Factors Affecting the Ascorbic Acid and Carotene Content of Broccoli

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The high ascorbic acid and carotene content of broccoli and the instability of these nutrients give significance to a study of factors affecting them. Investigations were made of the carotene content and of the oxidation and retention of ascorbic acid in broccoli as influenced by market form, length of storage, and cooking method. When cooked to optimum doneness, frozen and fresh broccoli retained about the same percentage of their original ascorbic acid, from 65 to 79% depending on the method of cooking used. Frozen broccoli contained only small amounts of dehydroascorbic and diketogulonic acids and no ascorbase enzyme. Frozen broccoli stored at 0° F. for 36 weeks showed only slight oxidation of ascorbic acid to dehydroascorbic and diketogulonic acids. No decrease in carotene content was found in frozen broccoli stored for 61 weeks at 0° F.

Work reported in previous publications describes the effect of cooking methods and other factors on the palatability and ascorbic acid and carotene content of fresh broccoli (*4*, *7*, *14*). Ascorbic acid was found to be rather evenly distributed between the head and the stem, but the smaller stems contained more ascorbic acid than those larger and more mature. Most of the carotene was found in the broccoli head, the content increasing with the maturity of the heads. The carotene present in the stems was concentrated chiefly in the outer portion.

In this paper are presented ascorbic acid retention values for fresh and frozen broccoli cooked by various methods and results of the effect of freezer storage and cooking of broccoli on the oxidation of ascorbic acid to dehydroascorbic acid and diketogulonic acid. Other factors investigated were the presence of reductones which interfere with the indophenol titration of ascorbic acid, and of ascorbase, an enzyme catalyzing the oxidation of ascorbic acid. The carotene content of fresh and frozen broccoli also was determined.

Experimental Methods

Materials. All broccoli was of the green sprouting type. The frozen broccoli was all of the same brand and was stored at 0° F. in a farm-type freezer. Most of it was obtained directly from the processor. That used for microwave cooking and for some of the carotene determinations was obtained at the retail market. The fresh broccoli was obtained at the retail market, iced, and stored overnight at 38° F.

Cooking Methods. A randomized block design [Cochran and Cox (*5*)] was followed for each cooking method. All cooking methods were replicated three times. Data from each exeriment were evaluated by analyses of variance. Differences between single means were examined for significance by applying the Duncan and Bonner test (*6*).

For determination of ascorbic acid retention, two 10-ounce packages of frozen broccoli were defrosted 1 hour at 72° F. Then 567-gram samples were weighed and cooked for various lengths of time by the following conventional methods:

Boiling in 236 grams of water heated to boiling before adding broccoli.

Boiling in 236 grams of water; broccoli added to cold water.

Boiling in 472 grams of water heated to boiling before adding broccoli.

Cooking by steaming (472 grams of water).

Cooking at a 15-pound steam pressure (118 grams of water).

In the study of the effect of cooking on reduced ascorbic acid, dehydroascorbic acid, and diketogulonic acid in frozen broccoli, a conventional method and a microwave method (*15*) were used. Two 10-ounce packages (567 grams) of frozen broccoli were cooked for various lengths of time by the two following methods: boiling in 236 grams of water; and microwave cooking, no water added.

Ascorbic Acid. In most of the analyses, reduced ascorbic acid was determined by the indophenol titration procedure (*2*). For investigation of the effect of cooking procedures and storage on reduced ascorbic, dehydroascorbic, and diketogulonic acids, the procedure of Roe *et al.* (*13*) was also used.

Reductones. Because any reductones present in the broccoli or produced by cooking would react with the indophenol dye used in titrating ascorbic acid, it was necessary to check for their presence. This was done by titrating in the presence of formaldehyde, as suggested by Robinson and Stotz (*12*). Results indicated that no reductones were present.

Ascorbase. Preliminary determinations of ascorbase were made by the procedure of McCombs (*9*) in which the unoxidized vitamin C is determined spectrophotometrically. This procedure was later modified by the substitution of a titration step for the photometric step, which made possible the use of larger samples for analysis.

Further work disclosed that the ascorbic acid was itself unstable in the citrate buffer used by McCombs. Accordingly, this buffer was replaced by a sodium oxalate phosphate buffer, pH 6, previously used by Ponting and Joslyn (*11*). The modified procedure used for ascorbase determination follows:

Twenty-five grams of broccoli were blended for 3 minutes with 100 ml. of cold 0.4*M* sodium chloride. The extract was diluted to 175 ml. with 0.4*M* sodium chloride, the suspension held for 2 hours at 0° C., and then centrifuged for 20 minutes at 3000 r.p.m. The supernatant liquid was decanted and kept in an ice bath until used.

The reaction mixture consisted of the following: 5 ml. of standard ascorbic acid solution containing 0.625 mg. of ascorbic acid per ml. in oxalate phosphate buffer, 35 ml. of buffer solution, and 10 ml. of centrifuged plant extract. The control mixture used was identical with the reaction mixture except for the substitution of 10 ml. of 0.4*M* sodium chloride for

10 ml. of the plant extract. The mixtures were held at 30° C. for 15 minutes. Five-milliliter aliquots were then withdrawn, diluted with 5 ml. of 1*M* oxalic acid, and titrated with 2,6-dichlorophenolindophenol in the usual manner.

Carotene. Carotene determinations were made by the procedure of Wiseman, Irvin, and Moore (*16*). So that larger samples might be tested, the following modification was used: Fifty-gram portions each of broccoli heads and stems were blended for 2 minutes at high speed with 100 grams of 95% ethyl alcohol. A 40-gram portion of the resulting slurry was then extracted as described in the Wiseman procedure. A 10-ml. aliquot was chromatographed and the eluate made to 25 ml.

Storage Study. Ascorbic acid and carotene determinations were made on frozen broccoli obtained directly from the processor and stored in a farm-type freezer at 0° F. for various periods of time. The storage time of the broccoli used in the ascorbic acid determinations ranged from approximately 2 to 36 weeks and that for carotene from 17 to 61 weeks.

Results and Discussion

Effect of Cooking Method on Ascorbic Acid. In Figure 1, *A*, are shown ascorbic acid retention values for 567-gram samples of frozen broccoli cooked by five methods. Best retention of ascorbic acid was found in methods using 236 grams of water or less. In Figure 1, *B*, are shown comparable values for ascorbic acid contained in the liquids, expressed as percentage of ascorbic acid originally in the broccoli. Retention values obtained on fresh broccoli cooked by four methods (*14*) are shown in Figure 1, *C*, for purposes of comparison. The amount of fresh broccoli used in each case was 1 pound (454 grams).

There were marked differences in the behavior of fresh and frozen broccoli with respect to retention of ascorbic acid. With frozen broccoli, the percentage of ascorbic acid retained in the solids cooked 5 minutes ranged from 66 to 77% (Figure 1, *A*). Losses of ascorbic acid in frozen broccoli cooked for periods longer than 5 minutes were not statistically significant. With fresh broccoli cooked 5 minutes in no more than 300 ml. of water, the percentage retained in the broccoli solids ranged from 80 to 91% (Figure 1, *C*). Further cooking, however, caused significant decreases in ascorbic acid content.

The relatively porous structure of the frozen broccoli resulting from the blanching and freezing seems to be responsible for the more rapid transfer of ascorbic acid in the early stages of cooking. Despite this difference in transfer rate, however, fresh and frozen broccoli, when cooked until the stems, as evaluated by panel (*7*), were tender yet firm (2 minutes by pressure and 10 minutes by the other methods), did not differ greatly in ascorbic acid retention. The range in mean values for ascorbic acid retention in broccoli was 65 to 79% for the frozen broccoli and 72 to 75% for the fresh. However, the actual mean ascorbic acid content of 39 samples of fresh broccoli ranged from 85 to 129 mg. per 100 grams with a mean of 106 mg., whereas the ascorbic acid content of 15 samples of frozen broccoli used in this portion of study ranged from 81 to 86 mg. per 100 grams with a mean of 84 mg. Therefore, the fresh broccoli was actually higher in ascorbic acid after cooking than was the frozen broccoli.

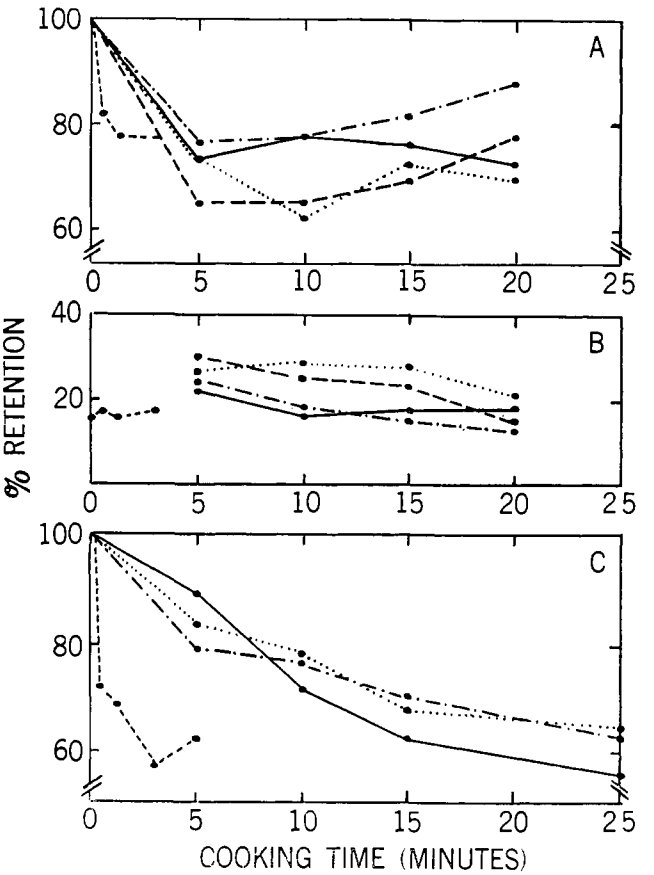

Figure 1. Retention of ascorbic acid

A. In frozen broccoli
B. In cooking liquid of frozen broccoli
C. In fresh broccoli

—— A and B, boiled in 236 grams of water (boiling start); C, 300 grams
– – A and B, boiled in 236 grams of water (cold start); C, 300 grams
— — A and B, boiled in 472 grams of water (boiling start)
– – – A, B, and C, cooked at 15-pound pressure (118 grams of water)
. . . A and B, steamed (472 grams of water); C, 600 grams

Effect of Cooking Methods on Reduced Ascorbic Acid, Dehydroascorbic Acid, and Diketogulonic Acid Content of Frozen Broccoli. Results of quantitative differentiation of reduced ascorbic acid, dehydroascorbic acid, and diketogulonic acid in frozen broccoli cooked by two methods for various periods of time are given in Table I. The values obtained for dehydroascorbic acid tended to be lower for frozen broccoli than those reported previously for fresh broccoli (*14*). Cooking, in general, caused decreases of all three compounds in both fresh and frozen broccoli. However, in some samples of fresh broccoli in which the dehydroascorbic acid content was very high there appeared to be some conversion of dehydroascorbic acid to reduced ascorbic acid on cooking, with a consequent increase in the reduced ascorbic acid content (*14*). In the frozen broccoli, the initial dehydroascorbic acid content was never great enough to cause a measurable increase in the reduced ascorbic acid content of the cooked broccoli.

Ascorbic Acid Oxidase. Ascorbic acid oxidase is shown to catalyze the oxidation from reduced to dehydroascorbic acid (*11*). Several studies have been conducted which show that maceration of plant tissue increases the dehydroascorbic acid content (*3, 9, 10*). As certain of the cooking methods investigated involved placing the fresh broccoli in cold water, it was believed advisable

Table I. Effect of Cooking Method on Content of Reduced Ascorbic Acid, Dehydroascorbic Acid, and Diketogulonic Acid in Frozen Broccoli

Sample and Cooking Method	Cooking Time, Min.	Reduced Ascorbic Acid, Mg./100 G.	Dehydroascorbic Acid, Mg./100 G.	Diketogulonic Acid, Mg./100 G.
Boiled in 236 g. water[a]				
Lot 1	0	30.8	5.2	6.2
	5	25.0	4.2	2.1
	20	22.7	6.2	2.3
Lot 2	0	79.8	5.3	8.8
	5	63.4	8.3	1.9
	10	60.6	7.9	3.8
	15	64.0	8.8	2.5
	20	56.9	7.3	4.2
Cooked by microwave, no water added				
Lot 1	0	61.9	9.1	8.5
	15	46.5	6.1	4.7
Lot 2	0	52.8	6.0	8.8
	15	37.8	5.9	4.9

[a] Water heated to boiling before adding broccoli.

Table II. Changes in Ascorbic Acid Content of Commercially Processed Frozen Broccoli Stored at 0° F. for Various Lengths of Time

Type of Sample	Storage Time at 0° F., Weeks	Reduced Ascorbic Acid, Mg./100 G.	Dehydroascorbic Acid, Mg./100 G.	Diketogulonic Acid, Mg./100 G.
Heads and stems	2	93.8	4.7	1.1
	13	79.8	5.3	8.8
	15	92.3	5.9	8.0
	17	81.0	10.9	6.0
	25	78.2	12.9	7.1
Heads	36	80.9	19.1	8.0
Stems	36	93.8	11.4	4.5

Table III. Carotene Content of Frozen and Fresh Broccoli

Description	Storage Conditions	Sample No.		Carotene Content, γ/100 G.
Frozen				
From processor	0° F., 17 weeks	1		1020
		2		1030
		3		1100
		4		1355
	0° F., 61 weeks	1		1020
		2		1070
		3		1110
		4		1150
			Mean	1107
From retail market	Overnight at 0° F.	1		640
		2		780
		3		780
		4		830
		5		840
		6		1040
		7		1060
		8		1180
			Mean	894
Fresh				
From retail market	Iced and stored overnight at 38° F.	1		620
		2		680
		3		680
		4		710
		5		740
		6		770
		7		770
		8		870
		9		890
		10		1170
		11		1180
		12		1280
			Mean	863

to determine the content of ascorbic acid oxidase so that its effect on reduced ascorbic acid might be evaluated.

Four samples of fresh broccoli tested contained sufficient ascorbase to oxidize 2.1 to 2.2 mg. of ascorbic acid. Tests on two samples of frozen broccoli showed no measurable amount of ascorbase. The absence of ascorbase in the frozen broccoli indicated that this enzyme had been destroyed by the blanching prior to freezing. The presence of ascorbase in fresh broccoli did not significantly affect ascorbic acid retention in broccoli cooked by the cold start method (Figure 1, *C*).

Stability of Ascorbic Acid and Carotene in Frozen Broccoli. As vegetables, when stored, are known to undergo changes in diketogulonic acid and in reduced and dehydroascorbic acid content, determinations of these substances were made on freshly processed frozen broccoli and on samples stored at 0° F. and withdrawn at various time intervals. The results are summarized in Table II. There appeared to be an increase in dehydroascorbic acid in the interval between 15 and 17 weeks of storage. There was an increase in diketogulonic acid between 2 and 13 weeks and a gradual decrease in reduced ascorbic acid with increased length of storage time.

As both dehydroascorbic acid and reduced ascorbic acid are biologically available, the decrease in reduced ascorbic acid is partially compensated for by the increase in dehydroascorbic acid, and the results of this study would indicate only slight decreases in ascorbic acid value after 36 weeks storage at 0° F. Determinations made separately on heads and stems after 36 weeks of storage indicated that the dehydroascorbic acid and diketogulonic acid content of the heads was higher and the reduced ascorbic acid lower in the heads than in the stems.

In Table III are shown results of carotene determinations made on frozen and fresh broccoli. Determinations on 4 samples of frozen broccoli obtained directly from the processor and stored for 17 weeks at 0° F. and 4 samples stored 61 weeks showed a range of 1020 to 1355 γ of carotene per 100 grams with a mean of 1107 and no apparent decrease in the period between 17 and 61 weeks. For eight samples obtained at the retail market the range was from 640 to 1180 γ per 100 grams with a mean of 894. The carotene content of 12 samples of fresh broccoli procured at the retail market ranged from 620 to 1280 γ per 100 grams with a mean of 863 γ.

Carotene is water insoluble and fairly stable to heat—almost 100% was retained in broccoli cooked by the methods studied (*14*). However, it is sensitive to light, auto-oxidation, and atmospheric oxygen (*1*, *8*). The lower carotene values of fresh and frozen broccoli obtained on the retail market demonstrate

this instability and indicate that carotene determinations are of importance in quality evaluation.

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BIOASSAY OF CAROTENOIDS

Vitamin A Activity of Beta-apo-8′-carotenal

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β-Apo-8′-carotenal in oil solution was assayed for vitamin A activity in a series of U.S.P. vitamin A curative rat-growth assays. Combining the data from two assays yielded an average potency of 1,200,000 U.S.P. units of vitamin A per gram of all-*trans* β-apo-8′-carotenal with a range of 1,070,000 to 1,330,000 units at $P = 0.05$ or 72 ± 8% of the activity of all-*trans* β-carotene on a weight basis. Dry stabilized beadlets of β-apo-8′-carotenal were also assayed in three separate tests. Within the limits of the assay procedure no significant difference was seen in vitamin A potency. The possibility is discussed that β-apo-8′-carotenal is an intermediate product in the biological conversion of β-carotene to vitamin A.

Euler, Karrer, and Solmssen (*4*) reported β-apo-8′-carotenal to be the main product resulting from the partial oxidation of β-carotene with calcium permanganate. Daily supplements of 5 γ fed to vitamin A–deficient rats stimulated growth. Glover and Redfearn (*6*) prepared β-apo-8′-, β-apo-10′-, and β-apo-12′-carotenal and fed them to vitamin A–deficient rats. All were transformed into vitamin A. It was noted that β-apo-10′-carotenal undergoes β-oxidation to β-apo-12′-carotenal. The structural relationship of vitamin A, β-carotene, and β-apo-8′-carotenal is shown on page 391. The commonly accepted vitamin A potency of β-carotene is 1,667,000 I.U. per gram as compared to 3,333,000 for vitamin A alcohol. On the basis of the formulas of the two molecules, conversion of β-carotene to vitamin A has been postulated to proceed via hydrolytic cleavage at the central double bond. Theoretically this would be expected to yield two molecules of vitamin A from one of β-carotene. However, attempts to carry out this unusual reaction chemically have generally been very inefficient. Conversion in the animal body under most conditions yields only one rather than two molecules of vitamin A as indicated by the relative potencies given above, which are based on numerous bioassays. Under certain conditions, however, a much more efficient conversion, approaching closely the theoretically possible two molecules of vitamin A, has been reported (*3, 7, 11*).

The stepwise oxidation of β-carotene from one end, yielding successively β-apo-8′-carotenal, β-apo-10′-carotenal, β-apo-12′-carotenal, and subsequently vitamin A aldehyde, which is reduced to vitamin A, has been postulated by Glover and Redfearn (*6*) as a possible mode of its conversion to vitamin A. In support of this they observed that the hitherto unidentified substances related to the carotenoids found by Festenstein (*5*) in horse intestine had spectroscopic and chromatographic properties identical to those of the β-apo-10′- and β-apo-12′-carotenals. Winterstein (*16*) isolated β-apo-8′-carotenal from oranges, tangerines, and spinach and demonstrated the identity of the naturally occurring substance with synthetic β-apo-8′-carotenal.

Isler *et al.* (*8*) synthesized β-apo-8′-carotenal as part of an intensive program to produce synthetically many of the naturally occurring carotenoids. This carotenoid has very desirable properties for coloring foods and beverages. Also, as a feed ingredient, it has been reported by Steinegger, Streiff, and Zeller (*14*), Steinegger and Zanetti (*15*), and Marusich, Kadin, and Bauernfeind (*13*) to be an efficient pigmenter for coloring egg yolks uniformly with a pleasing yellow equal to that obtained with high quality ingredients rich in natural xanthophylls.