Degradation of 3-Chloro-*p*-toluidine Hydrochloride in Watermelon Bait. Identification and Chemical Characterization of Novel *N*-Glucoside and Oxopropanimine

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Stability of the avicide 3-chloro-*p*-toluidine hydrochloride (CPTH) in watermelon bait was assessed. When exposed to light, the presence of CPTH accelerated nonenzymic browning (Maillard) reactions and degradation of the watermelon matrix. The addition of potassium metabisulfite appeared to hinder bait degradation. These experiments resulted in the identification and chemical characterization of two novel CPTH derivatives: *N*-β-D-glucopyranosyl-3-chloro-4-methylaniline and *N*-(3-chloro-4-methylphenyl)-2-oxopropanimine.

Keywords: *3-Chloro-p-toluidine hydrochloride; CPTH; DRC-1339; N-β-D-glucopyranosyl-3-chloro-4-methylaniline; N-(3-chloro-4-methylphenyl)-2-oxopropanimine; nonenzymic browning; Maillard reaction*

INTRODUCTION

Extensive damage to grapefruits and oranges are associated with large populations of the great-tailed grackle (*Quiscalus mexicanus*) in the Rio Grande Valley of Texas. Losses of $2.2 million in citrus damage were reported in 1987 (Glahn et al., 1995). Trapping and frightening techniques have been used to reduce crop damage. While trapping provides little relief, the birds adapt to the scare tactics, and heavy damage persists. An effective avicide, 3-chloro-*p*-toluidine hydrochloride (CPTH, Figure 1), has been registered by the EPA since 1967. USDA Animal Damage Control personnel use CPTH widely to control localized bird populations. CPTH is effective on target species including grackles, starlings, blackbirds, ravens, crows, gulls, and pigeons (Schafer, 1984).

In Texas, CPTH is formulated in dry dog food baits (Glahn et al., 1995). These baits, however, are poorly accepted by the birds during the summer months, when damage to citrus crops is severe. Attempts to increase bait acceptance have included formulation of CPTH into a watermelon matrix. Favorable results from initial trials led to field studies on the effectiveness and safety of 0.2% (w/w) CPTH treated watermelon.

During these field studies, fresh watermelon bait was prepared daily. Within hours of exposure to sunlight and heat, the bait discolored and developed an unpleasant odor. As the watermelon bait degraded, less acceptance of the bait was evident. Field personnel suggested that formation of the brown color and odor may have been responsible for the decreased acceptance (Glahn, personal communication). As a result of the field trials, preliminary laboratory studies with watermelon baits were performed under simulated field conditions. In these experiments, browning of the watermelon bait and decreases in CPTH concentrations resulted within hours of exposure to light (Goodall, unpublished report).

Watermelon pulp consists of approximately 93% water and 6% total carbohydrates (Watt and Merrill, 1963). While ultraviolet (UV) and ionizing radiation may stimulate enzymic browning of carbohydrates (Richardson, 1976), the presence of a primary or secondary amine accelerates complex nonenzymic browning (Maillard) reactions of carbohydrates (Hodge and Osman, 1976). Because the major saccharides in the watermelon pulp are sucrose, fructose, and glucose (Brown and Summers, 1985) and since CPTH is a primary amine (Figure 1), browning reactions of the sugars were presumably responsible for the bait degradation. To determine whether enzymic or nonenzymic processes caused the discoloration, heated and frozen CPTH-fortified watermelon baits were monitored for color changes when exposed to light.

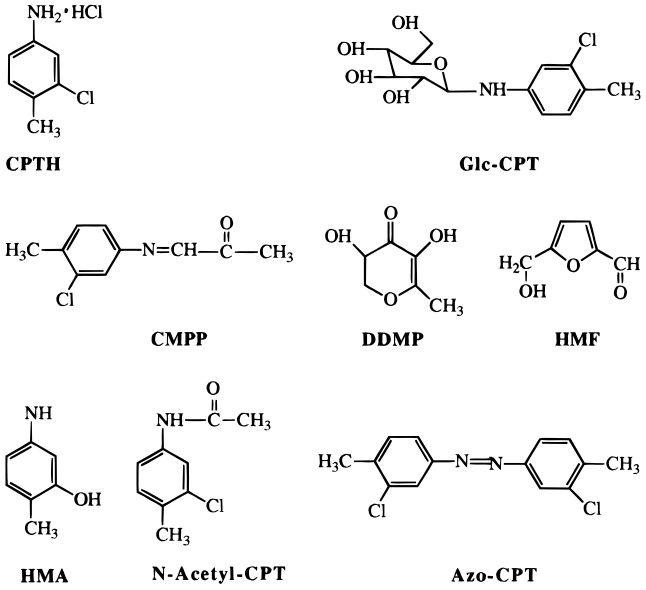

Figure 1. Structures of CPTH and proposed reaction products.

Sulfur dioxide and sulfites hinder browning due to nonenzymic processes (McWeeney et al., 1969). Potassium metabisulfite (PMB) has been used extensively as a fruit preservative. In the presence of metabisulfite, reducing sugars form sulfonated compounds that retard the browning process (Burton et al., 1963; Ingles, 1966).

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To further test if nonenzymic browning was responsible for discoloration of the watermelon matrix, additions of 1.5% and 3% (w/w) PMB to the watermelon bait were assessed as preservatives. In addition, extracts of light-exposed CPTH-fortified and control watermelon baits were analyzed by electrospray mass spectrometry (EMS), gas chromatography−mass spectrometry (GC−MS), high-performance liquid chromatography (HPLC), high-performance liquid chromatography−mass spectrometry (HPLC−MS), and nuclear magnetic resonance (NMR) spectroscopy for CPTH degradates and Maillard reaction products.

METHODS AND MATERIALS

Chemicals and Materials. All solvents and chemicals were reagent grade unless otherwise noted: CPTH, P. M. Resources, Inc.; D-fructose and α-D-glucose, Aldrich Chemical Co.; 5-(hydroxymethyl)-2-furfural (HMF), Aldrich; 5-nitro-*o*-cresol, TCI America; PMB, Aldrich; pyruvic aldehyde, 40 wt % solution in water, Aldrich; iron filings, 40 mesh, Fisher Scientific; C-18 silica gel, Alltech, 30−70 μm; NMR solvents, Aldrich.

Instrumentation. *EMS.* EMS analyses were performed on a Fisons VG Quattro-SQ mass spectrometer in the negative (0.1% NaOH) and positive (0.1% formic acid) ion modes with a cone voltage of 45 V. The samples were dissolved in 50:50 acetonitrile/water.

Environmental Chamber. A Revco Model PG-8-1045-A environmental chamber was used to expose bait samples to light. UV light, as well as incandescent light bulbs in the chamber, was engaged during the experiments. When the chamber was unavailabe for use, a sun lamp (General Electric, UV bulb, 275 W) and an incandescent bulb (General Electric, 200 W) were suspended above the samples contained in a box with reflective (aluminum foil lined) sides. The bulbs were situated approximately 40 cm above the samples. Since all the samples were sealed in quartz test tubes, the humidity was not monitored.

GC−MS. The GC−MS analyses were performed on an Hewlett-Packard (HP) 5890 gas chromatograph coupled to an HP 5970 mass selective detector. The GC was equipped with a DB-1 (J & W Scientific) 30 m × 0.25 mm i.d., 0.25 μm film thickness capillary column. Operating conditions included the following GC oven temperature program: 50 °C initial temperature, ramped at 10 °C/min to 160 °C (0.10 min) followed by a second oven program of 30 °C/min to a final temperature of 290 °C (2.5 min). Ionization was achieved by electron impact (70 eV) with an ion source temperature of 280 °C. Analyses were conducted in the scan mode (*m*/*z* 70−400).

HPLC. The watermelon extracts were analyzed on an HP 1090M HPLC equipped with an UV diode array detector (240 nm), a 250 × 4.6 mm Keystone Deltabond 5 μm C-8 analytical column, and a Keystone Deltabond C-8 guard column (15 × 3 mm). Injection volumes were 10 μL, the mobile phase was 70:30 acetonitrile/water with a flow rate of 1 mL/min, and the column was maintained at 40 °C. Chromatogram run times varied from 12 to 35 min depending on the samples analyzed.

HPLC−MS. HPLC−MS analyses were performed on an HP 1090 liquid chromatograph and an HP 5989 mass spectrometer. The system was equipped with an HP 5990A particle beam interface and operated in the electron impact (EI) ionization mode. Mass spectrometry conditions were as follows: helium nebulizer pressure, 40 psi; ionization potential, 70 eV; column temperature, 40 °C; desolvation chamber temperature, 40 °C; source temperature, 250 °C; transfer line temperature, 250 °C. Analyses were conducted in the scan mode (*m*/*z* 100−400). Chromatographic separation was performed using a 250 × 3 mm Keystone ODS/H column along with a Keystone Deltabond C-8 guard column (15 × 3 mm). The mobile phase consisted of methanol/water (65:35), and the flow rate was 0.5 mL/min.

Ion Chromatography (IC). The IC analyses were performed on a Dionex 4000i ion chromatograph equipped with a pulsed amperometric detector. The detector consisted of the following: gold working electrode; Ag/AgCl reference electrode; 150 mM NaOH filling solution, stainless steel counter electrode, thin cell gasket; 10 000 nA output range. Separations were performed using a Dionex CarboPak PA1 column, 4 mm i.d. × 25 cm, and a Dionex CarboPak PA1 guard column, 3 mm i.d. × 2.5 cm.

NMR Spectroscopy. NMR spectra were collected on a Bruker-ACE spectrometer operating at 300 (^{1}H) and 75 MHz (^{13}C).

Isolations, Syntheses, and Chemical Characterizations of Novel Compounds. *N-β-D-Glucopyranosyl-3-chloro-4-methylaniline* (Glc-CPT, Figure 1) was isolated from watermelon extracts by vacuum liquid chromatography (VLC) on C-18 silica gel with an acetonitrile/water gradient. The VLC fractions were screened by HPLC. The compound of interest eluted in the 20−30% acetonitrile fractions. These fractions were combined and evaporated in vacuo to approximately 1−2 mL. The neat compound was unstable, so the sample was completely evaporated just prior to collecting NMR spectra.

Glc-CPT was synthesized by the addition of CPTH (0.6 mmol) to α-D-glucose (0.6 mmol) dissolved in 10 mL of distilled water. The spontaneous formation of Glc-CPT was monitored by HPLC over a period of several days. The same reaction procedures were performed with D-fructose/CPTH and sucrose/CPTH.

N-(3-Chloro-4-methylphenyl)-2-oxopropanimine (CMPP, Figure 1) was prepared by the dropwise addition of pyruvic aldehyde (2.9 mmol) to an aqueous solution of CPTH (2.8 mmol). The reaction mixture was stirred for approximately 2 min. PMB (1.4 mmol) dissolved in 1 mL of water was added slowly, and a golden precipitate formed. After the solution was stirred for 3 min, the precipitate was filtered off.

CMPP was isolated as a binary mixture of CMPP and CPTH from the synthetic reaction products. All attempts to isolate CMPP were hindered by its unstable nature. By GC−MS analyses, the synthetic CMPP and that present in the watermelon extracts eluted at the same retention time with nearly identical fragmentation patterns. The NMR assignments of CMPP were deduced by subtraction of the resonances due to CPTH.

The synthesis of *3-hydroxy-4-methylaniline* (HMA, Figure 1) was performed following the procedure of Hazlet and Dornfeld (1944) for the reduction of 5-nitro-*o*-cresol with the following modifications. After the mixture was refluxed for 6 h, the reaction was stopped by filtering off the Fe filings and extracting the benzene solution with 1 N NaOH (4 × 50 mL). The NaOH extracts were combined and back-extracted with benzene (2 × 40 mL). Then the pH of the aqueous extracts was adjusted to approximately 8.5, and the mixture was extracted with ethyl acetate (3 × 75 mL). The ethyl acetate extracts were combined, dried over sodium sulfate, and evaporated, leaving a white solid. No other purification procedures were necessary.

Sample Preparation. The watermelon samples were prepared by blending the pulp to a watery consistency (60 s in a Waring blender) prior to treatment by heating, freezing, or addition of PMB. Heat-treated watermelon samples were prepared from blended watermelon pulp boiled at 99 °C for 5 min, while frozen watermelon samples were prepared from the blended pulp frozen for 24 h at −26 °C and thawed at room temperature. PMB (1.5% and 3% w/w) was added to the blended pulp and mixed for approximately 2 min with a spatula. The CPTH (0.2% w/w) was mixed similarly into the blended pulp immediately prior to commencement of each experiment. For each of the three treatments, a series of control samples (without CPTH) were prepared and treated identically. Triplicate 3.0 g watermelon samples (control and CPTH fortified) for each time interval were accurately weighed into quartz test tubes. While exposed to light, the tubes were aligned horizontally and turned periodically to allow for uniform exposure of each sample. The measurement of time intervals was based on the initial addition of CPTH (as opposed to when samples were placed under light) and included 0, 4, 8, and 24 h. For time zero analyses, the sample preparation time precluded an analysis reflecting an exact measurement of the compounds present in the watermelon extracts.

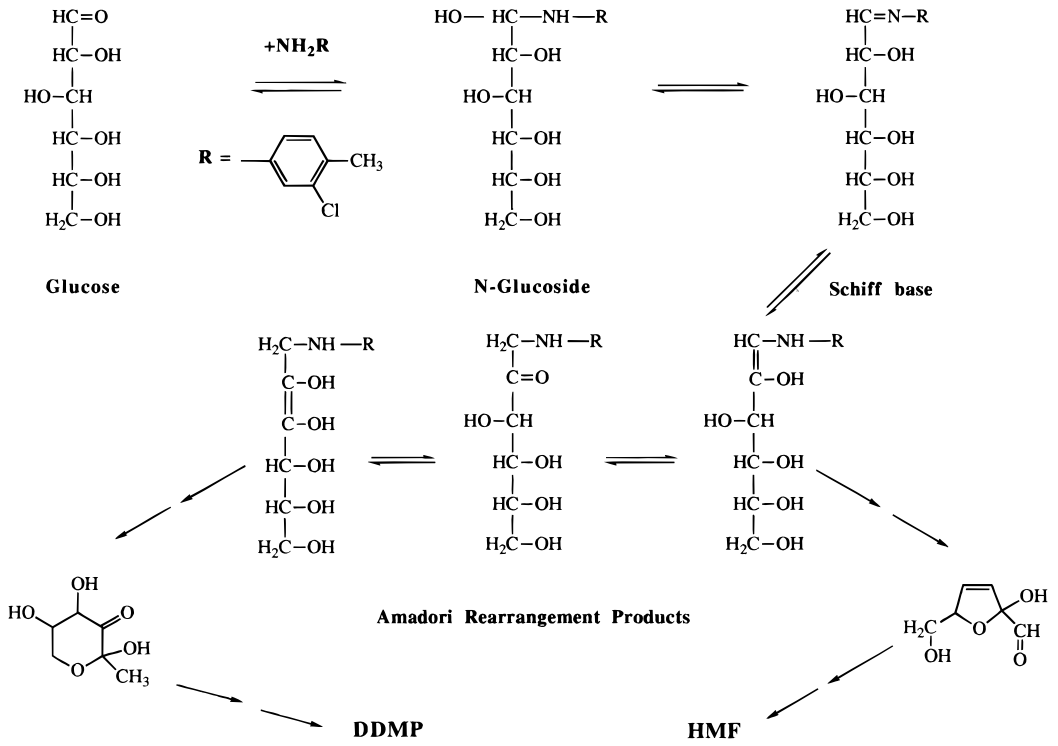

Figure 2. Amadori rearrangement products of Glc-CPT and proposed Maillard reaction schemes for the formation of HMF and DDMP in watermelon bait.

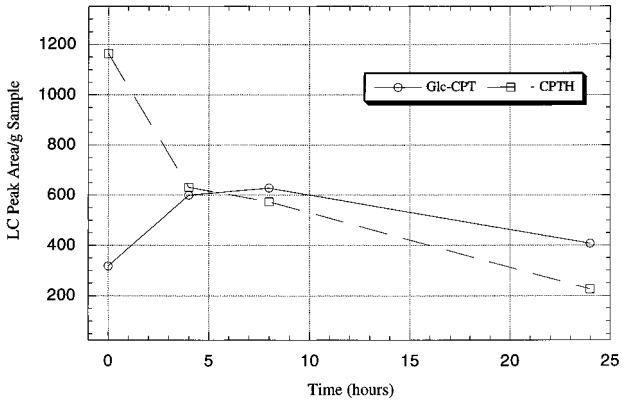

Figure 3. Glc-CPT and CPTH in untreated watermelon bait exposed to UV and incandescent light.

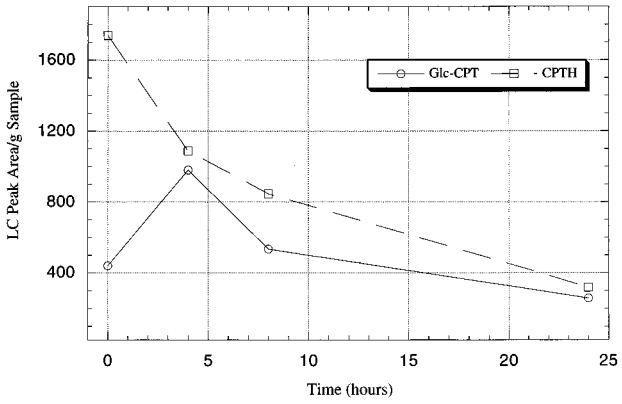

Figure 4. Glc-CPT and CPTH in heated watermelon bait exposed to UV and incandescent light.

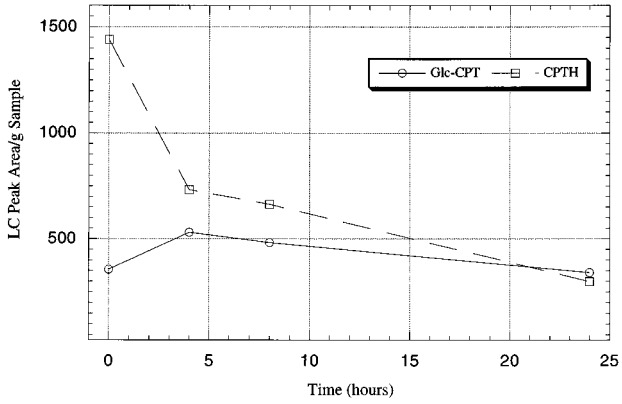

Figure 5. Glc-CPT and CPTH in frozen watermelon bait exposed to UV and incandescent light.

Watermelon samples were extracted with 15 mL of mobile phase (70:30 acetonitrile/water). The samples were vortexed and shaken mechanically for 10 min (Eberbach shaker). The samples were centrifuged for 5 min, and the supernatants were filtered (0.45 μm nylon filters) into sample vials for HPLC analyses.

Graphical Analysis. The unstable nature of Glc-CPT prevented purification for use as a calibration standard. Therefore the peak areas for Glc-CPT and CPTH were plotted to compare the relative variations of each compound in the samples analyzed (Figures 3−6). Each line represents the mean of the triplicate values for each time interval plotted. Quantitation of the loss of CPTH in each sample (Figure 7) was possible by using a single-point calibration, since the HPLC UV diode array detector response for CPTH is proportionally linear in the range from 20 to 200 ppm.

RESULTS

Chemical Characterizations of Glc-CPT: HPLC−MS *m/z* 303 (M^+, <1), 185 (6), 183 (17), 156 (30), 154 (100), 143 (7), 141 (22), 140 (21), 125 (13), 106 (19); EMS (ES^+) M + H = 304, 306; (ES^-) M − H = 302, 304; ^{1}H NMR (D_2O, 4.8 ppm) δ 2.26 (s, 3, CH_3), 3.39−3.62 (m, 4), 3.72 (dd, 1, J = 12, 5.4 Hz), 3.88 (dd, 1, J = 12, 2.1 Hz), 4.70 (d, 1, J = 8.8 Hz, anomeric H), 6.73 (dd, 1, J = 8.2, 2.4 Hz, H-6), 6.92 (d, 1, J = 2.3 Hz, H-2), 7.17 (d, 1, J = 8.3 Hz, H-5). Structurally, ^{1}H NMR data suggest the presence of the cyclic form of glucose in Glc-CPT,

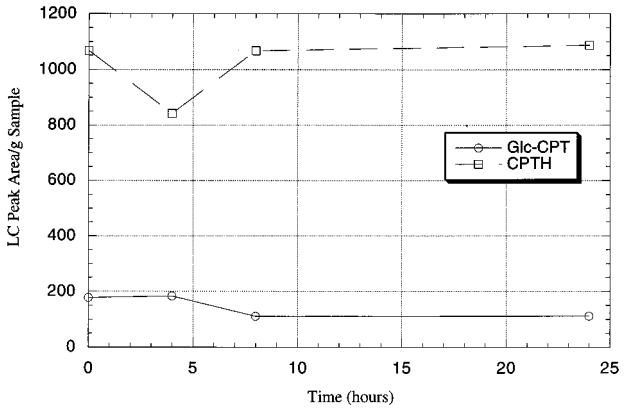

Figure 6. Glc-CPT and CPTH in 1.5% PMB treated watermelon bait exposed to UV and incandescent light.

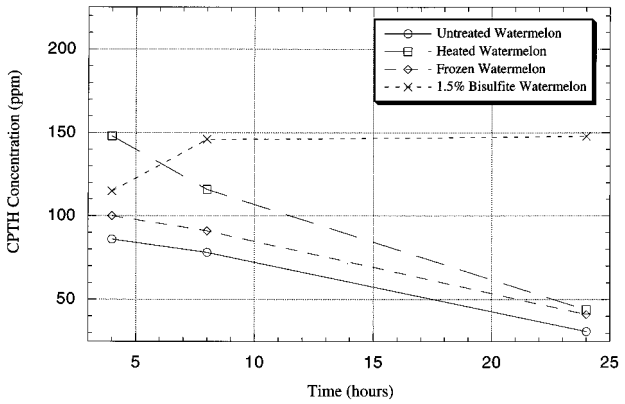

Figure 7. Comparison of CPTH recovered from untreated, heated, and frozen watermelon bait extracts versus the 1.5% PMB treated extract.

and the large coupling constant of the anomeric proton ($J = 8.8$ Hz) indicates a β-coupling (Collins and Ferrier, 1995) between the glucose and CPT moieties, as indicated in Figure 1. The ^{1}H NMR data are very similar to that reported for *N*-(3,4-dichlorophenyl)glucosylamine (Gareis et al., 1992): ^{13}C NMR (D_2O) δ 20.9, 63.5, 72.5, 75.4, 79.3, 79.6, 87.7, 116.1, 117.3, 129.5, 134.4, 137.1, 147.5. Glc-CPT degraded in D_2O during NMR analyses. After the NMR spectra were collected, HPLC analysis of the sample revealed that Glc-CPT had decomposed into CPTH and several undetermined compounds. The HPLC retention times and UV absorption spectra of the synthesized and the isolated Glc-CPT compounds were nearly identical.

Chemical Characterizations of CMPP: GC−MS *m*/*z* 195 (M^+, 11), 197 (M + 2, 3), 154 (33), 152 (100, M − $COCH_3$), 127 (24), 125 (78, M − $NCHCOCH_3$), 89 (53); ^{1}H NMR ($CDCl_3$, 7.24 ppm) δ 1.98 (s, 3, acetyl-CH_3), 2.39 (s, 3, CH_3), 5.14 (s, 1, N=C*H*), 6.42 (dd, 1, $J = 8.3$, 2.3 Hz, H-6), 6.57 (d, 1, $J = 2.3$ Hz, H-2), 6.76 (d, 1, $J =$ 8.1 Hz, H-5); ^{13}C NMR ($CDCl_3$, 77.0 ppm) δ 19.4 (CH_3), 29.3 (CH_3), 78.6 (CH), 118.8 (CH), 120.8 (CH), 131.1, 131.4 (CH), 134.4, 134.7, 201.9 (CO). Instability of CMPP prevented complete NMR characterization. Since CMPP was a minor component of the watermelon extracts, its relative concentrations were not monitored during the experimental procedures.

Chemical Characterizations of HMA: GC−MS *m*/*z* 123 (M^+, 100), 122 (93), 106 (13), 94 (39), 77 (28); ^{1}H NMR (CD_3OD, 3.30 ppm) δ 2.04 (s, 3, CH_3), 6.17 (dd, 1, $J = 7.8$, 2.4 Hz, H-6), 6.21 (d, 1, $J = 2.4$ Hz, H-2), 6.77 (d, 1, $J = 7.8$ Hz, H-5).

GC−MS analyses of the watermelon extracts confirmed the presence of **5-(hydroxymethyl)-2-furfural** (HMF, Figure 1) and **2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one** (DDMP, Figure 1). A standard reference solution of HMF and one compound of interest from the watermelon extracts had virtually identical retention times and fragmentation patterns. The fragmentation pattern for the other compound was nearly identical to that for DDMP (Mills et al., 1970).

DISCUSSION

Dramatic changes in the physical appearances of the untreated, heated, and frozen CPTH-fortified watermelon baits occurred during the course of the experiment. After exposure to light for 24 h, the bait samples were dark brown and the extracts were golden yellow. Also, unpleasant odors were detected in all these samples during the extraction procedure. The control samples which contained no CPTH appeared faded but not brown. No odor was apparent in the control samples, and the extracts were clear to slightly yellow in color. Because the heating and freezing treatments should have denatured the enzymes responsible for enzymic processes and the browning occurred only in CPTH-fortified samples, it appears the discoloration was due to nonenzymic processes. The rapid discoloration of the watermelon samples in the presence of CPTH was consistent with Maillard reactions for monosaccharides (Ledl and Schleicher, 1990).

Two major compounds present in the untreated, heated, and frozen watermelon baits were HMF and DDMP, reaction intermediates of numerous browning products from the degradation of glucose and fructose (Ledl and Schleicher, 1990). In general, the presence of DDMP serves as an indicator for the occurrence of the Maillard reaction. This supports the hypothesis that nonenzymic browning was primarily responsible for bait degradation.

The watermelon extracts also contained several undetermined compounds, one of which was a major component of the extracts and was identified as Glc-CPT, a novel CPTH derivative (Figure 1). Glc-CPT formation is typical of the reaction between a primary amine and glucose (Hodge and Osman, 1976). Although IC analyses of the watermelon samples revealed the presence of glucose, fructose, and sucrose as the major saccharides, only the formation of Glc-CPT was observed. One minor component of the extracts, another novel CPTH derivative, was identified as CMPP (Figure 1). Presumably CMPP was formed by the reaction between CPTH and pyruvic aldehyde, a decomposition product of glucose (Kort, 1990).

Although other studies revealed the formation of CPTH derivatives in biological or environmental samples, comparisons between the watermelon extracts and standard samples of these compounds were unsuccessful in identification of other undetermined compounds. The standard samples included the *cis* and *trans* isomers of 3,3′-chloro-4,4′-methylazobenzene (azo-CPT, Figure 1, Primus et al., 1996); *N*-(3-chloro-4-methylphenyl)acetamide (*N*-acetyl-CPT, Figure 1; Tweedy et al., 1970; Lee and Kim, 1978; Engelhardt et al., 1979) and HMA (Figure 1; Yao and Mill, 1993).

Figures 3−5 depict relative amounts of CPTH and Glc-CPT in untreated, heated, and frozen CPTH watermelon baits, respectively. The general trend in Figures 3−5 reveals that within the first 4 h CPTH levels decreased rapidly, while the Glc-CPT levels increased. The early appearance of Glc-CPT in the watermelon extracts implicates the spontaneous forma-

tion of the amino sugar as suggested by Winkler and Sandermann (1992). The CPTH levels decreased further with time, as did the Glc-CPT. Mutarotation at the anomeric carbon of *N*-glucosides (Collins and Ferrier, 1995) occurs commonly, and the glucose−amine bond cleaves easily in neutral and slightly acidic aqueous media (Pigman, 1957) which may account for the decrease in Glc-CPT levels. Presumably Glc-CPT degraded to HMF, DDMP (Figure 2), and other browning products.

Additional losses of CPTH depicted in Figures 3−5 may have resulted from CPTH complexation with the matrix, including lignins and other complex carbohydrates (Marco and Novak, 1991). In previous studies involving aniline-based pesticides, *N*-glucosides were found commonly in plants exposed to the aniline (Still, 1968; Yih et al., 1968; Schmidt et al., 1995). As a result of metabolism in the plants, the pesticides were found associated with polymeric lignin components of the plant matrices (Yih et al., 1968; Bartha and Pramer, 1970). The aniline lignin complexes have been referred to as bound nonextractable residues (Schmidt et al., 1995), as analyses of these plant materials precluded recovery of the analyte of interest.

In contrast to the untreated, heated, and frozen baits, the PMB-treated CPTH watermelon samples, as well as the respective controls, were salmon pink in color compared to the original reddish-pink pulp. Furthermore an odor was not detected, and the extracts ranged from clear to slightly yellow. It appears that the addition of PMB to the CPTH watermelon bait minimized color changes and production of undesirable odors.

In the Maillard reaction scheme (Figure 2), Glc-CPT and the Schiff base appear to be the key intermediates to the integrity of the watermelon bait, since Amadori rearrangement of the Schiff base leads to sugar degradation (Ledl and Schleicher, 1990). The observed preservation of PMB−CPTH watermelon bait may correspond to hindered formation of the *N*-glucoside by PMB through sulfonation of the aldehyde functionality of glucose (Burton et al., 1963).

The 1.5% PMB−CPTH watermelon samples showed a marked difference in CPTH and Glc-CPT levels (Figure 6) from those observed in untreated, heated, and frozen CPTH samples (Figures 3−5). Data from the 3% PMB−CPTH watermelon samples reflected the results of the 1.5% samples; therefore only the 1.5% PMB−CPTH results are depicted (Figure 6). The amount of CPTH remained relatively constant, while the formation of Glc-CPT was maintained at a lower level. The initial loss of CPTH noted in the PMB-treated samples may be accounted for in the formation of Glc-CPT, as well as in nonextractable residues, while the decomposition of Glc-CPT could account for the gradual decrease in Glc-CPT and the increase in CPTH during the 4−24 h range. The amount of recoverable CPTH in the PMB-treated extracts was greater than that observed in the CPTH samples without PMB (Figure 7).

Although light exposures differ from laboratory to field conditions, these data suggest that degradation of CPTH watermelon bait occurs primarily through nonenzymic browning reactions. Further field studies are necessary to assess bait acceptance and efficacy, as well as the effectiveness of PMB as a preservative. Other sulfhydryl compounds, such as cysteine, *N*-acetylcysteine, and reduced glutathione, also prevent nonenzymic browning in fruit (Friedman, 1996) and may be considered in future studies.

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LITERATURE CITED

Bartha, R.; Pramer, D. Metabolism of acylanilide herbicides. *Adv. Appl. Microbiol.* **1970**, *13*, 317−341.

Brown, A. C., Jr.; Summers, W. L. Changes in carbohydrate concentration during watermelon juice storage. *Hortic. Sci.* **1985**, *20*, 896−897.

Burton, H. S.; McWeeny, D. J.; Biltcliffe, D. O. Non-enzymic browning: the role of unsaturated carbonyl compounds as intermediates and of SO_2 as an inhibitor of browning. *J. Sci. Food Agric.* **1963**, *14*, 911−920.

Collins, P.; Ferrier, R. *Monosaccharides, Their Chemistry and Their Roles in Natural Products*; Wiley: West Sussex, England, 1995.

Engelhardt, G.; Wallnofer, P. R.; Ziegler, W. Degradation of the phenylamide herbicides Monolinuron and Solan by *Fusarium oxysporum* Schlecht. *Chemosphere* **1979**, *10*, 725−730.

Friedman, M. Food browning and its prevention: an overview. *J. Agric. Food Chem.* **1996**, *44*, 631−653.

Gareis, C.; Rivero, C.; Schuphan, I.; Schmidt, B. Plant metabolism of xenobiotics. Comparison of the metabolism of 3,4-dichloroaniline in soybean excised leaves and soybean cell suspension cultures. *Z. Naturforsch., A* **1992**, *47*, 823−829.

Glahn, J. F. USDA APHIS Denver Wildlife Research Center, Denver, CO, personal communication, 1995.

Glahn, J. F.; Palacios, J. D.; Garrison, M. V. Evaluation of methods for reducing great-tailed grackle damage to citrus in the lower Rio Grande Valley. Unpublished report. Denver Wildlife Research Center, Denver, CO, 1995.

Goodall, M. J. USDA APHIS Denver Wildlife Research Center, Denver, CO, unpublished report, 1995.

Hazlet, S. E.; Dornfeld, C. A. The reduction of aromatic nitro compounds with activated iron. *J. Am. Chem. Soc.* **1944**, *66*, 1781−1782.

Hodge, J. E.; Osman, E. M. Carbohydrates. In *Principles of Food Science, Part I, Food Chemistry*; Fennema, O. R., Ed.; Dekker: New York, 1976.

Ingles, D. L. Prevention of non-enzymic browning. *Food Preserv. Q.* **1966**, *26*, 39−44.

Kort, M. J. Reactions of free sugars with aqueous ammonia. *Adv. Carbohydr. Chem. Biochem.* **1990**, *25*, 311−349.

Ledl, F.; Schleicher, E. New aspects of the Maillard reaction in foods and in the human body. *Angew. Chem.* **1990**, *29*, 565−594.

Lee, J. K.; Kim, K. C. A study on the degradation of 3,4-dichloroaniline by a soil fungus, *Chaetomium globosum* (part I) with special emphasis on acetylation. *J. Korean Chem. Soc.* **1978**, *21*, 197−203.

Marco, G. J.; Novak, R. A. Natural product interactions during aniline metabolism including their incorporation in biopolymers. *J. Agric. Food Chem.* **1991**, *39*, 2101−2111.

McWeeney, D. J.; Biltcliffe, D. O.; Powell, R. C. T.; Spark, A. A. The Maillard reaction and its inhibition by sulfite. *J. Food Sci.* **1969**, *34*, 641−643.

Mills, F. D.; Weisleder, D.; Hodge, J. E. 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, a novel nonenzymatic browning product. *Tetrahedron Lett.* **1970**, *15*, 1243−1246.

Pigman, W. Nitrogenous derivatives. In *The Carbohydates, Chemistry, Biochemistry, Physiology*; Pigman, W., Ed.; Academic Press: New York, 1957.

Primus, T.; Tawara, J.; Johnston, J.; Cummings, J.; Volz, S.; Goodall, M.; Hurlbut, D.; Griffin, D.; Turnipseed, S. Degradation of the avicide 3-chloro-*p*-toluidine hydrochloride (CPTH) in Louisiana rice fields. *Environ. Sci. Technol.* **1996**, in press.

Richardson, T. M. Enzymes In *Principles of Food Science, Part I, Food Chemistry*; Fennema, O. R., Ed.; Dekker: New York, 1976.

Schafer, E. W. *Proc. Vertebr. Pest Conf.* **1984**, *11*, 217−222.

Schmidt, B.; Rivero, C.; Thiede, B. 3,4-Dichloroaniline *N*-glucosyl- and *N*-malonyltransferase activities in cell cultures and plants of soybean and wheat. *Phytochemistry* **1995**, *39*, 81−84.

Still, G. G. Metabolism of 3,4-dichloropropionanilide in plants: the metabolic fate of the 3,4-dichloroaniline moiety. *Science* **1968**, *159*, 992−993.

Tweedy, B. G.; Loeppky, C.; Ross, J. A. Metobromuron: acetylation of the aniline moiety as a detoxification mechanism. *Science* **1970**, *168*, 482−483.

Watt, B. K.; Merrill, A. L. *Composition of Foods, Agriculture Handbook 8*; USDA: Washington, DC, 1963.

Winkler, R.; Sandermann, H., Jr. *N*-Glucosyl conjugates of chlorinated anilines: spontaneous formation and cleavage. *J. Agric. Food Chem.* **1992**, *40*, 2008−2012.

Yao, D.; Mill, T. Aquatic photolysis study of 3-chloro-*p*-toluidine hydrochloride. Unpublished Report-QA-268. SRI, Menlo Park, CA/Denver Wildlife Research Center, Denver, CO, 1993.

Yih, R. Y.; McRae, D. H.; Wilson, H. F. Metabolism of 3′,4′-dichloropropionanilide: 3,4-dichloroaniline-lignin complex in rice plants. *Science* **1968**, *161*, 376−377.

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