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Assessment of Antioxidant Activity of Cane Brown Sugars by ABTS and DPPH Radical Scavenging Assays: Determination of Their Polyphenolic and Volatile Constituents

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Seven cane brown sugars (four from La Réunion, two from Mauritius, and one from France) were investigated for their polyphenol content and volatile composition in relation to their free radical scavenging capacity determined by ABTS and DPPH assays. The thin layer coated on the sugar crystal was extracted by Soxhlet extractor with dichloromethane. The volatile compounds of brown sugars were studied by GC-MS, and 43 compounds were identified. The total phenolic content of brown sugars was determined according to the Folin−Ciocalteu method. Phenolic compounds were quantified in the brown sugar extracts by LC-UV-ESI-MS. Brown sugar aqueous solutions exhibited weak free radical scavenging activity in the DPPH assay and higher antioxidant activity in the ABTS assay at relatively high concentration. The brown sugar extracts showed interesting free radical scavenging properties despite the low concentration of phenolic and volatile compounds. Sugar is a common foodstuff traditionally used for its sweetening properties, which might be accompanied by antioxidant properties arising from molecules (polyphenols, Maillard products) other than sucrose of the cane brown sugars.

KEYWORDS: Brown sugars; free radical scavenging capacity; total phenolic content; phenolic acids; volatile composition; Maillard reaction compounds

INTRODUCTION

Sugar is an important foodstuff in the world trade, and its consumption remains high despite the rise of synthetic sweeteners. Indeed, the nutritional, gustative, and preservative properties of sugar make it an essential nutrient in the world diet. White sugars consist of >99% of sucrose, whereas brown sugars are traditionally composed of 88−93% of sucrose and are characterized by a unique odor and flavor. Traditional brown sugars can be classified into two types, "boiled" and "coated" brown sugars. Boiled brown sugars are obtained by the crystallization of dark refined syrups, which are responsible for their color and flavor. Coated brown sugars are refined sugars that have been sprayed with a thin film of highly colored syrup, caramel, or molasses, which gives them a characteristic color and flavor (*1*). The color of brown sugars results from the inclusion of macromolecules present in the syrup (*2*).

Industrial sugars are obtained from sugar cane (~70% of the production) and sugar beet. Sugar cane contains phenolic acids, polyphenols, and flavonoids (*3*−*8*). These compounds have also been found in sugar products such as syrup or molasses and in the brown sugars themselves (*9*, *10*). More recently, Takara isolated several phenolic compounds from Kokuto, a noncentrifuged cane sugar, and showed their antioxidative activity (*11*). The interest in polyphenols, including flavonoids and phenolic acids, has considerably increased in recent years because of their biological properties, their antioxidant effects, and their possible role in the prevention of several chronic diseases involving oxidative stress, as well as their protective effect against low-density lipoprotein (LDL) oxidation (*12*, *13*).

The sugar process also involves the development of Maillard reaction compounds such as macromolecules (melanoidins, polymers) and heterocyclic aromatic compounds, which are strongly involved in the color and aroma of the sugar products. For a few years, the antioxidant properties of Maillard reaction compounds have been reported (*14*−*17*). Yanagimoto showed in several studies that aromatic compounds formed during the Maillard reactions also exhibit an interesting antioxidant activity (*18*, *19*).

The aim of this study was to investigate the free radical scavenging capacity of brown sugars due to the residual thin film coating on the sugar crystal. ABTS and DPPH assays were applied to brown sugar aqueous solutions and dichloromethane extracts. The molecules that might be responsible for the activity, phenolic compounds, and Maillard reaction products were identified by GC-MS and LC-MS.

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MATERIALS AND METHODS

Sugar Material. Seven industrial brown sugars were purchased at local markets; four were produced in La Reunion (sugars **1**, **2**, **4**, and **5**), two were produced in Mauritius Island (sugars **6** and **7**), and one was produced in France (sugar **3**). Sugars **2**, **4**, **6**, and **7** were common brown sugars. Sugar **1** was a "blond de canne" (brown cane caster sugar), sugar **3** a "cassonade", that is, a brown sugar extracted from cane juice, and sugar **5** a deep brown sugar.

Chemicals. All of the solvents used were of high-performance liquid chromatography (HPLC) grade. The water used for all experiments was ultrapure, supplied by a Milli-Q water purification system from Millipore (Bedford, MA). ABTS [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt], DPPH (2,2-diphenyl-1-picrylhydrazyl), Folin−Ciocalteu phenol reagent, gallic acid, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), *p*-hydroxybenzoic acid, vanillic acid, homovanillic acid, vanillin, *p*-coumaric acid, acetosyringone, ferulic acid, quercetin, apigenin, flavone, kaempferol, 1-methyl-2-pyrrolidinone, 1-octanol, 2,4-decadienal, 2-acetylpyrrole, 2-furanmethanol, 2-hydroxybenzaldehyde, 4′-hydroxyacetophenone, 4-hydroxybenzaldehyde, α-toluic acid, benzaldehyde, benzophenone, benzyl alcohol, dimethyl sulfoxide, dimethyl sulfone, dodecanoic acid, furfural, hydroquinone, nonanal, octanoic acid, pantolactone, and γ-butyrolactone were obtained from Fluka (St Quentin Fallavier, France). Syringic acid, benzoic acid, coniferyl alcohol, (+)-catechin, (−)-epicatechin, rutin, luteolin, 3-hydroxyflavone, 2,3-butanenediol, 2-furancarboxylic acid, 3-hydroxy-4-methoxybenzoic acid, 4-vinylguaiacol, 5-(hydroxymethyl)-dihydro-2(3*H*)-furanone, 5-(hydroxymethyl)-2(5*H*)-furanone, 5-hydroxymethylfurfural, acetovanillone, cinnamaldehyde, cinnamyl acetate, sodium carbonate, and potassium persulfate were obtained from Sigma-Aldrich (St Quentin Fallavier, France). All other chemicals and reagents were of analytical grade.

Extraction Procedure. Two hundred and fifty grams of sugar in an extraction thimble (Macherey-Nagel, Germany) were extracted with 500 mL of dichloromethane in a Soxhlet extractor for 8 h. The resulting extractive solution was evaporated to ∼1 mL using a rotary evaporator (Laborota 4003 control, Heidolph, Schwabach, Germany) without vacuum at 35 °C to minimize the loss of volatile compounds. The solvent was then further removed under a purified nitrogen stream until the volume was reduced to 0.2 mL. A blank of extraction (without brown sugar) was also realized. The extraction of brown sugars led to extracts **1**−**7**, obtained respectively from sugars **1**−**7**. All extractions were triplicated.

Spectroscopic Instrumentation. Assays were conducted on 96-well microtiter plates (Nunc, Germany), and the absorbance was measured on 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.

GC-MS Analysis. The volatile compounds of the sugar extracts were analyzed on a gas chromatograph (HP 6890 series; Hewlett-Packard, Wilmington, DE) equipped with a mass selective detector (HP 5972; Hewlett-Packard) for qualitative and semiquantitative analysis. Volatiles were separated with two columns, one polar [Supelcowax 10, 60 m × 0.32 mm i.d., 0.25 μm film thickness (Supelco, Bellefonte, PA)] and one nonpolar [SPB5, 60 m × 0.32 mm i.d., 0.25 μm film thickness (Supelco)]. The interface GC-MSD was at 180 °C. The carrier gas was helium at 0.8 mL/min. Injector temperature was 250°C. The injector port was in the splitless mode. One microliter of extract was injected with a solvent delay of 8 min. The temperature program with the polar column was as follows: initial temperature, 60 °C; raised from 60 to 180 °C at 4 °C/min; raised from 180 to 250 °C at 2 °C/min; and held for 60 min at 250 °C. The temperature program with the nonpolar column was as follows: initial temperature, 60 °C; raised from 60 to 220 °C at 4 °C/min; and held for 60 min at 220 °C. Spectra were produced in the electron impact (EI) mode at 70 eV. The range of fragments (*m*/*z*) detected was from 25 to 350 uma between 0 and 30 min, then from 25 to 500 uma until the end of the analysis. The initial identification of compounds was accomplished by matching spectra using the NIST02 library. Confirmation and identification were made by comparison of sample retention index with those of authentic standards and those reviewed by Jennings and Shibamoto and by Kondjoyan and Berdagué (*20*, *21*). Semiquantification was applied to the molecules identified with vanillin as external standard assuming that all response factors were of the same order.

Total Polyphenol Content. Estimation of the total polyphenol content of the brown sugars was performed according to the Folin−Ciocalteu method (*22*). Thirty microliters of sample and 150 μL of Folin−Ciocalteu reagent diluted 10 times with water were first pipetted in each well of a 96-well plate. Between 1 and 8 min, 120 μL of 7.5% in water Na_2CO_3 must be added. The plate was placed in the reader and incubated for 1 h at 30 °C, and the absorbance at 765 nm was measured. A blank measure, for which sample was replaced by appropriate solvent, 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. The results were expressed in milligrams of gallic acid equivalent per kilogram of sample (GAE/kg of sample).

For the study of brown sugars, the procedure was slightly modified. Aqueous solutions of brown sugars at 40% (w/w) were tested. The blank was carried out with sucrose at the same concentrations as the brown sugars, and the gallic acid standard solutions were prepared in the same sucrose concentration, that is, 40% (w/w). All assays were conducted in triplicate.

LC-MS Analysis. The polyphenol analysis was performed according to the method of Sakakibara (*23*) 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. For the column, a LiChrospher 100 RP-18 (250 × 4.6 mm i.d., S-5, 5 μm) (Merck, Darmstadt, Germany), joined with a guard column LichroCART 4-4 (Merck), was used at 35 °C. Gradient elution was performed with solution A, composed of 93% water at 0.1% acetic acid (pH 3.3) and 7% methanol, and solution B, comprising 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 injection volume for the extract was 10 μL.

For the polyphenol analysis, a library including 65 phenolic acids, catechins, flavonoids, and simple polyphenols was first made. The library was composed of HPLC retention times and UV-DAD spectra of aglycons, and a calibration table was constructed for each compound. The mass spectrum of each reference compound was also recorded and used to confirm identification. The internal standard used was 4-formylbenzoic acid methyl ester (360 μmol). The sugar extracts were analyzed using the same HPLC system. The polyphenols were identified on the basis of their retention times, UV-DAD spectra, and electrospray mass spectra and quantified according to the calibration table.

DPPH Radical Cation Decolorization Assay. The DPPH• assay was used to measure the free radical scavenging capacity of the brown sugars and their extracts. Used as reagent, DPPH• obviously offers a convenient and accurate method for titrating the oxidizable groups of natural or synthetic antioxidants (*24*, *25*). 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, or solvent for the blank. The mixture was incubated at 30 °C for 1 h, and the absorbance at 515 nm was measured with a microplate 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 515 nm.

All assays were conducted in triplicate.

ABTS Radical Cation Decolorization Assay. The $ABTS^{\bullet+}$ was generated through a chemical oxidation reaction with potassium persulfate as described by Re et al. (*26*). The concentration of the blue-

Table 1. Molecules Identified in the Sugar Extracts by GC-MS

compound	Rip[a]	Rinp[b]	sugar **1**[c]	sugar **2**[c]	sugar **3**[c]	sugar **4**[c]	sugar **5**[c]	sugar **6**[c]	sugar **7**[c]
1-methyl-2-pyrrolidinone	1680		+	+	+	+	+	+	+
1-octanol	1548		+	+	+		t		
2,3-butanediol	1569		t	+	+	t	+	+	++
2,3-dihydro-3,5-dihydroxy-6-methylpyran-4-one[d]		1146	+++	++	t		++	+++	
2,3-dihydrothiophene[d]	2333							++	+
2,4-decadienal	1815							+	
2,5-dihydrothiophene[d]	2386							+	
2-acetylpyrrole	1973				++			+	
2-butyltetrahydrofuran[d]	2635			t	++	t	t		
2-furancarboxylic acid		1065	++		++		+	++	
2-furanmethanol	1656		++						
2-hydroxybenzaldehyde	1692								+
3-hydroxy-4-methoxybenzoic acid		1589	t	t				t	
4′-hydroxyacetophenone	>2800				+			++	+
4-hydroxybenzaldehyde	>2800		++	+	++	++	+	++	++
4-vinylguaiacol		1319	++		t			t	
5-(hydroxymethyl)dihydro-2(3H)-furanone	2478	1198					+++		
5-(hydroxymethyl)-2(5*H*)-furanone		1186			+				
(5-methylpyrazin-2-yl)methanol[d]	2068	1143			++	t	+++		
5-hydroxymethylfurfural	2492				++			++	
5-methyl-2-furanmethanol[d]	1718		t						t
α-toluic acid		1252	++	+	++			++	
acetosyringone	>2800							++	
acetovanillone	2632	1495	t	t	++	+		+++	+
benzaldehyde	1532								+
benzoic acid		1168	+++	++	++	+		+++	++
benzophenone	2483	1640	++	++	++	++	++	++	++
benzyl alcohol	1875	1036	+	+	+	++	+	+	+
cinnamaldehyde	2052								++
cinnamyl acetate	2155								+
dihydro-4-hydroxyfuran-2(3*H*)-one[d]	2591	1155	++	+	++	t	+++	t	
dimethyl sulfoxide	1573	826	+	+	+++	+	+	++	++
dimethyl sulfone	1908				+			+	
dodecanoic acid	2500	1550	+++	+++	t	+++	+++		+++
furfural	1467		t		t				
hydroquinone		1285						++	
nonanal		1102	t	++	++				
octanoic acid	2085				++		t		
pantolactone	2039	1036			++			++	
syringaldehyde	>2800	1664	++	++	++	t	t	++	++
vanillic acid		1570	++	++	+++		++	+++	++
vanillin	2566	1406	++	++	+++	++	++	+++	++
γ-butyrolactone	1640		+		+			+	

[a] Retention index of the compounds identified on polar column. [b] Retention index of the compounds identified on apolar column. [c] t, traces; +, 1–10 μg/kg of sugar; ++, 10–100 μg/kg of sugar; +++, 100–500 μg/kg of sugar. [d] Tentatively identified.

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 the absorbance at 734 nm was measured with a microplate 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 734 nm.

The radical stock solution was freshly prepared, and all analyses were made in triplicate.

Statistical Analysis. Data are reported as mean ± standard deviation (SD) of triplicate determination. The statistical analysis was carried out using the Microsoft Excel software package (Microsoft Corp.) and *XL*STAT add-on (Addinsoft). The Pearson correlation coefficient was calculated at a confidence level of 95% ($p < 0.05$).

RESULTS AND DISCUSSION

The sugars were extracted according to a solid−liquid extraction technique, using a Soxhlet extractor with dichloromethane. Sucrose is insoluble in dichloromethane, so it was chosen as solvent to extract volatile and nonvolatile molecules coated on the sugar crystal. Continuous extraction (8 h) was adopted to optimize the recovery of compounds.

Chemical Composition. *Volatile Compound Fraction.* Forty-three compounds were identified in the sugar extracts by GC-MS (**Table 1**). The richest sugar was **3**, for which 30 components were reported, whereas 14 molecules were detected for the extract of sugar **4**. Eight constituents were present in all of the extracts: 1-methyl-2-pyrrolidinone, 2,3-butanediol, 4-hydroxybenzaldehyde, benzophenone, benzyl alcohol, dimethyl sulfoxide, syringaldehyde, and vanillin. Acetovanillone, benzoic acid, dihydro-4-hydroxyfuran-2(3*H*)-one, dodecanoic acid, and vanillic acid were identified in six different sugar extracts.

The volatile compounds reported in the sugar products came mainly from two sources, the sugar cane plant and the sugar manufacture process and storage. The degradation of metabolites from the cane plant gave rise to phenols and phenolic acids that was quantitatively considerable in the volatile composition of sugars. Vanillic acid, vanillin, benzoic acid, and syringaldehyde were among the most abundant constituents reported in the extracts. Octanoic and dodecanoic acids were also found in the extracts, resulting probably from microbial activity during storage. The main source of volatile compounds in sugar

Table 2. Total Polyphenol Content of the Brown Sugars

sugar	GAE[a]	sugar	GAE[a]
1	197.5 ± 0.4	5	343.9 ± 7.4
2	108.1 ± 2.5	6	418.1 ± 2.7
3	259.6 ± 3.5	7	198.0 ± 4.0
4	117.4 ± 1.0		

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

manufacture products is the acid or thermal degradation of carbohydrates via caramelization reactions or Maillard reactions during the process (*27*, *28*). Most of the molecules identified in the extracts result from these reactions, for instance, furans, furanones, 2-furancarboxaldehyde (furfural), 2-acetylpyrrole, thiophenes, or 5-(hydroxymethyl)furfural. Yanagimoto showed that pyrroles, furans, and thiophenes exhibited antioxidant activity by inhibiting hexanal oxidation at a concentration range of 50−500 μg/mL (*18*). The volatile composition of sugars showed that potent antioxidants were present in the sugar extracts, that is, phenols and Maillard reaction products.

Polyphenolic Fraction: Total Polyphenol Content. The brown sugars were investigated for their content in polyphenols according to the Folin−Ciocalteu method. Sugars such as glucose, fructose, or sucrose do not react appreciably at room temperature with Folin reagent on a molar basis, but they may interfere with the result of the test by enhancing the development of the blue color. This interference increases with a rise in temperature (*22*). To compensate this effect, the test was carried out at 30 °C; the blank incorporated sucrose at the same concentrations as the brown sugars, and the gallic acid standard solutions were prepared in the same sucrose concentration, that is, 40% (w/w). Sucrose enhanced the absorbance at 765 nm of gallic acid by 9.70% at 50 mg/kg and by 6.75% at 300 mg/kg.

The total phenolic content of the brown sugars ranged from 108.1 to 418.1 mg of GAE/kg of sample (**Table 2**). Sugar **6** contained ∼4 times more polyphenols than sugars **2** and **4** and ∼2 times more than sugar **7**, although they were all common brown sugars. The influence of both the different raw materials and the different manufacturing processes could explain those variations. Sugar **5**, a highly colored brown sugar, contained 343.9 mg of GAE/kg total phenols. That relatively high value was not surprising as phenolic compounds are strongly involved in the color formation of sugar products (*2*, *10*).

Polyphenolic Fraction: Identification of Polyphenolic Compounds. Polyphenol contents of the extracts were assessed more accurately by LC-MS according to the method of Sakakibara (*23*) (**Table 3**). Sixty-five phenolic acids, catechins, flavonoids, and phenols already identified in sugar cane and sugar products were searched for in the extracts. (+)-Catechin and (−)-epicatechin were not found in the extracts. No flavonoids (quercetin, apigenin, rutin, luteolin, flavone, 3-hydroxyflavone, kaempferol) were present. Mainly phenolic acids were detected in the sugar extracts. Vanillin, two cinnamic acid derivatives, and four benzoic acid derivatives were found in all of the extracts. Sugar **6** also contained homovanillic acid at 141.1 μg/kg and acetosyringone at 83.9 μg/kg. Coniferyl alcohol was present in sugar **1** at 23.5 μg/kg. Syringic acid was the polyphenol with the greatest concentration for all brown sugars except for sugar **4**, for which it was vanillin.

The differences in polyphenol concentration between brown sugars were greater for LC-MS than with the Folin−Ciocalteu method. In addition, the values obtained in LC-MS were much lower. The Folin−Ciocalteu assay gives a measure of all the polyphenols present in brown sugars, whereas 65 polyphenols were targeted in LC-MS. Moreover, the Folin−Ciocalteu assay is not specific for polyphenols; the assay, in fact, measures all compounds readily oxidizable under the test condition, and nonphenolic compounds such as 2-furanmethanol, furfural, or 5-(hydroxymethyl)furfural react with the reagent (*10*, *22*). The values obtained with the Folin−Ciocalteu assay were certainly overestimated. Besides, only the molecules on the surface of the sugar crystal were extracted. During the crystallization of sugar, molecules originally present on the crystal surface can migrate toward the inside because of vibrations and the presence of pores or fissures on the crystal surface (*29*, *30*). The total phenolic content of these molecules was measured by Folin−Ciocalteu assay because they were released in solution; however, according to the nondestructive method employed, they were not quantified by LC-MS in the extracts.

Free Radical Scavenging Assays. *DPPH Assay.* Antioxidant activity tests were performed on brown sugars and their dichloromethane extracts (extracts **1**−**7**). A concentration response curve of Trolox to DPPH assay was obtained (final concentrations ranging between 5 and 30 μM, r^2 = 0.9761). Trolox at 10 μM inhibited DPPH oxidation by 32.5% and Trolox at 30 μM, by 86.56%. Aqueous solutions of brown sugar exhibited weak free radical scavenging capacity to DPPH assay (**Table 4**). Sugar **6** inhibited DPPH oxidation by only 26.9% at 10 g of sugar/kg of solution, equivalent with 8.16 μM Trolox, whereas sucrose at the same concentration had no activity. Increasing the concentration of sugar from 10 to 45 g/kg of solution did not increase significantly the inhibition of DPPH oxidation by the brown sugars. The very low content of phenolic compounds in the brown sugars (400−4000 μg per kg) (**Table 3**) may explain the weak activity of the aqueous solution of brown sugar against the DPPH radical.

Sugar extracts showed higher activity, especially for sugar **1** (extract **1**) and **6** (extract **6**), which inhibited, respectively, DPPH oxidation by 69.3 and 60.2%, corresponding to 22.6 and 19.5

Table 3. Contents of Polyphenolic Constituents by LC-MS of Sugar Extracts[a]

compound	RT[b] (min)	1	2	3	4	5	6	7
p-hydroxybenzoic acid	12.61	41.6	7.2	96.5	1.9	13.2	240.9	37.7
vanillic acid	15.83	451.3	120.5	427.3	29.4	118.3	750.4	201.1
homovanillic acid	16.42						141.1	
syringic acid	18.11	833.7	255.7	892.5	71.3	241.2	1610.1	450.9
vanillin	19.40	194.5	218.8	290.9	214.4	203.0	529.8	219.5
coniferyl alcohol	20.89	23.5						
p-coumaric acid	22.57	103.3	18.5	104.9	7.8	46.1	332.0	285.6
acetosyringone	25.45						83.9	
ferulic acid	26.04	160.6	73.6	77.2	8.6	37.0	117.5	137.1
benzoic acid	28.53	134.6	90.7	111.7	62.4	98.8	221.2	106.0
total		1943.1	785.1	2001.1	395.7	757.6	4026.8	1437.8

[a] Expressed in micrograms per kilogram of sugar, means (n = 3). Standard deviation was always <10%. [b] Retention time of compounds expressed in minutes.

Table 4. DPPH Antioxidant Activity of Brown Sugars and Their Extracts

sugar[a]	% inhibition[b]	extract[c]	% inhibition[d]
1	22.1 ± 3.5	**1**	69.3 ± 3.5
2	14.5 ± 4.0	**2**	41.1 ± 3.1
3	22.1 ± 1.6	**3**	39.9 ± 4.6
4	15.8 ± 3.3	**4**	22.4 ± 4.7
5	22.5 ± 4.6	**5**	45.1 ± 2.6
6	26.9 ± 2.2	**6**	60.2 ± 3.2
7	16.2 ± 1.8	**7**	48.3 ± 5.7
sucrose	–0.5 ± 1.4	blank	–6.6 ± 1.4

[a] Aqueous solution of brown sugar, final concentration = 10 g of sugar/kg of solution. [b] Means ± SD ($n = 3$). [c] Soxhlet extract of brown sugar. [d] Means ± SD ($n = 9$).

μM Trolox. Extract **4** had weak free radical scavenging activity, and the extracts of sugars **2**, **3**, **5**, and **7** showed moderate activity.

ABTS Assay. A concentration response curve of Trolox to ABTS assay was obtained (final concentrations ranging between 2 and 20 μM, $r^2 = 0.9987$); Trolox at 2 μM inhibited ABTS oxidation by 9.07% and Trolox at 20 μM, by 87.58%. **Table 5** presents the free radical scavenging response to ABTS of aqueous solutions of brown sugars and their dichloromethane extracts (extracts **1**−**7**). Sucrose had no activity at all tested concentrations. Sugar **6** inhibited ABTS oxidation by 48.0% at 5 g of sugar/kg of solution and by up to 88.5% at 45 g of sugar/kg of solution, respectively equivalent to 10.7 and 20.1 μM Trolox. Sugar **4** was the weakest at all concentrations, inhibiting ABTS oxidation by 53.8% at 45 g of sugar/kg of solution. At 45 g of sugar/kg of solution, all of the brown sugars showed interesting activity. The extracts had moderate free radical scavenging activity toward the ABTS assay; sugars **1** and **6** led to the most active extracts as for DPPH assay.

Correlation between Brown Sugar Chemical Composition and Free Radical Scavenging Assays. The correlation coefficients between the total phenolic content of the sugars measured by the Folin−Ciocalteu assay, the total concentration of the compounds identified by LC-MS in the extracts, and DPPH and ABTS activities of the brown sugars were calculated (**Table 6**). The two determination methods of free radical scavenging activity, ABTS and DPPH assays, were significantly correlated concerning the brown sugar extracts (0.864; $p < 0.05$). The results were also significantly correlated for the aqueous solutions of brown sugar (10 g/kg of solution) (0.815; $p < 0.05$).

Total polyphenol contents of the sugars obtained by Folin assay were correlated at 0.713 (significant at $p < 0.08$) with the total polyphenols by LC-MS of the extracts, which was interesting when the interferences with the Folin method were taken into account.

The correlation between total polyphenols and antioxidant activity are in general good but depend on the nature of the sample and on the implication of other compounds in the antioxidant activity (*31*). In this study, total polyphenols and antioxidant activity were not significantly correlated. However, total polyphenols by LC-MS were more correlated with antioxidant tests (ABTS, 0.718; DPPH, 0.655) than Folin total polyphenols (ABTS, 0.614; DPPH, 0.462). Those correlation coefficients also pointed out the probable involvement of the phenolic compounds in the antioxidant activity of the brown sugars and also showed that other compounds certainly contribute to the free radical scavenging activity. Many nitrogenous and oxygenated heterocyclic compounds were found in the extracts; thus, Maillard reaction products formed during the sugar process (*2*, *27*, *28*), which present antioxidant properties (*14*−*18*), might contribute to the measured activity of the brown sugar dichloromethane extracts.

Sugar **6** was the brown sugar that had the richest volatile and polyphenol contents; it had also the greatest free radical scavenging capacity. Then, except for the latter, special brown sugars that claim aromatic and sensorial properties in terms of aroma, flavor, and taste (sugars **1** and **3** are extracted from cane juice; sugar **5** was highly colored) presented greater volatile and polyphenol compound concentration, and therefore their free radical scavenging properties were higher. Differences observed for all of the sugars may be explained by the fact that the polyphenolic compounds arise in the cane juice and may be modified by processing procedures. Different cane varieties, the maturity of the cane plant at harvest time, and the amount of leaves included in the cane when it is crushed can influence the constituents in the cane juice. Moreover, color formation and, therefore, its removal are important problems in sugar manufacture (*2*, *32*). The different techniques used in cane processing to remove color and impurities affect the amount of volatiles and polyphenolics that end up on the surface of the crystal. Finally, the volatiles and polyphenolics remaining on the surface syrup coating can negatively or positively affect the sensory quality of the sugar, the polyphenolics increasing the antioxidant activity. Sucrose is traditionally used at 8−12% in food as a sweetening agent, and the fact that brown sugars presented free radical scavenging activity at relatively high concentration is rather encouraging. Those antioxidant properties of brown sugars seemed to be related to polyphenol components native to the cane plant. Maillard reaction products formed during the process might also contribute to the antioxidant activity. The results may be significant for the consideration of brown sugars as food and in food applications; indeed, sugars have many nutritional and gustative properties that might be accompanied with antioxidant properties. The study of the phenolic content, the aromatic composition, and relative free

Table 5. ABTS Antioxidant Activity of Brown Sugars and Their Extracts

	% inhibition at final concn[a] of					
sugar[b]	1 g/kg[c]	5 g/kg[c]	10 g/kg[c]	45 g/kg[c]	extract[d]	% inhibition[e]
1	13.1 ± 1.6	31.4 ± 3.2	47.1 ± 2.4	60.5 ± 3.2	**1**	44.3 ± 3.0
2	5.4 ± 1.2	21.9 ± 0.9	36.3 ± 2.9	61.4 ± 2.4	**2**	31.2 ± 2.0
3	9.8 ± 1.2	33.7 ± 1.9	51.3 ± 5.5	86.2 ± 0.6	**3**	26.1 ± 3.6
4	4.9 ± 0.9	21.5 ± 0.7	34.5 ± 4.4	53.8 ± 5.5	**4**	25.6 ± 3.7
5	11.0 ± 1.0	35.9 ± 2.3	47.7 ± 3.6	73.4 ± 2.2	**5**	35.8 ± 4.9
6	15.4 ± 1.3	48.0 ± 3.1	54.4 ± 1.7	88.5 ± 1.0	**6**	48.4 ± 4.7
7	6.6 ± 0.7	28.2 ± 1.9	48.7 ± 5.1	72.2 ± 2.7	**7**	30.2 ± 4.7
sucrose	1.0 ± 0.5	0.97 ± 0.5	2.10 ± 0.9	3.1 ± 1.4	blank	1.5 ± 3.0

[a] Means ± SD ($n = 3$). [b] Aqueous solution of brown sugar. [c] Final concentration in grams of sugar per kilogram of solution. [d] Soxhlet extract of brown sugar. [e] Means ± SD ($n = 9$).

Table 6. Correlation Coefficient between Antioxidant Tests and Total Phenolic Contents

method	Folin[a]	LC-MS[b]	ABTS[c]	DPPH[c]
Folin[a]	1			
LC-MS[b]	0.713	1		
ABTS[c]	0.614	0.718	1	
DPPH[c]	0.462	0.655	0.864[d]	1

[a] Total phenolic content of the sugars. [b] Sum of all compounds identified by LC-MS in the extracts. [c] Antioxidant activity of the extracts. [d] Correlation coefficient significant at $p < 0.05$.

radical scavenging properties of cane sugar manufacturing products (juice, syrup, different sugars, and molasses) is in order.

ABBREVIATIONS USED

ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; 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; LC-MS, liquid chromatography coupled to a mass spectrometer.

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