amine was formed in samples of sewage and lake water treated with its precursors, dimethylamine and nitrite. Thus, the formation of NDPA from DPA could conceivably result from renitrosation in the environment. However, the corresponding nitrosation of NPA, if it should occur, would result in the formation of an unstable diazonium salt rather than an alkyl nitrosamine.

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Determination of Daminozide Residues on Foods and Its Degradation to 1,1-Dimethylhydrazine by Cooking

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Daminozide was determined by alkaline hydrolysis to dimethylhydrazine. The dimethylhydrazine was recovered by distillation, derivatized with pentafluorobenzoyl chloride, and determined by GLC. Recoveries from several commodities fortified from 0.10 to 40 ppm were generally greater than 80%. 1,1-Dimethylhydrazine was determined in apples by extraction with 0.01 N HCl, chromatography on a cation-exchange resin, and derivatization with pentafluorobenzoyl chloride. The resulting 1,1-dimethyl-2,2-bis(pentafluorobenzoyl)hydrazine was cleaned up on a silicic acid and quantitated by GLC. Recoveries averaged 88% from 0.10 to 12 ppm. Daminozide was found to decompose to dimethylhydrazine when boiled in apple homogenate. The amount of decomposition increased with the duration of boiling from 10 to 60 min and with the amount of daminozide from 5 to 30 ppm.

Daminozide (succinic acid 2,2-dimethylhydrazide) is a plant growth regulator registered for use on several crops including apples, peaches, grapes, plums, and tomatoes. It is water soluble and readily translocated to all parts of the plant. With apples, the recommended preharvest interval is 60–70 days and significant residues persist for over 100 days (Edgerton and Greenhalgh, 1966; Edgerton et al., 1967). Long persistence of daminozide residues has also been observed in cherries (Ryugo, 1965).

Daminozide has been implicated in the production of tumors when fed as a 2% solution in the drinking water of mice (Toth et al., 1977). The possibility also exists that hydrolysis of daminozide would yield 1,1-dimethylhydrazine, itself a carcinogen in mice (Roe et al., 1967).

Methods for the determination of daminozide in foods involve hydrolysis in strong alkali and distillation of the resulting dimethylhydrazine (Edgerton et al., 1967). The dimethylhydrazine is determined with a colorimetric reagent either directly (Edgerton et al., 1967; Lane, 1967) or after oxidation to formaldehyde (Lynch, 1969). No methods have been reported in the literature for the determination of free dimethylhydrazine in foods although it has been determined in tobacco (Schmeltz et al., 1977). Since colorimetric methods are insufficiently specific for regulatory purposes and since it was necessary to determine whether free dimethylhydrazine occurred in foods, the following method was developed. It is based on the derivatization of dimethylhydrazine with pentafluorobenzoyl chloride and subsequent determination of the derivative by GLC with electron-capture detection.

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EXPERIMENTAL SECTION

Materials. 1,1-Dimethylhydrazine was purchased from Aldrich Chemical Co., Milwaukee, WI, and was labeled as being 95% pure. A stock standard solution containing 1 mg/mL was prepared in 1 N HCl. Working standards containing 0.5 and 5.0 μg/mL were prepared by dilution of the stock in 0.01 N HCl.

Succinic acid 2,2-dimethyl hydrazide (Daminozide) was obtained from Aldrich Chemical Co.

Cation-exchange resin, Dowex 50W × 8, 100–200 mesh (Sigma Chemical Co., St. Louis, MO), was purified before use by washing with alkali and acid as described previously (Newsome, 1974). Ion-exchange columns containing 3 mL

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settled volume of resin were also prepared as described earlier (Newsome, 1974) and were washed before use by eluting sequentially with 1 N NaOH (15 mL), water until neutral, 1 N HCl (15 mL), and water until neutral.

Silicic acid for adsorption chromatography (Woelm, activity I, 100-200 μm) was obtained from ICN Pharmaceuticals Inc., Cleveland, OH, and was deactivated by the addition of 5% water.

Pentafluorobenzoyl chloride reagent was prepared by diluting 0.1 mL of pentafluorobenzoyl chloride (PCR Research Chemicals, Inc., Gainsville, FL) to 10 mL with dichloromethane.

Analytical Procedure. (*i*) *Dimethylhydrazine.* Samples (5 g) of previously homogenized crop were blended with ice-cold 0.01 N HCl (30 mL) for 30 s in a Sorvall Omni-Mixer. The mixture was filtered using vacuum on a 4.25-cm i.d. Büchner funnel containing a 1-g bed of Celite 545 supported by Whatman No. 1 filter paper. The filtrate was diluted to 100 mL with water. A standard was prepared by adding dimethylhydrazine working standard (1.0 mL; 5 μg) to 30 mL of 0.01 N HCl and diluting to 100 mL with water.

Aliquots (10 mL) of the diluted filtrate or standard were transferred to cation-exchange columns and permitted to run through the columns at a rate of ca. 45 mL/h. The columns were washed with 0.5 M sodium acetate (10 mL) and the eluate discarded. Dimethylhydrazine was recovered by eluting with 1 M sodium acetate (15 mL).

An aliquot (1.0 mL) of the 1 M sodium acetate eluate was added to 2 M K_2CO_3 (9.0 mL) in a conical tube. After addition of pentafluorobenzoyl chloride reagent (1.0 mL), the tube was stoppered tightly and shaken vigorously for 1 h. The reaction mixture was extracted with dichloromethane (4 mL) and the extract passed through a small bed of anhydrous Na_2SO_4 in a glass funnel into a 50-mL, round-bottomed flask. After the Na_2SO_4 was rinsed with a small volume of dichloromethane, the solvent was removed on a rotary evaporator with the water bath at room temperature.

The residue was dissolved in hexane (1 mL) and transferred to a column of deactivated silicic acid (2 g) prepared in hexane in a 190 × 7 mm chromatographic tube. The flask was rinsed with a further portion of hexane (1 mL) and the hexane solution permitted to flow onto the column. The column was eluted with 15% toluene in hexane and the first 15 mL discarded while the next 10 mL were collected and subjected to analysis by GLC.

(*ii*) *Daminozide.* A subsample (5 g) of previously chopped and homogenized sample was weighed into a 125-mL boiling flask. After 50% NaOH (40 mL) was added, the flask was placed on a hot plate and connected to a Liebig condenser with a 75 °C connecting tube. Teflon sleeves were employed at the joints to prevent freezing. The flask was heated to boiling and distillation conducted at such a rate that 10 mL was collected over a period of ca. 1 h. Concentrated HCl (0.1 mL) was added to and mixed with the distillate, and an aliquot (1.0 mL) was added to 2 M K_2CO_3 (9.0 mL) and reacted for 1 h with pentafluorobenzoyl chloride as described for dimethylhydrazine. Standard working solution (1.0 mL; 0.5 μg) was diluted to 10 mL with water and acidified with concentrated HCl (0.1 mL), and an aliquot (1.0 mL) was allowed to react with pentafluorobenzoyl chloride in the presence of K_2CO_3 as for the samples. Both samples and standards were extracted and subjected to cleanup on silicic acid as described for dimethylhydrazine.

Gas–Liquid Chromatography. Analyses of the final 15% toluene in hexane eluate were performed on a Varian 1400 fitted with a ^3H electron-capture detector and a 6 ft × 4 mm i.d. glass column. The column was packed with 5% butanediol succinate on 100–120 mesh Chromosorb W, HP and was conditioned for 4 days at 195 °C with a 30 mL/min flow of nitrogen carrier gas before use. Typical operating parameters were as follows: injector, 170 °C; column, 160 °C; detector, 195 °C; nitrogen carrier flow rate, 30 mL/min; electrometer range 10^{-10} amp/mV; attenuation 8. Under these conditions, the retention time of 1,1-dimethyl-2,2-bis(pentafluorobenzoyl)hydrazine was 7 min. Injections of 5 μL were made, and 2 pg of derivative resulted in a peak with 80% full-scale deflection. Samples were quantitated by comparison of the peak height with that of the standard. The amount of daminozide was then calculated from the ratio of the molecular weight of daminozide to dimethylhydrazine.

Table I. Recovery of Dimethylhydrazine Added to Apple

dimethylhydrazine added, ppm	dimethylhydrazine found, ppm	mean recov, %
0.10	0.08	85
	0.09	
0.20	0.19	100
	0.21	
0.40	0.33	82
	0.32	
1.0	0.88	100
	1.11	
2.0	1.98	91
	1.65	
4.0	2.96	75
	3.04	
8.0	6.82	90
	7.61	
12.0	9.54	82
	10.1	

Table II. Recovery[a] of Daminozide Added to Various Commodities

daminozide added, ppm	daminozide recovered, %				
	apple	peach	grape	plum	tomato
0.10	96	85	111	91	89
0.20	94	99	92	89	88
0.40	92	80	91	98	81
5.0	106	99	93	94	
10	84	95	87	107	
20	90	86	80	100	
40	72	79	83	90	

[a] Recoveries are of dimethylhydrazine produced by the hydrolysis of daminozide and are expressed in terms of daminozide. They are the means of duplicate determinations.

RESULTS AND DISCUSSION

1,1-Dimethylhydrazine in aqueous solution was found to react with pentafluorobenzoyl chloride when it was shaken with a dichloromethane solution of the latter in the presence of K_2CO_3. The 1,1-dimethyl-2,2-bis(pentafluorobenzoyl)hydrazine was formed as shown by high-resolution mass spectrometry of the derivative. The reaction conditions result in a 93% yield of derivative when standards are used. Approximately 50% loss of dimethylhydrazine occurs when standards are subjected to ion-exchange chromatography. The reason for this low yield is unclear, and attempts to improve it by changing the eluting solution were unsuccessful. Thus, for quantitation, it was necessary to include a standard before this step.

The recoveries of dimethylhydrazine added to apple homogenate before extraction are given in Table I. The overall average recovery from 0.10 to 12 ppm was 88%. On

Table III. Formation of Dimethylhydrazine by Boiling Apple Fortified with Daminozide

sample	dimethylhydrazine found, ppm
boiled[a] apple	<0.025
boiled apple + 1.85 ppm dimethylhydrazine	1.9
boiled apple + 30 ppm daminozide	0.29
apple + 30 ppm daminozide boiled	1.53

[a] Apple homogenate (5 g) was boiled in H_2O (10 mL) for 30 min.

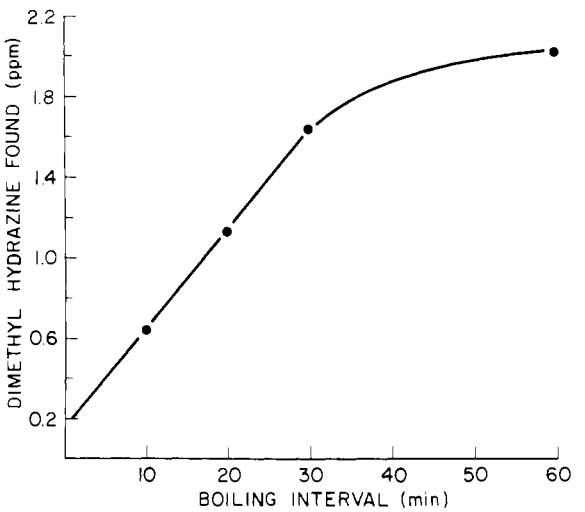

Figure 1. Effect of boiling time on the production of dimethylhydrazine from apple fortified with 30 ppm daminozide. The data are uncorrected for 1% dimethylhydrazine impurity in the daminozide.

the basis of a 2:1 signal-to-noise ratio, the minimum detectable level of dimethylhydrazine was estimated to be 0.01 ppm.

The recoveries of daminozide added to various commodities prior to extraction are presented in Table II. The data shows that recoveries are generally greater than 80% from 0.10 to 40 ppm of added daminozide. Care must be taken to distill the sample at a sufficiently slow rate to prevent losses. Crop blanks were less than 0.025 ppm of daminozide.

Daminozide was found to decompose to dimethylhydrazine when boiled in the presence of apple homogenate as shown by the data in Table III. Apple fortified with daminozide at the tolerance level of 30 ppm and boiled for 30 min contained 1.53 ppm of dimethylhydrazine. Similar results were obtained with peaches. Daminozide used to fortify the samples contained approximately 1% dimethylhydrazine as an impurity. The recovery of dimethylhydrazine from boiled samples was quantitative.

The level of dimethylhydrazine produced from apple fortified with daminozide was found to increase with boiling time as indicated by the data plotted in Figure 1. The amount of dimethylhydrazine also increased with the

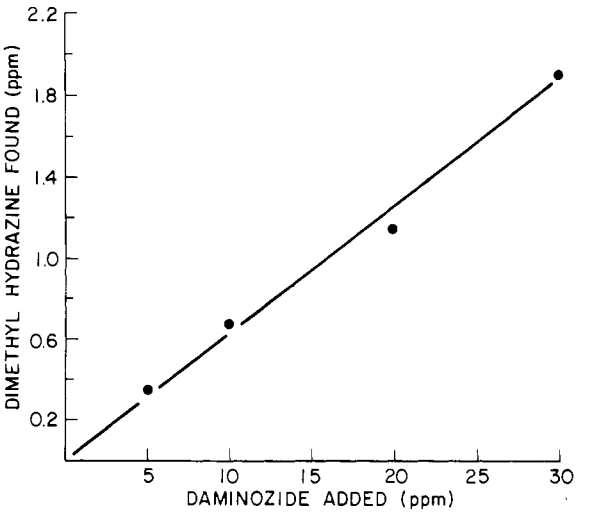

Figure 2. Effect of the fortification level of daminozide on the concentration of dimethylhydrazine formed by boiling apples for 30 min. The data are uncorrected for 1% dimethylhydrazine impurity in the daminozide.

level of daminozide added up to 30 ppm as seen in Figure 2. The average conversion on a weight basis was 6.4%.

To examine whether bio-incurred daminozide would hydrolyze to the same extent as that added directly to apple homogenate, a sample of MacIntosh apples treated in the field with daminozide at a rate of 3 lb/acre was analyzed. Eight weeks after treatment, the fruit sample was found to contain 9.1 ppm of daminozide. No free dimethylhydrazine was detected. When boiled for 30 min, 0.70 ppm of free dimethylhydrazine was found. Thus, a similar percentage conversion to that observed with fortified samples occurred.

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