Stability and Nutrient Contribution of β-Carotene Added to Selected Bakery Products[1]

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ABSTRACT

Three bakery products—yellow cake, sugar cookies, and bagels—were prepared with β-carotene added to the formula shortening. Products were sampled at various intervals during processing and during storage typifying their normal shelf life. An extraction method, using minimal equipment and ambient temperature, preceded high-performance liquid chromatography carotene characterization. For all products, prebaking processing steps had no adverse effect on the stability or isomeric distribution of carotene. Carotene losses during baking ranged from ~20% in bagels and cake to ~30% in cookies. During baking, the all-*trans* isomer was reduced from 91% in the spiked shortening to 85, 77, and 74% in bagels, cake, and cookies, respectively. No significant additional losses or isomeric transformations occurred during the typical shelf life of the products. A serving of each product provided about 1 mg of β-carotene.

Natural carotenoids in foods are important sources of vitamin A activity. β-Carotene is the most significant of these carotenoids. Both *cis* and *trans* isomers of β-carotene provide vitamin A activity; however, the highest activity is derived from the all-*trans* isomer (Beecher and Khachik 1984, NRC 1989). In the American diet, about one-third of the total vitamin A activity is derived from carotenoids (NRC 1989). Carotenoids, unlike vitamin A, are nontoxic at high intake levels. They are also potent antioxidant agents and, thus, may play a significant role in cancer prevention (Connett et al 1989).

Carotenoids, including β-carotene, undergo *trans*-to-*cis* isomerism. The 9-*cis* and 13-*cis* isomers are the major new forms produced when *trans*-carotenoids are exposed to light or heat (as during food processing) (Sweeney and Marsh 1971; Bushway 1986; Khachik et al 1986, 1989; Chandler and Schwartz 1987, 1988; Khachik and Beecher 1987; Park 1987; Mejia et al 1988; Speek et al 1988).

Beta carotene is only a trace constituent in wheat and in most other grains (Heinonen et al 1989). Therefore, bakery foods, unless they contain butter, margarine, or eggs, are insignificant sources of β-carotene. Synthetic β-carotene, commercially introduced in the United States in the mid 1950s, can now be added to foods, including bakery foods, to provide colors typical of butter, margarine, and eggs. This addition would also increase the intake of β-carotene, which, according to current estimates, is less than 2 mg per day (LaBell 1988). A carotene intake of 5–6 mg per day is recommended for both its provitamin A activity and its antioxidant potential (LaBell 1988). Adding β-carotene to bakery foods cannot achieve this goal unless the additive remains reasonably stable as an essentially all-*trans* isomer in the fortified products. This study was undertaken to examine that stability.

MATERIALS AND METHODS

Carotene

A food-grade, heat-stable form of β-carotene (22% oil, Roche code: 65644-0003) was obtained from Hoffman-La Roche (Nutley, NJ). At levels normally used, β-carotene provides a rich, yellow color to products. As analyzed, this carotene contained 91.1% all-*trans* and 8.9% *cis* isomers (Table I). Although *cis* isomers show lower provitamin A activity than *trans* isomers do (Beecher and Khachik 1984), their antioxidant properties presumably remain unaltered. Carotene, premixed with shortening (spiked shortening), was added to each product at levels calculated to result in about 1 mg of β-carotene per serving.

Product Formulation, Preparation, and Sampling

Three products were made—yellow layer cake, sugar cookies, and bagels. Typical in-house formulas were used; the ingredients are expressed as true percentages in Table II, but they can easily be converted into baker's percentage (flour = 100%). Carotene-fortified products were prepared with spiked shortening and control products with unspiked shortening. Product sampling protocols were followed to pinpoint gradual as well as potentially sudden changes in carotene stability, both during processing and during the typical shelf life of the product.

Cakes were prepared using cake flour (General Mills, Minneapolis, MN), emulsified shortening (Bunge, Bradley, IL), and mono- and diglyceride emulsifiers (Mallot and Co., Carnegie, PA). All dry ingredients were blended first for 1 min on low speed in a Hobart N-50 mixer with a paddle (Hobart Corp., Troy, OH). Other ingredients, including part of the water (14.89%), were added and mixed 1 min on low speed and 3 min on medium speed. More water (7.15%) was added. The batter was mixed for 1 min on low speed and 2 min on medium speed. Finally, the remaining water (11.32%) was added. The batter mixed again for 2 min on low speed. Batter (400 g) was scaled into 8-inch, round, cake pans and baked 33 min at 182.2°C. Samples were taken after the second stage of mixing (half mixed, 5 min), at the end of mixing, and after baking and cooling. Baked cakes were stored for 1, 3, or 6 days before sampling for storage stability.

TABLE I
Distribution of β-Carotene Isomers in Spiked Shortening

Isomer	Amount (% of total)
All-*trans*	91.1
13-*cis*	5.3
9-*cis*	3.6

TABLE II
Product Formulas

Ingredient	Amount, %		
	Yellow Cake	Sugar Cookies	Bagel
Flour	23.82	45.50	62.64
Sugar	28.59	22.75	1.88
Shortening	7.15	20.47	1.88
Nonfat dry milk	1.79	2.27	...
Salt	0.71	0.45	1.25
Compressed yeast	...	...	0.94
Dried whole eggs	2.39	...	...
Baking powder	1.49	0.45	...
Baking soda	...	0.37	...
Emulsifiers	0.60	...	...
Flavor	0.12	...	...
Calcium propionate	...	...	0.08
Water/ice	33.36	7.73	31.32
Total	100.02	99.99	99.99

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Cookies were made using pastry flour (General Mills) and all-purpose shortening (Bunge). Sugar and shortening were creamed for 2 min on low speed while the mixer bowl was frequently scraped. Additional ingredients were added and mixed for 2 min at low speed. Dough was rolled 3/16 in. thick, cut with a 3-in. diameter cookie cutter, and baked 14 min at 193.3°C. Samples were taken at the end of creaming, at the end of mixing, after rolling and cutting, and after baking and cooling. Baked cookies were stored for 1, 7, 14, 21, or 28 days before being sampled for storage stability.

Bagels were made using high-protein bread flour (General Mills) and all-purpose shortening (Bunge). All ingredients were mixed with an XTS-20 spiral bench mixer (Excelsior Industrial Equipment, Paramus, NJ) for 11 min. Coming from the mixer at 28.9°C, dough (90 g) was shaped in a bagel former (BMD Inc., Bohemia, NY), proofed for 25 min at 43.3°C and 75% rh, boiled 2 min per side in 98.3°C water, and baked 20 min at 232.2°C. Samples were taken after 5 min of mixing (half mixed), at the end of mixing, at the end of proofing, after boiling, and after baking and cooling. Baked bagels were stored for 1, 3, 6, or 9 days before being sampled for storage stability.

All baked samples were stored at room temperature (25 ± 1°C), either in a dark cabinet or on top of the bench, exposed to fluorescent light and incidental sunlight. At appropriate times, samples were frozen and held at −20°C until analyzed (within eight weeks). The bagel dough samples were freeze-dried before analysis. Freezing appears to have no adverse effect on either total or isomeric β-carotene content (Bushway and Wilson 1982; Broich et al 1983; Park 1987; Chandler and Schwartz 1988; Craft et al 1988, 1993).

Carotene Methodology (Analytical)

The official AACC (1983) and AOAC (1990) methods for extracting carotene from food and feed materials include hot saponification or overnight, cold saponification. The AACC method also requires multiple extractions and repeated washings. Several studies have been conducted to develop a rapid method of extracting carotene. One such method (Livingston 1986) was used in the current study. This method uses minimal equipment and ambient-temperature stirring techniques, thereby avoiding possible losses caused by oxidation and heat isomerization or by transfer error. The column (polymeric C_{18}) and mobile-phase solvent (100% methanol; Milne and Botnen 1986) used in the high-performance liquid chromatography (HPLC) system were selected to improve carotene recovery and selectivity (Epler et al 1992). In all steps, care was taken to minimize exposure of samples to oxygen, light, heat, and acid. All containers were briefly flushed with nitrogen and kept covered.

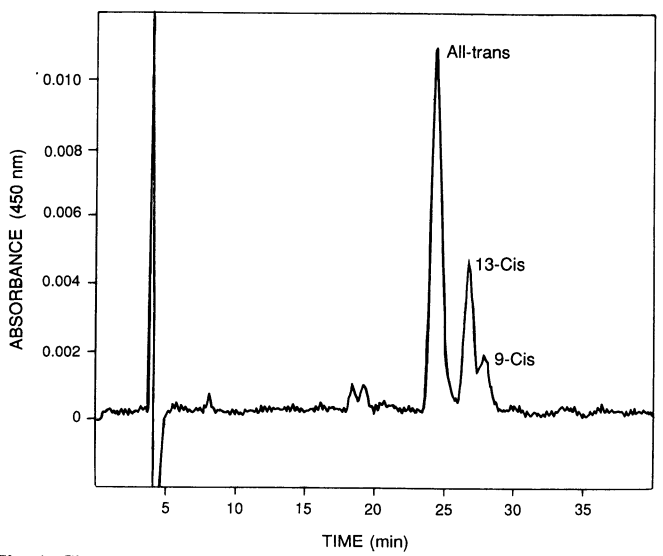

Fig. 1. Chromatogram of β-carotene extracted from fresh-baked cookie. Peaks were identified as all-*trans* (24.3 min), 13-*cis* (26.7 min), and 9-*cis* (27.8 min).

Frozen cake batters (2 g) were weighed into volumetric flasks and diluted with 1–3 ml of water before extraction. The following procedure was used, with the exception that no water was added in later steps to these samples. All other frozen samples were homogenized in a blender before extraction. Aliquots (2 g) were weighed into volumetric flasks and solubilized in 23 ml of extractant (hexane, acetone, ethanol, 10:7:6 with 0.00087% w/v butylated hydroxytoluene) by swirling for 1 min before 1 hr of saponification with 4 ml of KOH in methanol (40%, w/v). Water (1 ml) was added, and samples were stirred 30 min. A mixture of toluene and hexane (37 ml, 7:30) was added, and samples were stirred for 6 min. The samples were brought to 100-ml volume with 10% sodium sulfate (w/v, in water), shaken vigorously for 3 min, and allowed to stand for 1 hr. Aliquots (generally 3 ml) were taken from the clear upper phase and placed into an auto sampler vial. They were evaporated in a nitrogen stream and redissolved in a measured amount of ethanol. All reagents used were HPLC-grade.

Trans and *cis* isomers of β-carotene were determined by HPLC. The system (Waters, Milford, MA) consisted of the following components: model 600 pump, model 600E controller, WISP 700 automatic sample injector, model 991 photodiode array detector, and associated IBM-compatible computer. The HPLC column (Vydac 201TP54, C_{18} polymeric column without end-capping) was operated at room temperature (26 ± 1°C). The mobile phase was isocratic methanol at a flow rate of 0.7 ml/min. Injections of 20 μl were used. The wavelengths for detection were 190–600 nm. The elution time for all-*trans* β-carotene was 23.5 min at 26°C. Single extractions were run on at least two separate days; duplicate HPLC determinations were made of each extraction.

Concentrations of the standard stock solutions in ethanol were determined by measuring the absorbance at 453 nm with a Beckman recording spectrophotometer. The absorptivity constant was:

$$E^{1\%}_{1cm} = 2{,}620 \text{ (DeRitter 1981).}$$

Peak identification was based on absorption spectra obtained from photodiode array data, Q-ratios, and retention times. Figure 1 illustrates the chromatogram of the cookie extract. Cake and bagel extracts were similar. Current data were compared with published results (Jenson et al 1982, Tsukida et al 1982, Quackenbush 1987, Craft et al 1990, O'Neil et al 1991).

RESULTS AND DISCUSSION

Carotene Stability

No detectable amounts of β-carotene were found in the control (prepared with unspiked shortening) products, including the yellow cake that contained dried eggs. For all three carotene-spiked products, prebaking processing steps had little or no adverse effect on the stability or isomeric distribution of added

TABLE III
Retention of Added β-Carotene in Cake[a]

Batter/Cake	Amount Retained (ppm)[b]	Amount Retained (% of added)	Isomers (% of total)		
			All-*trans*	13-*cis*	9-*cis*
Half mixed	29.2 ± 1.0	101.4	92.0	4.8	3.2
Fully mixed	29.2 ± 1.0	101.4	93.1	4.2	2.6
Fresh baked	23.4 ± 1.2	81.3	77.2	19.1	3.7
Dark storage					
1 day	22.0 ± 0.4	76.4	76.1	19.9	4.0
3 day	23.1 ± 0.8	80.2	76.7	19.9	3.4
6 day	23.0 ± 0.7	79.9	77.7	19.2	3.1
Light storage					
1 day	23.5 ± 1.3	81.6	76.5	19.7	3.8
3 day	23.3 ± 0.6	80.9	77.9	18.5	3.5
6 day	23.7 ± 1.2	82.3	78.5	17.6	3.9

[a]Expressed on dry basis.
[b]Amount added (as spiked shortening): 28.8 ± 1.0 ppm. Spiked shortening contained 254.6 ± 9.1 ppm.

carotene (Tables III–V). This changed, however, when the products were baked. For bagels, the baking losses were about 20%, slightly more than the 10–15% losses reported by Gordon and Bauernfeind (1982) for yeast-raised products. Carotene losses during baking were the highest for cookies (30%). This was either because of the product characteristics (more surface area) or because the cookies contained a higher concentration of added carotene. Bauernfeind et al (1958) and Bunnell et al (1958) reported 74–95% retention of total β-carotene in cookies, pie crust, and yellow cakes. Gordon et al (1985) reported in another study that, when stability was based only on color retention, β-carotene was stable in yellow cakes, sugar cookies, and yeast-raised sweet dough.

TABLE IV
Retention of Added β-Carotene in Cookies[a]

Dough/Cookie	Amount Retained (ppm)[b]	Amount Retained (% of added)	Isomers (% of total)		
			All-*trans*	13-*cis*	9-*cis*
Creamed	49.8 ± 0.9	97.8	90.7	5.5	3.8
Mixed	50.2 ± 2.2	98.6	91.6	5.2	3.2
Rolled	49.5 ± 0.9	97.2	91.9	4.6	3.5
Fresh baked	36.3 ± 0.7	71.3	71.5	22.5	6.0
Dark storage					
1 day	35.4 ± 0.7	69.5	70.8	21.5	7.7
7 day	36.7 ± 0.8	72.1	73.2	20.0	6.8
14 day	36.6 ± 0.3	71.9	74.0	20.0	6.0
21 day	36.2 ± 1.1	71.1	74.2	18.5	7.4
28 day	35.0 ± 1.3	68.8	75.0	18.2	6.8
Light storage					
1 day	36.7 ± 0.5	72.1	71.7	19.6	8.7
7 day	37.1 ± 0.5	72.9	74.7	18.7	6.6
14 day	38.4 ± 1.3	75.4	75.9	16.5	7.6
21 day	37.5 ± 0.9	73.7	78.6	13.6	7.9
28 day	36.2 ± 0.9	71.1	79.1	13.2	7.7

[a]Expressed on dry basis.
[b]Amount added (as spiked shortening): 50.9 ± 2.5 ppm. Spiked shortening contained 215.3 ± 10.5 ppm.

TABLE V
Retention of Added β-Carotene in Bagels[a]

Dough/Bagel	Amount Retained (ppm)[b]	Amount Retained (% of added)	Isomers (% of total)		
			All-*trans*	13-*cis*	9-*cis*
Half mixed	34.5 ± 0.3	100.6	91.7	5.2	3.1
Mixed	32.5 ± 0.6	94.8	92.2	5.0	2.8
Proofed	32.9 ± 0.5	95.9	91.2	5.8	3.0
Boiled	30.4 ± 0.9	88.6	88.3	7.8	3.8
Fresh baked	27.8 ± 1.0	81.0	84.7	10.4	4.9
Dark storage					
1 day	27.1 ± 1.0	79.0	84.3	10.9	4.8
3 day	28.5 ± 1.4	83.1	86.1	9.9	4.0
6 day	26.7 ± 0.9	77.8	85.8	10.0	4.2
9 day	23.9 ± 0.6	69.7	85.3	10.2	4.5
Light storage					
1 day	28.5 ± 1.5	83.1	86.1	9.6	4.3
3 day	28.1 ± 1.3	81.9	86.0	9.7	4.3
6 day	27.1 ± 0.8	79.0	86.6	9.2	4.2
9 day	25.6 ± 1.6	74.6	85.7	9.4	5.0

[a]Expressed on dry basis.
[b]Amount added (as spiked shortening): 34.3 ± 1.0 ppm. Spiked shortening contained 1092.1 ± 32.1 ppm.

TABLE VI
Contribution of β-Carotene from Products Tested[a]

Product[b]	Serving Size (oz)	Carotene (mg)
Cake	2.5	1.2
Cookie	1.0	1.0
Bagel	2.0	1.0

[a]Based on the average of all values after baking and storage.
[b]Based on products as consumed. Moisture content: cake, 25%; cookies, 5%; and bagel, 33%.

Baking also shifted the isomeric distribution of carotene. This shift was most noticeable in cakes and cookies; a 15–20% decrease in all-*trans* isomer followed an increase of a similar magnitude in 13-*cis* isomer. Such shifts were of lesser magnitude in bagels. A notable increase in 9-*cis* isomer content occurred in cookies. The combination of product characteristics (large surface area and low final moisture content) results in cookies being the most dehydrated of the baked products studied. The degree of isomeric shift may be related to earlier studies (Livingston et al 1966, 1968; Park 1987; Speek et al 1988), which have shown that dehydration of plant tissue results in severe losses of β-carotene or shifts to 9-*cis* and 13-*cis* forms.

The anticipation that additional losses or unfavorable shifts in isomeric distribution of carotene may occur during the typical shelf life of these products appeared unfounded. This was true throughout the storage period of the products, irrespective of the light condition. The current study is the first to monitor isomer formation in baked products.

Nutrient Contribution of Added Carotene

As no further apparent loss occurred in carotene after baking, all values obtained after baking were averaged to arrive at typical carotene contents of each product: 2.31 mg (cake), 3.65 mg (cookies), and 2.69 mg (bagels) per 100 g of the product on a dry basis; and 1.73 mg (cake), 3.47 mg (cookies), and 1.80 mg (bagels) per 100 g of the product on an as-consumed basis. Each serving of these products would provide about 1 mg of carotene (Table VI). Collectively, consumption of all three products would provide 3 mg of carotene; that, combined with the current estimated intakes, would achieve the goal of 5–6 mg carotene in the daily diet.

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