Table IV. Effect of Added Nitrite on β-Carotene Destruction in Sheep Abomasum Juice (Experiment 6)

Treatment	β-Carotene Found μG.	β-Carotene Found % of theoretical[a]
Abomasum juice + corn oil + water	1	..
Abomasum juice + corn oil + sodium nitrite	2	..
Abomasum juice + β-carotene in corn oil + water	219	90
Abomasum juice + β-carotene in corn oil + sodium nitrite	18	7

[a] 243 μg. added.

in the juice. In experiments involving the oral administration of nitrites and carotene (or possibly vitamin A) to monogastric animals, this effect cannot be neglected. Its importance in ruminants, however, is not clear. Wang *et al.* (*13*) report that nitrite nitrogen probably passes rapidly and directly from the rumen to the blood. Whether, however, nitrite might yet reach the abomasum in sufficient concentrations to cause significant carotene destruction needs study.

The studies discussed here indicate that β-carotene is not affected by the presence of nitrate nitrogen under a variety of conditions. Furthermore, the presence of nitrite nitrogen at about neutral or slightly alkaline pH levels apparently causes only very slow destruction of β-carotene. That provitamin A activity has not been affected cannot, however, be concluded. Copper enhances the rate of destruction when nitrite is present, and it is possible that other substances present in biological materials may do the same. As pH is decreased below 6, destruction in the presence of nitrite occurs rapidly and can result in serious losses of the provitamin. The nitrite effect in acid solutions appears to be the result of the decomposition of nitrous acid to yield gaseous oxides of nitrogen which cause rapid destruction of β-carotene.

Destruction of carotene in the rumen of animals fed forages containing nitrates does not appear to present a problem of practical consequence. On the other hand, high levels of nitrates in ensiled forages may enhance carotene losses considerably, and more study of this phase of the problem seems justified. Furthermore, in experimental work with monogastric animals, the rapid destruction of carotene in acid fluids to which nitrites are added must be considered. Since no biological tests for vitamin A activity were made in this work, it can only be assumed that the carotene destruction noted did not yield compounds with vitamin A activity. In view of the considerable amount of work done on this in the past, however, the assumption appears a valid one.

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STORAGE EFFECTS ON WINTER SQUASHES

Varietal Differences and Storage Changes in the Ascorbic Acid Content of Six Varieties of Winter Squashes

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TABLES OF FOOD VALUES usually consulted when estimating the nutrient intake of groups or individuals indicate that winter squash contains a relatively small amount of ascorbic acid. In such tables, winter squash is generally not identified by variety. The amount of data on specific varieties elsewhere in the literature is very limited.

Since increasing numbers of different varieties are becoming available to the consumer, this investigation was made to provide additional information on varietal differences and on the effect of storage on ascorbic acid content of six varieties of winter squashes.

Studies by Holmes and coworkers are of particular interest. Ascorbic acid determinations (fresh basis) on five varieties 6 weeks after harvest showed distinct varietal differences. Buttercup had a mean value of 39.6 mg. per 100 grams compared with 3.4 mg. per 100 grams for Des Moines (*3*). In addition, their findings suggest that changes in ascorbic acid content of winter squashes during storage are not necessarily the same for different varieties (*4, 5*).

Experimental

The varieties studied during 1957–58 were Baby Blue, Buttercup, Blue Hubbard, Silver Bell, and Sweet Meat of the *Cucurbita maxima* species and Butternut of the *C. moschata* species. The six squash varieties were grown in four replications on the University Horticultural Farm. The squashes were harvested between September 17 and 20, and placed in storage on slatted racks, keeping the fruit from the 24 plots separated.

Table I. Mean Values[a] and Significance ($P = 0.01$) of Changes[b] in Ascorbic Acid of Winter Squashes During Storage

Ascorbic Acid

	Baby Blue					*Blue Hubbard*				
Dry basis	(0)[c] 165.2	(10) 157.0	(5) 137.0	(25) 134.2	(15) 123.0	(0) 150.2	(5) 132.2	(15) 115.0	(10) 102.0	(25) 70.0
Fresh basis	(0) 40.2	(5) 36.7	(10) 34.6	(25) 27.6	(15) 27.2	(0) 35.0	(5) 23.4	(10) 14.6	(15) 14.2	(25) 9.2
Total solids[d]	(5) 26.9	(0) 24.1	(10) 22.2	(15) 21.8	(25) 20.8	(0) 23.2	(5) 17.9	(10) 13.7	(15) 12.6	(25) 12.6
	Buttercup					*Silver Bell*				
Dry basis	(0) 185.5	(15) 176.2	(10) 156.0	(5) 150.2	(25) 128.8	(0) 126.2	(25) 119.8	(15) 119.0	(10) 107.2	(5) 94.8
Fresh basis	(0) 57.0	(10) 46.5	(5) 42.5	(15) 40.3	(25) 28.0	(0) 31.9	(15) 25.5	(10) 24.6	(25) 23.6	(5) 21.7
Total solids	(0) 30.6	(10) 30.0	(5) 28.4	(15) 22.8	(25) 21.7	(0) 25.2	(5) 22.9	(10) 22.7	(15) 21.3	(25) 19.4
	Butternut					*Sweet Meat*				
Dry basis	(0) 154.2	(5) 83.8	(10) 75.8	(25) 70.5	(15) 66.8	(0) 135.5	(5) 107.0	(10) 101.5	(15) 90.0	(25) 70.2
Fresh basis	(0) 29.5	(5) 16.8	(10) 13.9	(15) 12.3	(25) 10.8	(0) 35.0	(5) 24.4	(10) 23.0	(15) 18.7	(25) 12.5
Total solids	(5) 20.0	(0) 19.2	(15) 18.4	(10) 18.2	(25) 14.8	(0) 25.9	(5) 22.6	(10) 22.1	(15) 20.4	(25) 16.4

Dehydroascorbic Acid plus Diketogulonic Acid

	Baby Blue					*Blue Hubbard*				
Dry basis	(25) 46.5	(0) 34.8	(10) 29.2	(5) 28.2	(15) 27.5	(0) 44.0	(5) 26.5	(10) 20.2	(25) 19.0	(15) 18.0
Fresh basis	(25) 9.5	(0) 8.4	(5) 7.6	(10) 6.4	(15) 5.9	(0) 10.2	(5) 4.7	(10) 2.8	(25) 2.5	(15) 2.4
	Buttercup					*Silver Bell*				
Dry basis	(25) 30.8	(0) 28.5	(15) 23.0	(5) 22.5	(10) 19.2	(25) 37.8	(0) 37.0	(15) 29.5	(5) 27.8	(10) 21.0
Fresh basis	(0) 8.7	(25) 6.6	(5) 6.4	(10) 5.8	(15) 5.3	(0) 9.4	(25) 7.3	(5) 6.4	(15) 6.3	(10) 4.8
	Butternut					*Sweet Meat*				
Dry basis	(0) 31.8	(5) 21.2	(15) 21.0	(25) 15.2	(10) 14.0	(0) 41.0	(5) 17.5	(10) 16.2	(15) 15.8	(25) 14.2
Fresh basis	(0) 6.1	(5) 4.2	(15) 3.8	(10) 2.6	(25) 2.4	(0) 10.8	(5) 3.9	(10) 3.4	(15) 3.4	(25) 2.2

Total Vitamin C-Like Compounds[e]

	Baby Blue					*Blue Hubbard*				
Dry basis	(0) 200.0	(10) 186.2	(25) 180.8	(5) 165.2	(15) 150.5	(0) 194.2	(5) 158.8	(15) 133.0	(10) 122.2	(25) 89.0
Fresh basis	(0) 48.6	(5) 44.3	(10) 41.0	(25) 37.1	(15) 33.1	(0) 45.2	(5) 28.1	(10) 17.0	(15) 17.0	(25) 11.6
	Buttercup					*Silver Bell*				
Dry basis	(0) 214.0	(15) 199.2	(10) 175.2	(5) 172.8	(25) 159.5	(0) 163.2	(25) 157.5	(15) 148.5	(10) 128.2	(5) 122.5
Fresh basis	(0) 65.7	(10) 52.3	(5) 48.9	(15) 45.6	(25) 34.5	(0) 41.2	(15) 31.8	(25) 30.9	(10) 29.4	(5) 28.1
	Butternut					*Sweet Meat*				
Dry basis	(0) 186.0	(5) 105.0	(10) 89.8	(15) 87.8	(25) 85.8	(0) 176.5	(5) 124.5	(10) 117.8	(15) 105.8	(25) 84.5
Fresh basis	(0) 35.6	(5) 21.0	(10) 16.4	(15) 16.1	(25) 13.3	(0) 45.7	(5) 28.3	(10) 26.4	(15) 22.0	(25) 14.8

[a] Mean of four replications, expressed as mg. per cent.
[b] Any two means underscored by the same line are not significantly different from each other at the 1% level.
[c] Number of weeks of storage.
[d] Mean of four replications, expressed as per cent. Same percentages apply also for DHA + DKA and total vitamin C-like compounds.
[e] Sum of ascorbic acid and dehydroascorbic plus diketogulonic acids.

Six varieties of winter squashes were studied to determine varietal differences and effect of storage on ascorbic acid content. Buttercup and Baby Blue varieties were considerably higher than Blue Hubbard and Butternut in ascorbic acid content and in total vitamin C–like compounds (i.e., sum of ascorbic acid and dehydroascorbic acid plus diketogulonic acid). Ascorbic acid averaged 82.0 ± 6.9% of the total vitamin C–like compounds. Ascorbic acid and the total vitamin C–like compounds decreased during storage with two thirds of the loss occurring in the first 5 weeks. High ranking varieties retained a larger proportion of the harvest value than low ranking varieties. The findings indicate that variety and length of storage are factors to be considered when estimating the antiscorbutic value of winter squashes.

Table II. Ascorbic Acid Values of Six Varieties of Winter Squashes[a]

Variety	Dry Basis[b]	Fresh Basis[b]	Fresh Basis Adjusted[b]
	Ascorbic Acid		
Buttercup	159.4 a	42.8 a	38.6 a
Baby Blue	143.3 ab	33.2 a	29.2 b
Silver Bell	113.4 bc	25.5 b	23.4 bc
Sweet Meat	100.8 c	22.7 bc	21.1 cd
Blue Hubbard	113.9 bc	19.3 bc	18.1 cd
Butternut	90.2 c	16.6 c	14.7 d
	Total Vitamin C–Like Compounds[c]		
Buttercup	184.2 a	49.4 a	44.4 a
Baby Blue	176.6 ab	40.8 b	35.8 b
Silver Bell	144.0 bc	32.3 c	29.7 bc
Sweet Meat	121.8 c	27.4 cd	25.5 cd
Blue Hubbard	139.4 bc	23.8 cd	22.4 cd
Butternut	110.8 c	20.5 d	18.1 d

[a] Mean of determinations made at harvest and after 5, 10, 15, and 25 weeks of storage. Values are mg. per 100 grams.
[b] Means within a column followed by the same letter or letters are not significantly different from each other at the 1% level.
[c] Sum of ascorbic acid and dehydroascorbic plus diketogulonic acids.

Storage conditions, sampling procedures, and total solids determinations have been described in detail in previous papers (*6–8*) dealing with concurrent studies of the β-carotene, starch, and sugar content of these varieties.

At harvest and following 5, 10, 15, and 25 weeks of storage, a representative fruit or fruits of each variety from each replication were taken from storage, and the weight loss was determined. The raw edible portion was cubed, and 700-gram samples were blended with 350 ml. of a 3% oxalic acid–6% thiourea solution and 1050 ml. of glass-distilled water.

The vitamin C–like compounds were determined by the 2,4-dinitrophenylhydrazine method published by the Association of Vitamin Chemists (*1*), in which the ascorbic acid values (frequently referred to in the literature as reduced ascorbic acid) are obtained by the difference between the determined total vitamin C–like compounds and dehydroascorbic acid plus diketogulonic acid. Dehydroascorbic acid plus diketogulonic acid is subsequently referred to as DHA + DKA.

During 1958–59, as part of another investigation, the Baby Blue, Silver Bell, and Sweet Meat varieties were grown in four replications and stored under comparable conditions. Ascorbic acid content was obtained at harvest and after 6, 10, and 16 weeks of storage, using the 2,4-dinitrophenylhydrazine method modified by Schaffert and Kingsley (*9*). Determinations are made of the ascorbic acid plus dehydroascorbic acid and of the dehydroascorbic acid; ascorbic acid is the difference between these values.

Results and Discussion

The mean values for ascorbic acid, DHA + DKA, total vitamin C–like compounds, and percentage of total solids of the four replications of each variety at each sampling date in 1957–58 are presented in Table I. Since certain decreases and differences in total solids content during storage and between varieties influenced the fresh basis values, findings are expressed on both the fresh and dry basis. Storage changes and varietal differences in total solids have been presented in previous publications (*6, 8*).

Analysis of variance showed highly significant differences among varieties and among sampling dates, but no significant differences among replications in the content of ascorbic acid, DHA + DKA, and total vitamin C–like compounds. Duncan's multiple range test (*2*) was used to examine specific differences between varieties and sampling dates. The resulting significant differences are indicated in the tables. Significance refers to the 1% level of probability unless otherwise stated.

The fresh basis figures were also adjusted for weight loss of squashes during storage to evaluate absolute changes. The significance of this adjustment has been discussed elsewhere (*6*). The weight loss of squashes occurring during storage, particularly during the first 5 weeks after harvest, accentuated the decrease of the ascorbic acid, DHA + DKA, and total vitamin C–like compounds content expressed on an absolute basis. Varietal differences in weight loss will be discussed in a separate paper.

Values for ascorbic acid and total vitamin C–like compounds shown in Table II are the means of all determinations made on each variety on the dry, fresh, and adjusted-fresh basis, respectively.

Ascorbic Acid. The findings on ascorbic acid are of particular interest. The majority of the antiscorbutic agents in plants occurs as ascorbic acid, and the vitamin C content of foods is, therefore, usually expressed in this form.

The varietal differences are striking. In Table II, the values on the fresh basis for Buttercup and Baby Blue were significantly different from those for Blue Hubbard and Butternut, the first two varieties containing about double the amount of the latter two. Adjusting the figures for weight loss did not change the rank order of the six varieties, and changed only slightly the magnitude of the differences in the ascorbic acid content. On the dry basis, similar differences between varieties existed. The values reflect the fact that the six varieties differed in total solids content at harvest and/or in the rate and extent of change in total solids during storage. The ascorbic acid value for Buttercup was again significantly higher than that for Butter-

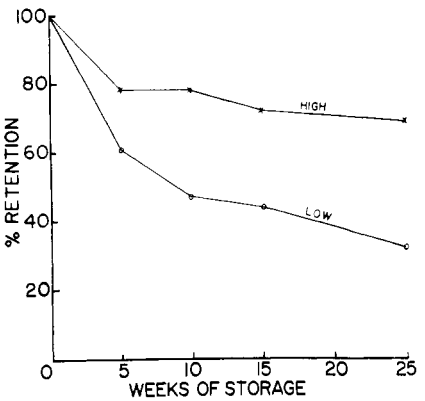

Figure 1. Retention of total vitamin C–like compounds during storage

High = mean of Buttercup, Baby Blue, and Silver Bell. Low = mean of Sweet Meat, Blue Hubbard, and Butternut

nut. The findings did not vary to any great extent when varietal differences were examined at each sampling date.

The ascorbic acid content decreased during storage. The rate and extent of this decrease varied among varieties. On the fresh basis, the loss became statistically significant in Blue Hubbard after 10 weeks of storage, in Buttercup and Butternut after 15 weeks, and in Sweet Meat after 25 weeks. As could be expected, on the adjusted-fresh basis, the values decreased more rapidly, and differences were already significant for Buttercup and Butternut after 5 weeks of storage. On the dry basis, storage changes were less pronounced, becoming significant in Butternut after 5 weeks and in Blue Hubbard after 25 weeks of storage.

No significant differences existed between varieties or sampling dates in the proportion of ascorbic acid to total vitamin C–like compounds. Ascorbic acid averaged 82.0 ± 6.9% of the total vitamin C–like compounds (fresh, dry, and adjusted-fresh bases). The positive association between the two were highly significant, the correlation coefficients (r) ranging from +0.942 to +0.995.

Dehydroascorbic Acid plus Diketogulonic Acid (DHA + DKA). Varieties did not differ in their content of DHA + DKA on the fresh, dry, or adjusted-fresh basis during the first four sampling periods. After 25 weeks of storage, Baby Blue contained significantly more than Butternut and Sweet Meat.

During storage, the DHA + DKA content tended to decrease. This decrease became significant in Blue Hubbard and Sweet Meat as shown in Table I.

When the DHA + DKA values were expressed as percentage of the total vitamin C–like compounds, no significant difference existed between varieties or sampling dates. On the average, 18.0 ± 6.9% of the total vitamin C–like compounds was in these two forms. Wide variation among varieties and replications were apparent on the last sampling date. The corresponding mean for the first four sampling dates was 17.2 ± 4.8%.

Total Vitamin C–Like Compounds. Since the ascorbic acid values are the difference between the values of the total vitamin C–like compounds and DHA + DKA, and since varietal differences and storage changes in DHA + DKA were minor, the findings on the content of total vitamin C–like compounds were similar to those on the ascorbic acid.

Buttercup and Baby Blue were again the high-ranking varieties, and Blue Hubbard and Butternut the low-ranking varieties (Table II). Significant differences between varieties and between sampling dates, on the three bases of expression, followed a pattern comparable to that for ascorbic acid. Similarly, the content of total vitamin C–like compounds decreased during storage. The sampling date when such decreases became significant was not always the same as for ascorbic acid. No biological importance, however, is attached to this behavior, considering the variability among samples of the different varieties.

Figure 1 shows the mean percentages of the harvest values of total vitamin C–like compounds retained at each sampling date by the three high-ranking varieties (Buttercup, Baby Blue, Silver Bell) and the three low-ranking varieties (Sweet Meat, Blue Hubbard, Butternut). During storage, the high-ranking group retained a significantly higher proportion than the low ranking group.

Seasonal Comparisons. In 1958–59, the ascorbic acid content of Baby Blue, Silver Bell, and Sweet Meat was obtained by the method of Schaffert and Kingsley (*9*). The ascorbic acid values of these three varieties did not differ significantly from the values obtained in 1957–58. When the varieties were ranked according to their ascorbic acid values, their relative positions were the same in both years. The total solids content of the varieties did not differ between the two years.

As during 1957–58, the ascorbic acid content of these three varieties decreased. The statistical significance of this loss was not always consistent among sampling dates in both years. However, these differences in storage changes between the seasons is probably more a change effect of variability among the samples than an actual seasonal effect.

Because of the difference in the methods of analysis, seasonal comparisons have been presented only for ascorbic acid.

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