JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Comparison of the Concentrations of Phenolic Constituents in Cane Sugar Manufacturing Products with Their Antioxidant Activities

BERTRAND PAYET,* ALAIN SHUM CHEONG SING, AND JACQUELINE SMADJA

Laboratoire de Chimie des Substances Naturelles et des Sciences des Aliments, Faculté des Sciences et Technologies, Université de la Réunion, 15 avenue René Cassin, B.P. 7151, 97715 Saint Denis Messag. Cedex 9, La Réunion, France

Polyphenol content and free radical scavenging capacity of seven kinds of sugar manufacturing products (A sugars, clear juices, syrups, massecuite, and A, B, and C molasses) were studied. Seventy-two samples were collected at different stages of the process during two sugar harvests from a local sugar factory (Bois-Rouge, La Réunion). The total phenolic content of sugar products was determined according to the Folin−Ciocalteu assay. Polyphenols of sugar products were extracted with ethyl acetate and quantified by LC-UV-ESI-MS during all of the process. ABTS and DPPH assays were applied to aqueous solution of sugar products, which exhibited interesting free radical scavenging activity. Comparatively, ethyl acetate extracts exhibited higher antioxidant activity. Multivariate analyses (principal component analysis and canonical discriminant analysis) demonstrated a significant correlation between polyphenols and antioxidant activity. Moreover, it was observed that the sugar process results in an increase of the phenolic content and the free radical scavenging capacity of the different products. These products and especially molasses proved to be a rich source of natural antioxidants and may represent an interesting alternative to synthetic food antioxidants.

KEYWORDS: Sugar cane; free radical scavenging capacity; total phenolic content; phenolic acids; cane sugar products; multivariate analysis

INTRODUCTION

Polyphenols including flavonoids and phenolic acids are secondary metabolites that constitute one of the most widely occurring groups of phytochemicals. These compounds have considerable physiological and morphological roles in plants (*1*). In addition, polyphenols exhibit many biological properties such as anti-inflammatory, antiallergic, antibacterial, antimicrobial, cardioprotective, and antioxidant activities (*2*). Moreover, they have been associated with health benefits afforded by the consumption of fruits and vegetables (*3*). Recently, more attention has been paid to their possible role in the prevention of several chronic diseases involving oxidative stress (*4*−*6*).

Food-processing products derived from plants and fruits are valuable natural sources of bioactive compounds including phenolic compounds (*7*, *8*). Numerous studies have been devoted to the bioactive capacity of agricultural byproducts (rice hulls, almond hulls, citrus peels and seed residue, apple peels, olive mill wastes, wine industry byproducts, etc.), in particular, to find some alternatives to synthetic food antioxidants (*9*). Cane sugar products may contain polyphenols as is the case for other fruits and plants. This assumption arose from previous studies dealing with phenolic acids, polyphenols, and flavonoids in sugar cane (*10*−*12*), phenolic compounds in liquid sugar from cane molasses or in brown sugars (*13*, *14*), and antioxidative phenolic compounds from a noncentrifuged sugar cane (*15*). In a previous work, we have identified phenolic compounds in several commercial cane brown sugars and assessed their free radical scavenging activities (*16*). To the best of our knowledge, the bioactive compounds from sugar products have not been investigated yet.

The aim of this study was to investigate the free radical scavenging capacity to 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays of sugar products collected at different stages of the sugar process. The total phenolic content of sugar products was determined, and polyphenol compounds were identified by liquid chromatography−mass spectrometry (LC-MS) in all of the studied fractions. Multivariate analyses were applied to all of the results to compare all of the samples and find the most relevant product in terms of bioactive compounds.

MATERIALS AND METHODS

Materials. Seventy-two samples of cane sugar products obtained from a local sugar factory (Bois-Rouge, La Réunion, France) were collected during two harvests. Samples were taken at different stages

* Author to whom correspondence should be addressed [telephone (00) 262 93 81 94; fax (00) 262 93 81 83; e-mail bpayet@univ-reunion.fr].

10.1021/jf060808o CCC: $33.50
Published on Web 08/29/2006

of the sugar manufacturing process, which essentially consists of the separation of pure sucrose from the other materials of the sugar cane (*17*). The ground canes are treated by diffusion of hot water (85 °C) to produce the "raw juice". The latter is then subjected to several chemical treatments and filtrations ("carbonatation" in the presence of lime and carbon dioxide, "sulfitation" with sulfur dioxide, and clarification by the addition of flocculents) to eliminate all impurities such as proteins, polysaccharides, and high molecular weight and color-forming compounds, thus leading to the "clear juice"(10−15% of dry content). Boiling the clear juice by heat exchange in evaporators results in a highly concentrated juice (65−70% of dry content) called the "syrup". Sucrose crystallization is then achieved by concentration of the syrup under vacuum to afford the "massecuite" (mixture of sucrose and highly concentrated syrup called molasses). A sursaturation state is maintained by the addition of seed crystals and sugar materials to the massecuite. Sucrose is separated from molasses by centrifugation and dried to give commercial raw sugar. Crystallization, centrifugation, and drying are repeated three times (cycles A, B, and C) to extract all of the sucrose from the molasses. A mixture of B and C sugars and C molasses constitutes the sugar materials added to A massecuite.

Seven classes of cane sugar manufacturing products (72 samples) were studied: clear juices (CJ), syrups (SI), A massecuite (MC), A, B, and C molasses (AM, BM, and CM, respectively), and A sugars (AS). Total soluble solids of samples were characterized by means of a handheld refractometer (Atago DR A1) to determine the dry matter.

Chemicals. All of the solvents used were of high-performance liquid chromatography (HPLC) grade, and water was supplied by a Milli-Q water purification system from Millipore (Bedford, MA). ABTS, DPPH, Folin−Ciocalteu phenol reagent, gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), *p*-hydroxybenzoic acid, vanillic acid, homovanillic acid, vanillin, *p*-coumaric acid, acetosyringone, ferulic acid, quercetin, apigenin, flavone, and kaempferol were purchased from Fluka (St Quentin Fallavier, France). Syringic acid, benzoic acid, coniferyl alcohol, (+)-catechin, (−)-epicatechin, rutin, luteolin, 3-hydroxyflavone, 4-formylbenzoic acid methyl ester, sodium carbonate, sodium sulfate, and potassium persulfate were obtained from Sigma-Aldrich (St Quentin Fallavier, France). All other chemicals and reagents were of analytical grade.

Extraction Procedure. A solution of sugar products (0.3 g) in 2 mL of ethyl acetate was extracted with sodium hydroxide solution (2 × 1 mL; 10%, w/w) using Vortex mixing (Heidolph Top-Mix 94323, Schwabach, Germany) and centrifugation (4000 rpm, 5 min; Bioblock Scientific 55702, Illirch, France). The pH of the resulting aqueous layer was adjusted to 4 by the addition of 5 N hydrochloric acid and extracted with ethyl acetate (3 × 2 mL). The combined organic layer was dried over anhydrous sodium sulfate and concentrated to ∼0.2 mL using a rotary evaporator (45 °C, 90 mbar; Laborota 4003 control, Heidolph, Schwabach, Germany). Ten microliters of 4-formylbenzoic acid methyl ester (3 mg/mL) was added as internal standard, and the volume was reduced to 0.5 mL with ethyl acetate.

All extractions were triplicated.

Spectroscopic Instrumentation. Assays were performed on 96-well microtiter plates (Nunc), and absorbance was measured with a Biotek Powerwave XS microplate reader (Winooski, VT). A Shimadzu Pharmaspec UV-1700 spectrophotometer (Kyoto, Japan) was also used for the measurement of larger solutions.

Total Polyphenol Content. Estimation of the total polyphenol content of the sugar products was performed according to the Folin−Ciocalteu method (*18*). Thirty microliters of sample and 150 μL of Folin−Ciocalteu reagent diluted 10 times with water were first pipetted into each well of a 96-well plate. Between 1 and 8 min, 120 μL of 7.5% in water Na_2CO_3 (w/w) must be added. The plate was placed in the reader and incubated for 1 h at 30 °C, and the absorbance was measured at 765 nm. A blank measure, for which the sample was replaced by water, was subtracted from the absorbance at 765 nm. Quantification was obtained by reporting the absorbance in the calibration curve of gallic acid used as standard phenol ($r^2 = 0.9962$). The results were expressed in grams of gallic acid equivalent per kilogram of sample (GAE/kg of sample). All assays were conducted in triplicate.

LC-MS Analysis. The polyphenol analysis was performed according to the Sakakibara procedure (*19*) with slight modifications. The LC system employed was an Agilent CPL/SM 1100 series (Massy, France) equipped with LC-MSD chemstation software, degasser G1322A, binary pump G1312A, autosampler G1313A, thermostated column oven G1316A, diode array detection system G1315B to monitor at all wavelengths from 200 to 400 nm, and MSD/VL mass spectrometer with electrospray source. LiChrospher 100 RP-18 column (250 × 4.6 mm i.d., S-5, 5 μm; Merck Darmstat, Germany) joined with a guard column LichroCART 4-4 (Merck, Darmstadt, Germany) was used at 35 °C. Gradient elution was performed with solution A (93% water at 0.1% acetic acid, pH 3.3−7% methanol) and solution B (70% methanol) delivered at a flow rate of 1.0 mL/min as follows: initially 100% of solution A; for the next 15 min, 70% A; for another 30 min, 65% A; for another 20 min, 60% A; for another 5 min, 5% A; and finally 0% A for 25 min. The electrospray mass spectrometer conditions were as follows: negative ion mode, fragmentor voltage, 70 V; capillary voltage, 4000 V; drying gas (nitrogen), flow, 11 mL/min; nebulizer pressure, 60 psig; drying gas temperature, 350 °C; mode scan, 50−400 uma.

The volume injected was 10 μL.

For the polyphenol analysis, a library including 65 phenolic acids, catechins, flavonoids, and simple polyphenols was first built up with retention times and UV-DAD spectra of aglycons. Besides, a calibration table was constructed: response factor was calculated for each reference compound (concentration range = 100−1000 $\mu mol.L^{-1}$) at a specific determination wavelength (250, 280, 320, or 370 nm depending on the compound class of polyphenol). The mass spectrum of each reference compound was also recorded and used to confirm identification. The internal standard was 4-formylbenzoic acid methyl ester (180 $\mu mol\ L^{-1}$). The sugar product extracts were analyzed according to the above procedure. The polyphenols were identified on the basis of their retention times, UV-DAD spectra, and electrospray mass spectra and then quantified according to the calibration table using the equation

$$C_i = (A_i/A_{is}) \times RF_i \times C_{is}$$

where C_i is the concentration ($\mu mol\ L^{-1}$) of compound i, A_i is the peak area of compound i, RF_i is the response factor of compound i, C_{is} is the concentration ($\mu mol\ L^{-1}$) of the internal standard, and A_{is} is the area of the internal standard peak.

DPPH Radical Cation Decolorization Assay. The DPPH• assay was used to measure the free radical scavenging capacity of sugar product aqueous solutions and their corresponding ethyl acetate extracts. DPPH• offers a convenient and accurate method for titrating oxidizable groups of both natural and synthetic antioxidants (*16*, *20*). Two hundred and eighty microliters of 0.004% DPPH• methanolic solution was pipetted into each well of a 96-well plate followed by 20 μL of sample or Trolox (solvent for the blank). The mixture was incubated at 30 °C, and absorbance was measured at 515 nm with a microplate reader after 60 min for diluted samples and every minute during 1 h for ethyl acetate extracts. The inhibition percentage of the radical scavenging activity was calculated using the equation

$$\text{inhibition (\%)} = 100 - 100 \times (\mathbf{A}_S \div \mathbf{A}_0)$$

where A_0 is the absorbance of the blank and A_S is the absorbance of the sample at 515 nm.

The radical scavenging activity was also expressed in terms of IC_{50} (concentration that provides 50% inhibition) or t_{IC50} (time necessary to reach 50% inhibition).

All assays were conducted in triplicate.

ABTS Radical Cation Decolorization Assay. $ABTS^{\bullet+}$ reagent was generated through a chemical oxidation reaction with potassium persulfate as described by Re et al. (*16*, *21*). The concentration of the blue-green ABTS radical solution was adjusted with methanol to an absorbance of 0.700 ± 0.020 (mean ± SD) at 734 nm. To 280 μL of this solution of $ABTS^{\bullet+}$ was added 20 μL of sample or Trolox or solvent upon a 96-well plate. The mixture was incubated for 5 min at 30 °C, and absorbance was measured at 734 nm using a microplate reader. The inhibition percentage of the radical scavenging activity

Table 1. Total Polyphenol Content of Cane Sugar Manufacturing Products

sample	GAE[a]	sample	GAE[a]
clear juices	5.52 ± 0.40	A molasses	13.88 ± 1.08
syrups	4.40 ± 0.48	B molasses	22.34 ± 1.18
massecuite	6.06 ± 0.89	C molasses	27.21 ± 1.39
A sugars	0.70 ± 0.25		

[a] Grams of gallic acid equivalent per kilogram of dry content of sample, means ± SD ($n = 6$).

was calculated using the equation

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

where A_0 is the absorbance of the blank and A_S is the absorbance of the sample at 734 nm.

For diluted samples, the radical scavenging activity was expressed in terms of IC_{50}.

The $ABTS^{\bullet+}$ radical solution was prepared daily, and all analyses were made in triplicate.

Statistical Analysis. Data are reported as mean ± standard deviation (SD) of triplicate determinations. The statistical analyses were carried out using the Microsoft Excel software package (Microsoft Corp.) and *XL*STAT add-on (Addinsoft). Principal component analysis (PCA) based on the Pearson correlation matrix was applied to the analytical data (total polyphenol content, polyphenol concentrations determined by LC-MS, and ABTS and DPPH free radical scavenging activity) to find the relationships between the different analytical methods and to point out the differences among sugar products. The two principal components with greatest eigenvalues were selected. Canonical discriminant analysis (CDA) was performed with sugar product types as dependent variable and total polyphenol content, concentrations of vanillin, protocatechuic, syringic, *p*-coumaric acids, and ABTS and DPPH free radical scavenging activities as explanatory variables.

RESULTS AND DISCUSSION

Total Polyphenol Content. The total phenolic contents of the sugar manufacturing products were determined according to the Folin−Ciocalteu method, which proved to be a convenient, simple, and rapid method for the estimation of total phenolic content of various samples (*18*). The total phenolic content of sugar products ranged from 0.7 GAE/kg of dry content for A sugars to 27.21 GAE/kg of dry content for C molasses (**Table 1**). Relating to dry matter, C molasses contained ∼2 times more polyphenols than A molasses, ∼4 times more than massecuite, ∼6 times more than syrups, and ∼5 times more than clear juices. It is noteworthy that very high polyphenol contents were observed for sugar products because they are highly colored materials and phenolic compounds are strongly involved in the formation of this color (*22*). However, these values obtained with the Folin−Ciocalteu assay were certainly overestimated due to the presence of sucrose, glucose, fructose, and Maillard reaction compounds in the sugar products, which may interfere with the test by enhancing the development of the blue color (*16*, *18*).

Identification of Polyphenolic Compounds by LC-MS. Polyphenols from the sugar product extracts were identified and quantified using HPLC with a photodiode array detector coupled with a mass spectrometer, according to the reported procedure (*19*). The 65 phenolic acids, catechins, flavonoids, and simple phenols of the library were searched for in the extracts. This method allowed simultaneous detection of many classes of polyphenols, but in our case phenolic acids were the major components of the extracts (**Table 2**). Indeed, vanillin, four derivatives of hydroxycinnamic acid, and five derivatives of hydroxybenzoic acid were identified and quantified. However, catechins and flavonoids were not found in the extracts. Interestingly, B and C molasses exhibited the highest polyphenol content (>1 g/kg of dry content). For all sugar products, *p*-coumaric and ferulic acids were the preponderant compounds. The latter, which are native to the cane plant and result from the degradation of lignin and hemicellulose materials, are also the major polyphenols of sugar cane roots (*11*) and bagasse (∼1−2%) (*23*). Chlorogenic acid, involved in the enzymatic browning of cane juice (*24*), was detected in molasses. It is noteworthy that contents of hydroxybenzoic acid derivatives increased during the sugar process. This was probably due to the degradation of the hydroxycinnamic acid derivatives as indicated by the presence of benzoic acid from the crystallization step. In conclusion, the sugar process results in an increase of phenolic compound contents in its products.

Free Radical Scavenging Assays. Antioxidant activities assays were performed on sugar manufacturing products in aqueous solution and on their ethyl acetate extracts.

DPPH Assay. Concentration response curves of two standard antioxidants, Trolox and ascorbic acid, were obtained. Trolox had an IC_{50} of 4.02 mg/kg (final concentrations ranging from 1.25 to 7.5 mg/kg, $r^2 = 0.9789$). The IC_{50} of ascorbic acid was 5.31 mg/kg (final concentrations ranging from 1 to 10 mg/kg, $r^2 = 0.9891$). Free radical scavenging responses to DPPH of aqueous solutions of sugar products and their ethyl acetate extracts are listed in **Table 3**. Clear juices and A sugars showed the weakest activities (IC_{50} of 7633 and 8626 mg/kg). In contrast, C molasses were the most active sample (60 mg of C molasses is equivalent to 1.33 mg of Trolox or 1 mg of ascorbic acid with regard to inhibition of DPPH oxidation). Sugar product extracts strongly inhibited DPPH oxidation (>90% at $t = 60$

Table 2. Contents of Polyphenolic Constituents by LC-MS of Cane Sugar Products[a]

compound	t_R[b] (min)	clear juices	syrups	massecuite	A molasses	B molasses	C molasses	A sugars
protocatechuic acid	8.87	6.3	8.5	19.4	42.9	71.2	102.3	0.4
p-hydroxybenzoic acid	12.90	15.8	16.4	27.6	45.1	68.3	107.9	
chlorogenic acid	14.80				8.1	13.1	14.3	
vanillic acid	16.41		2.2	7.5	7.1	26.1	41.8	
caffeic acid	16.79	8.3	57.0	25.2	82.1	147.7	88.3	0.6
syringic acid	18.36	5.0	11.1	13.5	37.7	54.8	70.8	
vanillin	19.4	8.1	10.3	14.5	33.1	44.2	46.3	
p-coumaric acid	22.86	135.8	140.9	144.7	289.1	424.6	423.1	10.2
ferulic acid	26.24	69.8	114.4	71.3	168.4	255.1	169.5	11.2
benzoic acid	28.40			2.1	47.8	128.4	71.1	
total		249.2	360.9	325.6	761.4	1233.4	1135.5	22.4

[a] Expressed in milligrams per kilogram of dry content of sample, means ($n = 3$). Standard deviation was always <10%. [b] Retention time of the compounds expressed in minutes.

Table 3. Free Radical Scavenging Activity of Cane Sugar Products and Their Extracts

	diluted samples		ethyl acetate extracts			
	ABTS assay	DPPH assay	ABTS assay	DPPH assay		
	IC_{50}[a]	IC_{50}[a]	% inhibition[b]	% inhibition[c] ($t = 0$ min)	% inhibition[d] ($t = 60$ min)	t_{IC50}[e] (min)
clear juices	4202 ± 397	7633 ± 450	12.8 ± 5.8	9.8 ± 5.3	51.3 ± 9.7	>60
syrups	782 ± 60	1080 ± 101	47.4 ± 7.0	23.6 ± 3.7	93.0 ± 0.8	3.97
massecuite	319 ± 29	466 ± 27	60.1 ± 5.9	36.9 ± 5.0	93.3 ± 0.3	1.92
A molasses	308 ± 29	418 ± 45	75.4 ± 8.0	39.6 ± 6.7	94.1 ± 0.5	1.47
B molasses	211 ± 13	289 ± 34	88.3 ± 5.1	55.5 ± 8.6	94.5 ± 0.7	0.24
C molasses	151 ± 21	234 ± 27	89.0 ± 4.9	56.7 ± 6.5	94.8 ± 1.2	0.12
A sugars	6498 ± 349	8626 ± 453	8.0 ± 3.4	5.8 ± 2.8	25.3 ± 2.5	>60

[a] Concentration in milligrams of sugar products per kilogram of aqueous solution providing 50% inhibition. Mean ± SD ($n = 3$ for each sample). [b] Percent inhibition of ethyl acetate extract diluted tenfold. Mean ± SD ($n = 3$ for each sample). [c] Percent inhibition of ethyl acetate extract at $t = 0$ min, i.e., when the sample was added. Mean ± SD ($n = 3$ for each sample). [d] Percent inhibition of ethyl acetate extract at $t = 60$ min. Mean ± SD ($n = 3$ for each sample). [e] Time to reach 50% inhibition.

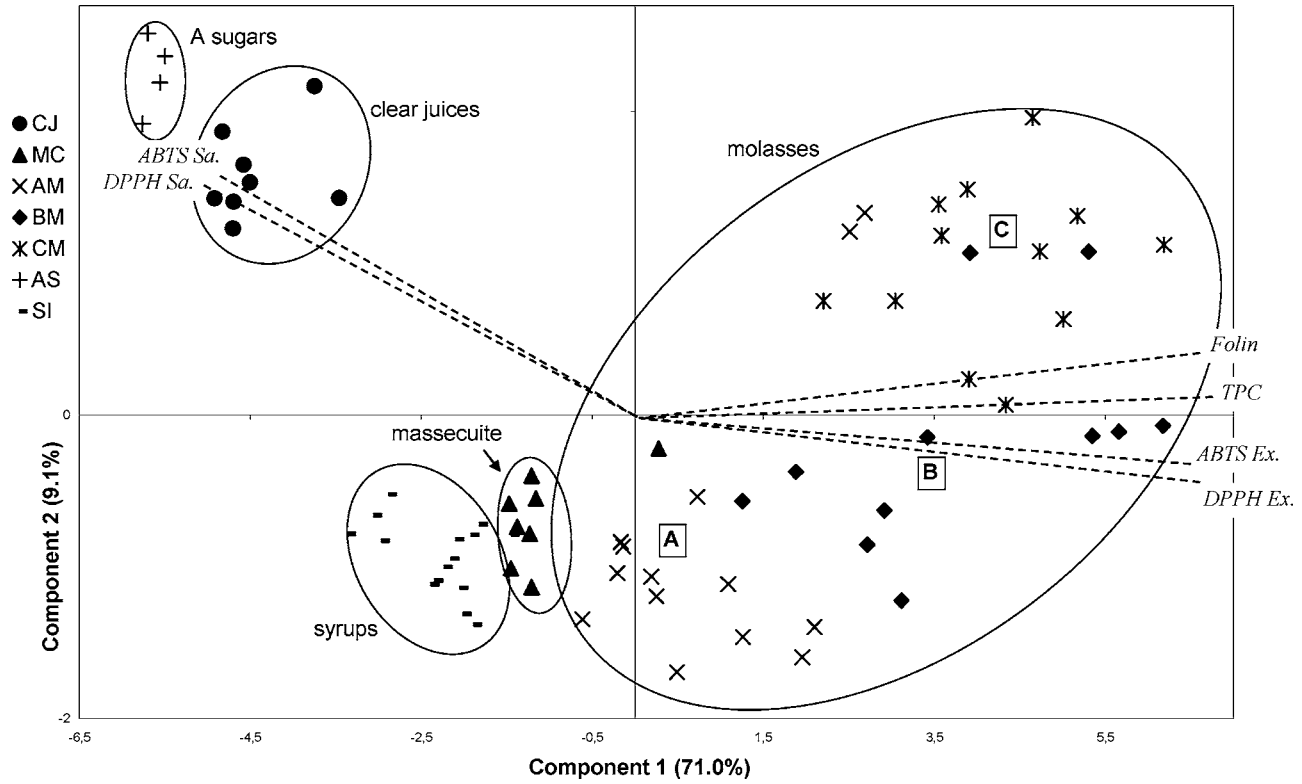

Figure 1. PCA of analytical data: biplot showing the projection of sugar products and variables onto the plane defined by the two principal components.

min) apart from A sugars and clear juices. Because antioxidant compounds react at different kinetic rates with DPPH radical (*25*), inhibition of DPPH oxidation when the sample was added ($t = 0$ min) and the time necessary to reach 50% inhibition were measured to differentiate sugar product extracts. Thus, we observed that extracts of B and C molasses reacted very quickly with DPPH radical ($t_{IC50} = 0.24$ and 0.12 min, respectively), whereas inhibition of DPPH by extracts of A molasses, massecuite, and syrups were slower but complete after 60 min. Clear juices extracts exhibited DPPH oxidation by 51.3% after 60 min and A sugars by only 25.3%.

ABTS Assay. Concentration response curves of two standard antioxidants, Trolox and ascorbic acid, were obtained. Trolox had an IC_{50} of 2.80 mg/kg (final concentrations ranging from 0.5 to 5 mg/kg, $r^2 = 0.9871$). The IC_{50} of ascorbic acid was 2.05 mg/kg (final concentrations ranging from 1 to 5 mg/kg, $r^2 = 0.9987$). Aqueous solutions of sugar products exhibited weak to moderate free radical scavenging capacity (**Table 3**). Clear juices and A sugars had very high IC_{50} values (respectively, 4202 and 6498 mg/kg), thus corresponding to weak activities, whereas the other sugar products showed greater activities. C molasses showed the strongest inhibition of ABTS oxidation ($IC_{50} = 151$ mg/kg), and 75 mg of C molasses is equivalent to 1.5 mg of Trolox or 1 mg of ascorbic acid. Ethyl acetate extracts of sugar products (diluted 10-fold) showed higher activities, in particular for B and C molasses, which inhibited ABTS oxidation by 90%. A molasses, syrups, and massecuite extracts exhibited moderate activity. Clear juices and A sugars extracts had no relevant free radical activity.

Finally, ABTS and DPPH free radical scavenging capacities of sugar manufacturing products increased during the sugar process, from clear juices that exhibited weak activities to C molasses which were more active.

Principal Component Analysis. A PCA was performed on analytical data. The two principal components with greatest eigenvalues accounted for 80.1% of the variance. **Figure 1** shows the projection of the 72 products and the variables (except for LC-MS polyphenol concentrations for clarity's sake) in the plane defined by the two principal components. PCA led to five differentiated groups of sugar products. A sugars clustered

Table 4. Correlation Coefficients ($p < 0.05$) between Antioxidant Tests and Total Phenolic Contents[a]

method	A	B	C	D	E	F
A: Folin	1	0.852	−0.563	−0.584	0.861	0.865
B: LC-MS		1	−0.554	−0.575	0.869	0.831
C: ABTS Sa			1	0.969	−0.754	−0.683
D: DPPH Sa				1	−0.780	−0.711
E: ABTS Ex					1	0.920
F: DPPH Ex						1

[a] Methods: A, total phenolic content of the sugar products; B, sum of all compounds identified by LC-MS in the extracts; C, ABTS assay on diluted samples; D, DPPH assay on diluted samples; E, ABTS activities of the extracts; F, DPPH activities of the extracts at $t = 0$ min.

together, similarly to clear juices, syrups, and massecuite. A, B, and C molasses were not totally differentiated and constituted the last group. Aqueous solutions of A sugars and clear juices were characterized by high IC_{50} values (variables ABTS Sa and DPPH Sa), and their weak activity clearly differentiates them from the other sugar products. The first principal component, accounting for 71.0% of the variance, was highly correlated with polyphenol content (Folin assay and total polyphenol identified by LC-MS) and free radical scavenging activity of ethyl acetate extracts. Distribution of the different groups along the first principal component established a strong correlation between the increase of polyphenol content of sugar products and the antioxidant activity during the sugar process.

Table 4 presents the correlation coefficients between antioxidant activity and total phenolic contents obtained from the Pearson correlation matrix. The expression of sugar product aqueous solutions activity in IC_{50} led to negative correlation with the other methods (high IC_{50} corresponding to weak activity). ABTS and DPPH assays were significantly correlated concerning the ethyl acetate extracts (0.920; $p < 0.05$) and the diluted samples (0.969; $p < 0.05$). Total phenolic contents obtained by Folin−Ciocalteu assay were significantly correlated with total polyphenols determined by LC-MS of the extracts (0.852; $p < 0.05$) despite the interferences with the Folin method. The correlation between the total polyphenol contents and antioxidant activity of the extracts were acceptable (up to 0.869 at $p < 0.05$ between ABTS assay and total polyphenol determined by LC-MS analyses). This result indicated that phenolic compounds were strongly involved in the free radical scavenging capacity of sugar product extracts. However, antioxidant assays applied to diluted samples in aqueous solution were not correlated to the activity of the extracts, therefore indicating the contribution of other compounds to the free radical scavenging activity of the sugar product aqueous solutions. It is known that Maillard reaction products are formed during the sugar process and may exhibit antioxidant activity (*26*); it would be therefore interesting to study their contribution to the sugar product activity. Finally, protocatechuic, syringic, and *p*-coumaric acids and vanillin concentrations were significantly correlated with the antioxidant activity of the extracts (>0.8; $p < 0.05$ data not shown). These antioxidant compounds (*27*, *28*) may represent markers for sugar product free radical scavenging activity.

Canonical Discriminant Analysis. Several parameters could influence the phenolic composition of sugar products because cane juice polyphenol content could be modified by the cane varieties, the maturity of the cane plant at harvest time, the amount of leaves crushed with the cane, and environmental factors such as weather conditions or infestation of the plant by pests (*11*, *29*). In addition, most phenolic compounds native to the cane plant are removed during sugar processing, in particular during the clarification and carbonatation steps, but these operations are not totally effective (*13*). Similarly, high-pressure and temperature conditions during the process could modify polyphenol nature and content. Thus, valorization of sugar products through their bioactive compounds requires homogeneity in terms of polyphenol content and free radical

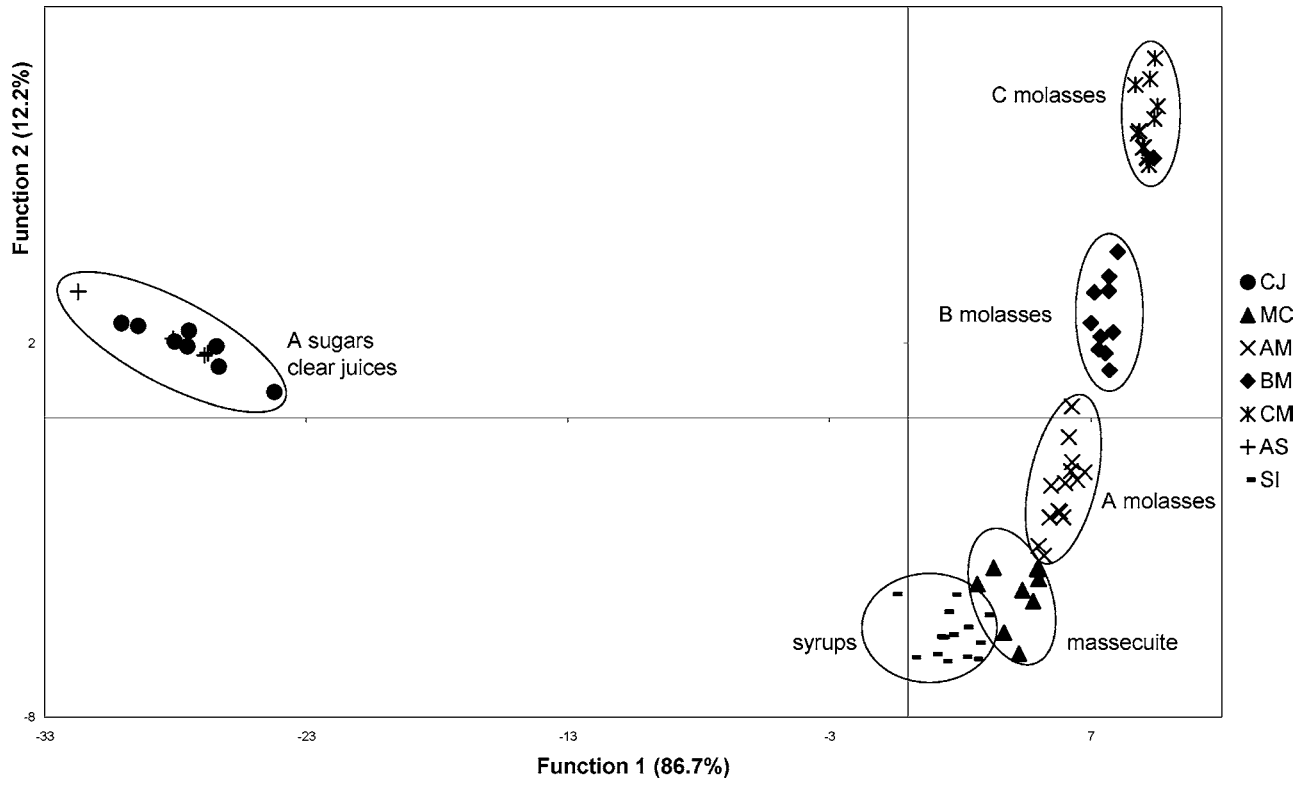

Figure 2. Discriminant plot of the sugar products.

Table 5. Classification of Sugar Products from Discriminant Analysis

	correct[a]	other[b]	%[c]
clear juices	8	0	100.0
syrups	14	1 (CM)	93.3
massecuite	8	0	100.0
A molasses	14	0	100.0
B molasses	10	1 (MC)	90.9
C molasses	12	0	100.0
A sugars	3	1 (CJ)	75.0
total	69	3	95.8

[a] Number of samples correctly classified. [b] Number of samples incorrectly classified. [c] Success rate.

scavenging capacity. For this purpose, the variability in polyphenol content and relative antioxidant activity of each kind of product was studied by a CDA because PCA is not appropriate to quantitative differentiation. CDA was used to classify sugar products on the basis of their total polyphenol contents, antioxidant activities, and concentrations of the most significant phenolic compounds (protocatechuic, syringic, and *p*-coumaric acids and vanillin). Sugar product type was used as grouping variable. The scatter plot of the two discriminant functions is presented in **Figure 2**. These two functions explained 98.7% of the variance. Projection of sugar products in the plane defined by these two functions led to a clear differentiation of each product type except for A sugars and clear juices, which clustered together. Moreover, classification of samples by comparison with the predefined sugar types provided a success rate of 95.8% (**Table 5**). Only 3 of the 72 samples were incorrectly assigned. These results showed a great homogeneity of each product of sugar manufacturing process concerning the polyphenol contents and their relative antioxidant activities.

Finally, sugar products, which are produced on a large scale, appeared to be a rich source of natural antioxidants, particularly the molasses. This study showed that the polyphenol contents increase along with the antioxidant activity throughout processing. B and C molasses were the products with the strong polyphenol content and relative free radical scavenging capacity. Polyphenols were naturally higher in the molasses samples, because the molasses is where the nonsugars concentrate. **Tables 1** and **2** show that there is not much significant change of the polyphenol content from clear juice through syrup to massecuite, reflecting the original amount present in the clear juice. The slight increases in the massecuite could be due to the interfering effect of Maillard reaction products; there is also the possibility of some phenolic groups, especially ferulic acid and vanillin, which could be hydrolyzed off the indigenous soluble cell wall polysaccharides naturally present in the cane juice. The very low level of polyphenols in raw sugar (A sugar) is a reflection of the purification process caused by crystallization, whereas the "impurities" went into the molasses. However, it is also likely that a fair amount of antioxidant activity may be caused by the large increase in colorant that is experienced throughout the process. The weak antioxidant activity of cane brown sugars (*16*) results obviously from the residual thin film of molasses coating on the sugar crystal. This study clearly established the possible valorization of cane sugar products as an alternative to synthetic food antioxidants, especially because they present interesting coloring and flavoring properties.

ABBREVIATIONS USED

ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; CDA, canonical discriminant analysis; DAD, diode array detector; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ESI, electrospray; GAE, gallic acid equivalent; GC-MS, gas chromatography coupled to a mass spectrometer; IC_{50}, concentration providing 50% inhibition of free radical; LC-MS, liquid chromatography coupled to a mass spectrometer; PCA, principal component analysis.

ACKNOWLEDGMENT

We are grateful to the Sucrerie de Bois-Rouge for its collaboration.

LITERATURE CITED

(1) Scalbert, A. *Polyphenolic Phenomena*; INRA Editions: Paris, France, 1993; 296 pp.
(2) Havsteen, B. H. The biochemistry and medical significance of the flavonoids. *Pharmacol. Ther.* **2002**, *96*, 67−202.
(3) Parr, A. J.; Bolwell, G. P. Phenols in plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *J. Sci. Food Agric.* **2000**, *80*, 985−1012.
(4) Fuhrman, B.; Aviram, M. Polyphenols and flavonoids protect LDL against atherogenic modifications. *Handbook of Antioxidants*, 2nd ed., revised and expanded; Cadenas, E.; Packer, L., Eds.; Dekker: New York, 2002; pp 167−187.
(5) Manach, C.; Mazur, A.; Scalbert, A. Polyphenols and prevention of cardiovascular diseases. *Curr. Opin. Lipidol.* **2005**, *16*, 77−84.
(6) Favier, A. Place des radicaux libres et des antioxydants dans la maladie cancéreuse. In *Radicaux Libres et Stress Oxydant Aspect Biologiques et Pathologiques*; Delattre, J., Beaudeux, J.-L., Bonnefont-Rousselot, D., Eds.; Lavoisier: Paris, France, 2005; pp 475−499.
(7) Moure, A.; Cruz, J. M.; Franco, D.; Dominguez, J. M.; Sineiro, J.; Dominguez, H.; Nunez, M. J.; Parajo, J. C. Natural antioxidant from residual sources. *Food Chem.* **2001**, *72*, 145−171.
(8) Antolovich, M.; Prenzler, P.; Robards, K.; Ryan, D. Sample preparation in the determination of phenolic compounds in fruits. *Analyst* **2000**, *125*, 989−1009.
(9) Balasundram, N.; Sundram, K.; Samman, S. Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. *Food Chem.* **2006**, *99*, 191−203.
(10) McGhie, T. K. Analysis of sugarcane flavonoids by capillary zone electrophoresis. *J. Chromatogr. A* **1993**, *634*, 107−112.
(11) Nutt, K. A.; O'Shea, M. G.; Allsopp, P. G. Feeding by sugarcane whitegrubs induces changes in the types and amounts of phenolics in the roots of sugarcane. *Environ. Exp. Bot.* **2004**, *51*, 155−165.
(12) Colombo, R.; Lanças, F. M.; Yariwake, J. H. Determination of flavonoids in cultivated sugarcane leaves, bagasse, juice and in transgenic sugarcane by liquid chromatography−UV detection. *J. Chromatogr. A* **2006**, *1103*, 118−124.
(13) Godshall, M. A.; Roberts, E. J. Phenolics in sugar products: their role in flavor and color production. *Proceedings of the 1982 Sugar Processing Research Conference*; 1982; pp 47−72.
(14) Palla, G. Isolation and identification of phenolic glucosides in liquid sugars from cane molasses. *J. Agric. Food Chem.* **1982**, *30*, 764−766.
(15) Takara, K.; Matsui, D.; Wada, K.; Ichiba, T.; Nakasone, Y. New antioxidative phenolic glycosides isolated from KOKUTO non-centrifugated cane sugar. *Biosci., Biotechnol., Biochem.* **2002**, *66*, 29−35.
(16) Payet, B.; Shum Cheong Sing, A.; Smadja, J. Assessment of antioxidant activity of cane brown sugars by ABTS and DPPH radical scavenging assays: determination of their polyphenolic and volatile constituents. *J. Agric. Food Chem.* **2005**, *53*, 10074−10079.
(17) Hugot, E. *La Sucrerie de Canne*; Lavoisier: Paris, France, 1987; 1056 pp.

(18) Singleton, V. L.; Orthofer, R.; Lamuela-Raventos, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin−Ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152−178.
(19) Sakakibara, H.; Honda, Y.; Nakagawa, S.; Ashida, H.; Kanazawa, K. Simultaneous determination of all polyphenols in vegetables, fruits and teas. *J. Agric. Food Chem.* **2003**, *51*, 571−581.
(20) Brand Williams, W.; Cuvelier, M. E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *Lebensmn. Wiss. Technol.* **1995**, *28*, 25−30.
(21) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* **1999**, *26*, 1231−1237.
(22) Godshall, M.-A.; Vercellotti, J. R.; Triche, R. Comparison of cane and beet sugar macromolecules in processing. *Int. Sugar J.* **2002**, *104*, 228−233.
(23) Xu, F.; Sun, R.-C.; Sun, J.-X.; Liu, C.-F.; He, B.-H.; Fan, J.-S. Determination cell wall ferulic and *p*-coumaric acids in sugarcane bagasse. *Anal. Chim. Acta* **2005**, *552*, 207−217.
(24) Paton, N. H. Sugar cane phenolics and first expressed juice colour. *Int. Sugar J.* **1992**, *94*, 99−102.
(25) Bondet, V.; Brand Williams, W.; Berset, C. Kinetics and mechanisms of antioxidant activity using the DPPH• free radical method. *Lebensmn. Wiss. Technol.* **1997**, *30*, 609−615.
(26) Yilmaz, Y.; Toledo, R. Antioxidant activity of water-soluble Maillard reaction products. *Food Chem.* **2005**, *93*, 273−278.
(27) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Structure−antioxidant activity relationship of flavonoids and phenolic acids. *Free Radical Biol. Med.* **1996**, *20*, 933−956.
(28) Santosh Kumar, S.; Priyadarsini, K. I.; Sainis, K. B. Free radical scavenging activity of vanillin and *o*-vanillin using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. *Redox Rep.* **2002**, *7*, 35−40.
(29) Qudsieh, H. Y. M.; Yusof, S.; Osman, A.; Rahman, R. A. Effect of maturity on chlorophyll, tannin, color, and polyphenols oxidase (PPO) activity of sugarcane juice (*Saccharum officinarum* Var. yellow cane). *J. Agric. Food Chem.* **2002**, *50*, 1615−1618.

Received for review March 23, 2006. Revised manuscript received June 20, 2006. Accepted June 25, 2006. We are grateful to the Conseil Régional of La Réunion for financial support.

JF060808O