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Antidiabetes and Antihypertension Potential of Commonly Consumed Carbohydrate Sweeteners Using *In Vitro* Models

Lena Galvez Ranilla,[1] Young-In Kwon,[2] Maria Ines Genovese,[1] Franco Maria Lajolo,[1] and Kalidas Shetty[2]

[1]*Laboratório de Química, Bioquímica e Biologia Molecular de Alimentos, Departamento de Alimentos e Nutrição Experimental, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, São Paulo, Brazil; and* [2]*Department of Food Science, University of Massachusetts, Amherst, Massachusetts*

ABSTRACT Commonly consumed carbohydrate sweeteners derived from sugar cane, palm, and corn (syrups) were investigated to determine their potential to inhibit key enzymes relevant to Type 2 diabetes and hypertension based on the total phenolic content and antioxidant activity using *in vitro* models. Among sugar cane derivatives, brown sugars showed higher antidiabetes potential than white sugars; nevertheless, no angiotensin I-converting enzyme (ACE) inhibition was detected in both sugar classes. Brown sugar from Peru and Mauritius (dark muscovado) had the highest total phenolic content and 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity, which correlated with a moderate inhibition of yeast α-glucosidase without showing a significant effect on porcine pancreatic α-amylase activity. In addition, chlorogenic acid quantified by high-performance liquid chromatography was detected in these sugars (128 ± 6 and 144 ± 2 μg/g of sample weight, respectively). Date sugar exhibited high α-glucosidase, α-amylase, and ACE inhibitory activities that correlated with high total phenolic content and antioxidant activity. Neither phenolic compounds or antioxidant activity was detected in corn syrups, indicating that nonphenolic factors may be involved in their significant ability to inhibit α-glucosidase, α-amylase, and ACE. This study provides a strong biochemical rationale for further *in vivo* studies and useful information to make better dietary sweetener choices for Type 2 diabetes and hypertension management.

KEY WORDS: • ***α-amylase inhibitor*** • ***angiotensin I-converting enzyme inhibitor*** • ***antioxidant activity*** • ***cane sugars*** • ***corn syrups*** • ***α-glucosidase inhibitor*** • ***hypertension*** • ***palm sugars*** • ***phenolics*** • ***Type 2 diabetes***

INTRODUCTION

POVERTY AND WEALTH have profound effects on the global dietary intake. Whereas chronic malnutrition results from poverty, economic growth gives rise to what has been called the nutrition transition.[1,2] As incomes rise and populations become more urban, diets high in complex carbohydrates and fiber give way to more energy-dense diets that are higher in refined carbohydrates and fats.[1,2] These global dietary trends are accompanied by changes in disease patterns, which move away from infectious and nutrient-deficiency disease toward higher rates of childhood obesity, coronary heart disease, and some types of cancer.[3]

Diabetes mellitus is one of the most common chronic diseases, and the predominant clinical form is Type 2 (non–insulin-dependent), which accounts for more than 90% of all cases[4,5] and in 2005 affected 19 million individuals in the United States, almost 7% of the population.[6] The postprandial phase in diabetes is characterized by a rapid increase in blood glucose levels, which may contribute to the onset of oxidation-linked cardiovascular complications through the overproduction of free radicals at the mitochondrial level.[7] Type 2 diabetes has been clearly associated with a substantial increase in the prevalence of hypertension and the accelerated development of atherosclerosis.[8]

Although the cause for Type 2 diabetes depends on several factors (genetic and environmental factors),[9] the scientific literature emphasizes a lot about the effects of specific dietary macronutrients on the risk of obesity and Type 2 diabetes. In this sense, recent reports indicate that the high consumption of refined carbohydrates, high-fructose corn syrups in beverages, and sugar-sweetened soft drinks with the concomitant decrease in intakes of fiber could be related with the prevalence of Type 2 diabetes and may play an important role in the epidemic of obesity.[10–12] Additionally, a diet high in sucrose (*i.e.*, >20% of energy) is associated with an elevation of plasma triglyceride concentrations. This increase is due to both increased hepatic secretion and

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Address reprint requests to: Kalidas Shetty, Department of Food Science, Chenoweth Laboratory, University of Massachusetts, Amherst, MA 01003, E-mail: kalidas@foodsci.umass.edu

impaired clearance of very-low-density lipoprotein. Triglyceride response to dietary sugar may, however, vary according to the amount of sugar and the presence of other nutrients.[13,14]

Replacing sucrose in processed food and beverages with alternative lower caloric and noncaloric sweeteners could be one of the long-term solutions. However, choosing the right sweetener with better health relevant functionality could be another effective option. Currently, there are wide varieties of sweeteners and sugars in the market, but research on their effect on the prevalence of Type 2 diabetes and hypertension is not available. The nutritional value of sugar (sucrose) is to provide calories; nevertheless, recent reports revealed that depending on the stage of processing, sugar from sugar cane can be an interesting source of other bioactive compounds. An important variety of polyphenolic constituents was found in different kinds of sugar products showing a significant correlation with their antioxidant activity assessed by the 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) and 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) assays.[15] The brown sugars had high free radical scavenging activity, and these antioxidant properties seemed to correlate to polyphenol components native to the cane plant.[16] Furthermore, Kwon *et al.*[17] and McCue and Shetty[18] have pointed out the potential of phenolic phytochemicals in diabetes and hypertension management through the *in vitro* inhibition of enzymes involved in carbohydrate hydrolysis and high blood pressure (α-glucosidase/α-amylase and angiotensin I-converting enzyme [ACE], respectively). This opens the possibility that sugars depending on the origin and type of processing have the potential to contribute to the management of diabetes and hypertension.

Therefore the objectives of this study were to evaluate whether commonly consumed carbohydrate sweeteners have anti-Type 2 diabetes and antihypertension functionality based on *in vitro* assays and to determine their correlation to total soluble phenolic content, phenolic profile, and free radical scavenging-linked antioxidant activities.

MATERIALS AND METHODS

Materials

Sugar cane derivatives and corn syrups were purchased from a local market in Hadley, MA. High-fructose corn syrups were provided by the Archer Daniels Midland Co. (Decatur, IL). Samples were classified in six groups (A–F) according to their origin, consumer preference, and raw material used for their processing (Table 1). Some physical characteristics such as pH, particle form, and color are included as well.

Porcine pancreatic α-amylase (EC 3.2.1.1), baker's yeast α-glucosidase (EC 3.2.1.20), and rabbit lung angiotensin-converting enzyme (EC 3.4.15.1) were purchased from Sigma Chemical Co. (St. Louis, MO). Unless noted, all chemicals also were purchased from Sigma Chemical Co.

Extract preparation

Sugar samples (1 g) were dissolved in 10 mL of distilled water. The syrups were diluted with distilled water to reach the same solid concentration (10°Brix, 25°C) of sugar extracts. The pH of each extract was adjusted to 6–8 and centrifuged at 9,300 *g* for 30 minutes. The supernatant was recovered and then used as the extract for *in vitro* assays. Extractions were performed in duplicate.

Total phenolics assay

The total phenolics were determined by the Folin-Ciocalteu method modified by Shetty *et al.*[19] Briefly, 1 mL of the sugar extract was transferred into a test tube and mixed with 1 mL of 95% ethanol and 5 mL of distilled water. To each sample 0.5 mL of 50% (vol/vol) Folin-Ciocalteu reagent was added and mixed. After 5 minutes, 1 mL of 5% Na_2CO_3 was added to the reaction mixture and allowed to stand for 60 minutes. The absorbance was read at 725 nm. The standard curve was established using various concentrations of gallic acid in 95% ethanol, and results were expressed as micrograms of gallic acid per gram of sample weight.

Antioxidant activity by DPPH inhibition assay

The DPPH scavenging activity was determined by an assay modified by Kwon *et al.*[17] To 1.25 mL of 60 μM DPPH in 95% ethanol, 250 μL of each sugar extract was added, and the decrease in absorbance was monitored after 1 minute at 517 nm. The percentage of inhibition was calculated by:

$$\% \text{ Inhibition} = \frac{A_{517}(\text{control}) - A_{517}(\text{extract})}{A_{517}(\text{control})} \times 100$$

α-Amylase inhibition assay

The α-amylase inhibitory activity was determined by an assay modified from the *Worthington Enzyme Manual*.[20] A total of 500 μL of each sugar extract and 500 μL of 0.02 *M* sodium phosphate buffer (pH 6.9 with 0.006 *M* NaCl) containing α-amylase solution (0.5 mg/mL) were incubated at 25°C for 10 minutes. After preincubation, 500 μL of a 1% starch solution in 0.02 *M* sodium phosphate buffer (pH 6.9 with 0.006 *M* NaCl) was added to each tube at timed intervals. The reaction mixtures were then incubated at 25°C for 10 minutes. The reaction was stopped with 1.0 mL of dinitrosalicylic acid color reagent. The test tubes were then incubated in a boiling water bath for 5 minutes and cooled to room temperature. The reaction mixture was then diluted after adding 15 mL of distilled water, and absorbance was measured at 540 nm. Sample blanks (buffer instead of enzyme solution) and a control (buffer in place of sample extract) were included as well. The α-glucosidase inhibitory activity was calculated according to the equation below:

$$\% \text{ Inhibition} = \frac{A_{540}(\text{control}) - A_{540}(\text{extract})}{A_{540}(\text{control})} \times 100$$

TABLE 1. CARBOHYDRATE SWEETENER CHARACTERISTICS

Group, code	*Product*	*Commercial name*[a]	*Origin*	*Source*	*Particle form/physical state*	*Color*	*pH at 25°C*[b]
A							
A1	Confectioners sugar, 10-x powdered, pure cane	Domino Sugar	USA	Sugar cane	Pulverized	White	6.17 ± 0.02
A2	Sugar, pure cane	Domino Sugar	USA	Sugar cane	Granulated	White	6.45 ± 0.02
A3	Superfine sugar, pure cane	Domino Sugar	USA	Sugar cane	Granulated	White	6.58 ± 0.05
A4	Light brown sugar, pure cane	Domino Sugar	USA	Sugar cane	Granulated	Light brown	5.91 ± 0.01
A5	Dark brown sugar, pure cane	Domino Sugar	USA	Sugar cane	Granulated	Dark brown	5.78 ± 0.02
B							
B1	Corn syrup	Aunt Jemima	USA	Corn	Viscous liquid	Dark brown	5.32 ± 0.01
B2	Corn syrup with 2% real maple syrup	Big Y	USA	Corn and maple	Viscous liquid	Dark brown	5.31 ± 0.03
B3	Corn syrup with 4% real maple syrup	Stop & Shop	USA	Corn and maple	Viscous liquid	Dark brown	5.17 ± 0.01
B4	Pure maple syrup grade A amber	Butternut Mountain Farm	USA	Maple sap	Viscous liquid	Dark amber	6.71 ± 0.01
B5	Light corn syrup + high-fructose corn syrup	Karo	USA	Corn	Viscous liquid	Clear	5.75 ± 0.01
B6	High-fructose corn syrup 42% fructose	ADM	USA	Corn	Viscous liquid	Clear	4.67 ± 0.02
B7	High-fructose corn syrup 55% fructose	ADM	USA	Corn	Viscous liquid	Clear	5.55 ± 0.01
B8	High-fructose corn syrup 90% fructose	ADM	USA	Corn	Viscous liquid	Clear	6.42 ± 0.01
C							
C1	Natural cane sugar, less processed	Florida Crystals	USA	Sugar cane	Granulated	White	6.42 ± 0.09
C2	Organic evaporated cane juice sugar	Whole Foods Market, 365 organic	South America	Sugar cane	Granulated	White	6.74 ± 0.05
C3	Sugar in the row, turbinado sugar Hawaii	Sugar in the raw	USA	Sugar cane	Granulated	Light brown	6.16 ± 0.07
C4	Evaporated cane juice turbinado raw sugar	Whole Foods market, 365 organic	Africa	Sugar cane	Granulated	Brown	6.31 ± 0.02
C5	Natural brown sugar	Whole Foods market, 365 organic	USA	Sugar cane	Granulated	Dark brown	6.16 ± 0.03
D							
D1	Evaporated cane juice organic sugar	Wholesome sweeteners	Paraguay	Sugar cane	Granulated	White	6.78 ± 0.06
D2	Organic turbinado raw cane sugar	Wholesome sweeteners	Brazil	Sugar cane	Granulated	Brown	6.93 ± 0.02
D3	Brown sugar	Ramela	Guyana	Sugar cane	Granulated	Brown	6.84 ± 0.01
D4	Brown sugar	La fe	Colombia	Sugar cane	Granulated	Brown	8.87 ± 0.09
D5	Dehydrated cane juice organic sucanat	Wholesome sweeteners	Costa Rica	Sugar cane	Granulated	Brown	6.4 ± 0.01
D6	Brown sugar	Cooperativa Oro Verde	Peru	Sugar cane	Granulated	Dark brown	5.86 ± 0.01
E							
E1	Caster sugar for baking (pure cane sugar)	Tate Lyle	England/Africa	Sugar cane	Granulated	White	6.72 ± 0.04
E2	Fondant and icing powdered cane sugar		India	Sugar cane	Pulverized	White	6.08 ± 0.08
E3	Raw cane sugar	Wholesome sweeteners	Malawi	Sugar cane	Granulated	Brown	6.34 ± 0.04
E4	Light muscovado		Mauritius	Sugar cane	Granulated	Brown	5.92 ± 0.01
E5	Dark muscovado		Mauritius	Sugar cane	Granulated	Dark brown	6.11 ± 0.01
F							
F1	Mishri cut sugar	Utsav	India	Sugar cane	Large particles	White	6.68 ± 0.05
F2	Rock candy (yellow lump)	Rock candy	China	Sugar cane	Solid	Yellow-brown	6.56 ± 0.04
F3	Palm sugar	R brand	Thailand	Palm sap	Solid	Light brown	5.97 ± 0.03
F4	Indian palm jaggery	Jaggery	India	Palm sap	Solid	Dark brown	8.42 ± 0.03
F5	Coconut sugar	Gula jawa	Indonesia	Palm sap	Solid	Brown	5.02 ± 0.04
F6	Sugar jaggery (Gur)	Swad	India	Sugar cane	Solid	Beige	6.54 ± 0.04
F7	Granulated date sugar	Chatfield's	USA	Palm date	Powder	Brown	5.55 ± 0.04

Group A, common consumed sugars in the United States; Group B, common consumed syrups in the United States; Group C, common consumed sugars in the United States (less processed and organic options); Group D, organic and less processed sugars from Central and South America; Group E, African sugars, Group F, special sugars (other sources and forms).

[a]Purchased at Whole Foods, Big Y, and Stop & Shop supermarkets (Hadley, MA). High-fructose corn syrups (42%, 55%, and 90%) were provided by the Archer Daniels Midland Co. (ADM) (Decatur, IL), and the Peruvian brown sugar was provided by the Oro Verde Cooperativa (Lamas, Peru).

[b]Aqueous sugar extracts (1 g/10 mL of distilled water). Syrups were diluted until 10°Brix at 25°C (the same for sugar extracts).

α-Glucosidase inhibition assay

A modified version of the assay described by the *Worthington Enzyme Manual*[21] was followed.[22] A volume of 50 μL of sugar extract diluted with 50 μL of 0.1 *M* potassium phosphate buffer (pH 6.9) and 100 μL of 0.1 *M* potassium phosphate buffer (pH 6.9) containing α-glucosidase solution (1.0 U/mL) was incubated in 96-well plates at 25°C for 10 minutes. After preincubation, 50 μL of 5 m*M* *p*-nitrophenyl-α-D-glucopyranoside solution in 0.1 *M* potassium phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25°C for 5 minutes. Before and after incubation, absorbance readings were recorded at 405 nm by a microplate reader (Thermomax, Molecular Devices Corp., Sunnyvale, CA) and compared to a control that had 50 μL of buffer solution in place of the extract. The α-glucosidase inhibitory activity was expressed as a percentage of inhibition and was calculated as follows:

$$\% \text{ Inhibition} = \frac{\Delta A_{402}(\text{control}) - \Delta A_{405}(\text{extract})}{A_{405}(\text{control})} \times 100$$

ACE inhibition assay

ACE inhibition was performed by a method modified by Kwon *et al.*[17] A volume of 50 μL of sugar extracts was incubated with 200 μL of 0.1 *M* NaCl-borate buffer (0.3 *M* NaCl, pH 8.3) containing 2 mU of ACE solution at 25°C for 10 minutes. After preincubation, 100 μL of a 5.0 m*M* substrate (hippuryl-histidyl-leucine) solution was added to

the reaction mixture. Test solutions were incubated at 37°C for 1 hour. The reaction was stopped with 150 μL of 0.5 *N* HCl. The hippuric acid formed was detected and quantified by the high-performance liquid chromatography (HPLC) method. A volume of 5 μL of sample was injected using an Agilent Technologies (Palo Alto, CA) ALS 1100 autosampler into an Agilent 1100 series high-performance liquid chromatograph equipped with a DAD 1100 diode array detector. The solvents used for the gradient were (A) 10 m*M* phosphoric acid (pH 2.5) and (B) 100% methanol. The methanol concentration was increased to 60% for the first 8 minutes and to 100% for 5 minutes and then decreased to 0% for the next 5 minutes (total run time, 18 minutes). The analytical column used was Supelco (Bellefonte, PA) INC Nucleosil 100-5C_{18} (250 × 4.6 mm i.d.) with packing material of 5 μm particle size at a flow rate of 1 mL/minute at room temperature. During each run the chromatogram was recorded at 228 nm and integrated using an Agilent Chemstation enhanced integrator for detection of liberated hippuric acid. Pure hippuric acid was used to calibrate the standard curve and retention time. The percentage of inhibition

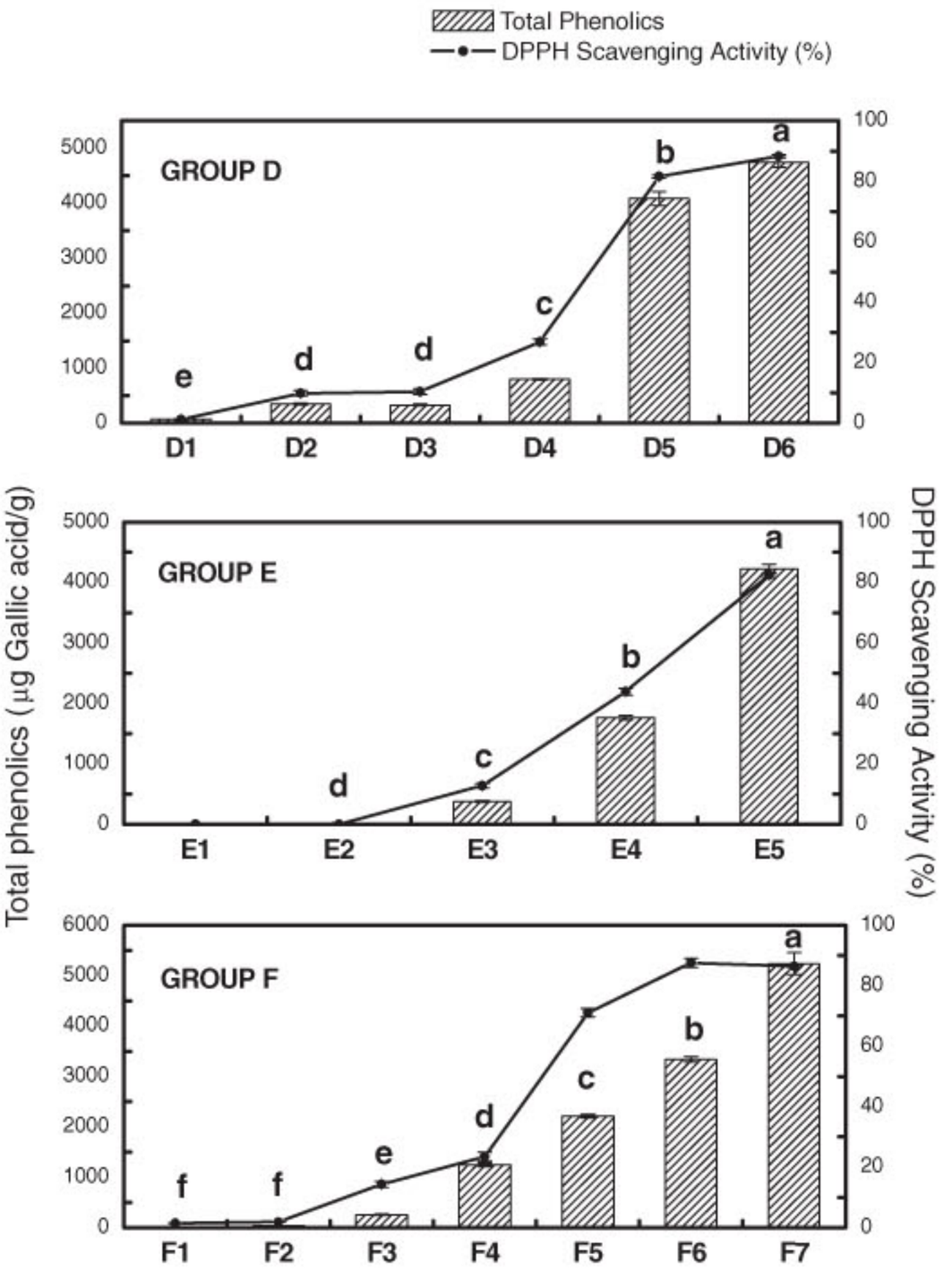

FIG. 2. Total phenolics and DPPH radical scavenging activity of sugars from groups D, E, and F. Bars with different letters are significantly different ($P < .05$).

was calculated considering the area of the hippuric acid peak according to the equation below:

$$\% \text{ Inhibition} = \frac{(Area_{\text{control}} - [Area_{\text{sample}} - Area_{\text{sample blank}}])}{(Area_{\text{control}} - Area_{\text{blank}})} \times 100$$

HPLC analysis of phenolic profiles

The sweetener extracts (2 mL) were filtered (pore size, 0.2 μm). A volume of 5 μL of sample was injected using an Agilent ALS 1100 autosampler into an Agilent 1100 series high-perfomance liquid chromatograph equipped with a DAD 1100 diode array detector. The solvents used for gradient elution were (A) 10 m*M* phosphoric acid (pH 2.5) and (B) 100% methanol. The methanol concentration was increased to 60% for the first 8 minutes and to 100% over the next 7 minutes, then decreased to 0% for the next 3 minutes, and was maintained for the next 7 minutes (total run time, 25 minutes). The analytical column used was Agilent Zorbax SB-C_{18} (250 × 4.6 mm i.d.) with packing material of 5 μm particle size at a flow rate of 1 mL/minute at room

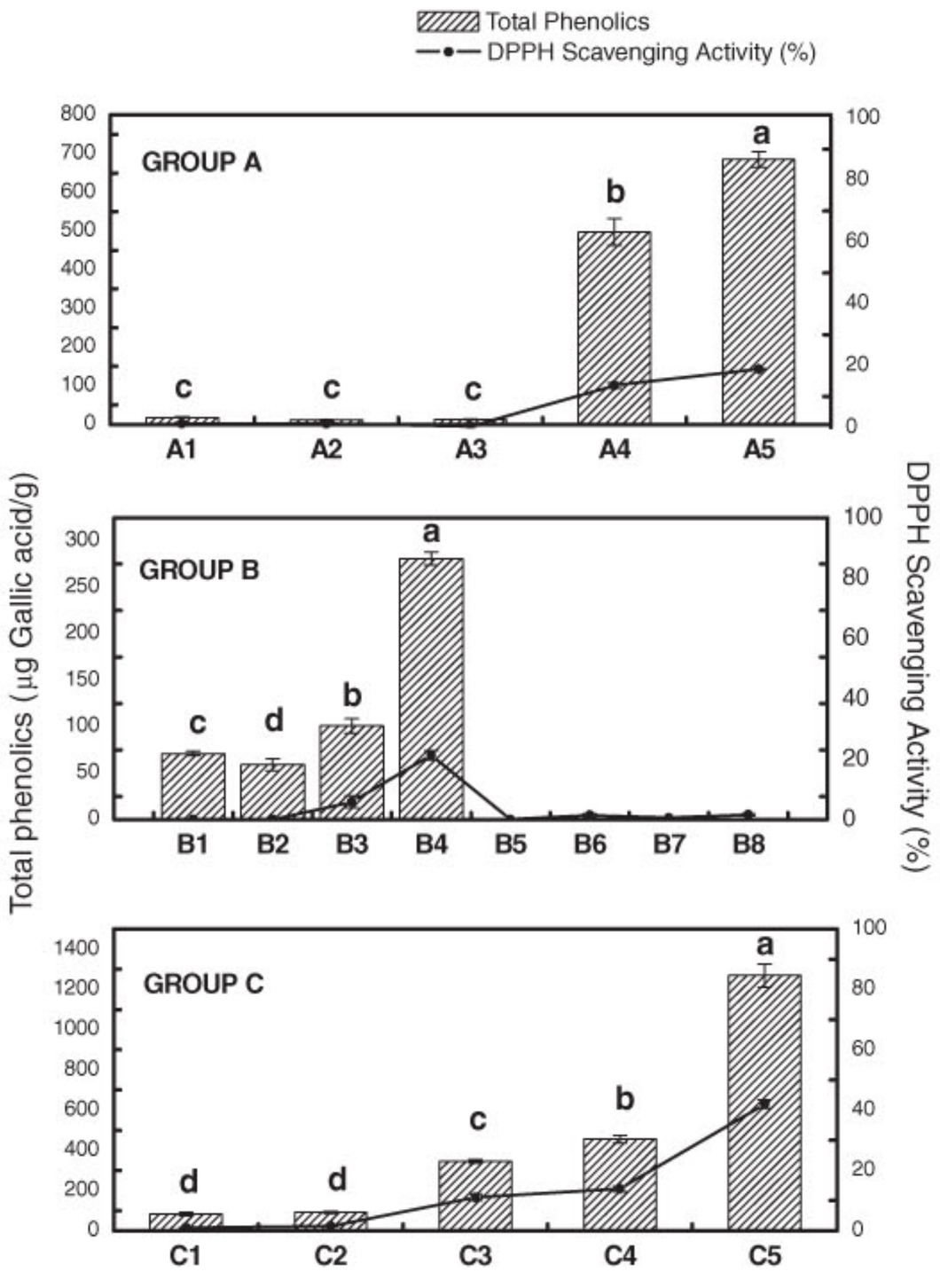

FIG. 1. Total phenolics and DPPH radical scavenging activity of sugars and syrups from groups A, B, and C. Bars with different letters are significantly different ($P < .05$).

temperature. During each run the chromatogram was recorded at 306 nm and 333 nm and integrated using an Agilent Chemstation enhanced integrator. A pure standard of chlorogenic acid in 100% methanol was used to calibrate the standard curve and retention time.

Statistical analysis

Two extractions were performed for each sample, and all *in vitro* analysis were carried out six times ($n = 12$). In the case of HPLC analysis, the experiments were performed at least in triplicates. Results were expressed as mean ± SD values. Data were subjected to analysis of variance analysis, means comparison to the Duncan test ($P < .05$), and Pearson correlations according to the Statistica software package version 5.0 (StatSoft, Tulsa, OK).

RESULTS

Total soluble phenolics and antioxidant activity

Figures 1 and 2 show the total phenolic content from sugar groups A–F related to their DPPH scavenging activity. In general, total phenolics and the antioxidant activity showed a significant correlation in all groups ($P < .05$) (Table 2).

In group A (common consumed sugars in the United States), which included sugars with the brand "Domino," the total phenolic content ranged from 18 to 684 μg of gallic acid/g, the dark brown sugar (A5) exhibited the highest total phenolic content (684 ± 20 μg/g) ($P < .05$) followed by the light brown sugar (A4) (497 ± 34 μg/g), whereas white sugars (A1–A3), independent of the particle size, had the lowest total phenolic content (<20 μg/g). The antioxidant activity based on the DPPH radical inhibition assay varied from 1% to 19% and had significant correlation with the total phenolics ($r = 0.99$).

In group B (common consumed syrups in the United States), maple syrup (B4) had the highest total phenolic content (281 ± 7 μg/g) and DPPH scavenging activity (21%), and these two functional characteristics decreased proportionally in syrups B3 and B2 according to their maple syrup content (5% and 2% real maple syrup, respectively). High-fructose corn syrups (B6–B8) with different proportions of fructose (42%, 55%, and 90%) were evaluated because these are the main ingredients in sweetener syrups commonly found in the market. High-fructose corn syrups did not contain total phenolics or show inhibitory activity against the DPPH radical. Pearson's correlation coefficient between DPPH scavenging activity and total phenolic content was 0.95 in this group.

Among sugars in Group C (common consumed sugars in the United States, less processed and organic options), the dark brown sugar (C5) was rich in total phenolics (1,268 ± 57 μg/g) and also inhibited significantly DPPH radical formation (42%). This sample had almost twofold higher inhibitory activity than Domino's brown sugar from group A. The total phenolic content and antioxidant activity had a significant correlation ($r = 0.99$) as also was observed in the first two groups.

Total phenolics and antioxidant activity from sugars belonging to Group D (organic and less processed sugars from Central and South America) showed a high correlation ($r = 0.99$), and their levels were in general higher than common sugar sources in the U.S. market. Total phenolics and DPPH inhibitory activities varied from 75 to 4,741 μg/g and from 1% to 88%, respectively. The Peruvian sugar (D6) exhibited the highest total phenolics and antioxidant activity (4,741 ± 85 μg/g and 88%, respectively), and, interestingly, it was the darkest sample among all sugars in this group.

Overall, brown sugars from Group E (African sugars) were richer in total phenolics and had higher antioxidant activity when compared with brown sugars from groups A and C. Sugar E5 (dark muscovado from Mauritius) had 4,221 ± 80 μg/g soluble phenolics and 82% antioxidant activity, which was the highest among sugars tested in this group.

TABLE 2. PEARSON CORRELATION COEFFICIENTS FOR CARBOHYDRATE SWEETENER GROUPS

	Group[b]					
Correlation[a]	A_1	B_2	C_3	D_4	E_5	F_6
TP versus DPPH	0.99*	0.95*	0.99*	0.99*	0.99*	0.93*
TP versus α–GLUC	0.50*	−0.61*	0.42*	0.79*	0.60*	0.91*
TP versus α–AMYL	0.66*	−0.84*	0.94*	0.70*		0.83*
TP versus ACE		−0.51*				0.68*
DPPH versus α-GLUC	0.49*	−0.70*	0.42*	0.77*	0.57*	0.78*
DPPH versus α–AMYL	0.67*	−0.82*	0.93*	0.66*		0.62*
DPPH versus ACE		0.41*				0.40*
α-GLUC versus α–AMYL	0.11	0.62*	0.33*	0.59*		0.94*
α-GLUC versus ACE		0.13				0.81*
α-AMYL versus ACE		0.76*				0.94*

[a]TP, total phenolics; DPPH, DPPH scavenging activity (%); α-GLUC, α-glucosidase inhibitory activity (%) (dose, 50 μL); α-AMYL, α-amylase inhibitory activity (%) (dose, 500 μL); ACE, ACE inhibitory activity (%) (dose, 50 μL).

[b]Subscripts indicate numbers of assays as follows: 1, $n = 60$; 2, $n = 60$; 3, $n = 60$; 4, $n = 72$; 5, $n = 51$; 6, $n = 62$.

*$P < .05$.

In group F (special sugars, from other sources and of other forms), total phenolic values and antioxidant activity were higher in palm derivatives than in sugar cane derivatives. Sugar F7 (obtained from date palm) contained 5,236 ± 216 μg/g total phenolics and showed 86% inhibitory activity against DPPH radical formation. These values were the highest not only when compared to other samples from group F, but also when compared to all analyzed sugar groups.

α-Glucosidase inhibition assay

All sugar extracts had the capacity to inhibit the yeast α-glucosidase enzyme, and a dose-dependency trend was observed when three different doses of aqueous sugar extracts were tested (10, 25, and 50 μL) (Figs. 3 and 4). The α-glucosidase inhibitory activities were moderately proportional to the total phenolic content and antioxidant activity, and Pearson correlations were statistically significant (Table 2).

The inhibition of α-glucosidase ranged from 26% to 32% in group A (dose of 50 μL). Samples A3–A5 (white, light brown, and dark brown, respectively) had the highest inhibition in this group, and no statistical difference was found among them ($P < .05$).

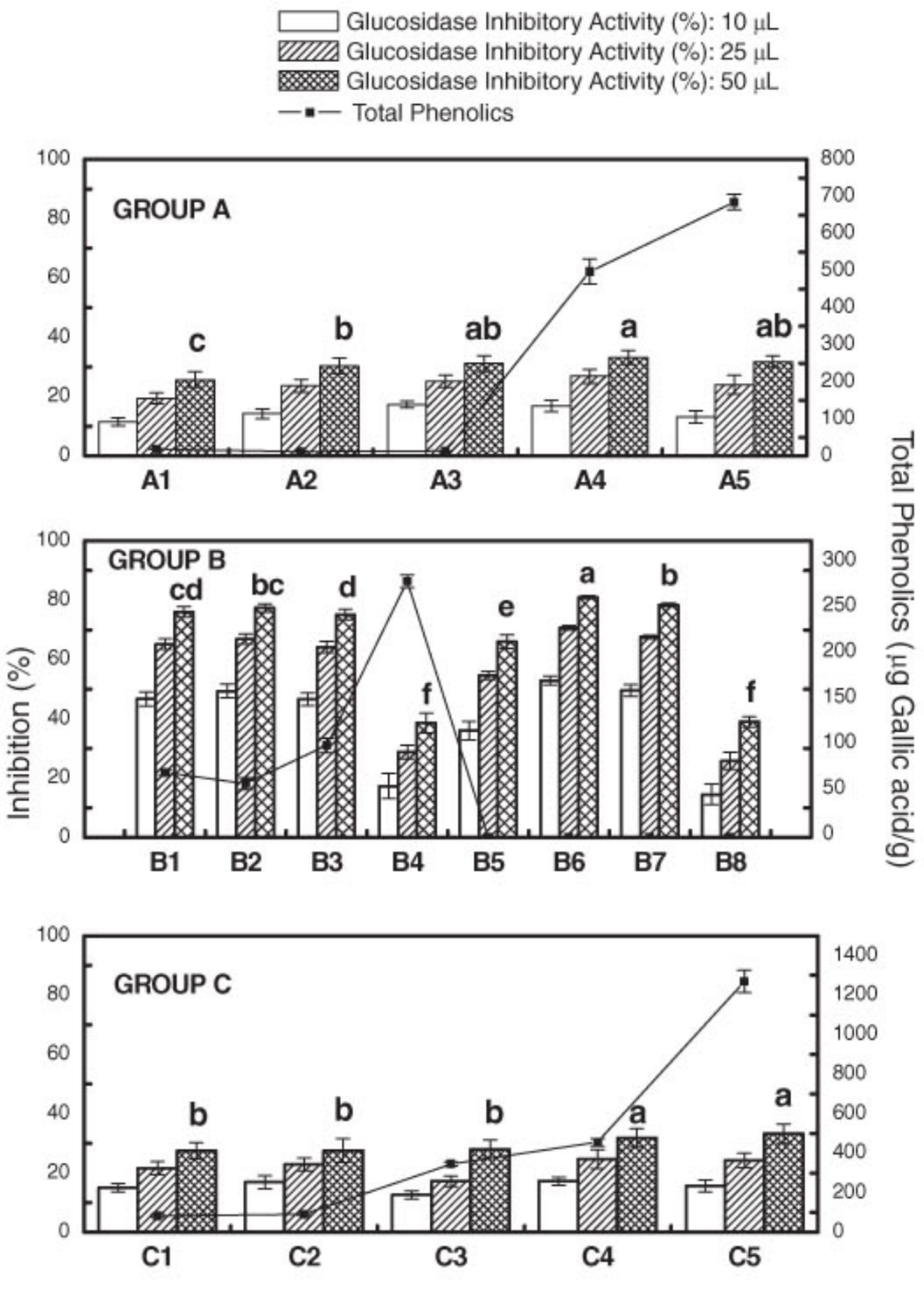

FIG. 3. α-Glucosidase inhibitory activity of sugars and syrups from Groups A, B, and C. Bars with different letters are significantly different ($P < .05$).

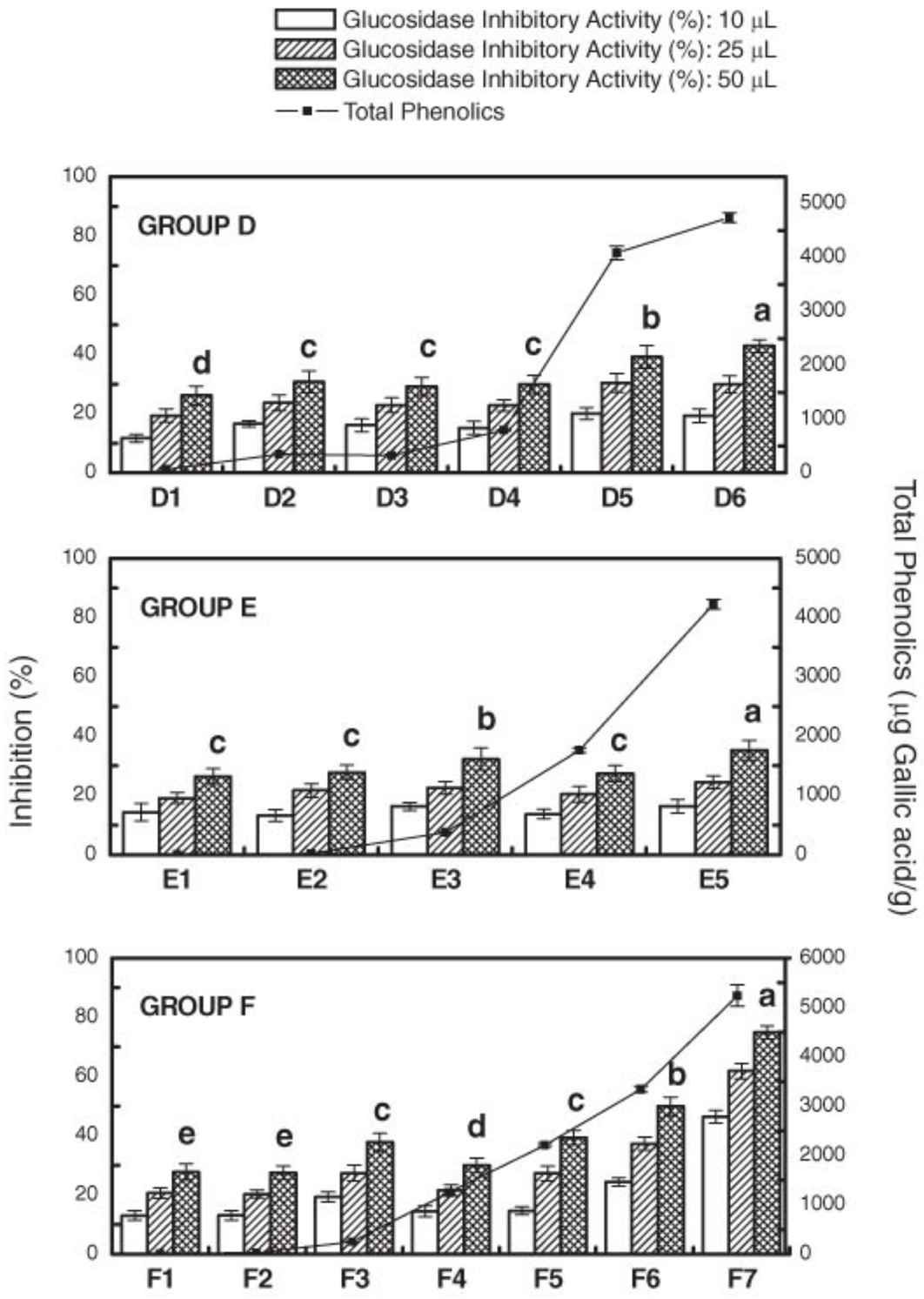

FIG. 4. α-Glucosidase inhibitory activity of sugars from Groups D, E, and F. Bars with different letters are significantly different ($P < .05$).

In group B, almost all syrups had a significant capacity for inhibitory activity at the three doses evaluated. Similar patterns were observed with syrups B1 (corn syrup, Aunt Jemima), B2 (corn syrup with 2% maple syrup, Big Y), B3 (corn syrup with 5% maple syrup, Stop & Shop), B6 (high-fructose corn syrup 42%), and B7 (high-fructose corn syrup 55%). In addition, syrups B3, B6, and B7 had the highest α-glucosidase inhibitory activity among all samples in this group (81%, 78%, and 77%, respectively, at 50 μL). The inhibitory activity in maple syrup (B4) was moderate (38% at 50 μL) and similar to that found in the high-fructose corn syrup 90% (39% at 50 μL). These corn derivatives showed an inverse correlation between total phenolics and DPPH scavenging activity versus α-glucosidase inhibitory activity ($r = -0.61$ and $r = -0.70$, respectively) in contrast to the trends observed in sugars derived from sugar cane.

Brown sugars C4 (raw sugar from Africa) and C5 (natural brown sugar from the United States) exhibited the high-

est inhibitory activities (32% and 33%, respectively, at 50 μL) in group C, and their correlation for total phenolic contents with antioxidant activity was moderate ($r = 0.42$).

The range of inhibition of yeast α-glucosidase by extracts of sugars from Group D was similar to that shown by groups A and C. Sample D5 (dehydrated cane juice organic sucanat from Costa Rica) and D6 (brown sugar from Peru) were the most interesting samples in this group, showing a high inhibitory activity (39% and 43%, respectively, at 50 μL). In addition, there was a better correlation between the total phenolic contents and antioxidant activity ($r = 0.79$ and $r = 0.77$, respectively).

Sample E5 (dark muscovado from Mauritius) was the sugar that had the highest α-glucosidase inhibitory activity (35% at 50 μL) among sugar extracts from group E, although the levels of inhibition were similar as those found in groups A, C, and D. Pearson correlation coefficients between α-glucosidase inhibitory activity and total phenolic contents and between α-glucosidase inhibitory activity and antioxidant activity were 0.60 and 0.57, respectively.

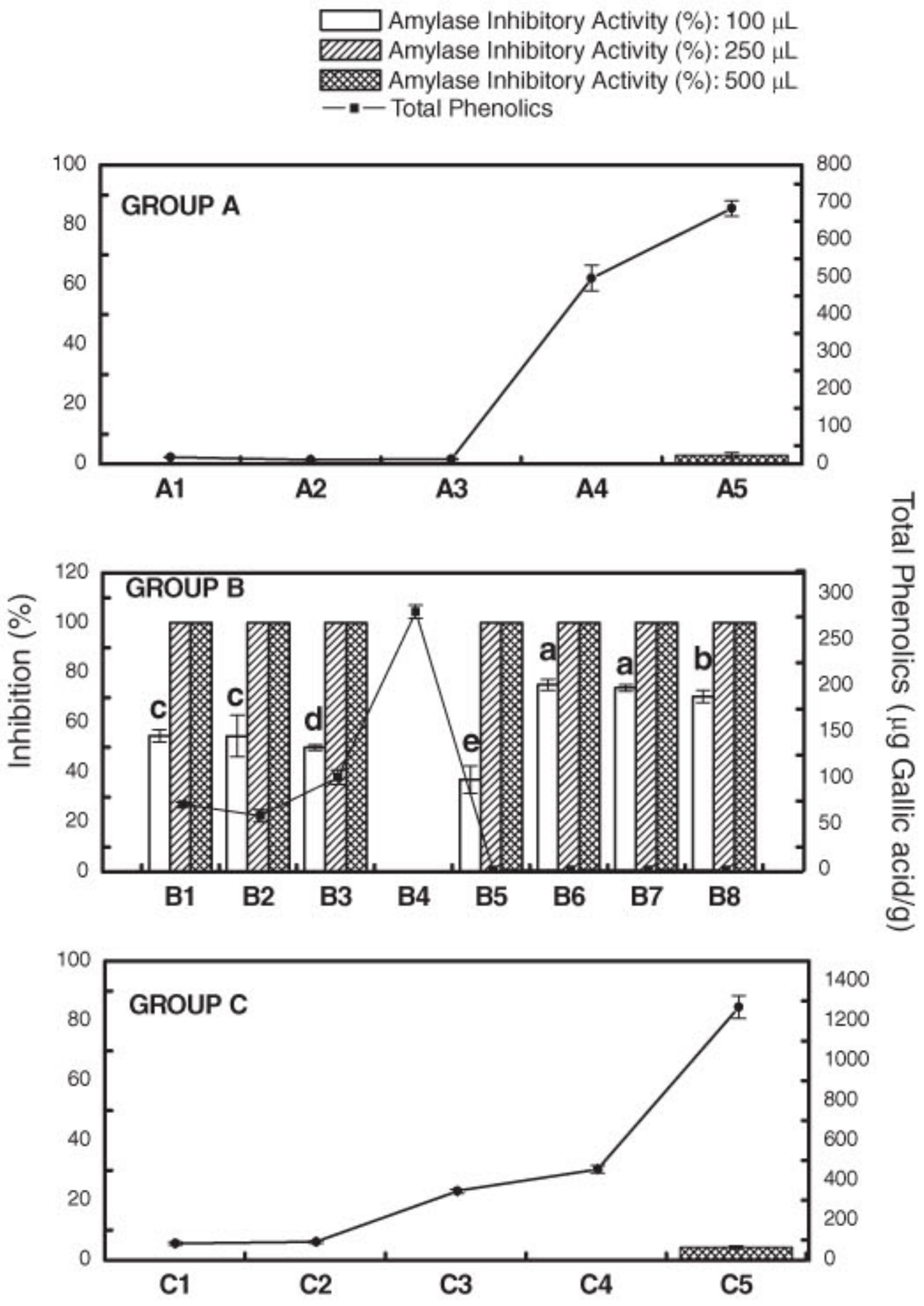

FIG. 5. α-Amylase inhibitory activity of sugars and syrups from Groups A, B, and C. Bars with different letters are significantly different ($P < .05$).

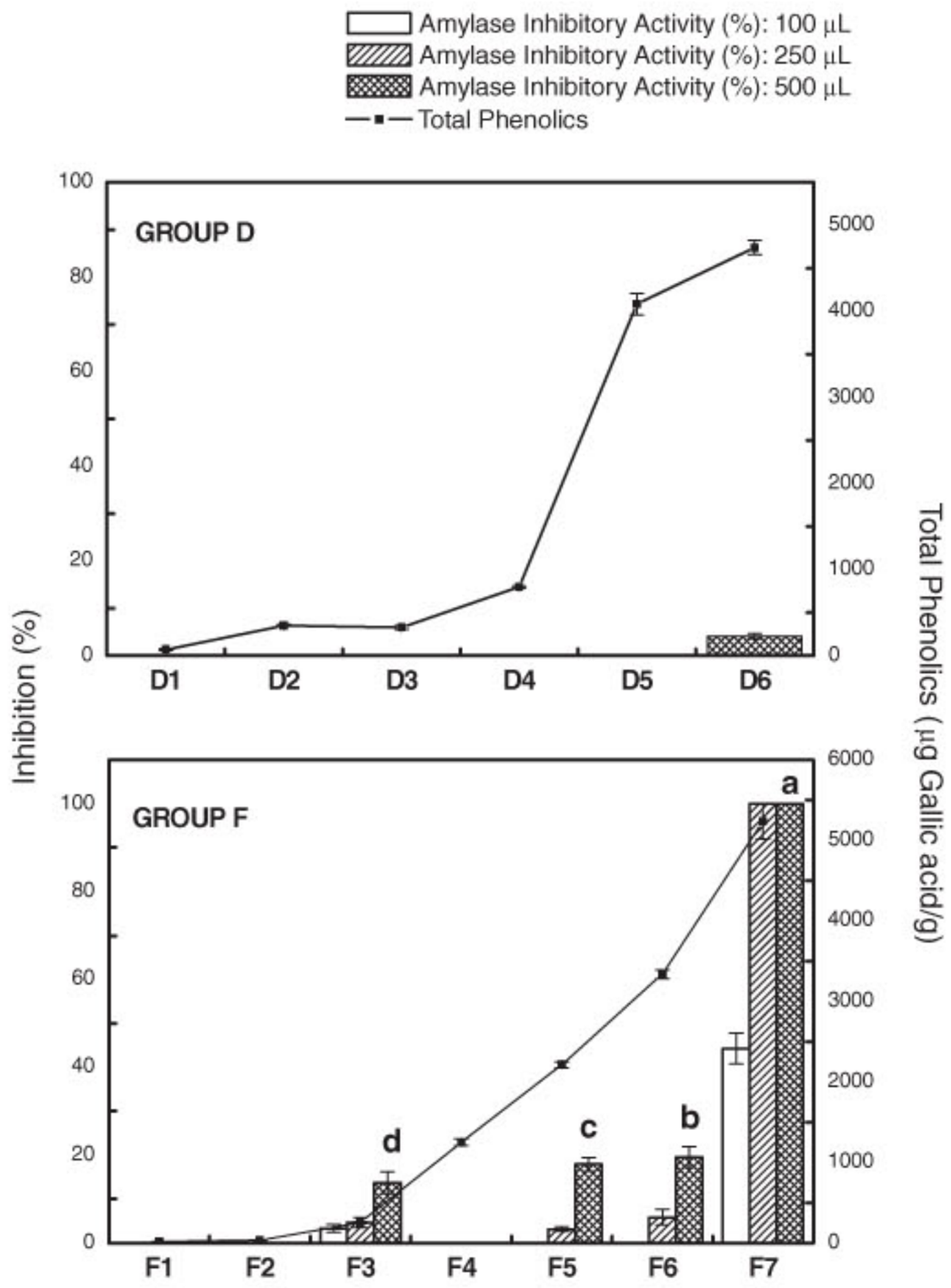

FIG. 6. α-Amylase inhibitory activity of sugars from groups D and F. Bars with different letters are significantly different ($P < .05$).

In Group F, sample F6 (sugar Jaggery Gur from India) and F7 (granulated date palm sugar from the United States) exhibited a strong inhibitory activity against yeast α-glucosidase (50% and 75%, respectively, at 50 μL) and had a significant correlation between their total phenolic contents and DPPH radical scavenging-linked antioxidant activity (Table 2).

α-Amylase inhibition assay

Since sugar extracts are often consumed with starch foods, their ability to inhibit porcine pancreatic α-amylase was evaluated at three doses (100, 250, and 500 μL). In contrast to α-glucosidase inhibitory activity results, α-amylase inhibitory activities were very low in several sugar groups (Figs. 5 and 6). Only brown sugars with the highest levels of total phenolics and DPPH scavenging activities in groups A, C, and D exhibited minor inhibition (A5, 3%; C5, 4%; D6, 4%) at the highest dose extract (500 μL). Furthermore, no α-amylase inhibition was detected in group E.

Syrups from group B had a particular trend, showing an inverse correlation with the total phenolics and antioxidant activity (-0.84 and -0.82, respectively) as also was ob-

served for α-glucosidase inhibitory activity. The syrup solutions had a significant inhibitory activity at 250 and 500 μL (~100%), and the inhibition ranged from 37% to 75% at 100 μL. At this dose, high-fructose corn syrups with 42% and 55% fructose inhibited strongly the α-amylase activity (75% and 74%, respectively), a higher value than with the high-fructose corn syrup with 90% fructose (70%) ($P < .05$). Corn syrups B1–B3 showed intermediate inhibition (55%, 54%, and 50%, respectively), whereas no inhibitory activity was detected in maple syrup (B4).

In group F, palm-derived sugars (F3, F5, and F7) and sugar F6 (sugar Jaggery Gur derived from sugar cane) were the only samples with α-amylase inhibitory activity, and inhibition was observed at all doses. The result with date sugar (F7) was particularly significant owing to its high α-amylase inhibitory activity (100% at 250 μL and 500 μL and 44% at 100 μL). The total phenolic content and antioxidant activity were correlated significantly with the inhibitory activity (Table 2).

ACE inhibition assay

The potential to inhibit the hypertension-related ACE was screened in all sugars. No inhibition was observed among sugars from groups A, C, D, and E (mainly sugar cane derivatives), while syrups (Group B) and some palm derivatives (Group F) exhibited significant inhibitory activities (Fig. 7).

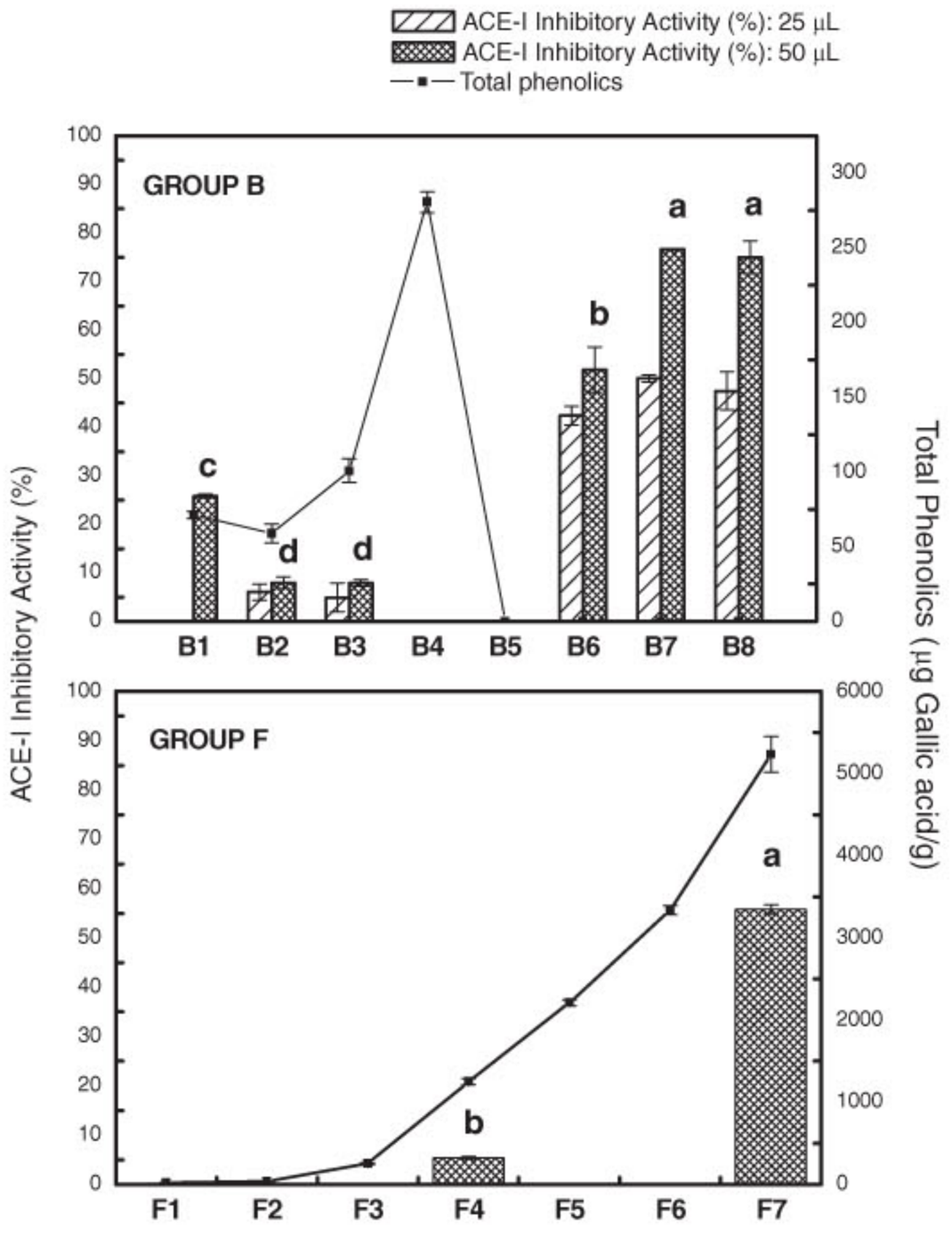

FIG. 7. ACE inhibitory activity (%) of groups B and F. Bars with different letters are significantly different ($P < .05$).

TABLE 3. CHLOROGENIC ACID CONTENTS IN BROWN CANE SUGARS QUANTIFIED BY HPLC WITH DIODE ARRAY DETECTOR

Sample	*Code*	*Chlorogenic acid (µg/g)*
Brown sugar (Peru)	D6	128 ± 6
Dark muscovado (Mauritius)	E5	144 ± 2

The content of total phenolics and antioxidant activity were proportional to the ACE inhibitory activity of palm derivatives (Group F), and the inhibition of ACE was strongly correlated with the α-amylase and α-glucosidase inhibitory activities (Table 2). The date sugar (F7) had the highest ACE inhibitory activity (56% at 50 μL) in this group, whereas the Indian Palm Jaggery (F4) had the lowest ACE inhibitory activity (5%).

In group B, high-fructose corn syrups showed significant inhibitory activities (from 52% to 75%), and the inhibition was in a dose-dependent manner. Corn syrups (B1–B3) exhibited a moderate inhibition of ACE, and sample B1 (without maple syrup in its composition) showed the highest inhibition (26%) among these three syrups. ACE inhibitory activities in syrups had an inverse correlation with the total phenolic contents and antioxidant activities, whereas a significant correlation with the α-amylase inhibition was found (Table 2).

HPLC profiles

The Folin-Ciocalteu method provides an overall measurement of total phenolic contents. However, the use of HPLC can give better information about specific phenolic profiles in each sample. Only chlorogenic acid was detected in sugars D6 (brown sugar from Peru) and E5 (dark muscovado from Mauritius) (Table 3). According to chromatograms (data not shown), other phenolic compounds were detected at 333 nm in sugar extracts (except in syrups); nevertheless, peaks corresponding to these phenolics were very small and did not allow a correct area integration in spite of the fact that initial sugar extracts were highly concentrated (10%).

Functionality based on color

Considering only sugar cane and palm derivatives, functionality data were regrouped according to color (in the solid state, Table 1) in order to compare all samples using a general approach. Figures 8 and 9 show that functionality properties such as total phenolic content, DPPH scavenging activity, and α-amylase and α-glucosidase inhibitory activities were high in light brown and brown sugars, rather than in white sugars. Total phenolics values were proportional

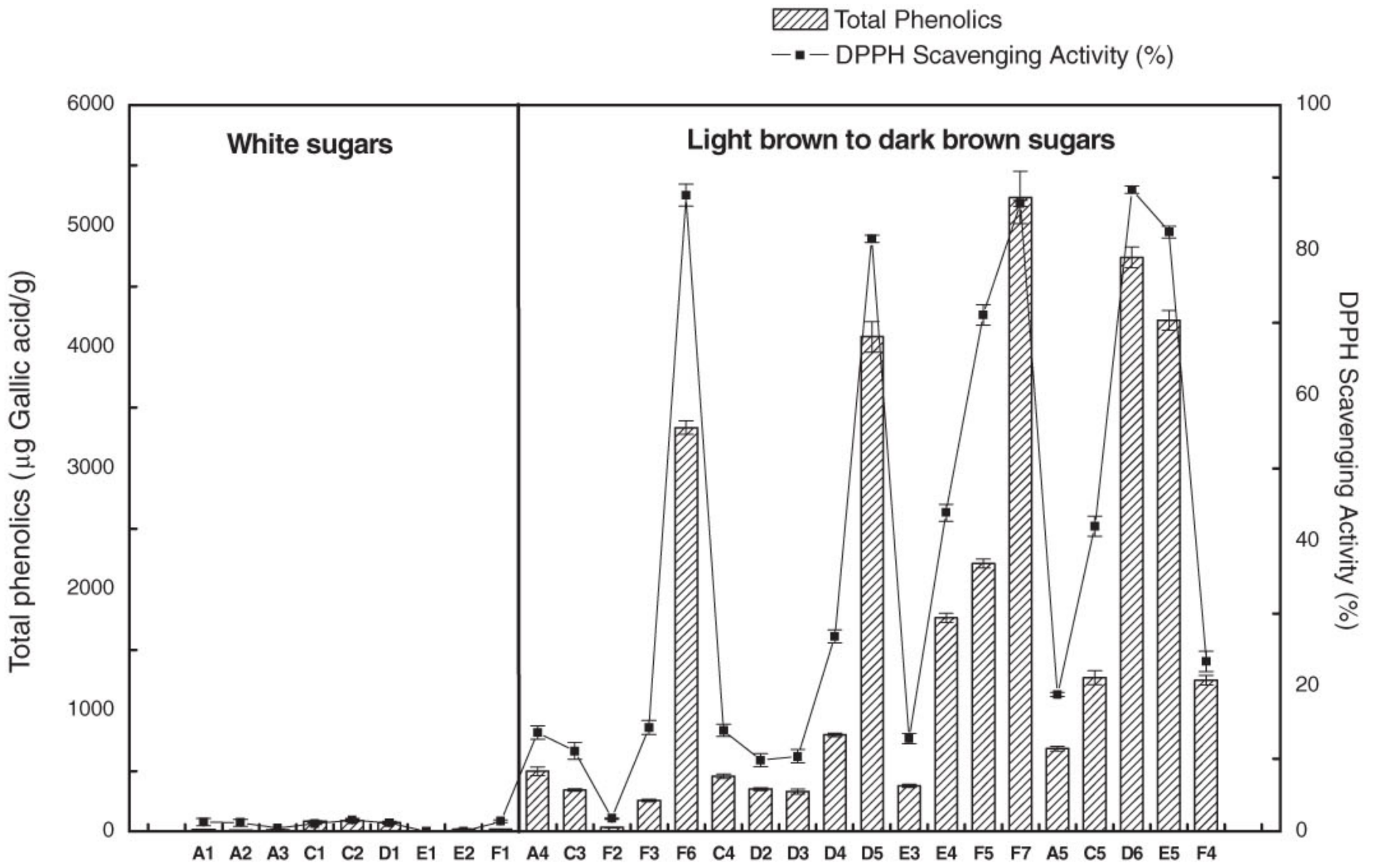

FIG. 8. Total phenolics and antioxidant activity of sugars based on color. Sample codes are indicated in Table 1. Pearson's correlation coefficient $r = 0.97$ ($P < .05$, $n = 321$).

to the α-amylase and α-glucosidase inhibitory activities (0.74 and 0.57, respectively), and correlation between α-amylase and α-glucosidase inhibitory activities was significant ($P < .05$).

DISCUSSION

Total soluble phenolics, antioxidant activity, and HPLC profiles

In general, sugars showed DPPH radical scavenging activity linked to the total phenolic contents. Brown sugars such as D6 (brown sugar from Peru), E5 (Dark muscovado from Mauritius), and F7 (date sugar from the United States) were the most relevant for their highest total phenolic content and antioxidant activity.

The phenolic components in the native cane plant and the modification during manufacturing process may be the key factors that exert a significant influence on phenolic profiles and antioxidant activity in sugar cane derivatives. Duarte-Almeida *et al.*[23] detected flavonoids (apigenin, luteolin, and tricin derivatives) and phenolic acids (hydroxycinnamic, caffeic, and sinapic acids) in sugar cane juice from Brazil. Similarly, other authors have reported the presence of phenolic acids in brown sugars,[16] but no flavonoids were detected in other cane sugar products.[15] Several phenolic glycosides were identified in liquid sugar from cane molasses[24] and antioxidative phenolic compounds in a noncentrifuged sugar cane.[25] In relation to the present study, this is the first time that chlorogenic acid has been reported in brown sugars as it was detected in Peruvian and dark muscovado (Mauritius) sugars.

Phenolic compounds seem to be involved in the formation of color in brown sugars and therefore may be responsible for their high antioxidant activity; nevertheless, Maillard reaction products formed during the sugar producing process may also contribute to the antioxidant activity.[26–28] In contrast, the lack of phenolic compounds in white sugars, probably lost during the refining process, was proportional to their low antioxidant activity.

Maple syrup (B4) is not only a source of sucrose, organic acids, vitamins, and minerals such as potassium, magnesium, and calcium,[29] but also has moderate amounts of phenolic compounds and free radical scavenging-linked antioxidant properties. The results reported in this study indicate that the use of maple syrup in different proportions in common corn syrups (B2 and B3) enhances the total phenolic content and antioxidant activity.

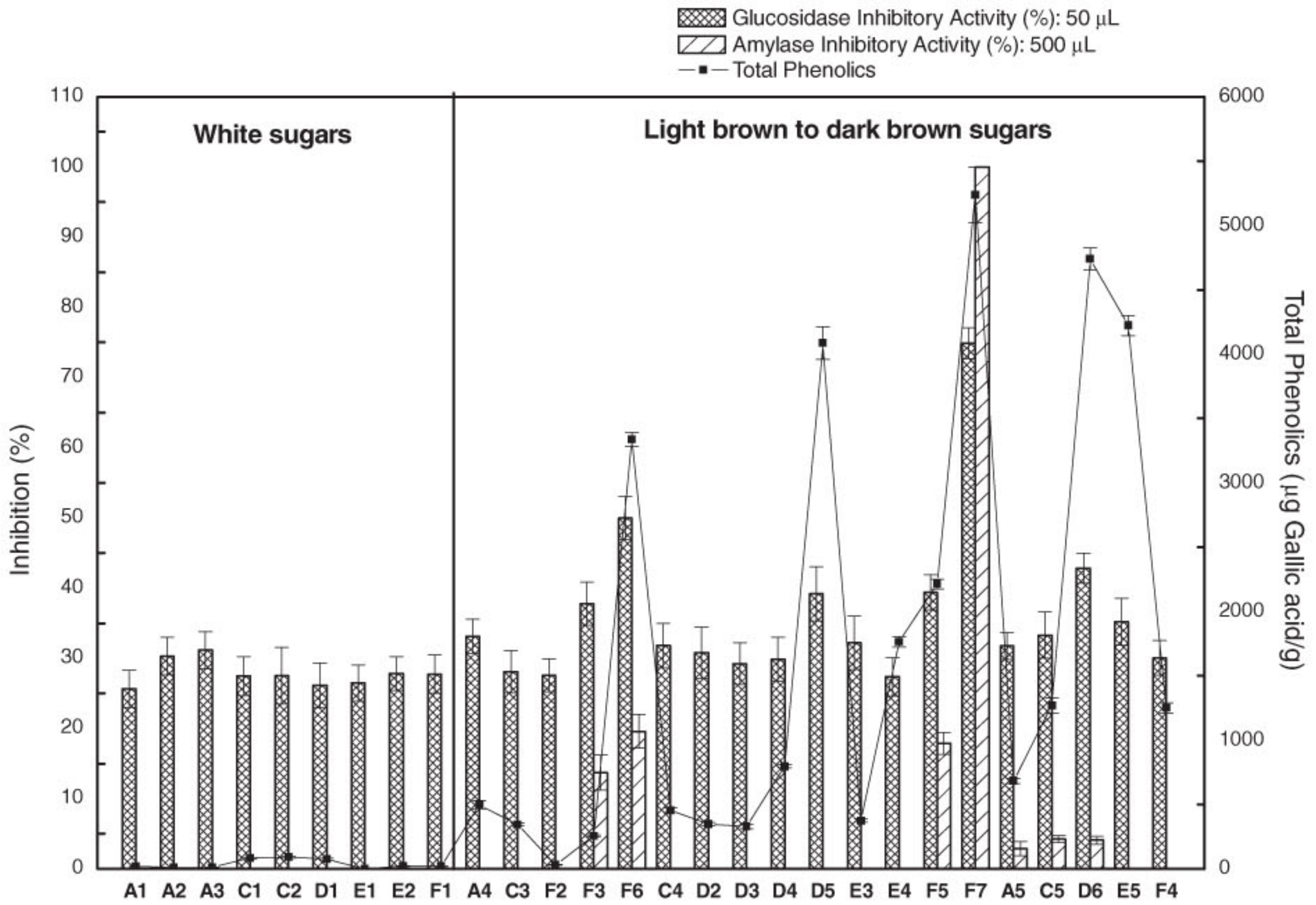

FIG. 9. α-Glucosidase inhibitory activity, α-amylase inhibitory activity, and total phenolics of sugars based on color. Samples codes are indicated in Table 1. Pearson's correlation coefficient between total phenolics and α-glucosidase inhibitory activity is $r = 0.74$ ($P < .05$, $n = 321$), between total phenolics and α-amylase inhibitory activity is $r = 0.57$ ($P < .05$, $n = 321$), and between α-glucosidase inhibitory activity and α-amylase inhibitory activity is $r = 0.87$ ($P < .05$, $n = 321$).

In vitro *α-glucosidase, α-amylase, and ACE inhibitory activities*

Type 2 diabetes is characterized by a rapid increase in blood glucose levels due to hydrolysis of starch by pancreatic α-amylase and absorption of glucose in the small intestine by α-glucosidase. This may be controlled by inhibition of these enzymes involved in the digestion of carbohydrates. The consumption of inhibitors naturally from constitutents in the diet could be an effective therapy for managing postprandial hyperglycemia with minimal side effects in contrast to traditional treatments with drugs such as acarbose.[17] Furthermore, one of the main macrovascular complications of diabetes is hypertension, which is a risk factor for many cardiovascular diseases. Control of hypertension via modulation of ACE by dietary antihypertensive ingredients is an important strategy to manage this risk factor.

Carbohydrate sweeteners derived from sugar cane inhibited moderately the yeast α-glucosidase, had almost no effect against α-amylase activity, and did not inhibit ACE. α-Glucosidase inhibitory activities ranged from 26% to 50%, and sugars that had the highest total phenolic and antioxidant activity levels (brown sugars) also showed the highest inhibition (A5, 32%; C5, 33%; D5, 39%; D6, 43%; E5, 35%; F6, 50%). These data suggest that total phenolic content may be associated with the potential of cane sugars to inhibit this enzyme. Hence, brown sugars might be an alternative sweetener to refined white sugar for diabetes management without side effects produced for high α-amylase inhibition,[30] if consumed in combination with a high starch diet.

Interestingly, sweeteners derived from corn hydrolysis did not contain phenolic compounds; however, they showed high α-glucosidase, α-amylase, and ACE inhibitory activities (Figs. 3, 5, and 7, respectively). It was observed that syrups with more glucose or dextrose in their composition such as high-fructose corn syrup samples B6 (42% fructose, 52% dextrose) and B7 (55% fructose, 41% dextrose) had a higher inhibition of both enzymes than syrups rich in sucrose (maple syrup derivative plus sucrose) and fructose (high-fructose corn syrup, 90% fructose, 5.6% dextrose) ($P < .05$). Syrups B1–B3 showed similar trends as syrups B6 and B7, and this may be related with the fact that these products contain high-fructose corn syrup at 42% or 55%. It has been reported that several inhibitors of α-glucosidases and α-amylases have structural similarities to sugars such as disaccharides/oligosaccharides that act as substrate analogs and bind to the enzyme catalytic sites.[31,32] Further-

more, unusual sugars such as L-fructose and L-xylulose showed competitive inhibition towards yeast α-glucosidase.[33] Such sugar analogs may be found in high-fructose corn syrups and similar derivatives.

Even though high-fructose corn syrup with 42% fructose (B6) had lower inhibition (52%) than syrups with 55% and 90% fructose (76% and 75%, respectively), the relevance of fructose in high ACE inhibition is not clear. Furthermore, the other corn syrups (B1–B3, except sample B5) exhibited low inhibitory activities against ACE. Probably, the ratio of monosaccharides and polymeric oligosaccharides contained in corn syrups (the main ingredient of these products) may influence ACE inhibition. These results indicate that the types of sugars contained in corn syrups (dextrose, fructose, and higher oligosaccharides) may be linked to the *in vitro* α-glucosidase and α-amylase inhibition, but their relevance to ACE inhibition is not clear. Further research will be needed in order to elucidate the mechanism and the specific compounds from syrups involved in the *in vitro* inhibition of key enzymes related to diabetes and hypertension.

Among palm derivatives, only date palm sugar had high α-glucosidase (75% at 50 μL), α-amylase (~100% at 500 μL), and ACE (56% at 50 μL) inhibitory activities that correlated to higher total phenolics and antioxidant activity (Figs. 4, 6, and 7, respectively). Thus, date palm sugar may be an interesting option for diabetes and hypertension management. However, when it is used with starch foods, care should be taken to avoid the potential side effects of undigested starch linked to high α-amylase inhibitory activity. Date palm sugar consists mainly of dried dates, which are a good source of dietary fiber[34] and other antioxidants such as anthocyanins, carotenoids, and condensed tannins.[35,36] These compounds could also contribute to the inhibitory effect on the digestion of starch, probably in a synergistic manner.[37–39]

An important influence of the sugar color was also observed (Figs. 8 and 9), especially among sugar cane derivatives. In general, brown sugars showed higher antidiabetes potential coupled to higher levels of total phenolics and antioxidant activity. This correlation between sugar color and functionality may be an interesting and simple tool for the selection of appropriate sugars for hyperglycemia-linked diabetes management and for functional food design. However, other external factors such as environmental, agronomic, and sugar cane processing conditions should be considered as well.

IMPLICATIONS

The global dietary trend is toward increased consumption of refined carbohydrates and fats. This has been linked to the rise of diet-linked chronic diseases such as postprandial hyperglycemia and diabetes complications associated with hypertension that contributes to subsequent cardiovascular diseases. Research to understand functional properties of foods linked to well-designed dietary strategies may counteract this problem. According to this study, commonly consumed carbohydrate sweeteners exhibited an interesting potential for *in vitro* inhibition of key enzymes related to Type 2 diabetes and hypertension depending on their origin and grade of refining. Less processed sugars (brown sugars) derived from sugar cane are important sources of phenolic compounds coupled to a high ability of free radical scavenging-linked antioxidant activity. These functional properties were well correlated with a moderate inhibition of α-glucosidase. However, α-amylase and ACE activities were not affected. Sugar derived from date palm is a good option for diabetes-linked hypertension management. In contrast, sweeteners derived from corn hydrolysis such as corn syrups and high-fructose corn syrups inhibited α-glucosidase, α-amylase, and ACE enzymes, indicating that nonphenolic compounds, such as polymeric oligosaccharides and their hydrolyzed derivatives, may play a role. These results indicate that a strategic choice of dietary source of sweeteners has potential in the development of strategies for better management of Type 2 diabetes and related complication of hypertension.

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