Nonenzymatic Discoloration in Dried Cabbage. III. Decomposition Products of Ascorbic Acid and Glycine

Ascorbic acid (AA) or dehydroascorbic acid (DHA), on heating in an oxygen atmosphere, yielded acetaldehyde and carbon dioxide in the alcoholic medium and mostly carbon dioxide in the aqueous medium. A pathway for acetaldehyde formation is proposed. The red condensation product of DHA and glycine (red chromogen B) turned brown on heating in the alcoholic medium, and yielded carbon dioxide, formaldehyde (arising *via* Strecker degradation), acetaldehyde (due to decomposition of DHA moiety), and 11 carbonyls, of which six were similar to the carbonyls found in the decomposition products of DHA. The brown color was mainly due to the breakdown products containing nitrogen. Gel elution of the brown coloring matter from DHA–glycine ethyl ester condensation product (red chromogen A) yielded two fractions having the composition $C_{20}H_{26}N_2O_{15}$ and $C_{12}H_{17}NO_{10}$.

The composition and properties of red chromogen A which had the same color characteristics as that of the cherry red color formed initially in the freeze-dried cabbage were discussed in the previous paper (Ranganna and Setty, 1974). The decompositions of AA, DHA, and the red chromogen are presented in this paper.

EXPERIMENTAL SECTION

AA (Merck USP grade), glycine (Merck), microcrystalline DHA, and red chromogen A prepared in this laboratory (Ranganna and Setty, 1974) were used. Red chromogen B was prepared in the same way as red chromogen A, but glycine was used in place of glycine ethyl ester and it was used without further purification. Aldehyde-free ethyl alcohol was prepared by the AOAC method (1970).

The apparatus used for studying the volatile decomposition products was the same as that described in the earlier paper (Ranganna and Setty, 1968) with the exception that the reaction flask was fitted with a water-cooled condenser and the heating mantle was replaced by a temperature controlled water bath. Oxygen was scrubbed through 20% sodium hydroxide solution before letting it into the reaction flask.

The reaction conditions are given in Table I. To prepare derivatives, the carbonyls were swept with the carrier gas and trapped in a saturated solution of 2,4-dinitrophenylhydrazine (2,4-DNPH) in 2 *N* HCl. The derivatives were filtered under suction, washed with 1 *N* HCl and water, dried in a vacuum desiccator, and recrystallized from ethanol. The total volatile carbonyls or the acetaldehyde produced was estimated by absorbing in a 1% solution of 2,4-DNPH in 50% alcohol containing 2% HCl. To estimate the formaldehyde produced when glycine was the source of amino nitrogen, the 2,4-DNPH traps were replaced by a series of four water traps cooled in ice.

Formaldehyde was estimated colorimetrically by reaction with chromotropic acid and the color measured at 575 nm (Alexander *et al.*, 1945). The total carbonyls were estimated according to Critchfield (1963) with the exception that 2 ml of methanol was added after addition of pyridine and methanolic potassium hydroxide to prevent phase separation. The color was measured at 480 nm. Carbon dioxide was absorbed in 1 *N* NaOH and estimated by titrating against 0.1 *N* HCl.

2,4-DNPH Derivatives of the Residual Carbonyls in the Reaction Mixture. Two or three days after the volatile carbonyls ceased to evolve, heating was discontinued and the ethanol present in the reaction mixture removed by vacuum distillation. A saturated solution of 2,4-DNPH in 2 *N* HCl was added to the residual solution and allowed to stand overnight at room temperature, and the derivatives were examined by tlc using silica gel G as adsorbent and the multiple development technique of Clegg and Morton (1965).

Conversion of Red Color to Brown. An ethanolic solution of red chromogen A was mixed with chromatographic grade Whatman cellulose powder, incubated at 70° for 7 days in a hot air oven, and packed in a glass column, and the color was eluted using alcohol and concentrated *in vacuo* at 40°. Unlike the red chromogen, when ether was added to the concentrated solution, the brown coloring matter did not precipitate but only a syrupy liquid separated out which was repeatedly triturated with ether and dried in a vacuum desiccator.

To isolate different fractions of the brown color, 50 mg of the dried powder was dissolved in 1 ml of water, placed on a Sephadex G-10 column, and eluted with distilled water (Ranganna and Setty, 1974). Two distinct bands separated on the column. Fractions from each band having similar uv absorptions were pooled, freeze-dried, and subjected to elemental analysis.

RESULTS AND DISCUSSION

Volatile Breakdown Products from AA and DHA. AA or DHA, when incubated in an alcoholic medium, formed a volatile carbonyl and carbon dioxide (Table I). The carbonyl was found to be acetaldehyde from the results of analysis of the 2,4-DNPH derivative: recrystallized from ethanol; long, yellow needles; mp 162°; uv λ_{max} in chloroform 260 and 354 nm; ir NH absorption band at 3290 cm^{-1} (Figure 1). (*Anal.* Calcd for $C_8H_8N_4O_4$: C, 42.89; H, 3.60; N, 24.99; O, 28.55. Found: C, 42.91; H, 3.63; N, 25.22; O, 28.27). No acetaldehyde was produced when aldehyde-free ethanol was similarly incubated. Alcohol used as the medium simulated the low moisture conditions prevailing in the dried cabbage (Ranganna and Setty, 1968, 1974).

Reynolds (1965) has reviewed the decomposition of AA under oxidative and nonoxidative conditions. Kurata and Sakurai (1967) heated DHA under oxidative conditions (5% H_2SO_4) and isolated furfural, ethyl glyoxal, 2-keto-3-deoxy-L-pentono-γ-lactone, and L-xylosone. Tatum *et al.* (1969) heated AA in unbuffered aqueous solution without excluding oxygen and isolated ten furan type compounds, two lactones, three acids, and 3-hydroxy-2-pyrone. Under anaerobic conditions, AA decomposes into furfural, carbon dioxide, and a number of acids (Huelin, 1953; Huelin *et al.*, 1971). Below pH 4.0, furfural is the main product of degradation. Acetaldehyde found in the present study had not been hitherto reported.

The extent of acetaldehyde and carbon dioxide formed from AA or DHA depended on the medium, the temperature, and the carrier gas (Table I). Alcoholic medium and oxygen (oxidative conditions) favored the formation of the aldehyde. One mole of AA and DHA yielded 0.87 and 0.75 mol of acetaldehyde and 0.23 and 0.37 mol of carbon dioxide, respectively. Nonoxidative conditions (carbon dioxide used as carrier gas) considerably reduced the aldehyde

Table I. Volatile Breakdown Products from AA, DHA, and Red Chromogen

Materials	Concn, mmol	Medium[a]	Carrier gas	Temp, °C	Duration, days[d]	Yield of volatile breakdown products, mmol: Formaldehyde	Acetaldehyde	Carbon dioxide
AA	10	70% A[b]	N_2	25	14		0.193[e]	1.312[e]
	10	70% A	N_2	40	10		0.293[e]	0.625[e]
	5	96% A	O_2	65	11		4.335	1.167
	5	96% A	CO_2	65	11		1.317	
	5	W[c]	O_2	65	4		0.147	2.278
	5	W	CO_2	65	10		0.335	
DHA	5	96% A	O_2	65	11		3.790	1.869
	5	96% A	CO_2	65	4		0.095	
	5	W	O_2	65	12		0.251	3.610
	5	W	CO_2	65	12		0.316	
Glycine + DHA	10 + 10	96% A	CO_2	75	5.5	0.670[f]	1.710	
	10 + 20	96% A	CO_2	75	5.5	1.380[g]	1.760	
Red chromogen A	5	96% A	O_2	65	9		2.190	4.680
	5	96% A	CO_2	65	9		0.540	
	5	W	O_2	65	9		0.260	1.750
	5	W	CO_2	65	9		0.280	
Red chromogen B	5	96% A	CO_2	75	0.02	0.062	0.320	
Red chromogen B	5	96% A	O_2	75	1	0.000	6.140	
stored in desiccator	5	96% A	CO_2	75	1	0.000	4.240	
for 30 days	5	96% A	O_2	75	4.5	0.000	11.700	
	5	96% A	CO_2	75	4.5	0.000	8.230	

[a] Volume of reaction mixture was 200 ml. [b] A = alcohol. [c] W = water. [d] When the aldehyde value was negative consecutively for 2 or 3 days, the experiment was discontinued. Hence, variations in duration. [e] pH adjusted to 5.0. [f] Yield as nanomoles (*i.e.* 10^{-9}) per hour = 5106. [g] Yield as nanomoles per hour = 10,440.

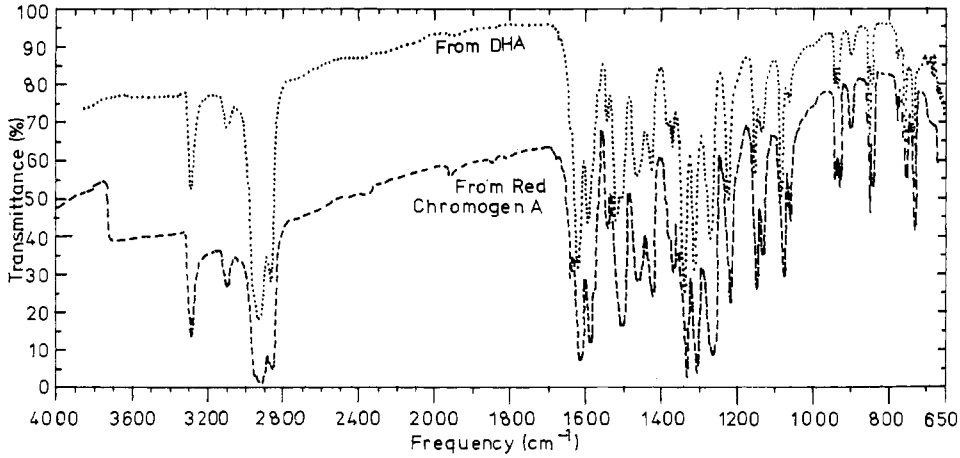


Figure 1. Spectrum (in Nujol) of 2,4-DNPH derivatives of acetaldehyde from DHA and from red chromogen.

formation and gave a yield only 2.5% of that obtained using oxygen (Table I). In the aqueous medium, under oxidative conditions, the yield of carbonyls was only 3 and 6% of that formed in the alcoholic medium using AA and DHA, respectively. The major breakdown product was carbon dioxide which was 15 times more than that of the volatile carbonyls. The rate of production of acetaldehyde and carbon dioxide is shown in Figure 2.

The mechanism proposed for acid-catalyzed decarboxylation of AA by Kurata and Sakurai (1967) can be extended to explain the influence of oxygen on the enhanced acetaldehyde formation from AA as shown in Scheme I. A similar oxidative mechanism may probably be operative for the observed formation of acetaldehyde from DHA under the experimental conditions employed. The $CHOHCH_2OH$ group may also be dehydrated to $CH_3C{:}O$ by hydroxyl elimination from C-6 when a carbonyl develops at C-4. The elimination of CO_2 from 2,3-diketogulonic acid would result in $HC{:}OC{:}OCHOHCHOHCH_2OH$ which could tautomerize to $HC{:}OCHOHC{:}OCHOHCH_2OH$ to promote β elimination of the terminal OH producing $HC{:}OCHOHC{:}OC{:}OCH_3$. Oxidation would promote the hydrolysis to acetaldehyde as found.

Volatile Breakdown Products from DHA and Glycine. DHA has the essential dicarbonyl structure required for the Strecker degradation of amino acid (Schönberg

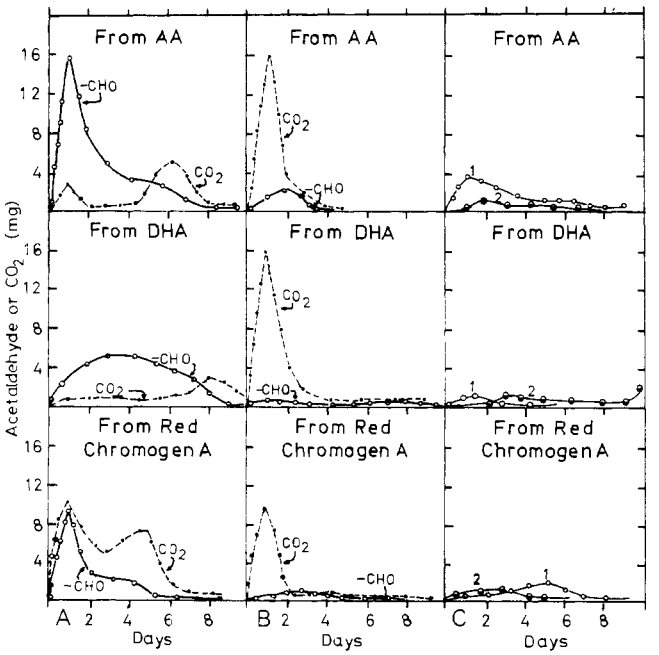
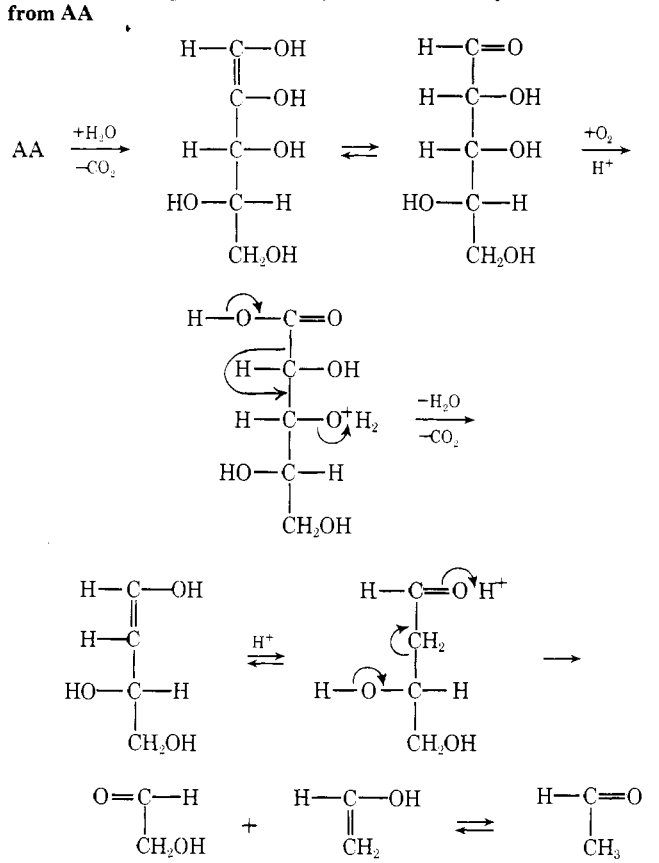


Figure 2. Formation of aldehyde and CO_2 from AA, DHA, and red chromogen A; 176 mg of AA, 174 mg of DHA, or 268 mg of red chromogen A used; acetaldehyde using alcoholic medium (1) and aqueous medium (2): (A) carrier gas, O_2; medium, 96% alcohol; temperature, 65°; (B) carrier gas, O_2; medium, aqueous; temperature, 65°; (C) carrier gas, CO_2; medium, 96% alcohol and aqueous; temperature, 65°.

and Moubacher, 1952). When DHA and glycine were incubated, acetaldehyde and formaldehyde (the product of Strecker degradation) were found (Table I). The yield of formaldehyde appeared to depend on the ratio of glycine and DHA in the reaction mixture. When the ratio was 1:2, the yield was twice that when the ratio was 1:1.

Scheme I. Proposed Pathway for Acetaldehyde Formation from AA



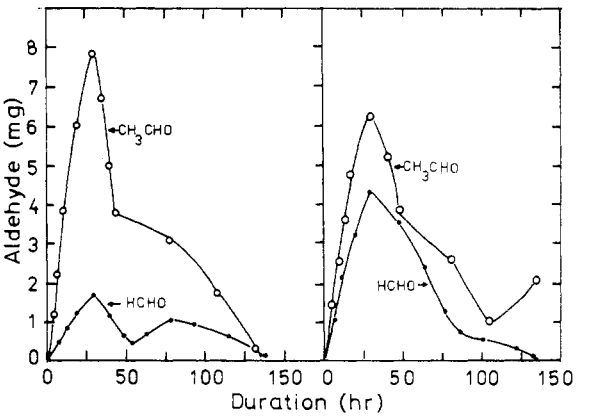


Figure 3. Formation of acetaldehyde and formaldehyde from glycine–DHA reaction mixture: (A, left) from 348 mg (10 mmol) of DHA and 150 mg (10 mmol) of glycine; (B, right) from 696 mg (20 mmol) of DHA and 150 mg (10 mmol) of glycine; medium, 96% alcohol; carrier gas, CO_2; temperature, 65°.

Casey *et al.* (1965), using 10 mmol of amino acid (alanine, valine, leucine, methionine, or α-aminobutyric acid) and 100 mmol of glucose or fructose, reported the yield of corresponding aldehyde *via* Strecker degradation by gas chromatographic method to vary from 0.05 to 57.7 nmol (*i.e.*, 10^{-9} mol) per hour from the straight line portion of the graph. As compared to heating in water, the yield increased when the medium was buffered, more at pH 7.5 than at 6.5. In the present study, using glycine and DHA in unbuffered alcoholic medium (pH 4.9), the yields were estimated at intervals (Figure 3) until the evolution was complete. From the total yield (Table I), formaldehyde formed was found to be 5106 and 10,440 nmol (*i.e.*, 10^{-9} mol) per hour for 10 and 20 mmol, respectively, of the DHA used along with 10 mmol of glycine in the reaction mixture. These results confirmed the earlier findings (Self, 1967) that DHA was a more efficient deamination and decarboxylation agent than fructose and glucose. A several-hundred-fold increased yield of aldehyde from the Strecker degradation obtained in the present study is attributed to the alcoholic medium used.

Volatile Breakdown Products from Red Chromogen. In the previous paper (Ranganna and Setty, 1974), it was shown that the red chromogen A synthesized using DHA and glycine ethyl ester was an initial dimeric condensation product of the reactants. When it was heated in the alcoholic medium with oxygen or carbon dioxide as a carrier gas, the only volatile carbonyl found was acetaldehyde (Table I). The identification was based on the results of elemental analysis, mixture melting point, and uv and ir spectra (Figure 1), which were similar to the acetaldehyde produced from DHA. No formaldehyde was found.

Red chromogen B prepared using DHA and glycine, when fresh, yielded formaldehyde in addition to acetaldehyde (Table I). After storage of the chromogen in a desiccator for about a month, no formaldehyde evolved but acetaldehyde was present, the yield of which was more when oxygen was used as the carrier gas instead of carbon dioxide, and which increased with the duration of heating. The formation of acetaldehyde and formaldehyde from the red chromogen B indicated that the former arose through the decomposition of the DHA moiety and the latter arose *via* Strecker degradation of the amino moiety of the chromogen.

Acetaldehyde formed from red chromogen using either oxygen or carbon dioxide as carrier gas was considerably more than when DHA alone was incubated (Table I). These results show that just as the Amadori rearrangement in the carbonyl amino mechanism renders the sugar moiety more labile than in the nonamino mechanism in-

Table II. R_f Values of 2,4-DNPH Derivatives of Carbonyls Formed from DHA and Red Chromogen

No.	From DHA refluxed in alcoholic medium at 65° with O_2 as carrier gas	From DHA refluxed in alcoholic medium at 65° with CO_2 as carrier gas	From red chromogen A refluxed in alcoholic medium at 65° with O_2 as carrier gas	From red chromogen A dissolved in alcohol, mixed with cellulose, and incubated at 40° for 7 days
1			0.84	0.84
2	0.80	0.80		0.80
3	0.71		0.71	0.70
4	0.68		0.68	0.68
5	0.64	0.64	0.64	0.64
6	0.57	0.57	0.57	0.57
7	0.46	0.46	0.46	0.46
8			0.40	0.40
9			0.33	0.33
10				0.30
11		0.28	0.28	0.28

volving 2,3-enolization of sugars (Hodge, 1953), the amino acid–DHA interaction renders the DHA more labile for decomposition than when present alone.

In the synthesis of the red chromogen A, the chromogen was precipitated from a concentrated alcoholic solution with ethyl ether (Ranganna and Setty, 1974). The alcohol–ethyl ether solvent mixture containing portions of unprecipitated chromogen was left in a refrigerator for a few days. During the recovery of ether, when the mixture was washed with water, the aqueous phase was yellow in color and gave a copious precipitate with 2,4-DNPH. Tlc of the derivative in different solvents gave only one spot. Recrystallization from ethanol yielded long, yellow needles and was found to be the 2,4-DNPH derivative of acetaldehyde (mp 161°. *Anal.* Calcd for $C_8H_8N_4O_4$: C, 42.89; H, 3.60; N, 24.99; O, 28.55. Found: C, 42.77; H, 3.66; N, 25.07; O, 28.50). This observation lends further support to the labile nature of the DHA moiety in the chromogen.

Nonvolatile Carbonyls from DHA and Red Chromogen. The carbonyls found in the residual solutions of DHA and red chromogen after incubation and stripping of the volatiles evolved are given in Table II. Of the 11 unidentified carbonyls found in red chromogen A, the R_f values of six were similar to the carbonyls found in the decomposition products of DHA. This similarity is analogous to the breakdown of sugar in the presence or absence of amino compounds (Hodge, 1953). The λ_{max} in methanol of the carbonyls found in DHA was between 265 and 275 nm and did not correspond to the λ_{max} of the carbonyls found under oxidative conditions in the aqueous medium by Kurata and Sakurai (1967).

Color Formation. The solution of AA or DHA after heating in alcohol (11 days) or water (4 days) at 65° in an atmosphere of oxygen or carbon dioxide was colorless or light straw yellow. On the contrary, the mixture of DHA and glycine or the purified red chromogen became dark brown in the alcoholic medium within 1–2 days of heating. The absorbance at 515 nm decreased whereas that at 420 nm increased which is typical of browning. The dry mixture of DHA, glycine, and Celite (inert carrier) left in a closed test tube became deep brown within a period of 3 months. The test for furfural at this stage was negative. From the results of the elemental analysis of the two fractions of the brown color eluted from Sephadex G-10 column, the compositions were found to be $C_{20}H_{26}N_2O_{15}$ (*Anal.* Calcd: C, 44.94; H, 4.91; N, 5.25; O, 44.90. Found: C, 44.77; H, 4.97; N, 5.30; O, 44.94) and $C_{12}H_{17}NO_{10}$ (*Anal.* Calcd: C, 42.99; H, 5.12; N, 4.18; O, 47.73. Found: C, 43.31; H, 5.00; N, 4.14; O, 47.55). The two fractions had an absorption maximum at 248 nm in the uv region but had no peak in the visible region. The carbonyls formed in the conversion of red color to brown might contribute to browning by polymerization or by condensation with amino compounds (Hodge, 1953). However, the results indicated that browning was mostly caused by the breakdown products containing nitrogen.

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