Stability and Contribution of Beta Carotene Added to Whole Wheat Bread and Crackers

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ABSTRACT

Whole wheat bread and crackers were fortified with beta carotene using three pure (all-*trans*) sources: one water-soluble and two oil-soluble. Two sources contained added antioxidants. Carotene in both bakery products was highly stable during the prebaking steps and also during the typical market shelf life of the products. However, carotene losses were appreciable during baking, ranging from 4–15% for bread products to 18–23% for crackers. Beta carotene sources with added antioxidants generally exhibited greater carotene stability, but only during baking and not during product storage. Baking caused a steric shift in carotene, with *cis* isomers averaging ~18% in bread products and ~34% in crackers. A serving of each product would provide over 500 IU of vitamin A activity (can be labeled a "good source" of vitamin A) and nearly 0.4 mg of carotene.

Antioxidant vitamins, reported to curb damaging oxidative reactions in the body, are increasingly viewed as a significant factor in reducing the risk of degenerative diseases such as cancer and cardiovascular disease (Temple and Basu 1988, Ziegler 1989, Jialal et al 1991, Byers and Perry 1992). Vitamins E and C, and beta carotene are good sources of antioxidants in food. Beta carotene is also a potent source of provitamin A activity.

In wheat and most other grains, beta carotene is present in only a trace amount (Heinonen et al 1989). Thus, it is only through fortification that grain-based foods, a dietary staple, can become a source of carotene in our diet. Beta carotene can be added as a pure source or through the use of ingredients such as butter and eggs. At levels normally used in grain-based foods, carotene-containing ingredients, however, may not contribute significant amounts of beta carotene to our diet, especially when daily intakes of 15–20 mg, which are several-fold higher than the current intake, are now suggested for the beneficial antioxidant effect (FDA 1993).

As a food-grade ingredient, beta carotene has traditionally played a role as a food colorant. To the consumer, the imparted yellow color, however, may imply that the products contain ingredients such as eggs and butter whose consumption is advised in moderation. Among bakery products, the coloring effect would be most obvious for products made with white flour. Such would usually not be the case when carotene is added to products made with whole wheat flour. Further, adding beta carotene to whole wheat products can accentuate the already impressive nutritional profile of these products, provided that the added carotene remains reasonably stable. This study focused on two whole wheat products (bread and crackers) and assessed the stability characteristics of beta carotene added to those products.

MATERIALS AND METHODS

Carotene Sources

Three food-grade carotene sources (one water-soluble and two oil-soluble) were used to fortify bread and crackers. All three sources were obtained from Hoffman-La Roche (Nutley, NJ). Beta carotene was present in these sources virtually as the all-*trans* isomer (Table I).

Level of Addition

In preliminary studies, whole wheat bread was made to contain 0.0, 0.25, 0.5, or 1.0 mg of carotene per serving (i.e., a 1-oz slice). The highest level imparted a slight yellow tinge to the product. To minimize this coloring effect, the level eventually used in the comprehensive study was 0.5 mg. The same level was used to fortify crackers where it imparted a slight, but appealing, yellow tinge.

As analyzed, carotene values in the sources used showed slight variations from label values. Additions were made based on label values, but carotene stability was assessed based on initial content in fully mixed doughs. Carotene was added to the dough premixed with formula water (spiked water) or formula shortening (spiked shortening).

Product Making and Sampling

Whole wheat bread was made by the sponge-and-dough method using an in-house formula (Table II). After a 2.5-hr sponge fer-

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TABLE I
Beta Carotene Sources Tested

Source	Antioxidants	Isomeric Distribution (% of total)		
		All-*trans*	13-*cis*	9-*cis*
Dry beadlets, 10% (water dispersible)	Alpha tocopherol, ascorbyl palmitate	98.8	1.0	0.2
Carotene in oil, 22%	Alpha tocopherol, ascorbyl palmitate	99.6	0.3	0.1
Carotene in oil, 30%	None	98.8	0.8	0.4

TABLE II
Bread and Cracker Formulas (Baker's Percent)

Ingredient	Bread Sponge	Bread Dough	Crackers
Whole wheat flour	70	30	50
Cookie flour	...	...	50
Vital wheat gluten	5	...	...
Mineral yeast food	0.5	...	...
Compressed yeast	2.5	...	...
Granulated sugar	...	8.5	7
Dextrose	...	...	1
Nonfat dry milk	...	3	...
Dry whey	...	...	1.5
Salt	...	2	1
Calcium propionate	...	0.2	...
All-purpose shortening	...	3	10
Sodium bicarbonate	...	...	0.75
Monocalcium phosphate	...	...	1.3
Ammonium bicarbonate	...	...	1
Sodium sulfite	...	...	0.04
Water	47	...	28
Water plus ice	...	18.5	...

mentation (76° F), dough was mixed to full gluten development (81° F), rested for 10 min at ambient temperature, scaled (526 g per loaf), pan proofed (110° F) to 100 mm height, baked for 19 min (435° F), cooled for 1 hr and stored at 75° F in polyethylene bread bags. Crackers were made with a 1:1 blend of whole wheat and cookie flours (Table II) by mixing all ingredients for 10 min, sheeting the dough to 1 mm thickness and baking rotary cut crackers for 6–7 min in a tunnel oven. Temperatures in the three heat zones of the oven, top and bottom, respectively, were: 440° F and 490° F (zone 1), 475° F and 460° F (zone 2), and 435° F and 465° F (zone 3).

Product sampling protocol was followed to pinpoint changes in carotene stability during product making and storage. Bread doughs were sampled both after full mixing and after pan proofing. Breads were sampled at 0, 1, 3, 5, and 7 days after baking. Cracker doughs were sampled after full mixing and after sheeting. Baked crackers were sampled at 0, 30, 60, and 90 days after baking. Both products were stored (bagged in polyethylene bags) in a lighted room (crackers in boxes), with temperature maintained at 25° C. Sampled products were frozen and held at −20° C until analyzed. The dough samples were freeze-dried overnight before analysis. Freezing, at least for short periods, appears to have no adverse effect on carotene stability (Park 1987).

Carotene Analysis

The procedure followed to determine beta carotene (*trans* and *cis* isomers) in spiked materials, in doughs, and in finished products was the same as described in our earlier study (Rogers et al 1993). Essentially, the product samples were thoroughly homogenized, suitable aliquots taken, extracted with appropriate solvents, saponified, more solvents added, brought to volume, shaken, and allowed to stand. Total extraction time was 160 min at room temperature. Aliquots were taken from the clear upper phase, evaporated in a nitrogen stream, redissolved in ethanol, and the amount of beta carotene (*trans* and *cis* isomers) was determined by high-performance liquid chromatography.

RESULTS AND DISCUSSION

Bakery Products and Quality

Breads tested were a 100% whole wheat product. Good quality crackers, however, could not be made with all whole wheat flour. Hence, half of the wheat flour was replaced with cookie flour in the cracker formula (Table II). Baked products were not scored, but a limited evaluation suggested no adverse effect of carotene on product quality, including flavor. When replacing yellow colors with carotene, Bauernfeind (1981) also reported no alteration in texture, volume, or organoleptic characteristics of baked goods.

Carotene Sources

An oil-soluble carotene source is recommended for products where it may be desirable to color the fat phase. However, where a variety of baked products (some containing little or no fat) are produced in a plant, a water-dispersible source may offer greater flexibility. Both types were tested in this study. Two of the three sources tested contained antioxidants, presumably added to improve carotene stability (Table I).

Carotene Stability During Product Making

As in our earlier studies (Rogers et al 1993), we found no beta carotene in the control products. Stability characteristics of the carotene-fortified (spiked) products are shown in Table III (10% carotene source), Table IV (22% carotene source), and Table V (30% carotene source).

For both bread and crackers, the added carotene showed a high degree of stability during mixing, proofing (breads), or sheeting (crackers) of the doughs, irrespective of the carotene source used (Tables III–V). In yeast-raised doughs, Gordon and Bauernfeind (1982) reported virtually no destruction of beta carotene during mixing and proofing. Carotene stability was reduced when products were baked, with losses ranging between 4.3 and 14.8% for bread products (proofed doughs vs. fresh breads) and between 17.9 and 22.8% for crackers (sheeted doughs vs. baked crackers). Losses in crackers were higher probably because of their greater relative surface area and more severe baking conditions (lower final product moisture).

TABLE III
Retention of 10% Water-Soluble Carotene Source in Bread and Crackers

	Amount[a] (ppm)	Amount Retained (%)[b]	Isomers (% of total)		
			All-*trans*	13-*cis*	9-*cis*
Bread					
Fully mixed dough	23.3 ± 0.2	. . .	94.1	5.1	0.8
Proofed dough	22.6 ± 1.8	97.0	90.6	6.9	2.5
Baked bread (fresh)	21.6 ± 0.7	92.7	78.2	16.5	5.3
Stored bread (1 day)	20.0 ± 0.7	85.8	80.4	15.5	4.1
Stored bread (3 day)	17.4 ± 0.9	74.7	80.1	15.7	4.2
Stored bread (5 day)	17.1 ± 1.1	73.4	75.8	17.5	6.7
Stored bread (7 day)	16.3 ± 0.3	70.0	76.5	18.5	5.0
Crackers					
Fully mixed dough	18.5 ± 0.5	. . .	93.8	4.6	1.6
Sheeted dough	18.4 ± 0.2	99.5	93.2	4.4	2.4
Baked crackers (fresh)	15.1 ± 0.7	81.6	63.0	24.5	12.5
Stored crackers (30 day)	14.8 ± 0.0	80.0	67.7	21.1	11.2
Stored crackers (60 day)	14.4 ± 0.9	77.8	67.0	20.9	12.1
Stored crackers (90 day)	14.5 ± 1.0	78.4	68.7	19.9	11.4

[a]Expressed on moisture-free basis.
[b]Percent of amount in mixed dough.

TABLE IV
Retention of 22% Oil-Soluble Carotene Source in Bread and Crackers

	Amount[a] (ppm)	Amount Retained (%)[b]	Isomers (% of total)		
			All-*trans*	13-*cis*	9-*cis*
Bread					
Fully mixed dough	24.8 ± 1.7	. . .	90.5	6.9	2.6
Proofed dough	24.7 ± 1.0	99.6	90.9	6.5	2.6
Baked bread (fresh)	22.4 ± 1.1	90.3	86.0	10.9	3.1
Stored bread (1 day)	22.2 ± 0.7	89.5	83.2	12.7	4.1
Stored bread (3 day)	22.2 ± 0.5	89.5	87.4	8.5	4.1
Stored bread (5 day)	22.8 ± 0.4	91.9	84.9	10.2	4.9
Stored bread (7 day)	22.1 ± 0.3	89.1	81.6	14.0	4.4
Crackers					
Fully mixed dough	18.9 ± 1.0	. . .	91.6	4.9	3.5
Sheeted dough	18.1 ± 1.2	95.8	92.4	5.1	2.5
Baked crackers (fresh)	13.8 ± 0.3	73.0	63.0	27.8	9.2
Stored crackers (30 day)	13.8 ± 0.9	73.0	64.5	22.9	12.6
Stored crackers (60 day)	13.8 ± 0.8	73.0	63.1	23.3	13.5
Stored crackers (90 day)	12.9 ± 0.6	68.3	68.6	23.1	8.3

[a]Expressed on moisture-free basis.
[b]Percent of amount in mixed dough.

TABLE V
Retention of 30% Oil-Soluble Carotene Source in Bread and Crackers

	Amount[a] (ppm)	Amount Retained (%)[b]	Isomers (% of total)		
			All-*trans*	13-*cis*	9-*cis*
Bread					
Fully mixed dough	23.0 ± 2.1	. . .	91.2	7.1	1.7
Proofed dough	22.6 ± 0.7	98.3	89.6	7.7	2.7
Baked bread (fresh)	19.2 ± 1.0	83.5	84.2	12.1	3.7
Stored bread (1 day)	18.6 ± 0.3	80.9	85.5	10.9	3.6
Stored bread (3 day)	18.3 ± 1.0	79.6	85.1	11.2	3.7
Stored bread (5 day)	19.5 ± 0.4	84.8	84.4	11.0	4.6
Stored bread (7 day)	18.7 ± 0.7	81.3	81.7	13.1	5.2
Crackers					
Fully mixed dough	18.1 ± 0.5	. . .	94.2	3.6	2.2
Sheeted dough	17.4 ± 0.4	96.1	90.7	6.9	2.4
Baked crackers (fresh)	13.4 ± 0.6	74.0	62.1	24.7	13.2
Stored crackers (30 day)	13.1 ± 0.9	72.4	66.3	22.4	11.3
Stored crackers (60 day)	13.5 ± 0.8	74.6	67.8	23.0	9.2
Stored crackers (90 day)	12.4 ± 0.3	68.5	66.8	29.0	4.2

[a]Expressed on moisture-free basis.
[b]Percent of amount in mixed dough.

TABLE VI
Effect of Antioxidants on Carotene Stability

Carotene Source[a]	Percent Carotene Loss During			
	Baking[b]		Storage[c]	
	Bread	Crackers	Bread	Crackers
10% (+ AOX)	4.4	18.0	18.0	3.6
22% (+ AOX)	9.3	23.8	0.3	2.2
30% (− AOX)	15.1	23.0	2.2	3.0

[a]Containing (+) or not containing (−) antioxidants (AOX).
[b]Proofed or sheeted dough vs. freshly baked product.
[c]Fresh product vs. all (average) stored products.

TABLE VII
Contribution of Beta Carotene from Test Products[a]

Product[b]	Serving Size		Carotene (mg)
	(g)	Household Unit	
Bread	28.5	1 Slice	0.35
Crackers	30.0	10 Pieces	0.39

[a]Based on average of all values after baking and storage (all three sources).
[b]As consumed. Moisture content: bread, 38%; and crackers, 5%.

Carotene Stability During Product Storage

During the 7-day storage period (the typical market shelf life of bread), carotene in bread showed a high degree of stability, especially in breads containing oil-soluble carotene sources (Tables IV–V). Some carotene losses occurred during storage in breads made with water-soluble carotene (Table III). For crackers, the water-soluble source was, however, as stable as the other two sources. Carotene losses during the 90-day storage of crackers were insignificant. Overall carotene losses averaged about one-fifth in bread products and one-third in crackers, with the bulk of this loss occurring during baking and not during product storage.

Effect of Antioxidants

Beta carotene sources with added antioxidants (Table I) exhibited greater carotene stability during baking, especially in bread products (Table VI). No such effect due to antioxidants was observed during product storage. Some carotene loss during product storage did occur, however, with significant changes only in bread products made with the water-soluble carotene source even though it contained antioxidants.

Isomeric Distribution of Carotene

The initial processing steps (dough mixing, proofing, and sheeting) affected the isomeric distribution of added carotene only slightly. That changed significantly when products were baked. *Cis* isomers, a trace component initially (Table I), became a major component of carotene in baked products (Tables III–V). That change was less pronounced in bread products, especially those containing oil-soluble carotene, as compared to cracker products. In our earlier study (Rogers et al 1993), we noted a similar trend when comparing bagels (less *cis* formation) with cookies and cakes. That transformation would affect vitamin A activity of carotene (NRC 1989), but probably not its antioxidant potential.

Carotene Contribution

In most cases, the stability of carotene changed only minimally after baking. As such, all values obtained after baking were averaged to arrive at carotene values typical for the two products tested. On a dry basis, those values were: 19.9 ppm for bread products and 13.8 ppm for crackers, based on average stability (doughs vs. other samples) values of 83.8% (breads) and 74.6% (crackers). On an as-consumed basis, a serving of those products would provide nearly 0.4 mg of beta carotene (Table VII). That contribution would allow labeling the product as a "good source" of vitamin A, a claim not usually possible for unfortified grain-based foods. Higher additions of carotene would obviously provide more carotene. However, it may be prudent to not over-fortify foods, as combined intakes of carotene from various fortified foods and supplements may become excessive, going well beyond levels suggested for the antioxidant effect.

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