Journal of Food Protection, Vol. 54, No. 3, Pages 225-229 (March 1991)
Copyright© International Association of Milk, Food and Environmental Sanitarians

The Decomposition of Daminozide (Alar) to Form Unsymmetrical Dimethylhydrazine (UDMH) in Heated, pH Adjusted, Canned Solutions

CHARLES R. SANTERRE*[1], JERRY N. CASH[2], and MATTHEW J. ZABIK[3]

[1]Department of Food Science and Technology, University of Georgia, Athens, Georgia 30602;
[2]Department of Food Science and Human Nutrition, Michigan State University, East Lansing, Michigan 48824;
[3]Department of Entomology, Pesticide Research Center, Michigan State University, East Lansing, Michigan 48824.

(Received for publication August 22, 1990)

ABSTRACT

Processing conditions were chosen to determine the influence of temperature, pH, and processing on model solutions containing daminozide residues. Daminozide (succinamic acid 2, 2-dimethylhydrazide) fortified solutions (12.5 ppm) containing 50mM NaH_2P0_4 and 24% sucrose (w/w) were adjusted to pH 3.0, 3.6, or 4.0 and either heated (100°C) for 0, 5, 10, or 15 min in sealed cans and cooled or heated (80°C) for 0, 5, or 10 min in open cans, sealed, heated (100°C) for 5 min, and cooled. Daminozide degradation due to heating was less than the HPLC detection limit (1.5 ppm) for all of the treatments. Unsymmetrical dimethylhydrazine (UDMH) concentration was significantly affected by heating time, pH, and processing. Heating of daminozide solutions in sealed cans produced approximately 1 ppm of UDMH for every minute of heating at 100°C. Heating of daminozide solutions in open cans at 80°C resulted in simultaneous production of UDMH in the solution and loss of UDMH through volatilization. Maximum degradation of daminozide was observed at pH 3.6.

Daminozide (succinamic acid 2, 2-dimethylhydrazide) is a plant growth regulator which was used in the production of apples, peanuts, cherries, pears, blueberries, cranberries, and grapes to improve crop yield and product quality (*10*). This agricultural chemical was introduced in 1962 and registered for use on food crops in 1968. In 1980, Newsome (*7*) demonstrated that daminozide can hydrolyze during the thermal processing of fruit products to form unsymmetrical dimethylhydrazine (UDMH; Fig. 1). Concern over the safety of foods containing UDMH has prompted studies to determine the toxicity of UDMH and the levels of UDMH in processed food products which contain daminozide residues.

Newsome (*7*) fortified applesauce with 30 ppm daminozide then boiled the sauce in an open container for 30 or 60 min and assayed 1.53 ppm and 1.92 ppm UDMH, respectively. A recent study by Saxton et al. (*8*) indicated that less than 1.1 ppm of daminozide was found in samples of fresh apples, applesauce, and apple juice. In addition, less than 62 ppb UDMH was found in these samples. Therefore, the 30 ppm daminozide fortification to applesauce used by Newsome (*7*) may be significantly higher than found in commercially processed apple products. In addition, the duration of heating applied to the applesauces was not representative of commercial practices. Therefore, further research is required to determine the dynamics of daminozide decomposition and UDMH formation. A first step would be to use heated model solutions which are free of interfering compounds which may affect assays which are used for determining daminozide and UDMH concentrations but which also are expected to be good predictors of food systems.

A sensitive assay using gas chromatography/mass spectrometry to determine daminozide concentrations in foods was reported by Conditt et al. (*2*). In this procedure, daminozide is first hydrolyzed with boiling sodium hydroxide to form UDMH. UDMH is then distilled into a collection vessel containing salicylaldehyde to form salicylaldehyde dimethylhydrazone (SDH; Fig. 2). GC/MS, selected ion monitoring (SIM) is then used to determine SDH concentration following extraction with methylene chloride. Free UDMH is generally present at much lower levels than daminozide and will only slightly affect the daminozide determinations. Free UDMH can be determined by eliminating the daminozide hydrolysis step and adding salicylaldehyde directly to the sample prior to methylene chloride extraction. The method of Conditt et al. (*2*) is time consuming (30 min/distillation) and subject to sample loss if the distillation system is not completely sealed. To avoid these problems a liquid chromatographic (LC) method was developed to assay for daminozide which would be acceptable for samples which contain few interfering compounds.

The objectives of this study were to determine the influence of heating time and pH on the decomposition of daminozide and the formation of UDMH in a canned sucrose syrup. The processing conditions were designed to predict the reaction kinetics of UDMH formation in food samples following standard processing regimens. An additional objective was to determine the influence of heating daminozide fortified samples prior to can sealing.

*Corresponding author.

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/54/3/225/1660212/0362-028x-54_3_225.pdf by guest on 24 October 2021

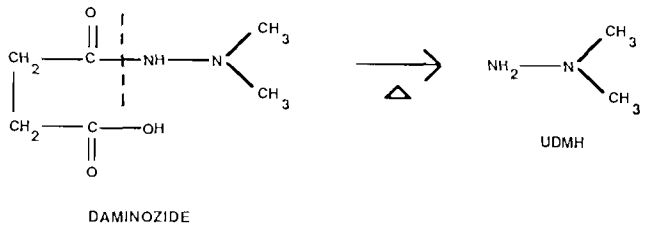

Figure 1. *Thermal decomposition of daminozide to yield unsymmetrical dimethylhydrazine (UDMH).*

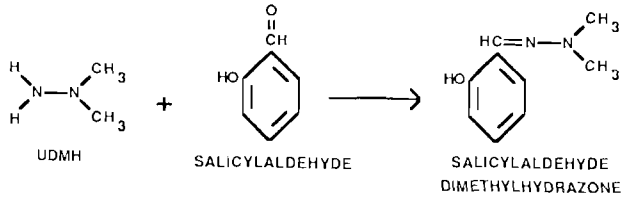

Figure 2. *Reaction between unsymmetrical dimethylhydrazine and salicylaldehyde to produce salicylaldehyde dimethylhydrazone.*

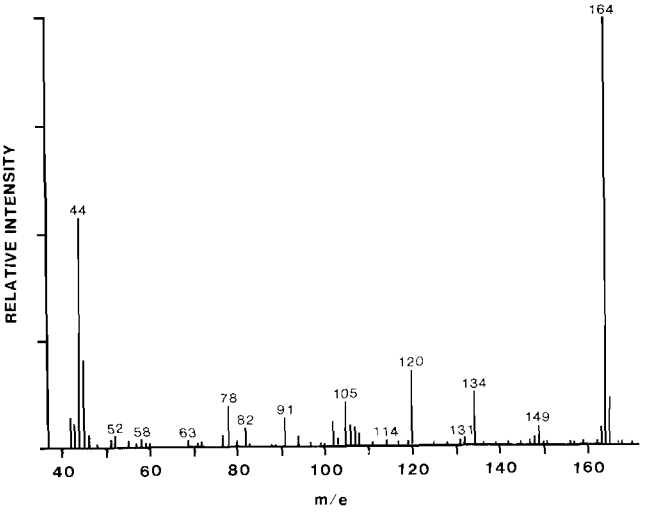

Figure 3. *Mass spectral fragmentation of salicylaldehyde dimethylhydrazone (MW = 160).*

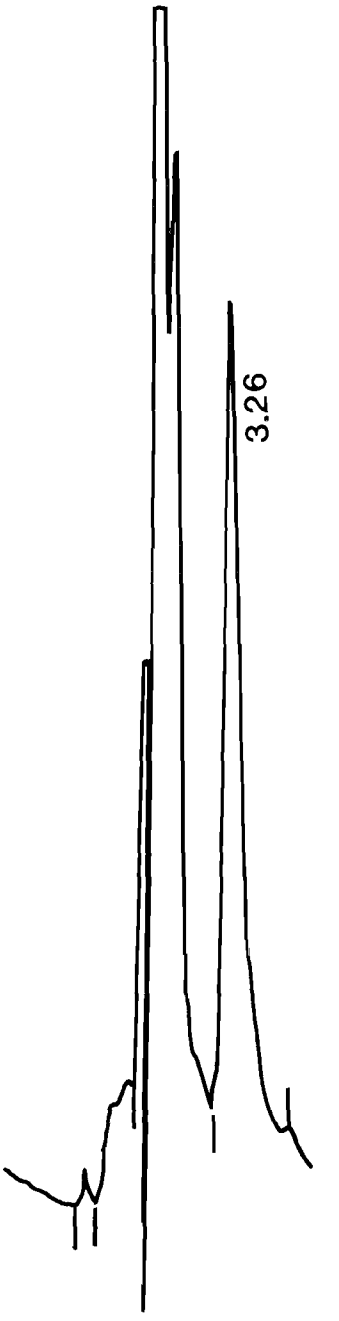

Figure 4. *LC chromatogram of daminozide (RT = 3.26 min).*

MATERIALS AND METHODS

Forty eight liters of 0.05 M NaH_2PO_4 buffered solution was prepared by combining 331.2 g NaH_2P0_4-H_20 (Mallinckrodt Inc., Paris, KY) with 48 L of distilled-deionized water. Sixty kilograms of 24% sucrose solution was prepared by adding 14.4 kg sucrose (GR grade; EM Science, Cherry Hill, NJ) to 45.6 kg of 0.05 M NaH_2PO_4 solution. Three solutions were prepared from the buffered sucrose solution by adjusting the pH to 3.0, 3.6, or 4.2. These pH values were selected to cover the range of pH's for processed fruits such as apples and cherries. A 20-kg volume of this buffered-sucrose solution was adjusted to pH 3.0 by adding 70 Ml of 1 N HCl. A 20-kg volume of this buffered-sucrose solution was adjusted to pH 3.6 by adding 14 ml of 1 N HCl. A 20-kg volume was adjusted to pH 4.2 by adding 4 ml of 1 NaOH. Each solution (i.e., pH 3.0, 3.6, and 4.2) was fortified with 100 ml of daminozide stock solution [125 g daminozide (Aldrich Chemical Co., Milwaukee, WI)/500 ml distilled-deionized water] to give a final concentration of 12.5 ppm daminozide in a 0.05 M NaH_2PO_4, 24% sucrose solution.

The daminozide-fortified solutions were filled (450 g per can) into No. 303 x 404 enamel-coated cans (American Can Co., Greenwich, CT), lidded, and sealed. Twenty cans per pH level were then divided into four groups and immersed in rapidly boiling water for a) 0 min; b) 5 min; c) 10 min; and d) 15 min. Following heating, cans were immediately cooled in 15.5°C water for 1 h and placed in 3.8°C storage prior to daminozide and UDMH analysis.

Fifteen cans per pH level were filled (450 g daminozide-fortified solution per can), heated to 80°C, held for a) 0 min; b) 5 min; and c) 10 min, lidded, and sealed. Following closure, cans were immersed for 5 min in rapidly boiling water, then cooled in 15.5°C water and stored at 3.8°C until daminozide and UDMH analysis. To compensate for evaporative losses, cans were opened and the fill weight was adjusted to 450 g with distilled-deionized water just prior to residue analysis.

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/54/3/225/1660212/0362-028x-54_3_225.pdf by guest on 24 October 2021

Daminozide determination

Canned samples were opened (adjusted to 450 g weight with distilled-deionized water if heated prior to can closure) and adjusted to pH 4.2 with 1 N NaOH. Five milliliters were filtered through a 0.45 µm filter (Type HA; Millipore Inc., Bedford, MA) attached to a Luer-lock syringe. Daminozide samples and standards were separated and quantified using high performance liquid chromatography (HPLC) with UV detection using the following parameters; Alltech Carbohydrate 10U column (Alltech Inc., Deerfield, IL), 300 mm x 4.1 mm i.d.; Anspec Model 2200 pump, flow rate = 1.5 ml/min; Milton Roy Spectro Monitor 3100 detector, Settings: 0.01 AUFS, 0.50 sec (response time), 218 nm (wavelength); Injector: Rheodyne Model 7125 (20 µl loop); solvent: 0.05 M NaH_2P0_4/methanol (90:10) degassed and filtered (0.45 µm Millipore filter, Type HA); Integrator: Spectra Physics Model SP4270, chart speed = 1.27 cm/min; Syringe: Hamilton #705 (blunt tip), 30 µl injection volume. Three cans for each heating time and pH level were assayed twice to provide 3 replicates and 2 subsamples per replicate.

Daminozide standards were prepared at 25, 10, 7.55, 5.0, 2.5, and 0 ppm daminozide concentrations by diluting a 1000 ppm daminozide stock solution [100 mg daminozide crystals/100 ml 0.05 M NaH_2P0_4- 24% sucrose solution (pH = 4.2)] to a 100 ppm daminozide stock solution (0.5 ml of 1000 ppm daminozide in 500 ml volumetric flask). Further dilutions were prepared by diluting the 100 ppm daminozide stock solutions with the 0.05 mM NaH_2P0_4 - 24% sucrose solutions (pH = 4.2).

UDMH determination

Canned samples were opened (adjusted to 450 g weight with distilled-deionized water if heated prior to can closure) and a 150-ml aliquot was combined with 150 µl salicylaldehyde (Aldrich Chem. Co.) (neat) in a 38 x 200-mm culture tube and sealed with a Teflon-lined screw cap (Kimax). Culture tubes plus sample were shaken, placed in a 50°C water bath for 90 min, and cooled to 3.8°C overnight. Sodium chloride (3 g) was added to each tube and the tubes were then sealed and shaken. The samples were then extracted two times with 4 ml of methylene chloride (Aldrich Chem. Co.) which contained 0.5 ml of 4-nitroanisole (Aldrich Chem. Co.)/1000 ml as an internal standard. After each addition of methylene chloride plus 4-nitroanisole, samples were sealed and shaken vigorously for 30 sec. The bottom layer was removed using a Pasteur pipet and combined with 1 g of Na_2S0_4 (anhydrous; Aldrich Chem. Co.) as a drying agent. Samples were then analyzed using GC/MS. GC/MS equipment and parameters were as follows: Gas Chromatograph - Delsi Di 700; oven temperature = 180°C, isothermal interface temperature = 250°C, source temperature = 250°C; column: DB-1; 30 m - fused silica, phase thickness = 0.25 µm; carrier gas: helium; injection: on-column (2 µL). Quadrapole Mass Spectrometer - Nermag R10-10C; electron ionization (EI), positive ion mode, selected ion monitoring; primary pressure: 1.3 x 10^{-3} Torr; IE = 0.198 amps; e^- = 70 eV; focal = -94.6; ions = -15.5; external = +39.1; multiplier = -2.81; polarizing voltage = -4.85V; monitor: Tektronix 4012; Printer: Okidata 250 printer; computer: Digital PDP 11/70, data acquisition delay time, 2 min.

Four spectral mass units were used to quantify UDMH and the internal standard, 4-nitroanisole. The mass spectrum of salicylaldehyde dimethylhydrazone (MW = 160) is shown in Fig. 3. The mass with the greatest intensity is the molecular ion peak m/e = 164 and the most intense fragment is the [N - 44] m/e = 120. For 4-nitroanisole, the parent peak is found at m/e = 153 with the most intense fragment at m/e = 123. Retention times for salicylaldehyde dimethylhydrazone and 4-nitroanisole are 4:37 min and 3:30 min, respectively. The ratio of m/e 164/153 was used to determine concentration of UDMH.

UDMH standards were prepared fresh daily at 500, 250, 100, 50, 25 and 0 ppb levels. A 10,000 ppm solution was prepared by mixing UDMH (Aldrich Chem. Co.) in a 100-L volumetric flask with 0.05 M NaH_2P0_4 - 25% sucrose solution (pH = 4.2) for 1 min. Serial dilutions of 10,000 ppm, 100 ppm, and 5 ppm were made. Final concentrations of UDMH were prepared from the 5 ppm stock solution using the buffered sucrose diluent.

Both sets of UDMH analysis data (i.e., samples heated following can closure and samples heated before and after can closure) were statistically analyzed using a two treatment, randomized complete block (RCB), factorial design with replicates (*2*). Tukey's LSD was used to determine significant differences between treatment means.

RESULTS AND DISCUSSION

Daminozide analyses

In the LC analysis, daminozide appeared as a descending shoulder of the large sucrose peak (Fig. 4). The retention time of daminozide was 206 sec (44 sec after sucrose) and could not be resolved further from the sucrose peak by changing the solvent polarity or flow rate. In order to reduce the variability of this analytical method, each of three replicate samples was analyzed twice. The sensitivity of the LC procedure was determined to be ±1.5 ppm daminozide for these experiments.

Many researchers (*1-9*) have analyzed for daminozide in food and environmental samples. None of these researchers have applied LC techniques to determine daminozide residues. Instead, most of these researchers hydrolyzed daminozide with heat and alkalai to produce UDMH. UDMH was then reacted with trisodium pentacyanoamine ferroate (*4,5*) or salicylaldehyde (*1,2*) and determined colorimetrically or spectrometrically. Another approach was to oxidize UDMH with selenium dioxide to yield formaldehyde which was then determined colorimetrically with 2-hydrazinobenzothiazole or the derivatization of UDMH with pentafluorobenzoyl chloride to produce 1,1-dimethyl-2-2-bis (pentafluorobenzoyl) hydrazine which was determined with GC (*7*). Steinbrecher et al. (*9*) was able to achieve a sensitivity of 50 ppb for daminozide in food samples when they used a derivatization procedure.

There were no statistical differences (p = 0.01) in the daminozide concentrations between samples heated in open or closed cans and those samples which were unheated at any of the pH levels (i.e., pH = 3.0, 3.6, and 4.2). The 12.5 ppm daminozide which was added to the buffered-sucrose solution was not degraded in any significant (p = 0.05) amount by heating at 100°C for 15 min. Daminozide was stable in buffered-sucrose solution during the experimental conditions and less than 1.5 ppm daminozide (level of sensitivity) was decomposed during the heating intervals applied. Since the food matrix has a major influence on the heating and cooling dynamics of canned food products, the degradation of daminozide may be different in other food matrices for the same time-temperature conditions.

UDMH determination

A mass spectral scan of a 10 ppm UDMH solution is shown in Fig. 5. The retention times of 4-nitroanisole (internal standard) and UDMH were 3:30 min and 4:37

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/54/3/225/1660212/0362-028x-54_3_225.pdf by guest on 24 October 2021

min, respectively. Samples containing less than 10 ppm UDMH did not have a peak at m/e = 120, due to limited intensity of this mass fragment. Concentrations were calculated directly from the area ratios of m/e 164: m/e 153. Standard curves for 0-500 ppb and 0-10 ppm UDMH gave correlation coefficients (R^2) for the linear regressions of these curves of 0.995.

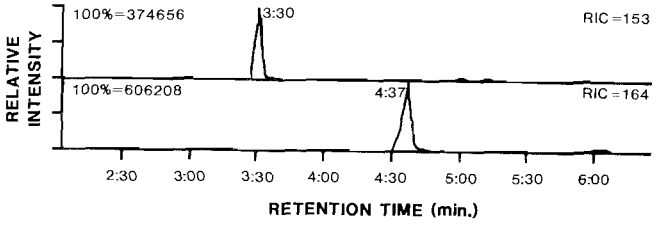

Figure 5. *GC/MS, SIM chromatograph of m/e = 153 (4-nitroanisole) and m/e = 164 (salicylaldehyde dimethylhydrazone) from a 10 ppm UDMH standard.*

TABLE 1. *Analysis of variance (AOV) describing the influence of pH and heating time on the concentration of UDMH in canned samples which were closed prior to heating (100°C).*

Source	df	SS	MS	F-Value	Sing.
Rep	1	0.37	0.368	0.15	n.s.
pH Level (A)	2	126.35	63.174	25.85	.000
Heating time (B)	3	974.74	324.912	132.96	.000
A X B	6	184.01	30.668	12.55	.000
Error	11	26.88	2.444		

TABLE 2. *Analysis of variance (AOV) describing the influence of pH and heating time on the concentration of UDMH in canned samples which were heated before (80°C) and after (100°C) can closure.*

Source	df	SS	MS	F-Value	Sing.
Rep	1	8.34	8.343	2.00	n.s.
pH Level (A)	2	53.01	26.507	6.37	.014
Heating time (B)	3	515.81	171.936	41.32	.000
A X B	6	92.16	15.359	3.69	.029
Error	11	45.78	4.161		

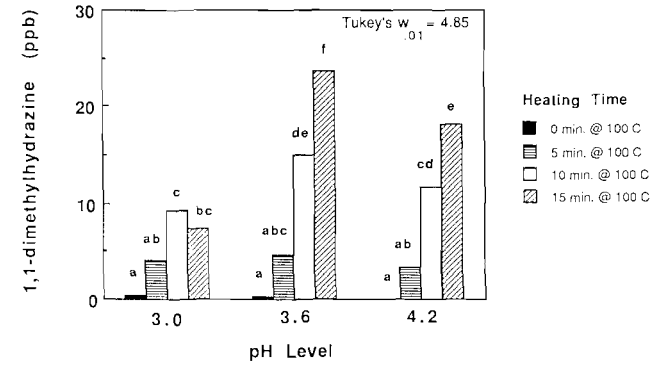

Figure 6. *UDMH residues in samples which were heated (100°C) following can closure as a function of heating time and pH. Bars with the same letter are not significantly different at the Tukey's $W_{0.01}$ level.*

UDMH production was significantly (p = 0.01) influenced by the interaction between heating time and pH for samples which were sealed prior to heating (Table 1). There were significantly greater UDMH concentrations in samples at pH 3.6 than samples at pH 3.0 or pH 4.2 (Fig. 6). This suggests that daminozide is hydrolyzed to UDMH at an optimal pH in food products which are heated. There was an increase in UDMH of about 5 ppb for every 5 min of heating at 100°C.

The level of UDMH found in these samples is much lower than the level reported by Newsome (7). He measured 600 ppb UDMH in applesauce which was fortified with 30 ppm daminozide and boiled in an open container for 10 min. After 20 min of boiling, 1.25 ppm of UDMH was detected in the applesauce. There is no indication that

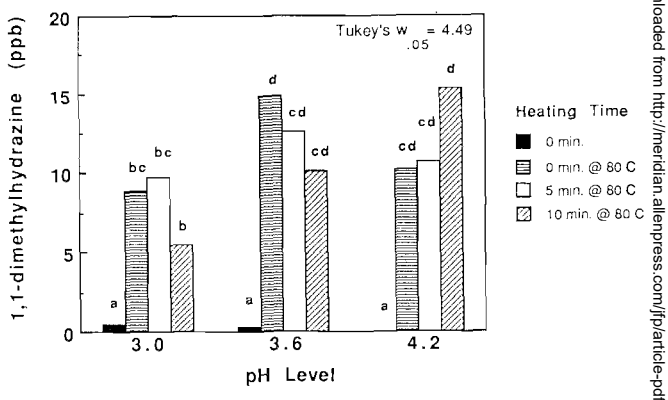

Figure 7. *UDMH residues as a function of heating time and pH in samples which were heated (80°C) prior to can closure in addition to 5 min heating (100°C) following closure. Bars with the same letter are not significantly different at the Tukey's $W_{0.01}$ level.*

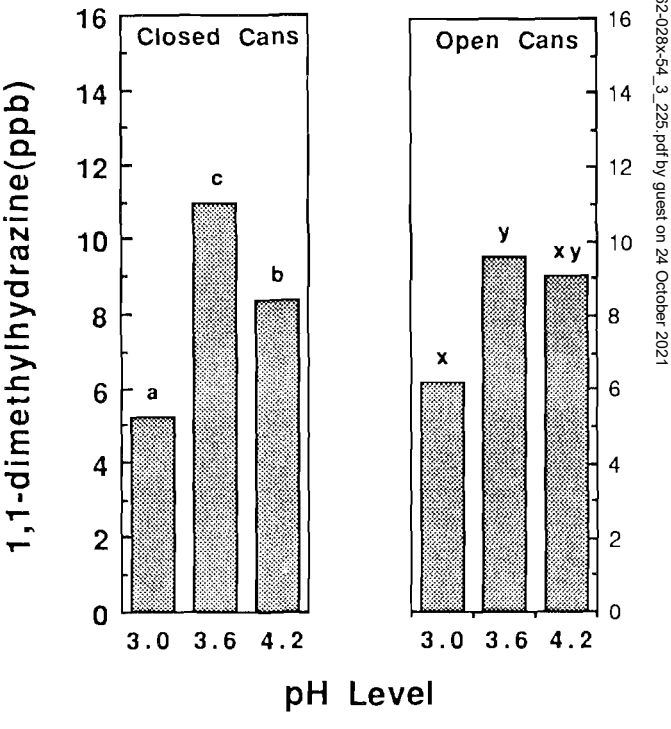

Figure 8. *Influence of pH on the production of UDMH in open and closed cans which were heated at 80°C and 100°C, respectively. Bars with the same letter are not significantly different at the Tukey's $W_{0.01}$ level.*

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/54/3/225/1660212/0362-028x-54_3_225.pdf by guest on 24 October 2021

the level of UDMH was assayed prior to heating. However, the level of UDMH, extrapolated to 0 min of boiling, was 200 ppb. There appears to be a large amount of residual UDMH in the daminozide stock solution or applesauce. The UDMH concentrations measured represent a 40-fold increase in daminozide decomposition rate compared to the data presented here. The difference between our results may be attributed to the analytical methods used to determine UDMH concentration or to differences in the sample matrix and processing conditions.

Daminozide-treated samples, which were heated to 80°C, held for 0, 5, and 10 min, then lidded, sealed and heated in 100°C water for 5 min, had significantly ($p = 0.01$) higher concentration of UDMH than control samples (Fig. 7). A higher concentration of UDMH was measured for closed and open heated samples at the pH 3.6 level for each experiment (Fig. 8). Lowest concentrations of UDMH were measured at pH 3.0 for both processing regimens.

There was no difference in UDMH accumulation between samples heated for greater than 5 min prior to can closure. This differs from the samples which were lidded and sealed prior to thermal processing. Since UDMH is an extremely volatile compound, it appears that further heating of daminozide-treated solutions will cause UDMH volatilization as rapidly as UDMH is generated. In contrast, daminozide-treated solutions which are heated following can closure have accumulated more UDMH than open-container heated samples.

Theoretically, for every 1 ppm daminozide hydrolyzed in a closed system, 375 ppb UDMH is generated. As discussed in the preceding section, all of the samples in this experiment had less than 25 ppb UDMH. This means that less than 1 ppm of daminozide was degraded during this experiment. In order to detect changes in the daminozide concentration of less than 1 ppm, a more sensitive assay for daminozide than used here would be required.

ACKNOWLEDGMENTS

Thanks to Yen Ling (Annie) Chai and Dr. Salah Selim for assistance in sample preparation and analysis. Special thanks to Margaret Conner and Kim Felver for assistance with manuscript preparation.

REFERENCES

1. Allen, J. G. 1980. Daminozide residues in sweet cherries, and their determination by colorimetric and gas-liquid chromatographic methods. Pest. Sci. 11:347-350.
2. Conditt, M. K., J. R. Baumgardner, and L. M. Hellmann. 1988. Gas chromatographic/mass spectrometric determination of daminozide in high protein food products. J. Assoc. Off. Anal. Chem. 71:735-739.
3. Dicks, J. W. 1971. Determination of daminozide residues in chrysanthemum tissues. Pest. Sci. 2:176-178.
4. Food and Drug Administration. 1975. Method of analysis for alar residue in various crops. *In* Pesticide analytical manual, vol. 2. FDA, Rockville, MD. Pest. Reg. Sec. 180-246.
5. Lane, J. R. 1970. General alar residue method. Uniroyal Chemical Co., Bethany, CN. pp. 1-4.
6. Liu, C.-H., G.C. Mattern, G.M. Singer, and J.D. Rosen. 1989. Determination of daminozide in apples by gas chromatography/chemical ionization mass spectrometry. J. Assoc. Off. Anal. Chem. 72:984-986.
7. Newsome, W. H. 1980. Determination of daminozide residues on food and its degradation to 1,1-dimethylhydrazone by cooking. J. Agr. Food Chem. 28:319-321.
8. Saxton, W. L., K. Steinbrecher, and E. Gunderson. 1989. Results of a survey for the presence of daminozide and unsymmetrical dimethylhydrazine in food. J. Agr. Food Chem. 37:570-573.
9. Steinbrecher, K., W.L. Saxton, and G.A. Oehler. 1990. Improved gas chromatographic method for determination of daminozide by alkaline hydrolysis and 2-nitrobenzaldehyde derivatization and survey results of daminozide in agricultural products. J. Assoc. Off. Anal. Chem. 73:512-515.
10. Unrath, C. R., A. L. Kenworthy, and C. L. Bedford. 1969. The effect of Alar, succinic acid 2,2-dimethylhydrazone, on fruit maturation, quality, and vegetative growth of sour cherries, *Prunus cerasus* L., cv. "Montmorency". J. Am. Soc. Hort. Sci. 94:387-391.

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/54/3/225/1660212/0362-028x-54_3_225.pdf by guest on 24 October 2021