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Sugar Cane and Sugar Beet Molasses, Antioxidant-rich Alternatives to Refined Sugar

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ABSTRACT: Molasses, the main byproduct of sugar production, is a well-known source of antioxidants. In this study sugar cane molasses (SCM) and sugar beet molasses (SBM) were investigated for their phenolic profile and in vitro antioxidant capacity and for their protective effect in human HepG2 cells submitted to oxidative stress. According to its higher phenolic concentration and antioxidant capacity in vitro, SCM exhibited an effective protection in cells, comparable to or even greater than that of α-tocopherol. Data herein reported emphasize the potential health effects of molasses and the possibility of using byproducts for their antioxidant activity. This is particularly important for consumers in developing countries, as it highlights the importance of consuming a low-price, yet very nutritious, commodity.

KEYWORDS: *sugar cane molasses, sugar beet molasses, phenolic compounds, antioxidant capacity, antioxidant protection, cultured HepG2 cells*

INTRODUCTION

Sugar cane (*Saccharum officinarum* L.) and sugar beet (*Beta vulgaris* L. ssp. *saccharata*) are the most important crops for the production of sugar. Molasses, the thick, dark syrup obtained as a byproduct from the processing of sugar cane and sugar beet into sucrose, consists of fermentable carbohydrates (sucrose, glucose, fructose) and several nonsugar organic materials (betaine and other amino acids; minerals and trace elements; vitamins, especially of the B-group, etc.). Although molasses is mainly used as a supplement for livestock feed and as a source of carbon in fermentation processes, for example, for the production of ethanol,[1] by tradition it also serves as a sweetener and colorant substitutes in cakes. Molasses is considered to be generally regarded as safe (GRAS) by the U.S. Food and Drug Administration, and people believe molasses has health benefits beyond its special taste and flavor due to it being rich in minerals. In addition, several studies evidenced that molasses is a rich source of phenolic compounds[2,3] having possible roles in the prevention of several chronic diseases involving oxidative stress.[4−6] Maillard browning carbohydrate−amino acid condensation products, formed during sugar processing, are also in very high concentration in molasses and range from low organic compounds to complex aromatic polymers. They are strongly involved in the color and aroma of molasses, and they have been reported to have antioxidant activities.[7−10] In the light of the recommendation of increasing the intake of antioxidant-rich foods,[11−14] the substitution of sugar with molasses could represent a potential extra source of antioxidants.

In this study we assessed the in vitro antioxidant capacity and phenolic composition of molasses from sugar cane (SCM) and from sugar beet (SBM), comparing them to other common sweeteners. Then, to go further in demonstrating the oxygen free radical inhibition by molasses, the biological activity of SCM and SBM was verified supplementing HepG2 cells with two different molasses concentrations. HepG2 cells, a human hepatoma cell line considered to be a good model to study in vitro cytotoxic agents,[15,16] were chosen as model system given that the liver is the organ mainly involved in xenobiotic metabolism.[17] SCM and SBM protection from the oxidative damage induced by cell exposure to hydrogen peroxide (H_2O_2) was assessed by measuring cell viability, reduced glutathione (GSH) and reactive oxygen species (ROS) intracellular contents, cytosolic total antioxidant capacity (TAC), lactate dehydrogenase (LDH) release, and thiobarbituric acid reactive substances (TBARS) content in the media. To compare the effect of molasses to the effect of a well-known antioxidant, some cells were supplemented with 8.6 μg/mL (20 μM) α-tocopherol (TC), considered to be the most important endogenous antioxidant in cells.[18]

Our results emphasize the potential health effects of molasses, adding functional properties and nutritional value to a sweetening agent and sustaining its use as a refined sugar substitute. Considering that refined sugar is the most common form of sugar in North America as well as in Europe,[19] the use of molasses as an alternative to refined sugar could increase antioxidant intake similar to replacement of refined grains with whole grains.[20]

MATERIALS AND METHODS

Chemicals. Dulbecco's modified Eagle's medium (DMEM), penicillin, streptomycin, and Dulbecco's phosphate-buffered saline (DPBS) were purchased from Lonza (Milan, Italy). Ethanol and 1-propanol were

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supplied by Carlo Erba (Milan, Italy), whereas HPLC grade solvents acetonitrile, water, methanol, and acetic acid were purchased from Merck KGaA (Darmstadt, Germany). All other chemicals were purchased from Sigma-Aldrich (Milan, Italy).

Sweeteners. White refined beet sugar, brown raw cane sugar, sugar cane molasses, sugar beet molasses, acacia honey, maple syrup, and fructose were purchased from local markets. Glucose was purchased from Sigma-Aldrich, and grape sugar and rebaudioside (60 and 98% purity, respectively) were a kind gift of Eridania Spa (Bologna, Italy).

Methods. *In Vitro Antioxidant Capacity (TAC) and Phenolic Composition. (a) In Vitro TAC of Different Sweeteners Using the ABTS Assay.* One gram of each sweetener was dissolved in 10 mL of water. TAC was measured using the method of Re et al.,[21] based on the capacity of antioxidant molecules in the sample to reduce the radical cation of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$). The decolorization of $ABTS^{\bullet+}$ was measured as the quenching of the absorbance at 734 nm. Values obtained were compared to the concentration−response curve of the standard Trolox solution and expressed as micromoles of Trolox equivalents (TE) per gram.

(b) In Vitro TAC of Different Sweeteners Using the 1-Diphenyl-2-picrylhydrazyl (DPPH) Assay. SBM and SCM TAC were also evaluated using the DPPH radical scavenging capacity assay according to the method of Cheng et al.[22] with some modifications. Solutions of molasses were prepared in ethanol/water 70:30 at different concentrations (0.1, 0.2, 0.5, 1, 2, 5, 10.0 mg/mL), and 100 μL of 0.208 mM DPPH in ethanol/water (70:30 v/v) was added to 100 μL of each solution. The obtained mixtures were left to stand in the dark for 60 min, and the absorbance at 515 nm was measured with a Tecan Infinite M200 microplate reader (Tecan, Männedorf, Switzerland). The radical scavenging activity was calculated by using the following formula: inhibition = $[(Abs_{blank} - Abs_{sample})/Abs_{blank}] \times 100$. The concentration required to obtain a 50% radical scavenging activity (IC_{50}) was calculated based on a dose−response curve correlating the concentration of molasses solution to the average inhibition percentage.[23]

(c) HPLC with Diode Array Detection Coupled to Electrospray and Mass Spectrometry (HPLC-DAD-ESI-MS) Analysis of Phenolic Compounds. Liquid chromatography (LC) analyses were performed using an Agilent 1100 series LC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a degasser, a binary pump, an autosampler, a column heater, a diode array detector (DAD), and a quadrupole mass spectrometer. Separation was carried out on a fused core type column Kinetex C18 (100 mm × 4.6 mm, 2.6 μm) (Phenomenex, Torrance, CA, USA). The gradient elution was programmed using as mobile phase A acidified water (1% acetic acid) and as mobile phase B 60% of phase A and 40% of acetonitrile. The program was developed as follows: from 5 to 7% phase B, 0−2 min; from 7 to 9% phase B, 2−4 min; from 9 to 12% phase B, 4−7 min; from 12 to 15% phase B, 7−8 min; from 15 to 16% phase B, 8−9 min; from 16 to 18% phase B, 9−12 min; from 18 to 20% phase B, 12−14 min; from 20 to 22% phase B, 14−15 min; from 22 to 25% phase B, 15−16.5 min; from 25 to 28% phase B, 16.5−18 min; from 28 to 30% phase B, 18−19 min; from 30 to 31% phase B, 19−20 min; from 31 to 32% phase B, 20−21.5 min; from 32 to 34% phase B, 21.5−23 min; from 34 to 35% phase B, 23−24 min; from 35 to 40% phase B, 24−25.5 min; from 40 to 50% phase B, 25.5−27 min; from 50 to 100% phase B, 27−30 min; 100% B, 30−33 min; and from 100 to 5% phase B in 2 min. The flow rate was constant at 0.8 mL/min, and the column temperature was maintained at 25 °C. The injection volume was 2.5 μL, and UV spectra were recorded from 200 to 600 nm, whereas the chromatograms were registered at 280 and 330 nm.

MS analyses were carried out using an electrospray (ESI) interface using the following conditions: drying gas flow, 9.0 L/min; nebulizer pressure, 35 psig; gas drying temperature, 350 °C; capillary voltage, 3000 V; fragmentor voltage, 80 V.

Biological Protective Activity of Molasses. (a) HepG2 Cell Culture and Supplementation. HepG2 cells were cultured in DMEM fortified with 10% (v/v) fetal calf serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. Cells were maintained at 37 °C in a humidified atmosphere containing 95% air and 5% CO_2; once a week cells were split 1:20 into a new 75 cm^2 flask, and culture medium was changed every 48 h. For experiments cells were seeded in 6-well plates, and after 24 h (75−80% confluence), they were randomly divided into two groups (supplemented and unsupplemented). Supplemented cells were grown in serum-free DMEM containing TC (8.6 μg/mL) or molasses (SCM or SBM) at two different concentrations (10^2 and 10^3 μg/mL medium); unsupplemented (US) cells received a corresponding amount of sterile water. Prior to supplementation, SCM and SBM were dissolved in water and filtered with a sterile 0.2 μm membrane. The total volume of the added molasses solution was <1% of the medium total volume.

Twenty-four hours after supplementation, cells were washed twice with warm DPBS. To cause an oxidative stress, cells were exposed to 0.2 mM H_2O_2 in Earle's balanced salt solution (EBSS) (116 mM NaCl, 5.4 mM KCl, 0.8 mM NaH_2PO_4, 26 mM $NaHCO_3$, 2.38 mM $CaCl_2$, 0.39 mM $MgSO_4$) for 1 h. Nonstressed US cells instead received EBSS without H_2O_2. After 1 h, EBSS was removed, centrifuged at 400*g* for 3 min, and used for TBARS assay and lactate dehydrogenase (LDH) release determination as described below. Cells were washed twice with cold DBPS and immediately used for further analysis.

(b) Measurement of Intracellular Reactive Oxygen Species (ROS) Concentration. Intracellular ROS concentration was monitored spectrofluorometrically according to the method of Jiao et al.[24] with slight modifications. DCFH-DA (2 mM) in absolute ethanol was kept in the dark at −20 °C until used, and 10 μL of DCFH-DA/mL medium was added to HepG2 cells 30 min prior to H_2O_2. DCFH-DA penetrates the cell membrane and is enzymatically hydrolyzed by intracellular esterases to the nonfluorescent DCFH, which can be rapidly oxidized to the highly fluorescent DCF in the presence of ROS. At the end of the oxidative stress, cells were washed twice with cold DPBS, lysed with 1 mL of cold Nonidet P-40 (0.25% in DPBS), incubated for 30 min on ice, and centrifuged at 14000*g* for 15 min. DCF fluorescence intensity was detected (λ_{ex} = 485 nm, λ_{em} = 535 nm) using a Tecan Infinite F200 microplate reader, normalized for protein content in the sample and expressed as percent of value in nonstressed US cells.

(c) TBARS Concentration. TBARS, the end-products of lipid peroxidation, were assayed in EBSS as reported.[25] One hundred microliters of EBSS buffer was added to a mixture containing 100 μL of TCA (30% in 0.25N HCl), 100 μL of TBA (0.75% in 0.25 N HCl), and 3 μL of BHT (1% in ethanol). The mixture was heated for 10 min in a boiling water bath and allowed to cool, and the TBA adducts were detected fluorometrically (λ_{ex} = 535 nm, λ_{em} = 595 nm).[26] TBARS level was expressed as relative fluorescence units (RFU) and normalized for milligrams of proteins in each well.

(d) LDH Release. LDH is a soluble cytosolic enzyme converting pyruvic acid to lactic acid through NADH oxidation. Because loss of membrane integrity causes LDH leakage, the level of enzyme activity in extracellular fluids is used as an indicator of membrane damage. LDH activity in the EBSS buffer was assessed spectrophotometrically at 340 nm for 1 min by measuring the rate of NADH oxidation.[27] The assay mixture contained 100 μL of 1.4 mM NADH, 100 μL of 10 mM pyruvate, and 600 μL of DPBS; the reaction started with 200 μL of sample. Enzyme activity was calculated using the extinction coefficient of NADH (6.22 $mmol^{-1}$ cm^{-1}), expressed as milliunits per milliliter of medium and normalized for milligrams of protein in each well.

(e) Cell Viability. Cell viability was evaluated by measuring the conversion of the tetrazolium salt to its formazan product as previously reported.[28] 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was dissolved in RPMI-1640 medium without phenol red (final concentration = 0.5 mg/mL) and added to cells. After 1 h of incubation at 37 °C, medium was completely removed, 1-propanol was added to dissolve formazan product, and absorbance was measured against a propanol blank at 560 nm. Cell viability in stressed cells was expressed as percent of nonstressed US cells.

(f) Cytosolic TAC. Cells were lysed with 1 mL of cold Nonidet P-40 (0.25% in DPBS), incubated for 30 min on ice and centrifuged at 14000*g* for 15 min. Cytosolic TAC was measured on the supernatant

using the method of Re et al.,[21] as described above. Values were normalized for protein content in the sample and expressed as micromoles of trolox equivalents (TE) per milligram of protein.

(g) GSH Content. Cytosolic GSH content was determined as previously described.[29] Cells were lysed with 700 μL of cold Nonidet P-40 (0.25% in DPBS), incubated for 30 min on ice, and centrifuged at 14000*g* for 15 min. One hundred microliters of the supernatant was incubated with 100 μL of reagent buffer (80 mM sodium phosphate, pH 8.0, 2 mM EDTA, 2% SDS, and 250 μM DTNB) for 30 min. GSH was measured spectrophotometrically by reading the absorbance of the newly formed 5-thio-2-nitrobenzoic acid at 415 nm. The obtained results were compared to the concentration−response curve of standard GSH solutions, normalized for protein content in the sample, and expressed as nanomoles of GSH per milligram of protein.

(h) Protein Content. Protein content was determined according to the method of Bradford,[30] using bovine serum albumin as standard.

(i) Statistical Snalysis. Data on in vitro antioxidant activity and phenolic profile are reported as the mean ± SD (n = 3); data obtained in cell cultures are reported as the mean ± SD of at least six samples derived from three independent cell cultures.

The evaluation of DPPH and HPLC-MS data statistical significance was carried out by Student's t test. All other data were analyzed for statistical significance by one-way ANOVA, using Dunnett's post hoc test.

RESULTS

In Vitro TAC and Phenolic Composition. The in vitro TAC of SCM and SBM was higher than that of the other tested sweeteners; SCM had the highest TAC (Figure 1). The higher

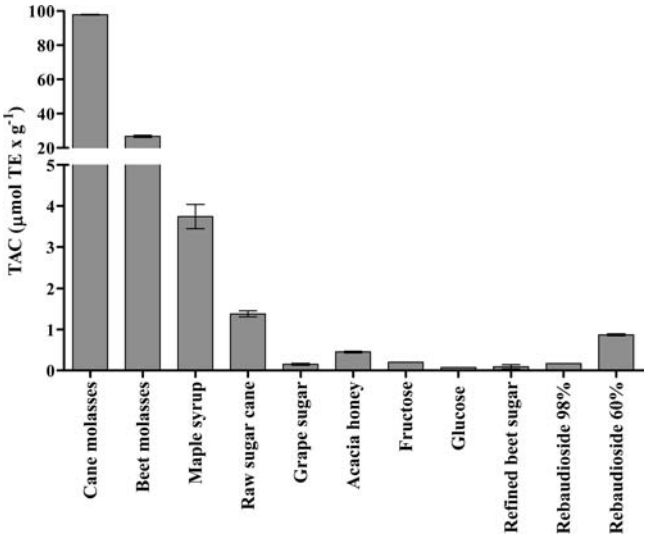

Figure 1. Total antioxidant capacity (TAC) of the different sweeteners. TAC is expressed as μmol of Trolox equivalents (TE)/g of sweetener. Data are the mean ± SD. Statistical analysis was by one-way ANOVA (p < 0.001).

antioxidant capacity of SCM than SBM was confirmed by the DPPH assay, because the molasses concentrations needed to reduce oxidation by 50% (IC_{50}) were 7.25 mg/mL for SBM and 1.47 mg/mL for SCM (p < 0.001).

Total phenolic content and SCM and SBM profiles showed significant differences between the two molasses, SCM possessing not only a 6 times higher total phenolic content but also a more complex and different profile (Table 1). Figure 2 shows the UV chromatogram at λ = 280 nm of the SCM and SBM phenolic compounds identified using UV and MS data. Syringic acid, the major phenolic component of SCM, was present in small amount in SBM, whereas vanillin, luteolin/kaempferol, and ferulic acid, the major components of SBM, were absent or present in smaller amounts in SCM.

Biological Protective Activity of Molasses. The biological activity of SBM and SCM was verified using HepG2 cells as model system. In preliminary experiments cells were supplemented with SCM and SBM at 10^{-2}−10^{5} μg/mL medium concentration, and possible cytotoxic effects were assessed by MTT and LDH assays. Neither SCM nor SBM up to the 10^{4} μg/mL medium concentration caused modifications in the tested parameters (data not shown), whereas the highest molasses concentration (10^{5} μg/mL medium) caused cell death.

The microscope observation of 10^{4} μg/mL supplemented cells highlighted appreciable changes in morphology, supplemented cells appearing less in number than US cells, mainly in clusters and with a well-rounded shape. For this reason the 10^{2} and 10^{3} μg/mL medium concentrations were used for supplementation in the following experiments.

To verify the onset of oxidative stress due to H_2O_2 treatment and its possible counteraction by SBM and SCM, intracellular ROS production and TBARS concentration were detected. As reported in Figure 3A, incubation with 0.2 mM H_2O_2 resulted in a significant increase in ROS production in unsupplemented and 10^{2} μg/mL SBM and SCM supplemented cells. Intracellular ROS concentration was unchanged in TC and 10^{3} μg/mL SBM supplemented cells and decreased in 10^{3} μg/mL SCM supplemented ones with respect to nonstressed unsupplemented HepG2. Treatment with H_2O_2 caused a significant increase of TBARS concentration in all tested conditions except for TC and SCM at the highest concentration (Figure 3B).

The strong increase in LDH leakage induced by oxidative stress in US cells was completely prevented by SBM and SCM even at the lower concentration used (Figure 4A). Exposure to H_2O_2 evoked a reduction of cell viability in unsupplemented and 10^{2} SBM supplemented cells, whereas 10^{2} SCM supplementation was slightly protective. TC and the higher SBM and SCM concentrations completely protected HepG2 cells, viability being even higher in molasses-supplemented than in nonstressed US ones (Figure 4B).

As shown in Figure 5A, cytosolic TAC did not change in any of the tested conditions compared to nonstressed US cells; similarly, no modification in GSH content was observed in US cells or in cells supplemented with SBM and SCM at the lowest concentration. On the contrary, TC and the highest SBM and SCM concentrations caused an increase of the antioxidant thiols (Figure 5B).

DISCUSSION

Byproducts of plant food processing represent a major disposal problem for the food industry, but they are also promising sources of compounds that may be used because of their favorable technological or nutritional properties.[1] Special attention has already been paid to agricultural byproducts, such as rice hulls, almond hulls, potato peel waste, olive mill wastewater, grape and citrus seeds and peels, and green-vegetable byproducts, that have been proven to be effective sources of antioxidants.[31,32]

The presence of phytochemicals in sugar is often undesirable, as they influence the quality and color of the final product; hence, these phytochemicals are removed through various purification procedures in the sugar industry.[19] Thus, molasses, the byproduct of sugar refining, is a very good source of

Table 1. SBM and SCM Phenolic Profile[a]

	phenolic compound	RT	$[M - H]^-$	μg/g SCM	μg/g SBM
1	5,7-dihydroxyflavanone	4.6	255	9.71 ± 1.05	
2	catechin	5.0	289	16.42 ± 0.20	
3	4-hydroxyphenylacetic acid	6.9	151	5.83 ± 0.14	
4	dicaffeoylquinic acid glucoside	7.4	677	2.08 ± 0.33	
5	vanillic acid	7.7	167	30.07 ± 0.20	
6	syringic acid	8.5	197	85.53 ± 1.38	2.26 ± 0.07
7	quercetin 3-*O*-glucosyl-xyloxide	9.7	515	25.27 ± 1.94	
8	vanillin	9.9	151		17.41 ± 0.51
9	feruoylquinic acid	10.0	367	5.32 ± 0.06	
10	diferuoylquinic acid	10.4	735	5.23 ± 0.20	
11	tricin 7-*O*-glucoside	10.8	491	16.45 ± 1.02	
12	*p*-coumaric acid	10.9	163	9.18 ± 0.91	
13	apigenin-hexoside-pentoside	11.6	563	53.66 ± 3.02	
14	ferulic acid	12.3	193	6.25 ± 0.63	14.83 ± 0.29
15	hydroxybenzaldehyde	13.4	121		2.93 ± 0.10
16	7-methylapigenin-6-*C*-glucoside	13.8	445	22.28 ± 0.96	
17	hydroxybenzoic acid	13.9	137		1.12 ± 0.11
18	caffeoyl-*O*-malonyl-*O*-coumaroylquinic acid	15.2	585	4.19 ± 0.41	
19	6,8-dihydroxykaempferol	15.8	287	22.35 ± 1.67	
20	tricin-7-*O*-*β*-(6-*p*-methoxycinnamate)-glucoside	16.6	651	15.52 ± 0.38	
21	luteolin/kaempferol	19.8	285		17.24 ± 0.49
22	caffeoylquinic acid	20.0	353	10.45 ± 0.71	
23	feruloyl-arabinose-arabinose	20.3	307	35.99 ± 1.31	4.51 ± 0.47
24	caffeoyltartaric acid	25.8	311		1.95 ± 0.15
	total			381.62 ± 6.82	62.25 ± 1.72

[a]Phenolic compound concentration is expressed in μg analyte/g. Data are the mean ± SD. Student's *t* test was used to determine the statistical differences for peaks 6 ($p < 0.001$), 14 ($p < 0.001$), and 23 ($p < 0.001$).

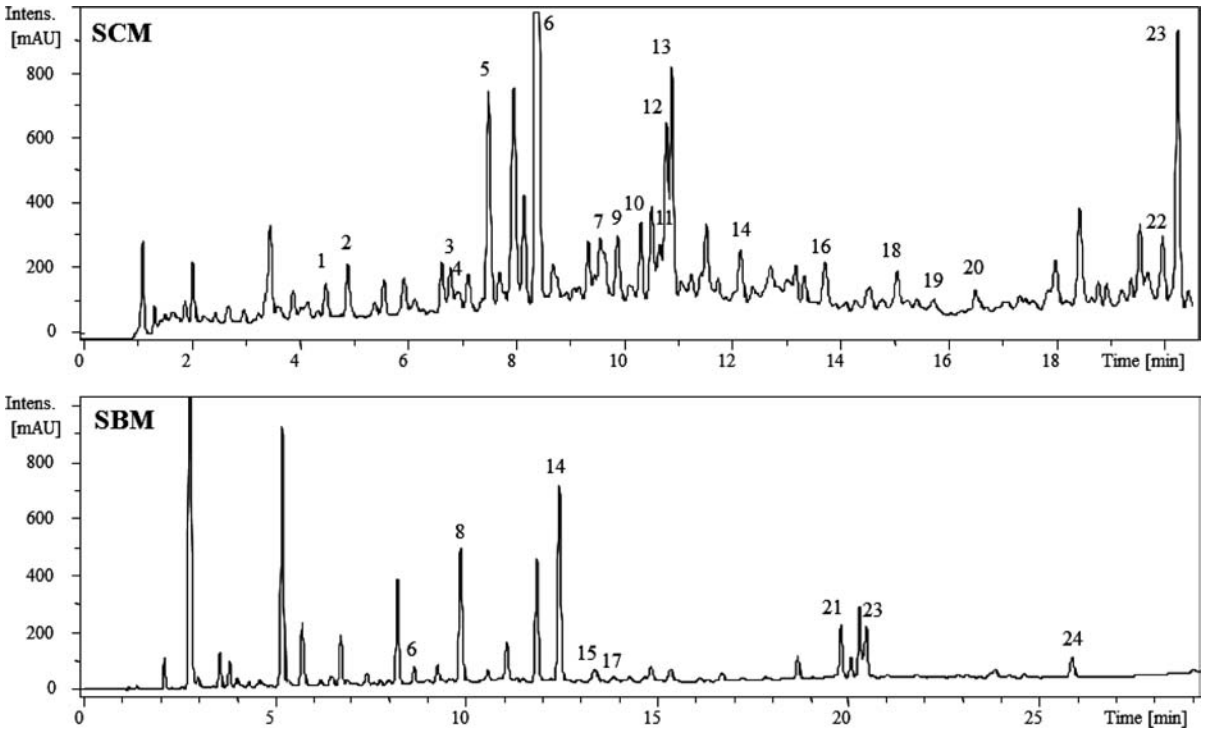

Figure 2. Chromatograms of the phenolic compounds of SCM and SBM at λ = 280 nm. Peaks: 1, 5,7-dihydroxyflavanone; 2, catechin; 3, 4-hydroxyphenylacetic acid; 4, dicaffeoylquinic acid glucoside; 5, vanillic acid; 6, syringic acid; 7, quercetin 3-*O*-glucosyl-xyloside; 8, vanillin; 9, feruoylquinic acid; 10, diferuoylquinic acid; 11, tricin 7-*O*-glucoside; 12, *p*-coumaric acid; 13, apigenin-hexoside-pentoside; 14, ferulic acid; 15, hydroxybenzaldehyde; 16, 7-methylapigenin-6-*C*-glucoside; 17, hydroxybenzoic acid; 18, caffeoyl-*O*-malonyl-*O*-coumaroylquinic acid; 19, 6, 8-dihydroxykaempferol; 20, tricin-7-*O*-*β*-(6-*p*-methoxycinnamate)-glucoside; 21, luteolin/kaempferol; 22, caffeoylquinic acid; 23, feruloyl-arabinose-arabinose; 24, caffeoyltartaric acid.

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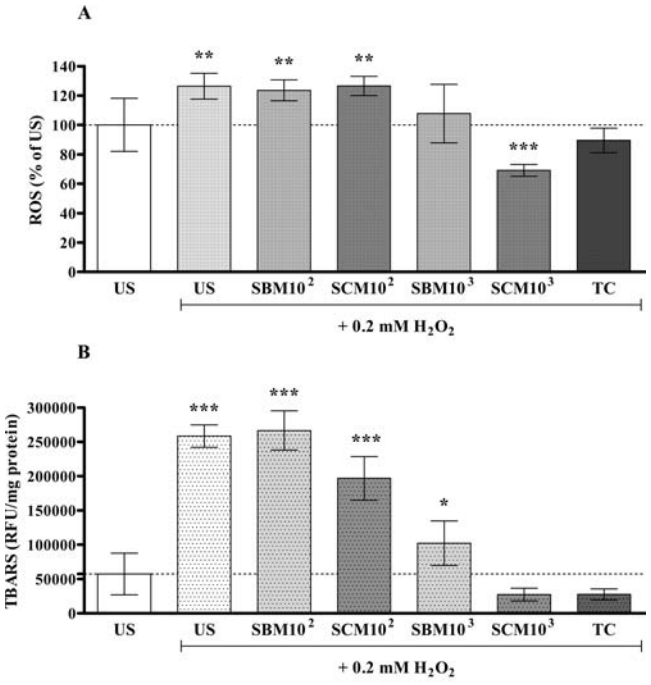

Figure 3. Cellular ROS (A) and TBARS (B) concentrations in unsupplemented and supplemented cells. ROS concentration (A) is expressed as percent of the concentration determined in nonstressed, unsupplemented (US) cells (assigned as 100%). TBARS concentration (B) is expressed as RFU/mg protein in the corresponding well. Data are the mean ± SD. Statistical analysis was by one-way ANOVA ($p < 0.001$) with Dunnett's post hoc test: (*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$ versus nonstressed US cells.

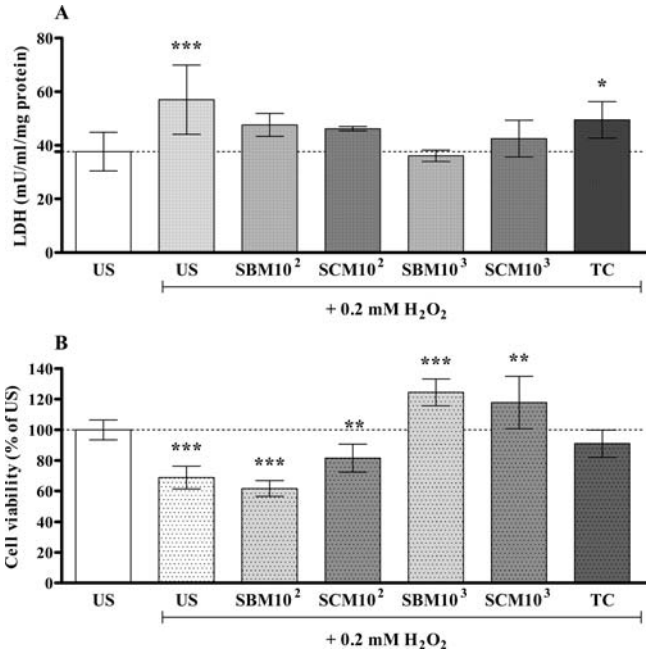

Figure 4. LDH release (A) and cell viability (B) in unsupplemented and supplemented cells. LDH activity in the medium (A) is expressed as mU/mL medium/mg protein in the corresponding well. Cell viability (B) is expressed as percent of nonstressed, unsupplemented (US) cells (assigned as 100%). Data are the mean ± SD. Statistical analysis was by one-way ANOVA ($p < 0.001$) with Dunnett's post hoc test: (*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$ versus nonstressed US cells.

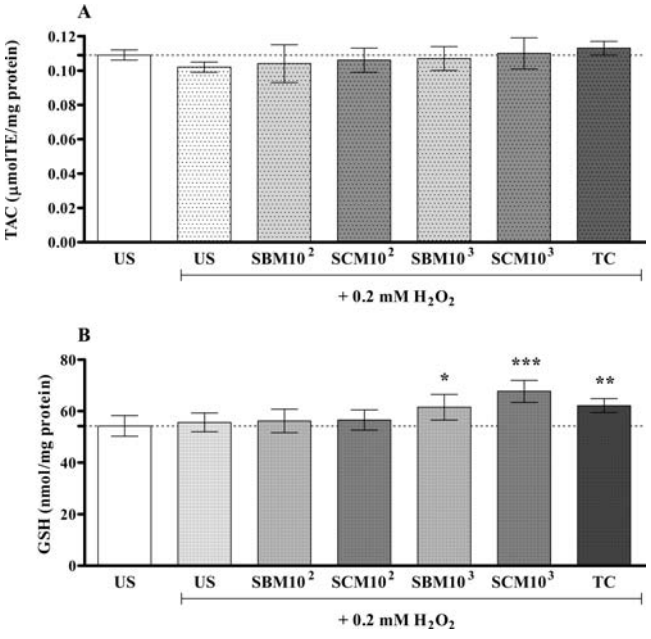

Figure 5. Cytosolic TAC (A) and reduced glutathione (B) concentration in unsupplemented and supplemented cells. Cytosolic TAC (A) is expressed as μmol TE/mg protein in the corresponding well and GSH concentration (B) as nmol/mg protein in the corresponding well. Data are the mean ± SD. Statistical analysis was performed by one-way ANOVA ($p < 0.001$) with Dunnett's post hoc test: (*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$ versus nonstressed US cells.

residual antioxidant components from the plant and of antioxidant molecules formed during the cooking of the juice.[20]

In this study cane and beet molasses were first evaluated in vitro for their antioxidant capacity and compared with other sweeteners. Our results are in accordance with those obtained by Phillips et al.,[20] who reported substantial differences in the TAC of several sweeteners, SCM having the highest one. In our study maple syrup, for which a high content of phenolics has been already reported,[33] showed a quite good TAC, whereas sugar cane had a low TAC. Dissimilarities among brown sugars have been reported[34] and are related to differences in cane varieties, in the maturity of the cane plant at harvest time, in the processing procedures, and mainly in the techniques used to remove color and impurities that affect the amount of volatiles and polyphenols that end up on the surface of the crystal. The observed low TAC of acacia honey is in agreement with Ghedolf and Engeseth,[35] who found a wide range of antioxidant capacity in honey from different sources, acacia honey having the lowest one. The higher TAC of rebaudioside 60% than rebaudioside 98% can be attributed to the lower purity of the former sweetener. TAC of other sweeteners was negligible.

Because the in vitro TAC of foods is only an approximate reflection of their biological protective activity, chemical assays and cell-based methods giving often contradictory results,[36,37] we evaluated the protective effect of SCM and SBM supplementation against an induced oxidative stress in HepG2 cells. α-Tocopherol, a well-known potent antioxidant acting as peroxyl radical scavenger that terminates chain reaction,[38] was used as positive control. In preliminary experiments possible cytotoxicity was evaluated using different SCM and SBM concentrations, and further experiments were performed using the highest molasses concentrations causing no sign of cell toxicity.

The effectiveness in the protection of HepG2 cells from the induced oxidative stress, as indicated by the different markers considered, was greater for SCM than for SBM and dependent on the concentration used: at the highest concentration, SCM protection was equal to or higher than the TC effect.

The higher biological effectiveness of SCM is in agreement with data on in vitro TAC and phenolic composition, which were higher for SCM than for SBM, emphasizing the importance of phenolic concentration and profile for molasses protective action. It is conceivable that molasses antioxidant properties are mainly ascribable to the phenolic content, although other molecules such as Maillard reaction products (MRP) could contribute to the overall effect. Indeed, MRP effective antioxidant protection against oxidizable substrates has already been evidenced in cell culture systems.[17,39,40] This strongly suggests the implication of MRP in the observed protective effects of molasses.

Extensive work has been carried out for the identification and quantification of the major macromolecules (including colorants) in cane and beet sugar processing at all stages.[41−43] In general, the colorants are believed to be produced during Maillard reaction, alkaline degradation reactions, and sugar degradation.[43] Godshall et al.[41] evidenced that beet and cane colorants are fundamentally different: beet colorants tend to be produced during processing, mainly from alkaline degradation of invert and melanoidin formation, whereas cane colorants enter the process in the cane juice as plant pigments associated with polysaccharide and change very little in process. In addition, cane polysaccharides involved in color formation have been shown to be associated with polyphenolic acids.[44] These differences could have contributed to the higher activity of SCM than SBM.

In this study we supplemented cells with the whole molasses, and not with molasses-derived compounds, so it was not possible to define which components were the most protective ones. Although it could appear to be a limitation of our study, to our aim, that is, the evaluation of the possible protective effect of molasses as food/food ingredient, it was the best approach, and it allowed us to consider the synergism between the different antioxidant molecules and the importance of the food matrix, well-recognized factors of the overall antioxidant effectiveness[45] that are ignored in studies evaluating the effect of pure compounds.

Few data are available in the literature on molasses bioactivity in in vivo or ex vivo systems: sugar molasses have been reported to have immunomodulatory activity in human whole blood cell cultures,[46] to raise HDL cholesterol level in rats,[47] and to have inhibitory effects on mutation and nitric oxide production in lipopolysaccharide-stimulated macrophages.[48] To our knowledge this work is the first one evidencing molasses effectiveness in the counteraction of the oxidative damage in cultured cells. We acknowledge that results in whole organisms may diverge from those in the cultured cells due to the bioavailability and metabolism of ingested phytochemical mixtures and agents, and therefore results in the cultured cells could be misleading if taken in isolation. After ingestion, most of the dietary (poly)phenolics appear in the circulatory system not as the parent compounds, but as phase II metabolites.[49] Although in our study the use of human hepatic cells able to metabolize the parent compounds reduced in part the distance between our approach and the physiological situation in humans, further investigations in vivo are needed before conclusions can be drawn. Further in vitro mechanistic studies are also needed to understand how molasses bioactive molecules interact with human physiological and pathological processes, particularly considering that it is becoming clear that the mechanisms of action of polyphenols go beyond the modulation of oxidative stress.[50] Particularly, Guimarães et al.[3] demonstrated cane molasses protection against DNA oxidative damage besides radical scavenging capacity.

Although the variability due to agronomical and technological factors among the different molasses must be taken into account, our results support a greater exploitation of molasses as a food ingredient considering it as a tasty extra source of antioxidants. In this light the broad quality of molasses sources must be carefully considered, because some impurities (particularly plant growth regulators, pesticides, clarification polymers such as polyacrylamide, heavy metals, and plant electrolyte salts) concentrated in the sugar syrups could be present in the magma from which molasses originates.[51] Consequently, the relative quality of the molasses must be assessed before they are marketed to the public.

Besides all of these considerations, data herein reported emphasize the potential health effects of molasses, adding functional properties and nutritional value to a sweetening agent and sustaining its use as a refined sugar substitute. Furthermore, they emphasize the possibility of using by-products for their antioxidant activity. This is particularly important for consumers in developing countries, as it highlights the importance of consuming a low-price, yet very nutritious, commodity.

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Notes

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ABBREVIATIONS USED

ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); BHT, 3,5-di-*tert*-4-butylhydroxytoluene; DCFH, 2′,7′-dichlorofluorescein; DCFH-DA, 2′,7′-dichlorofluorescein diacetate; DMEM, Dulbecco's modified Eagle's medium; DPBS, Dulbecco's phosphate-buffered saline; DPPH, 1,1-diphenyl-2-picrylhydrazyl; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); EBSS, Earle's balanced salt solution; EDTA, ethylenediaminetetraacetic acid; GSH, reduced glutathione; HDL, high-density lipoprotein; HPLC-DAD-ESI-MS, high-performance liquid chromatography with diode array detection coupled to electrospray and mass spectrometry; IC_{50}, inhibitory concentration of 50%; LC, liquid chromatography; LDH, lactate dehydrogenase; MRP, Maillard reaction products; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NADH, reduced β-nicotinamide adenine dinucleotide; RFU, relative fluorescence units; ROS, reactive oxygen species; RPMI, Roswell Park Memorial Institute; SBM, sugar beet

molasses; SCM, sugar cane molasses; SDS, sodium dodecyl sulfate; TAC, total antioxidant capacity; TBA, 2-thiobarbituric acid; TBARS, thiobarbituric acid reactive substances; TC, α-tocopherol; TCA, trichloroacetic acid; TE, trolox equivalent; US, unsupplemented

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