Diphenylamine Residues in Apples (*Malus domestica* Borkh.), Cider, and Pomace following Commercial Controlled Atmosphere Storage

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Diphenylamine (DPA) is used for scald suppression on apples held in commercial controlled atmosphere (CA) storage. The whole fruit tolerance in the United States for DPA is 10 ppm. This study was conducted to quantify terminal DPA residues in Red Delicious and Granny Smith apples and processed products including cider and wet and dried pomace following DPA treatments at maximum labeled use rates. Samples were analyzed at several intervals during an approximately 9-month CA storage period. Initial DPA residues in whole apples were <10 ppm and progressively declined under commercial CA storage. Only traces of DPA occurred in cider; however, DPA concentrated in both wet and dried pomace. Residues were less in dried pomace than in wet pomace, presumably due to loss by volatility during the drying process. DPA residues in cider and pomace also dissipated with time in CA storage.

Keywords: *Diphenylamine; apples; Malus domestica; controlled atmosphere storage; concentration factors*

INTRODUCTION

Apples can be held in controlled atmosphere (CA) storage for up to 10 months following harvest. Long-term exposure of fruit to low temperatures in these storages commonly induces a physiological disorder known as scald. Diphenylamine (DPA) is federally registered for application to apples for reducing scald damage while in CA storage. Most commonly, DPA is applied as a drench treatment to the fruit within 7 days following harvest and prior to storage; however, postharvest dipping and spraying treatments also are conducted. A national survey of postharvest chemical use on apples harvested for fresh market showed that 47% were treated with DPA for scald suppression (Kupferman, 1991). Red Delicious and Granny Smith varieties are both susceptible to scald development and were the most commonly treated varieties (62% and 54%, respectively).

A tolerance in the United States has been established for residues of DPA at 10 ppm in or on apples. Terminal DPA residues in/on Red Delicious and Granny Smith apples, cider, and pomace following treatment of whole apples using registered methods at maximum use rates and storage in commercial CA storage facilities have not previously been published. Objectives of this study were to (1) quantify terminal DPA residues in Red Delicious and Granny Smith whole apples, cider, and wet and dried pomace following postharvest application of DPA at maximum labeled use rates, (2) evaluate DPA dissipation rates under commercial CA storage conditions, and (3) calculate DPA concentration factors in cider and wet and dried pomace processed from treated apples. This study was conducted to satisfy U.S. Environmental Protection Agency (USEPA) DPA reregistration data requirements and followed then current USEPA residue chemistry guidelines and current Good Laboratory Practice standards.

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METHODS

Treatment. Study apples were harvested from commercial orchards in Grant County, WA, on September 27, 1993 (Red Delicious), and October 17, 1993 (Granny Smith), and treated within 2 days of harvest. Red Delicious apples for the whole apple residue study were treated with a commercial formulation containing 31% DPA at the maximum labeled rate of 2000 ppm active ingredient (AI), and Granny Smith apples were treated with a commercial formulation containing 15% DPA at the maximum labeled use rate of 2200 ppm AI. Apples to be processed into cider and pomace were treated with the same formulations at 10 times the maximum labeled use rates (20 000 ppm AI for Red Delicious and 22 000 ppm AI for Granny Smith). Exaggerated rates were used for apples to be processed because it was anticipated from apple metabolism study data that exaggerated DPA treatments would be required to detect residues of DPA metabolites in the processed products, if required for USEPA reregistration purposes. As is standard commercial practice, a commercial formulation of thiabendazole fungicide was added to each treatment solution at the labeled rate of 528.5 ppm AI to maintain apples while in storage.

Each individual sample was comprised of approximately 75 apples weighing approximately 14 kg. Following standard commercial practice, apple samples and treatment solutions were at ambient temperatures during the treatment. Red Delicious apples were dipped, whereas Granny Smith apples were drenched in DPA solutions. The most extensively used commercial treatment method is by drench application. Red Delicious samples were placed in plastic fruit picking lugs, and

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Table 1. Mean Recoveries of Diphenylamine in Red Delicious and Granny Smith Whole Apples, Cider, Wet Pomace, and Dried Pomace

cultivar/matrix	conctn Range (ppm)	mean recovery (%)	SD (%)
Red Delicious/whole apple	4.00−60.0	93.3	3.21
Granny Smith/whole apple	2.00−26.0	94.0	1.73
Red Delicious/cider	0.080−10.0	88.3	8.69
Granny Smith/cider	0.080−10.0	92.1	4.97
Red Delicious/wet pomace	20.0−200	92.3	10.8
Granny Smith/wet pomace	20.0−120	89.8	5.42
Red Delicious/dried pomace	0.400−100	75.2	7.03
Granny Smith/dried pomace	0.399−100	83.3	14.3

the lug was immersed in a 95-L dipping solution for 1 min while the solution was agitated. A treatment time of 1 min was selected to ensure residues were maximized; increases in dipping times over 30 s have not been shown to increase DPA residues (Lee et al., 1984). Granny Smith samples also were placed in fruit picking lugs and treated using an experimental drencher at the rate of 0.3 L of solution/kg of apples/30 s. Following treatment, all samples were allowed to drain dry. Samples of each application solution formulated to treat samples were analyzed to determine DPA concentration; mean DPA concentration ranged from 94.1% to 112.5% of the nominal concentration in all treatment solutions.

For each CA storage period, two replicate samples of Red Delicious and Granny Smith apples were treated for the whole apple residue study, and two replicate samples of each variety were treated for processing. Treatment solutions were duplicated to replicate treatments; half the samples were treated with each application solution made. Assignment of samples to storage period was random.

Storage. Treated apples were placed in a commercial CA storage facility located in Quincy, WA. Treated Red Delicious and Granny Smith apples were held in separate storages, with both storages maintained at temperature and atmosphere conditions standard for each variety. Red Delicious samples were stored at approximately −0.11 °C, 1.5% O_2, and 1.9% CO_2, and Granny Smith samples were stored at approximately 1.3 °C, 1.3% O_2, and 1.5% CO_2. Apples were removed from CA storage at approximately 0-, 3-, 6-, and 9-month intervals for the whole apple residue study and at 0-, 6-, and 9-month intervals for the processing study.

Processing. Raw apples were processed into cider and wet and dried pomace using procedures simulating standard industrial processing procedures. The apples were tub washed and sorted. Washed apples were crushed and pressed using a Suntech fruit press. The wet pomace sample fraction was removed, and the remaining wet pomace was dried at 79−93 °C to <10% moisture using a Bin air drier. The fresh cider recovered during crushing and pressing was filtered through a standard milk filter before sample collection.

Analysis. Two 25-g aliquots of homogenized whole apple, cider, and wet pomace and two 5-g aliquots of dried pomace were analyzed for each of the two replicate samples, resulting in four analyses for each sample matrix and CA storage period. Prepared samples were extracted with acetone in a blender cup and filtered. The filtrate was mixed with water and partitioned against hexane. The hexane extract was filtered through a bed of sodium sulfate (Na_2SO_4) to remove emulsions and traces of moisture. After exchange with dichloromethane (DCM) and concentration, the DCM solution containing diphenylamine was derivatized with trifluoroacetic anhydride. The acetylated DPA was determined by gas chromatography with mass selective detection in the selected ion monitoring mode. All extracts were analyzed using a Hewlett-Packard 5890 gas chromatograph/mass spectrometer system. The limit of quantitation was 0.08 ppm for whole apples, cider, and wet pomace and 0.4 ppm for dried pomace. Mean recoveries ranged from 75.2 ± 7.03% (SD) in Red Delicious dry pomace to 94.0 ± 1.73% in Granny Smith whole apples (Table 1).

Mean DPA residues and standard errors were calculated and plotted by day using the two replicates. A quadratic function was developed to model the relationship between length of CA storage period and DPA residues using SAS GRAPH software (SAS Institute Inc., 1988). DPA concentration factors in cider and pomace were calculated by dividing the DPA residue value in each processed product by the corresponding DPA residue value in whole apples treated at the same time and at the same application rate as those apples treated for processing.

RESULTS

DPA residues in Red Delicious samples treated with 2000 ppm DPA solutions averaged 5.86 ppm immediately following treatment. Following 90, 181, and 281 days of CA storage, DPA residues averaged 5.46, 3.96, and 3.18 ppm, respectively (Table 2). The highest DPA residue detected in any single assay was 8.36 ppm, which occurred in a sample collected following 90 days of CA storage. DPA residues in Granny Smith samples treated with 2200 ppm DPA solutions averaged 3.37 ppm on day 0, 2.41 ppm on day 91, 2.06 ppm on day 181, and 1.01 ppm following 260 days of CA storage (Table 2). The highest residue detected in any sample assay was 3.56 ppm in a sample taken immediately following application.

DPA residues in whole Red Delicious apples treated at 10 times the maximum labeled application rate averaged 29.6 ppm on day 0 and 19.6 ppm after 281 days in CA storage (Table 2). In Granny Smith apples treated at the 10× application rate, DPA residues averaged 28.5 ppm on day 0 and 5.16 ppm following 260 days of CA storage (Table 2).

DPA residues in cider processed from Red Delicious apples treated with the 20 000 ppm DPA solutions averaged 1.38 ppm on the day of treatment. DPA residues in these same samples averaged 1.02 ppm following 181 days of CA storage and 0.755 ppm following 281 days of storage (Table 2). The highest residue detected in any single assay was 1.48 ppm on day 0. Mean DPA residues in cider processed from Granny Smith apples treated with the 22 000 ppm DPA solution were 0.661 ppm on day 0, 0.702 ppm on day 181, and 0.421 ppm on day 260 (Table 2). The highest DPA residue detected in any Granny Smith cider assay (0.911 ppm) occurred following 181 days of CA storage.

DPA residues in wet pomace processed from Red Delicious samples averaged 144 ppm on day 0, 83.5 ppm on day 181, and 64.5 ppm following 281 days of storage (Table 2). On day 0, DPA residues in wet pomace processed from treated Granny Smith samples averaged 94.3 ppm. Residues averaged 62.3 ppm after 181 days and 31.1 ppm after 260 days of storage (Table 2). The highest DPA residue detected in any single assay was 167 ppm in Red Delicious and 102 ppm in Granny Smith samples, both of which occurred immediately following treatment.

Red Delicious dried pomace DPA residues averaged 54.9 ppm on day 0, 54.1 ppm on day 181, and 37.2 ppm on day 281 (Table 2). DPA residues in dried pomace processed from Granny Smith samples averaged 69.1, 25.3, and 15.2 ppm following 0, 181, and 260 days of CA storage, respectively (Table 2).

DPA did not concentrate in cider expressed from Red Delicious or Granny Smith apples. DPA concentration factors for Red Delicious cider were 0.047 on day 0 and 0.039 on day 281. For Granny Smith samples, the DPA concentration factor in cider was 0.023 on day 0 and 0.082 on day 260 (Table 3).

DPA concentration factors in Red Delicious wet pomace and dried pomace on the day of treatment were calculated to be 4.86 and 1.85, respectively. Following

Table 2. Mean (±SD) DPA Residues (ppm) in Red Delicious and Granny Smith Apples, Cider, Wet Pomace, and Dried Pomace following Controlled Atmosphere Storage

		approximate time in controlled atmosphere storage			
cultivar/matrix	application[a] (ppm)	0 months	3 months	6 months	9 months
Red Delicious/whole apple	2 000 dip	5.86 ± 0.46	5.46 ± 1.19	3.96 ± 0.50	3.18 ± 0.47
Granny Smith/whole apple	2 200 drench	3.37 ± 0.02	2.41 ± 0.08	2.06 ± 0.11	1.01 ± 0.08
Red Delicious/whole apple	20 000 dip	29.6 ± 5.7	*b*	*b*	19.6 ± 13.1
Granny Smith/whole apple	22 000 drench	28.5 ± 4.5	*b*	*b*	5.16 ± 0.74
Red Delicious/cider	20 000 dip	1.38 ± 0.15	*b*	1.02 ± 0.12	0.755 ± 0.134
Granny Smith/cider	22 000 drench	0.661 ± 0.047	*b*	0.702 ± 0.294	0.421 ± 0.194
Red Delicious/wet pomace	20 000 dip	144 ± 23	*b*	83.5 ± 13.2	64.6 ± 3.0
Granny Smith/wet pomace	22 000 drench	94.3 ± 6.8	*b*	62.3 ± 14.6	31.1 ± 10.9
Red Delicious/dried pomace	20 000 dip	54.9 ± 12.2	*b*	54.1 ± 2.2	37.2 ± 4.5
Granny Smith/dried pomace	22 000 drench	69.1 ± 43.5	*b*	25.3 ± 14.6	15.2 ± 1.9

[a] Maximum labeled application rate is 2 000 ppm for Red Delicious and 2 200 ppm for Granny Smith cultivars. [b] Samples of matrix not collected for this storage interval.

Table 3. Calculated DPA Concentration Factors in Cider, Wet Pomace, and Dried Pomace Processed from Red Delicious and Granny Smith Apples

	time in controlled atmosphere storage	
cultivar/processed fraction	0 months	9 months
Red Delicious/cider	0.047	0.039
Granny Smith/cider	0.023	0.082
Red Delicious/wet pomace	4.86	3.30
Granny Smith/wet pomace	3.31	6.03
Red Delicious/dried pomace	1.85	1.90
Granny Smith/dried pomace	2.42	2.95

Table 4. Estimated DPA Residues (ppm) in Cider and Pomace Processed from Apples Treated at Maximum Labeled Use Rates

	controlled atmosphere storage period	
cultivar/processed fraction	0 months	9 months
Red Delicious/cider	0.275	0.124
Granny Smith/cider	0.078	0.083
Red Delicious/wet pomace	28.5	10.5
Granny Smith/wet pomace	11.2	6.09
Red Delicious/dried pomace	10.8	6.04
Granny Smith/dried pomace	8.16	2.98

281 days in CA storage, DPA concentration factors were calculated to be 3.30 in wet pomace and 1.90 in dried pomace. For Granny Smith samples, DPA concentration factors on the day of treatment were 3.31 in wet pomace and 2.42 in dried pomace. Following 260 days in storage, concentration factors were 6.03 in wet pomace and 2.95 in dried pomace (Table 3).

To estimate DPA residue levels expected in cider and pomace processed from apples treated at the maximum labeled use rates, rather than the exaggerated rates used in this study, DPA residue values in whole apples treated at maximum labeled use rates were multiplied by the concentration factors calculated above for cider and pomace processed from apples treated at the 10× rate. Based on these calculations, the highest estimated DPA residues in products processed from apples treated at maximum labeled use rates were 0.275, 28.5, and 10.8 ppm in Red Delicious cider, wet pomace, and dried pomace, respectively, and 0.083, 11.2, and 8.16 ppm in Granny Smith cider, wet pomace, and dried pomace (Table 4).

DISCUSSION

Treatment of Red Delicious and Granny Smith apples with DPA at maximum labeled use rates and following standard commercial practices resulted in initial mean DPA residues in whole fruit well below the 10 ppm U.S.

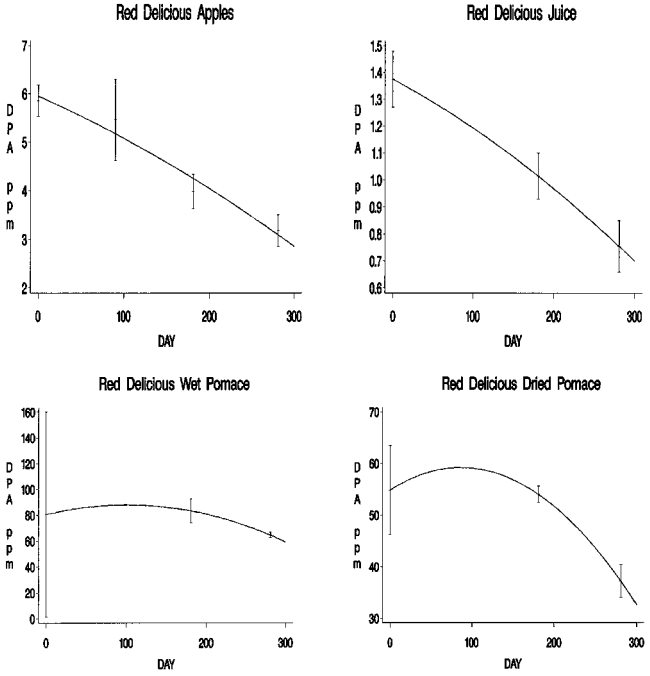

Figure 1. Mean (±SE) DPA residues in Red Delicious apple matrices under commercial controlled atmosphere storage.

tolerance for DPA. DPA residues in all Red Delicious (Figure 1) and Granny Smith (Figure 2) whole fruit and processed matrices progressively declined under temperature and atmospheric conditions present in commercial CA storage units. Similar rates of DPA residue dissipation under commercial storage conditions were documented in Starking apples treated with 2000 ppm DPA dip solutions (Combrink et al., 1987). Residues in Red Delicious apples were higher than those in Granny Smith apples, even though DPA concentration was 10% higher in solutions used to treat Granny Smith samples. This difference may have been due to differences in treatments (dip vs drench); however, differences in cultivar also may affect terminal DPA residues. Of five apple cultivars treated by Lee et al. (1984) with a 2000 ppm DPA dip, DPA residues were highest in Red Delicious samples.

DPA did not concentrate in cider expressed from fresh or stored Red Delicious and Granny Smith apples treated with 10× exaggerated rates of DPA, and DPA residues in cider were well below those found in whole apples treated at maximum labeled (1×) use rates. DPA did concentrate in wet and dried pomace. These results are consistent with those of Gutenmann et al. (1990), who found that essentially all DPA remained in the

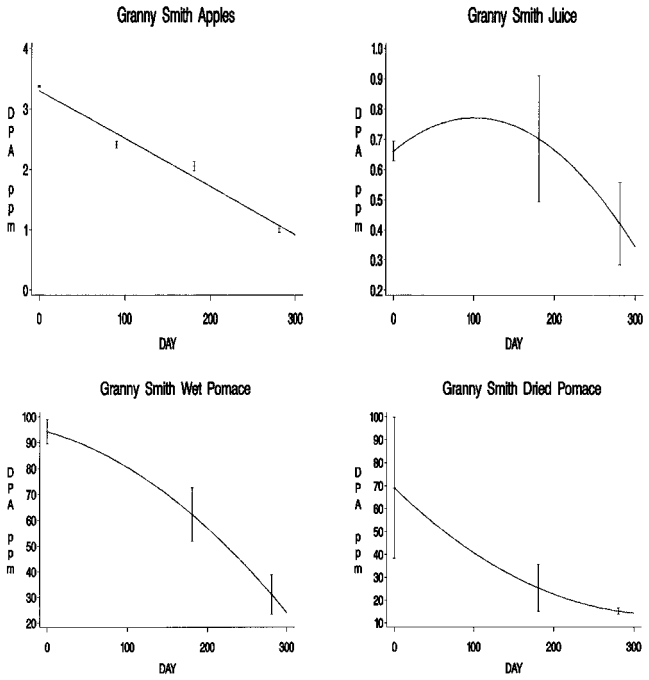

Figure 2. Mean (±SE) DPA residues in Granny Smith apple matrices under commercial controlled atmosphere storage.

pomace following fog applications of DPA to Empire, MacIntosh, Red Delicious, Rome Beauty, and Spartan apple varieties. DPA residues in dried pomace were lower than in wet pomace, presumably due to loss of DPA through volatility during the drying process. Estimated DPA residues in pomace processed from apples treated with DPA at maximum use rates exceeded the whole apple tolerance in Red Delicious wet and dried pomace and Granny Smith wet pomace immediately following treatment. By the end of the approximate 9-month storage period, however, only residues in Red Delicious wet pomace were still greater than the whole apple tolerance.

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