carotene		(13*Z*)-β-carotene		(all-*E*)-β-carotene	
	raw	cooked	raw	cooked	raw	cooked	raw	cooked
0 days at 20 °C	ND	1.03	0.29	0.54	0.55	5.75	62.28	59.18
3 days at 20 °C	ND	1.32	0.34	0.75	0.49	7.12	68.50	64.05
10 days at 20 °C	ND	1.59	0.25	0.64	0.33	8.02	82.96	79.16
14 days at 20 °C	ND	1.46	0.22	0.50	0.35	7.83	83.68	78.24
21 days at 20 °C	ND	1.27	0.18	0.33	0.32	6.86	66.00	68.22
LSD (p = 0.05)		NS	NS	NS	0.13	NS	12.35	8.81
0 days at 4 °C	ND	1.03	0.29	0.54	0.56	5.75	61.69	59.18
3 days at 4 °C	ND	1.20	0.49	0.85	0.49	6.09	77.73	73.84
8 days at 4 °C	ND	0.87	0.27	0.46	0.39	4.54	74.21	81.09
10 days at 4 °C	ND	0.76	0.35	0.36	0.59	4.47	67.71	66.06
15 days at 4 °C	ND	0.69	0.15	0.26	0.42	3.86	67.72	64.95
21 days at 4 °C	ND	0.68	0.19	0.27	0.60	3.09	63.92	63.37
27 days at 4 °C	ND	0.54	0.26	0.37	0.92	3.27	65.33	64.84
35 days at 4 °C	ND	0.52	0.21	0.44	0.84	2.52	61.51	62.32
42 days at 4 °C	ND	0.49	0.25	0.38	1.23	3.51	60.12	61.82
56 days at 4 °C	ND	0.40	0.29	0.30	1.17	2.80	63.55	57.54
LSD (p = 0.05)		0.28	0.14	0.15	0.31	1.68	8.81	9.36

[a] Cooked carrots were boiled for 15 min after indicated storage times. LSDs are shown at p = 0.05. ND = not detected. NS = not significant.

a decline in 9*Z* after 10 days. After 56 days of storage at 4 °C, cooking resulted in 2.6% of the (all-*E*)-β-carotene being converted to (13*Z*)-β-carotene, 0.6% to (15*Z*)-β-carotene, and 0.02% to (9*Z*)-β-carotene. While the bioavailability of the *Z* isomer is considered less than that of the all-*E* form (*8*), the amount of (13*Z*)-β-carotene relative to (all-*E*)-β-carotene was approximately 1.8% after 56 days at 4 °C and it is unlikely that the observed increase would have a significant impact on the overall bioavailability of β-carotene in stored carrots. Storage at 4 °C for >10 days, therefore, significantly reduced the proportion of *Z* isomers formed during cooking. Thus, storing carrots may enhance the bioavailability of β-carotene, provided that the carrots are boiled before consumption.

Dietz et al. (*29*) reported a 40% decrease in total β-carotene after boiling carrots for 30 min, while steaming did not affect carotene content significantly. On the other hand, low-pressure boiling for 21 min was determined by Sant'Ana et al. (*12*) to be the cooking method that resulted in the best retention of carotenoids and resultant provitamin A activity compared to steaming and high-pressure boiling. Levels of *Z* isomers in raw carrots reported in the literature are very low, with reports generally 0−2% of the total β-carotene (*11*, *30*). Cooked carrots, however, are reported to possess significantly higher levels of *Z*-β-carotene isomers (*11*) in part because of the thermal isomerization of (all-*E*) isomers (*31*). Our results agree with Chen et al. (*32*), who investigated the effects of thermal processing on β-carotene in carrot juice and reported that, under canning conditions of 121 °C for 30 min, (13*Z*)-β-carotene was the major *Z* isomer present, followed by (9*Z*)- and (15*Z*)-β-carotene. Conversely, microwave cooking (9 min at 700 W) had no significant effect on (all-*E*)-β-carotene content in fresh carrots (*23*), while steam blanching also had no effect (*33*).

Von Doering et al. (*14*) concluded that thermal treatment of (all-*E*)-β-carotene below 100 °C favored the formation of (13*Z*)- and (15*Z*)-β-carotene, whereas (9*Z*)-β-carotene production was favored in temperatures above 100 °C. Supporting this hypothesis, Lessin et al. (*11*) reported a 33% increase in *Z* isomers in carrots after canning at 121 °C, with (9*Z*)-β-carotene the predominant isomer followed by (13*Z*)-β-carotene. The relatively gentle processing conditions employed in the present study (boiling for 15 min), however, may account for the formation of higher levels of (15*Z*)-β-carotene relative to (9*Z*)-β-carotene. Cooking per se may also result in greater carotenoid extraction because of the denaturing of carotene-binding proteins, which may raise all carotene values after cooking (*34*).

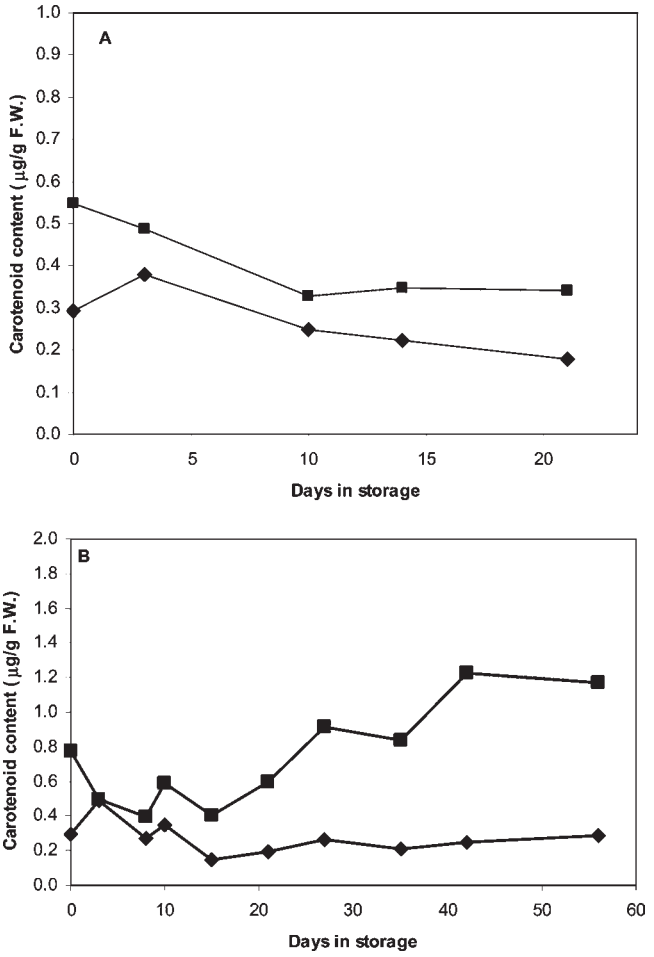

Figure 4. Effect of storing raw carrots at (A) 20 °C on (■) (13*Z*)-β-carotene ($LSD_{(p = 0.05)}$ = 0.13) and (◆) (9*Z*)-β-carotene ($LSD_{(p = 0.05)}$ = not significant) or (B) 4 °C on (■) (13*Z*)-β-carotene ($LSD_{(p = 0.05)}$ = 0.31) and (◆) (9*Z*)-β-carotene ($LSD_{(p = 0.05)}$ = 0.14).

In conclusion, carrots appear to be an ideal vehicle for the delivery of provitamin A via (all-*E*)-β-carotene. Carrots are one of the highest plant sources of β-carotene, with very little if any occurring as the less bioavailable *Z* isomers both before and after boiling for 15 min. Carrots store extremely well, with storage both short term at room temperature and long term at low temperature, resulting in increased β-carotene content. Boiling carrots for 15 min generated relatively low levels of *Z* isomers, and carrots stored for extended periods at reduced temperatures retain even higher levels of (all-*E*)-β-carotene upon cooking.

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