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Postharvest Accumulation of Resveratrol and Piceatannol in Sugarcane with Enhanced Antioxidant Activity

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ABSTRACT: A new plant source, sugarcane, was used to produce the stilbenes piceatannol and resveratrol. Both stilbenes were identified in sugarcane billet stalks (12 mm) after incubation at room temperature for 3 days. Low concentrations of piceatannol (30.6 μg/g) and resveratrol (12.3 μg/g) were detected at day 3. At day 7 of incubation higher concentrations of piceatannol (1659 μg/g) and resveratrol (73 μg/g) were produced. Sugarcane juice obtained from billets that were incubated for 7 days contained high levels of piceatannol (8.5 mg/L) and resveratrol (1.2 mg/L). Although high stilbene concentrations were determined in the sugarcane variety L 97-128, two other varieties (Ho 95-988 and LCP 85-384) displayed lower stilbene concentrations after incubation for 7 days. The total phenolic content (TPC) and antioxidant activities of incubated sugarcane extracts were determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP). The TPC and antioxidant activities were highest in sugarcane extracts that were incubated for 7 days. This study details a postharvest method to produce stilbene-enriched sugarcane with increased levels of piceatannol and resveratrol.

KEYWORDS: *piceatannol, resveratrol, stilbene, sugarcane, total phenolic content, antioxidant activity*

INTRODUCTION

Resveratrol and piceatannol (Figure 1) are naturally occurring phenolic compounds belonging to the stilbene family.

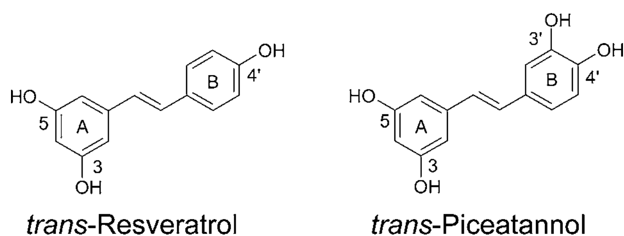

Figure 1. Stilbenes in cut sugarcane.

Resveratrol has been shown to have cancer preventive properties[1−5] and anti-inflammatory properties,[6] as well as a role in the prevention of atherosclerosis and coronary diseases.[7,8] More recently, resveratrol has been implicated in lifespan extension of several organisms.[9−11] Structurally similar to resveratrol is piceatannol (Figure 1), a metabolite of resveratrol by the enzyme CYP1B1 in the cytochrome P450 family, with a polyhydroxystilbene core structure containing one additional hydroxyl group at the 3′-position of the B-ring. Piceatannol has many biological activities including anticancer and antileukemic properties.[12,13] Research has linked the antitumor activities of piceatannol to its ability to inhibit cell proliferation and arrest cells in the S phase.[14] When compared to resveratrol, piceatannol is a stronger antioxidant and inducer of apoptosis.[15]

Resveratrol is found in many different plant sources in significant quantities, including grapes and red wine,[16−18] Japanese knotweed,[19] peanuts,[20] cocoa,[21] strawberries,[22] *Vaccinium* berries,[23,24] and tomato.[25] Piceatannol is found in only a limited number of natural sources. Piceatannol was first isolated as an antileukemic agent from the seeds of *Euphorbia lagascae*[26] and is also present in grape[27] and *Vaccinium* berries.[23] Other plants, such as *Rheum* spp.,[28] *Machura pomifera*,[29] and *Senna* spp.,[30] have also been reported to contain piceatannol in very low concentrations. The amount of resveratrol and piceatannol in berries and fruits can be affected by different treatments or stresses. Therefore, both stilbenes are considered phytoalexins that are synthesized de novo and accumulate in plants in response to infection or stress due to wounding, freezing, ultraviolet light exposure, and exposure to microorganisms.[16,31−37] Phytoalexin biosynthesis can be manipulated by application of abiotic (nonliving) or biotic (living) factors that stress the plant into producing or releasing greater phytoalexin concentrations. Piceatannol and resveratrol are synthesized as phytoalexins in response to fungal attack[37,38] or through use of UV irradiation.[31−36]

Piceatannol has also been isolated from sugarcane (*Saccharum* spp.) infected with *Collectotrichum falcatum*, but not from healthy or wounded sugarcane.[39,40] Sugarcane red rot is one of the major diseases of sugarcane and is named because of the

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large red lesions that develop in affected stalks.[41,42] The causal agent is the fungus *C. falcatum*; however, reddening in sugarcane is a common infection response mentioned in the disease description of about half the leaf or stalk diseases in *Sugar-Cane Diseases of the World*.[43,44] It is reported that sugarcane plants respond to fungal attack by means of hypersensitive response, which often involves the accumulation of colored pigments at the infection site.[45] Earlier release of a red compound referred to as red rot pigment in cells and intercellular spaces near invading *C. falcatum* in sugarcane showed the presence of the 3-deoxyanthocyandins luteolidin, apigeninidin, and caffeic acid ester of 5-*O*-apigeninidin.[46]

Although piceatannol has been reported in *C. falcatum* infected sugarcane stalks, previous research indicated that uninfected stalks did not contain piceatannol. Here, we report the detection and quantitation of resveratrol in sugarcane. To the best of our knowledge, the presence of resveratrol in sugarcane has not been reported. The objectives of this research were (1) to identify and quantitate resveratrol and piceatannol in postharvest sugarcane, (2) to determine optimum postharvest conditions necessary to produce stilbenes, and (3) to determine total phenolic content and antioxidant activity of cut sugarcane extracts with various incubation times.

■ EXPERMENTAL PROCEDURES

Materials. Sugarcane (*Saccharum* spp.) plants of variety L 97-128 were grown at the Southern Regional Research Center (New Orleans, LA, USA) and harvested in 2010. Two other varieties tested, Ho 95-988 and LCP 85-384, were grown at the Sugarcane Research Unit (Houma, LA, USA) and harvested in 2011. The authentic standard of *trans*-piceatannol was purchased from Alexis Biochemicals (San Diego, CA, USA). *trans*-Resveratrol was purchased from Sigma (St. Louis, MO, USA). HPLC grade solvents acetonitrile and methanol were purchased from J. T. Baker (Phillipsburg, NJ, USA). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), iron(III) chloride hexahydrate, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), Folin−Ciocalteu reagent, iron(II) sulfate heptahydrate, and gallic acid were purchased from Sigma. Water was obtained using a Millipore system and used during sample preparation procedures and HPLC analyses.

Preparation of Sugarcane and Extraction of Stilbenes. Experiments were conducted using freshly harvested sugarcane using the lower 91.4 cm stalk section. A 10% bleach solution was used for surface sterilization. Sugarcane billets (12 mm) were cut using pruning shears. Cutting and extraction of billet samples were carried out in low light or in a room with fluorescent lights equipped with UV-absorbing shields, to prevent isomerization of stilbenes. Billets were placed on flat trays, covered with aluminum foil, and incubated at room temperature (24 °C). Six to eight billets were harvested at each time point after incubation to minimize variability. Billets were frozen at −80 °C after incubation before lyophilizaton. Lyophilized billets were ground in a Tekmar A10 analytical mill (Janke and Kunkel GmbH & Co., Staufen, Germany). Samples (0.2 g) were extracted using 2 mL of methanol with sonication for 1 h. Methanol extracts were filtered using 0.45 μm filters and analyzed by HPLC.

Stilbene Analysis of Sugarcane Juice. A Carver (Wabash, IN, USA) hydraulic press model C and stainless steel cage equipment (at 15000 lb) was used to press juice from sugarcane billets. Aliquots of sugarcane juice (30 mL) obtained at days 0 and 7 of incubation were lyophilized. Lyophilized samples (0.2 g) were extracted using 1 mL of methanol with sonication for 1 h. Methanol extracts were filtered using 0.45 μm filters and analyzed by HPLC. Analyses were conducted on triplicate samples.

Phytoalexin Isolation and Identification. For semipreparative isolation of resveratrol and piceatannol, cut sugarcane billets (0.5 kg) were incubated for 7 days. Billets were then frozen, lyophilized, and ground using a Retsch (Haan, Germany) SM100 mill using a 1 mm screen. The extraction solvent was 2 L of methanol. Rotary evaporation was used to concentrate the methanolic sugarcane extract almost to dryness before semipreparative HPLC. The HPLC was a Waters 600 pump combined with a Waters 996 photodiode array detector. The column was a Whatman ODS-2 10 mm × 500 mm using a flow rate of 3.0 mL/min with the following solvent system: A, acetonitrile; B, water; 5% A for 15 min, then from 5 to 90% A in 40 min followed by holding at 90% A for 20 min. Piceatannol and resveratrol, shown in Figure 1, were confirmed by UV−vis spectrophotometry, APCI mass spectrometry (MS and MS/MS), and NMR (^{1}H and ^{13}C) analyses. ^{1}H and ^{13}C spectra were recorded in deuterated acetone with a Bruker DMX-500 spectrometer (Billerica, MA, USA).

For the identification of stilbenes, the LC-MS/MS analyses were performed in negative ionization mode on an LCQ ion trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA) fitted with an atmospheric pressure chemical ionization (APCI) probe. The ion trap was connected to a Waters 2695 HPLC and a Waters 996 photodiode array detector. APCI conditions were optimized using piceatannol as a standard. The full APCI-MS spectrum of crude methanol extract was first obtained followed by the collision-induced dissociation (CID) spectra of select ions. HPLC effluent at 1 mL/min was introduced directly into the interface without splitting using a source temperature of 500 °C and a capillary temperature of 220 °C. The sheath gas flow was set to 60 arbitrary units. The full scan mass spectra of the stilbenes from m/z 100 to 1000 were measured using 500 ms for collection time, and three microscans were summed. Tandem mass spectrometry was performed using a collision energy of 35% for MS/MS analyses.

Analysis of Resveratrol and Piceatannol. The quantity of piceatannol and *trans*-resveratrol in sugarcane billets was determined using a Waters 2695 HPLC combined with a Waters 996 photodiode array detector. Stilbenes were separated using a Luna (Phenomenex, Torrance, CA, USA) C_{18} reverse-phase column (250 × 4.6 mm, 5 μm particle size). A guard column containing the same packing was used to protect the analytical column. The injection volume of sample was 10 μL with a flow rate of 1.0 mL/min with the following solvent system: A, 3 mM ammonium acetate/water; B, acetonitrile; from 0 to 45% B in 17 min, then from 45 to 90% B in 10 min followed by holding at 90% B for 6 min. The spectra were collected between 220 and 400 nm by PDA, and stilbenes were quantified at 306 nm for resveratrol and at 325 nm for piceatannol.

To quantify the *trans*-piceatannol and *trans*-resveratrol contents in sugarcane, calibration curves were constructed prior to sample analysis using authentic *trans*-piceatannol and *trans*-resveratrol. The linear range of the quantitative analyses for piceatannol was 0.025−25.0 μg/mL. The linear range of quantitative analyses for resveratrol was 0.025−20.0 μg/mL. The corresponding correlation coefficients (R^2) were 0.9974 and 0.9980, indicating excellent correlations between peak areas and standard concentrations.

Total Phenolic Content (TPC). The TPC of the sugarcane extracts was determined according to the Folin−Ciocalteu method with minor modifications using gallic acid as the standard.[47] Sample (30 μL) and 150 μL of Folin−Ciocalteu reagent diluted 10 times with water were first pipetted in each well of a 96-well plate. After 8 min, 120 μL of 7.5% Na_2CO_3 was added. The plate was placed in the reader and incubated for 1 h at 30 °C, and the aborbance at 750 nm was recorded. All determinations were carried out in triplicate, and the results are expressed as milligrams of gallic acid equivalent (GAE) per gram of extract.

DPPH Radical Scavenging Assay. The DPPH radical scavenging capacity of each sugarcane extract was determined according to the method of Brand-Williams[48] with some modifications. DPPH radicals have an absorption maximum at 517 nm, which disappears with reduction by an antioxidant compound. The DPPH radical solution at a final concentration of 0.1 mM in methanol was prepared daily, and 190 μL was added to each well of a 96-well plate followed by 10 μL of sample or methanol for blank. The mixture was incubated at 30 °C for 30 min, and the absorbance at 517 was measured with a microplate

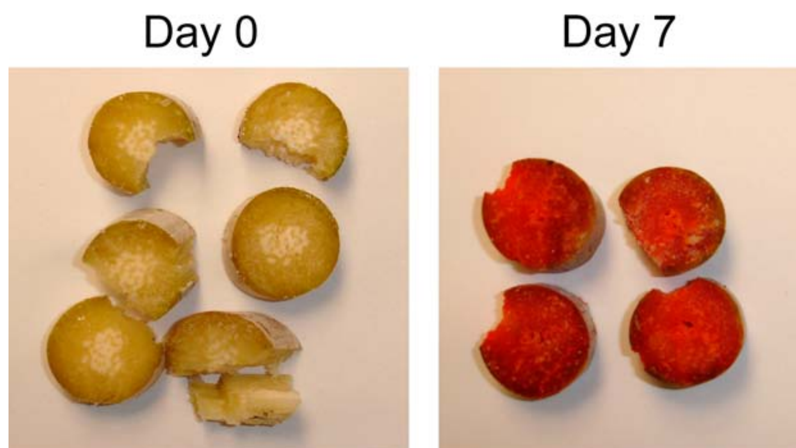

Figure 2. Cut sugarcane billets initially at day 0 and after incubation for 7 days. Color of billet changes from colorless to dark red at day 7.

reader. The inhibition percentage of the radical scavenging activity was calculated using the equation

$$\text{inhibition (\%)} = 100 - 100(A_S \div A_0)$$

where A_0 is absorbance of the blank and A_S is absorbance of the sample at 517 nm. All assays were conducted in triplicate.

Ferric Reducing Antioxidant Potential (FRAP) Assay. The ferric reducing power of each sugarcane extract was determined using a modified version of the FRAP assay.[49] This method is based on the reduction, at low pH, of a colorless ferric complex (Fe^{3+}–tripyridyltriazine) to a blue-colored ferrous complex (Fe^{2+}–tripyridyltriazine) by the action of electron-donating antioxidants. The FRAP reagent was prepared by mixing 10 volumes of 250 mM acetate buffer (pH 3.6) with 1 volume of 10 mM TPTZ in 40 mM HCl and with 1 volume of 20 mM $FeCl_3 \cdot 6\ H_2O$. A total of 10 μL of sample and 30 μL of distilled water was added to 260 μL of freshly prepared FRAP reagent in a well of a 96-well plate. The mixture was incubated at 37 °C throughout the reaction. After 8 min, the absorbance at 593 nm was read using a microplate reader against a reagent blank. A standard curve was prepared using various concentrations of $FeSO_4 \cdot 7H_2O$. The FRAP value was calculated and expressed as millimoles of Fe^{2+} equivalents per gram extract (mmol Fe^{2+} equiv/g). All assays were conducted in triplicate.

Carbohydrate Analyses in Sugarcane Juice. For the analysis of soluble sugars, 10 μL of diluted sugarcane juice (1:20) was injected onto a Rezex RCM monosaccharide Ca^+ column, 300 mm × 7.8 mm diameter, 8 μm particle size (Phenomenex; part 00H-0130-K0), with a Carbo-Ca^+ guard column, 4 mm × 3 mm diameter (Phenomenex; part AJ0-4493). The column temperature was maintained at 75 °C using a Waters post column reaction module. The mobile phase was HPLC grade water at a flow rate of 0.6 mL/min. Eluted carbohydrates were monitored using a refractive index detector (Waters 2410, Milford, MA, USA) connected to the Waters 2695 HPLC system. The presence and abundance of fructose, glucose, and sucrose were calculated by comparing sample peak area to standards. Sample analyses were performed in triplicate. Sucrose, fructose, and glucose contents were calculated as grams per 100 mL for each sample.

Statistical Analysis. TPC and antioxidant activity data were analyzed using XLSTAT (version 2007.6; Addinsoft, Inc., New York, NY, USA). Statistical evaluation of results was performed by using Tukey's multiple-comparison test. Differences are considered significant at $p < 0.05$. Each value is presented as the mean ± standard deviation (SD).

RESULTS

Identification of Resveratrol and Piceatannol. The color of the cut surface of sugarcane billets changed from colorless to light red (beginning at day 2–3) to dark red (beginning at day 5–6). Figure 2 displays a photograph of cut sugarcane billets at days 0 and 7. HPLC analysis was conducted to determine the polyphenolic components of the cut sugarcane billets. Figure 3 displays the HPLC chromatogram

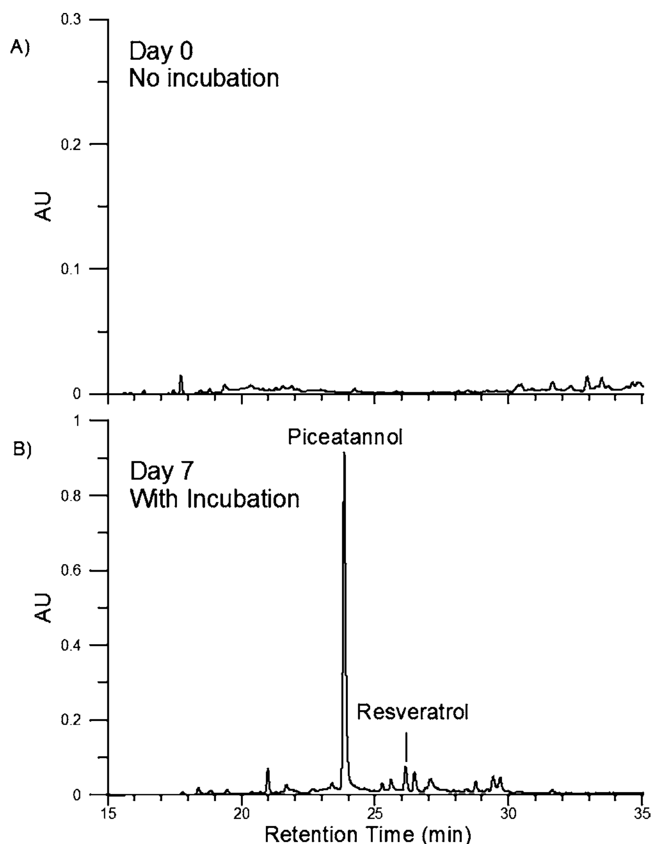

Figure 3. HPLC chromatograms obtained at 325 nm of cut sugarcane (A) at initial conditions at day 0 and (B) after incubation for 7 days. Piceatannol and resveratrol are detected at day 7.

of cut sugarcane at (A) day 0 and (B) day 7. Stilbenes were not detected in sugarcane samples analyzed at day 0. Samples at day 7 contained two stilbenes with retention times of 23.9 min for *trans*-piceatannol and 26.2 min for *trans*-resveratrol.

The initial identification of resveratrol and piceatannol in sugarcane billets was conducted using LC-UV spectra and LC-MS. Evaluation of the retention times and UV spectra for the peaks corresponding to resveratrol and piceatannol in sugar-

cane extracts revealed identical spectra compared with those in standards. Also, LC-MS data confirmed that the compounds identified were resveratrol and piceatannol. The compounds identified had identical retention times and mass spectra compared with those of resveratrol and piceatannol standards and published data.[28,50,51]

Tandem mass spectrometry (LC-MS/MS) was used to confirm the identities of the two stilbenes in sugarcane samples. Figure 4 displays the MS/MS spectra of both piceatannol and resveratrol from sugarcane after an incubation period of 7 days. The MS/MS spectrum of piceatannol is shown in Figure 4A. The ion at m/z 243 corresponds to the deprotonated $[M - H]^-$ of piceatannol. Piceatannol, which contains 3′,4′-dihydroxyl groups, eliminates one molecule of H_2O to produce the $[M - H_2O]^-$ ion at m/z 225. The ion at m/z 201 is due to neutral loss of C_2H_2O from the parent ion. The MS/MS spectrum of resveratrol is reported in Figure 4B. The spectrum contains the $[M - H]^-$ at m/z 227, which confirms the molecular weight. The two product ions at m/z 185 $[M - H - CH_2CO]^-$ and the ion at m/z 143 $[M - H - 2CH_2CO]^-$ involve the sequential loss of two ketene molecules C_2H_2O. The MS/MS spectra of piceatannol and resveratrol were consistent with previous studies.[28,50,51] Unequivocal identification of the two stilbene structures was provided by NMR spectroscopic analysis.

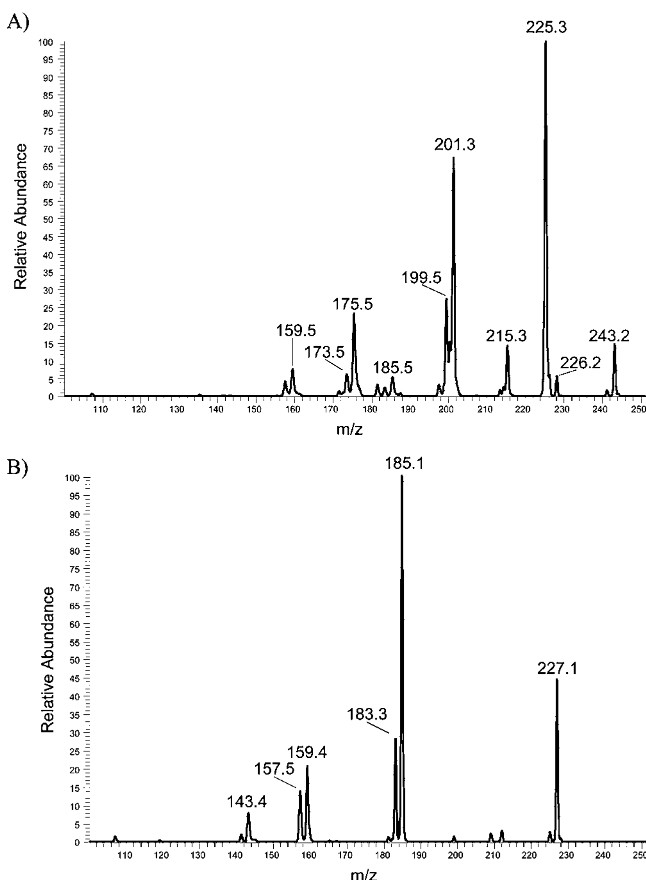

Figure 4. Tandem mass spectrometry of sugarcane stilbenes: (A) MS/MS spectrum of piceatannol from cut sugarcane at day 7; (B) MS/MS spectrum of resveratrol from cut sugarcane at day 7.

Structure Determination of Isolated Stilbenes. *trans-Piceatannol:* APCI m/z 243 $[M - H]^-$; MS/MS spectrum Figure 4A; ^1H NMR δ 6.97 (d, 1H, J = 2 Hz, H6′), 6.89 (d, 1H, H-α or H-β), 6.83 (dd, 1H, $J_{2,6}$ = 2 Hz, $J_{5,6}$ = 8 Hz, H-6), 6.73 (d, 1H, J = 16 Hz, H-α or H-β), 6.73 (d, 1H, J = 8 Hz, H-5), 6.43 (d, 2H, J = 2 Hz, H2′ and H-6′), 6.15 (t, 1H, J = 2 Hz, H-4′); ^{13}C NMR δ 159.6 (C3, 5), 146.3 (C3′, 4′), 140.8 (C1), 132.9 (C1′), 129.7 (C-β), 126.9 (C-α), 119.9 (C-6′), 116.2 (C2′), 113.8 (C5′), 105.6 (C2, 6), 102.6 (C4).

trans-Resveratrol: APCI m/z 227; MS/MS spectrum Figure 4B; ^1H NMR δ 7.42 (d, 2H, J = 8.25 Hz, H2′ and H6′), 7.03 (1H, H-β), 6.89 (1H, H-α), 6.84 (d, 2H, J = 8.2 Hz, H3′ and H5′), 6.53 (2H, d, H-2, 6), 6.26 (1H, d, H4); ^{13}C NMR δ 159.6 (C3, 5), 158.2 (C4′), 140.9 (C1), 130.0 (C1′), 129.1 (C-β), 128.8 (C2′, 6′), 126.9 (C-α), 116.4 (C3′, 5′), 105.7 (C2, 6), 102.7 (C4).

Production of Piceatannol and Resveratrol with Various Incubation Times. To determine the incubation time necessary to produce optimal amounts of stilbenes in cut sugarcane billets, a timecourse experiment was conducted. The postharvest production of piceatannol (Figure 5A) and resveratrol (Figure 5B) is displayed over a 7 day period. Both piceatannol (30.6 μg/g) and resveratrol (12.3 μg/g) were first detected at day 3 after incubation. Piceatannol reached a maximum concentration (1659 μg/g) at day 7. The highest level of resveratrol (73.0 μg/g) was also detected at day 7.

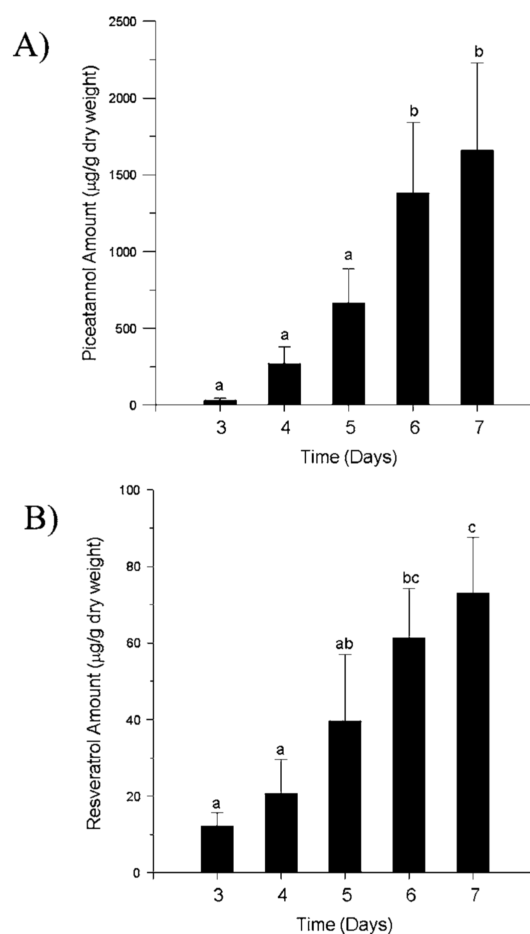

Figure 5. Changes in content of (A) piceatannol and (B) resveratrol in cut sugarcane during incubation for 7 days. Different letters correspond to significant differences at $p < 0.05$.

Total Phenolic Content. Cut sugarcane after incubation for up to 7 days was extracted in methanol and evaluated for phenolic content according to the Folin−Ciocalteu method.

The TPC of the extracts ranged from 14.09 mg GAE/g extract at day 0 to 50.24 mg GAE/g extract at day 7 (Table 1). The TPC at day 3 increased 71% versus initial day 0 extract; at day 5, increased 148% versus initial extract; and at day 7, increased 257% versus initial day 0 extract.

Table 1. Total Phenolic Content, DPPH Radical Scavenging Activity, and Ferric Reducing Capacity of Cut Sugarcane Extracts after Different Incubation Times[a]

incubation time	total phenolics (mg GAE[b]/g)	DPPH (% inhibition)	FRAP (mmol Fe^{2+} equiv/g)
day 0	14.09 ± 0.24a	22.44 ± 1.69a	1.94 ± 0.44a
day 3	24.08 ± 0.93b	27.32 ± 1.13a	3.65 ± 0.56a
day 5	34.94 ± 1.34c	58.99 ± 1.08b	7.37 ± 0.69b
day 7	50.24 ± 0.76d	71.46 ± 8.48b	11.75 ± 0.93c

[a]Cut sugarcane (variety L 97-128) billets incubated; results are expressed as the mean ± standard deviation (n = 3). Different lower case letters within a column correspond to significant differences at $p < 0.05$. [b]GAE gallic acid equivalents.

DPPH Radical Scavenging Activity. Antioxidant activity was performed on the cut sugarcane extracts with various incubation times using the DPPH radical scavenging assay (Table 1). The DPPH radical is a stable organic free radical that after accepting an electron or hydrogen in the presence of a hydrogen-donating antioxidant is reduced to a nonradical form. The initial sugarcane extract at day 0 inhibited DPPH oxidation by 22.44%. Increasing levels of inhibition were observed after incubation at day 3 (27.32%) and day 5 (58.99%). The highest DPPH radical scavenging activity was observed after cut sugarcane incubation for 7 days with 71.46% inhibition.

Ferric Reducing Potential. Results of ferric reducing capacities of the cut sugarcane extracts at different incubation times are also presented in Table 1. The trend for the ferric ion reducing activities of the sugarcane extracts was similar to that obtained for their DPPH radical scavenging activities. The initial sugarcane extract at day 0 was the lowest (1.94 mmol Fe^{2+}/g), followed by increased ferric reducing capacities for the extract at day 3 (3.65 mmol Fe^{2+}/g) and for the extract at day 5 (7.37 mmol Fe^{2+}/g). In this study, the cut sugarcane extract at day 7 of incubation possessed the highest ferric reducing capacity at 11.75 mmol Fe^{2+}/g.

Antioxidant Activity and Correlation Coefficient. The pattern observed for the antioxidant activity of sugarcane samples closely resembled those recorded for TPC. Both DPPH and FRAP assays appear to have similar trends, with high antioxidant activity observed with longer incubation times. The TPC was higher using longer incubation times. To analyze the correlative relationships among total antioxidant activity (DPPH and FRAP) and total phenolics, a Pearson correlation analysis was conducted. The antioxidant activity determined by DPPH assay is positively correlated (R^2 = 0.87; $p < 0.01$) to that detected by the FRAP assay. Moreover, both DPPH and FRAP assays are significantly in positive correlation (R^2 = 0.88 and 0.96, respectively; $p < 0.01$) with TPC, suggesting that higher concentrations of phenolic compounds may be responsible for a large proportion of the antioxidant activity observed.

Varietal Differences. Initial stilbene analysis was performed on the sugarcane variety L 97-128. Two additional sugarcane varieties were analyzed for stilbenes after cutting and incubating for 7 days. Stilbenes were not detected in freshly cut sugarcane samples (day 0). Figure 6 displays the results for the quantification of piceatannol and resveratrol in cut billets from

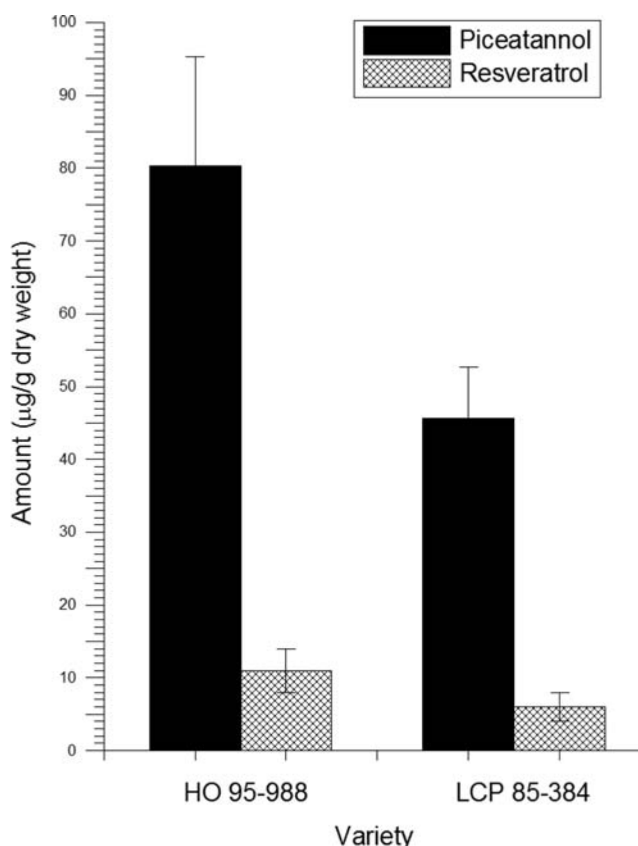

Figure 6. Content of piceatannol and resveratrol in cut sugarcane after incubation for 7 days in varieties HO 95-988 and LCP 85-384.

varieties Ho 95-988 and LCP 85-384 at day 7 after incubation. Ho 95-988 contained slightly higher amounts of piceantannol (80.3 μg/g) and resveratrol (11 μg/g) when compared to variety LCP 85-384 (piceatannol, 45.7 μg/g; resveratrol, 6 μg/g). However, the concentrations of stilbenes in both varieties were significantly lower than levels detected in sugarcane variety L 97-128.

Stilbene and Carbohydrate Analysis of Sugarcane Juice. Sugarcane juice was prepared from cut sugarcane (variety L97-128) billets at days 0 and 7. For the analysis of stilbenes, juice samples were first lyophilized. Table 2 displays the results of the stilbene and carbohydrate analysis of sugarcane juice at days 0 and 7. As expected, no stilbenes were detected in juice samples at day 0. Low levels of both piceatannol (8.5 mg/L) and resveratol (1.2 mg/L) were detected in juice samples at day 7. Carbohydrate analysis showed only sucrose (18.4%) in juice samples at day 0; however, after incubation for 7 days, glucose (3.4%) and fructose (3.4%) were detected, with a decrease in sucrose content to 5.9%.

DISCUSSION

Sugarcane is used primarily as a source of sucrose (table sugar) used by consumers throughout the world. Sugarcane harvested under normal conditions does not contain stilbenes. However, under conditions of stress or fungal infestation, piceatannol has been shown to be present at infection sites on sugarcane

Table 2. Piceatannol, Resveratrol, and Carbohydrate Contents in Sugarcane Juice Obtained at Day 0 and after Incubation for 7 Days[a]

sample	piceatannol (mg/L)	resveratrol (mg/L)	sucrose (g/100 mL)	glucose (g/100 mL)	fructose (g/100 mL)
sugarcane juice, day 0	N/D	N/D	18.4	N/D	N/D
sugarcane juice, day 7[a]	8.5 ± 1.7	1.2 ± 0.17	5.9	3.4	3.4

[a]Cut sugarcane (variety L 97-128) billets were incubated for 7 days before pressing for juice production. N/D, not detected. Piceatannol and resveratrol content results are expressed as the mean ± standard deviation (n = 3).

stalks.[39,40] Piceatannol was identified at infection sites in sugarcane inoculated with *C. falcatum*, but not in healthy or wounded sugarcane.[39,40] Unfortunately, the fungus *C. falcatum*, causal agent of the main disease of sugarcane causing red rot, can inhibit sugarcane growth and decrease sugarcane yields. Typically, fungus-infected sugarcane, depending on extent of infection, is not harvested but discarded.

Sugarcane contains a variety of fungi that have the potential to stimulate the production of phytoalexins (stilbenes) when present.[39,40] Initial sugarcane sampling in our laboratory identified *Penicillium* subgenus *Biverticillium* in sugarcane samples (variety L 97-128) that were incubated without initial bleach rinsing. Fungal growth on these billet samples was concentrated on the external rind. Sterilization using a 10% bleach solution inhibited fungal growth during incubation; however, incubation times longer than 7 days often produced visible fungal mycelia.

Our results showed that the stilbenes piceatannol and resveratrol can be produced postharvest in cut sugarcane after incubation. Although piceatannol production in sugarcane stalks has been reported, our study showed for the first time the occurrence of resveratrol in sugarcane. Variety L 97-128 showed the highest levels of stilbenes; however, two other varieties (Ho 95-988 and LCP 85-384) also displayed production of piceatannol and resveratrol at much lower concentrations. Variations in the ability of sugarcane varieties to produce stilbenes are to be expected, and more testing is needed to identify what these variations are. Our data showed that postharvest stilbene production in cut sugarcane that is incubated for at least 3 days is necessary for low levels of stilbenes to be detected. Longer incubation times allow higher stilbene concentrations to be produced. Also, TPC and antioxidant activity (DPPH radical scavenging and ferric reducing capacity) increased with longer incubation times. The concentrations of these stilbenes in sugarcane (L 97-128) are high when compared to other foods. Resveratrol concentrations in grapes have been reported to range from 0.356 to 237.83 μg/g of grape powder.[24] Higher resveratrol levels have been detected in the skin of mature grapes ranging from 10 μg/g[33−35] to 24 μg/g.[52] In grapes infected by powdery mildew the stilbene content was increased and positively correlated with fungal infection.[52]

In this study quantification was conducted on lyophilized sugarcane billet samples to aid in the extraction of stilbenes (dry samples were ground to fine powder). Sugarcane typically contains up to 50% water, and results herein using cut billets confirmed this water content. Fresh weight stilbene concentrations can be approximated by reducing dry weight concentrations by half. However, the concentrations of stilbenes detected using fresh weight amounts would still be significantly higher compared to those found in many other food sources.

Sugarcane juice was also shown to contain the stilbenes piceatannol and resveratrol. For comparison, Concord grape juice contained 1.5 μmol/L resveratrol in a study conducted by Stalmach et al.[53] In red grape juices the average resveratrol concentration was 0.50 mg/L (2.19 μmol/L), and lower amounts (0.05 mg/L) of resveratrol were detected in white grape juice.[54] Red and white wines are another source of resveratrol.[55,56] Resveratrol in white wines varied between 0.011 and 0.547 mg/L.[55] Spanish rosé wines were reported to vary in resveratrol content (0.05−1.19 mg/L).[55,56] Our results when compared to wine and fruit juices show comparable levels of resveratrol in sugarcane juice after 7 days of incubation at 1.2 mg/L (5.2 μmol/L). Additionally, high levels of piceatannol (8.5 mg/L) were detected. The analysis of sugars determined high levels of sucrose in juice samples from freshly harvested cut sugarcane, but degradation produced both fructose and glucose at day 7 after incubation. The degradation of sucrose in sugarcane has been well characterized in past studies,[57] and harvested sugarcane is typically processed quickly to avoid loss of sucrose.

Although research has shown piceatannol to be a component of fungus-infected sugarcane, few data exist on stilbenes produced in noninfected sugarcane. Also, direct evidence of resveratrol in sugarcane has not been reported. However, evidence for the accumulation of stilbenes was provided by Yu et al. using sorghum.[58,59] Biosynthesis of resveratrol is catalyzed by stilbene synthase (STS), which utilizes the same substrates as chalcone synthase, but a different cyclization mechanism is involved.[57] Resveratrol STS enzymes were originally described in grapes and peanuts. Yu and others reported the first example of a monocot STS gene, *SBSTS1*, isolated from sorghum.[56] SBSTS1 was expressed only following infection with fungal pathogens. Further research showed accumulation of *cis*-piceid, a glycoside of resveratrol, in transgenic *Arabidopsis* over-expressing *SbSTS1*. Other stilbenes, including resveratrol, piceatannol, and pinosylvin, were not detected in sorghum using HPLC-UV detection.[59] In further research, piceid (glycosylated resveratrol) was detected in sorghum seedlings infected with *Colletotrichum sublineolum*.[60] Also, piceid and resveratrol were authenticated for the first time in red sorghum grains.[61] Research has shown that the genomes of sorghum and sugarcane are very closely related,[62] and the expression of *SbSTS1* in sugarcane needs to be determined.

Other compounds including anthocyanins and flavonoids have been detected in infected sugarcane. The anthocyanidin luteolidin, along with an undetermined glycoside of luteolidin, was identified in extracts of *C. falcatum*-infected sugarcane[41,45,46] and is presumably responsible for the orange-red color, which appears at least 24 h before piceatannol is detected (day 3). Other compounds detected in sugarcane after *C. falcatum* were apigenidin and the caffeic acid ester of 5-*O*-apigenidin.[45,46] Other phenolics were also identified in sugarcane juice that was not incubated, including the flavones apigenin, luteolin, and tricin derivatives and the phenolic acids hydroxycinnamic, caffeic, and sinapic acids.[63] These flavonoids and phenolic acids contributed to increased phenolic content

(160 mg chlorogenic acid equiv/L) and exhibited both in vitro and in vivo antioxidant activities in sugarcane juice samples.[63] In our study, the day 0 sugarcane extracts displayed low-level phenolic content and antioxidant activities. However, increasing incubation time stimulated production of stilbenes and other phenolic components (Table 1). Further characterization is necessary to completely identify all phenolic components that may be increased or decreased during incubation.

The identification of resveratrol and piceatannol in sugarcane billets and extracted juice in this study points to the potential production of either isolated stilbenes or stilbene-enriched sugarcane. Recently, newer functional foods containing stilbenes have been proposed. In both the title and conclusion of the publication by Cantos et al.,[35] the authors ask if the resveratrol-enriched grape product produced by UV irradiation is a new "functional" fruit. In a perspective paper we introduced the concept of phytoalexin-enriched functional foods.[64] Although this study introduces stilbene-enriched sugarcane, more research is needed to examine the potential to create a functional food from stilbene-enriched sugarcane.

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Notes

The authors declare no competing financial interest.

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