Phenolic composition and antioxidant activity of culms and sugarcane 
(Saccharum officinarum L.) products

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A R T I C L E   I N F O

Article info

Article history:
Received 14 April 2010
Received in revised form 21 July 2010
Accepted 15 September 2010

Keywords:
Sugar cane products
Phenolic compounds
Antioxidant activity
Flavonoids
Tricin
Chlorogenic acid

A B S T R A C T

The present work reports amounts of flavonoids and phenylpropanoids of culms of three sugarcane varieties and of raw juice, syrup, molasses and VHP sugar. The antioxidant activity of those materials was evaluated by the DPPH and β-carotene/linoleic acid methods. The predominant phenolics in culms were phenylpropanoids (caffeic, chlorogenic and coumaric acids), while flavones (apigenin, tricin and luteolin derivatives) appeared in lower amounts. Differences were noted either among phenolic profiles of sugarcane culms or between culms and sugarcane products. The antioxidant activities of solutions from most samples were similar or higher than an 80 μM Trolox solution.

1. Introduction

Sugarcane (Saccharum officinarum L.) is a source not only of sugar and raw material for alcohol production, but also for a variety of other products. Raw juice is a liquid with 14–16 °Brix obtained upon squeezing of the culms. Concentration of the juice to 55–65 °Brix produces sugarcane syrup. Further evaporation up to about 80 °Brix leads to molasses, a dark product almost as thick as honey. “Rapadura” is a hard, dark green food consumed mainly in Brazilian rural areas, obtained by solidification of molasses. It is similar to kokuto, a sugarcane product consumed as candies in Japan. VHP (Very High Polarisation) sugar, a dark brown product, is sold in the sugar market for industrial production of refined, white sugar. VHP processing may lead either to crystal, amorphous or granulated refined sugar (Cosan, 2002). Conventional methods for sugar production include thermal and chemical treatment of the juice, syrup and molasses, aiming to increase the sucrose concentration and pigment removal.

Pigments present in sugarcane juice are mainly phenolic compounds. Paton and Duong (1992) reported the phenolic composition of sugarcane and its products. Such compounds are mainly phenylpropanoids and flavonoids, major representatives of the latter being derivatives of naringenin, tricin, apigenin and luteolin (Smith & Paton, 1986; Williams, Harborne, & Clifford, 1974). Nakasone, Takara, Wada, Tanaka, and Yogi (1996) isolated five antioxidant compounds from a kokuto extract; Takara et al. (2002) increased the number to thirteen, including several glycosylated phenolic compounds. Some compounds showed antioxidant activity higher than α-tocopherol. Payet, Cheong, and Smadja (2005, 2006) reported antioxidant activity by different samples of brown sugar and suggested that some phenolic acids and flavonoids may account for at least part of the observed activity. Phenolic substances in sugarcane juice may exert biological activities (Duarte-Almeida, Vidal Novoa, Fallarerino Linares, Lajolo, & Genovese, 2006). An acylated tricin glycoside isolated from sugarcane juice was shown to have antiproliferative activity (Duarte-Almeida, Negri, Salatino, Carvalho, & Lajolo, 2007).

The present work aims to analyse the phenolic composition of culms of three varieties of sugarcane and products obtained during sugar production, as well as the corresponding antioxidant activities.

2. Materials and methods

2.1. Materials

Samples of sugarcane culms and sugarcane products from progressive stages of sugar production were obtained from Cosan (Piracicaba, Brazil, a manufacturer enterprise of sugarcane products). Sugarcane culms analysed were of the varieties SP801842...
Sugar products analysed were raw juice (RJ), syrup (SY), molasse (MO) and VHP sugar.

2.2. Chemicals

All chemicals used were reagent or HPLC grade. DPPH (2,2-diphenyl-1-picrylhydrazyl free radical), Trolox, Tween 40, β-carotene, linoleic acid, apigenin, luteolin, and coumaric acids were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO). Caffeic, ferulic and chlorogenic acids were purchased from Apin Chemicals Ltd. (Abingdon, UK). Tricin was isolated as described elsewhere (Duarte-Almeida et al., 2007).

2.3. Extraction of phenolic substances

Extraction was performed in triplicate according to Duarte-Almeida et al. (2007), with slight modifications. Solid samples were thoroughly homogenised by powdering with liquid nitrogen. Duplicate samples of the fresh powder (20 g) were extracted three times with methanol/water 70:30, including the water conveyed by the samples, at speed five on a Brinkmann homogenizer (Polytron-Kinematica GmbH, Kriens-Luzern, Sweden) for 1 min in an ice bath. The homogenate was filtered under reduced pressure through filter paper (Whatman No. 6). Other liquid samples were extracted with methanol/water 70:30, including the volume of water contained in the sample, at speed five for 1 min, as above. The solvent of the extracts was evaporated almost to dryness at 40 °C under reduced pressure on a Rotavapor RE 120 (Büchi, Flawil, Sweden). The concentrated extracts were diluted with water to the final volume of 25 ml.

2.4. Solid-phase extraction

Purification of extracts, aiming to maximise phenolic contents, was carried out by fractionation of 10 ml aliquots of the extracts on polyamide columns (CC 6, Macherey–Nagel Gmbh and Co., Duren, Germany), soaked previously with 20 ml methanol and 60 ml water. The columns were washed with water (20 ml) and eluted with methanol (50 ml), followed by methanol/ammonia (99.5:0.5 v/v, 50 ml). Each eluate was evaporated to dryness under reduced pressure at 40 °C, resuspended in 1 ml methanol and filtered through a 0.22 μm PTFE filter (Millipore Ltd., Bedford, MA) prior to HPLC analysis and evaluation of antioxidant activities.

2.5. Analytical HPLC

Identification and quantification of phenolic substances in the eluates were carried out in duplicates, using analytical reversed-phase HPLC on an Agilent 1100 system with autosampler and quaternary pump coupled to a diode array detector. The column used was a Prodigy 5 μ ODS3 reversed phase silica (250 × 4.6 mm i.d., Phenomenex Ltd.) and elution solvents were: A, water:tetrahydrofuran:trifluoroacetic acid 98:2:0.1 and B, acetonitrile. Gradient used was based on Duarte-Almeida et al. (2007). Identification followed comparison of UV spectra and retention times with authentic standards, and quantification based on external calibration. Standards used for phenylpropanoid analyses were caffeic, coumaric, ferulic and chlorogenic acids, and for flavones, tricin, luteolin and apigenin. Results were expressed as mg/100 g of sample.

2.6. Antioxidant activity

Care was taken to avoid the influence of differences in phenolic concentrations among samples. After solid-phase extraction, all

<table>
<thead>
<tr>
<th>Phenoic compounds contents of sugarcane culm varieties determined by HPLC-DAD.</th>
<th>Total flavonoids</th>
<th>Total cinnamic acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apigenin</td>
<td>Luteolin</td>
<td>Tricin</td>
</tr>
<tr>
<td>V1</td>
<td>0.56 ± 0.01</td>
<td>0.67 ± 0.00</td>
</tr>
<tr>
<td>V2</td>
<td>0.26 ± 0.00</td>
<td>0.51 ± 0.00</td>
</tr>
<tr>
<td>V3</td>
<td>0.60 ± 0.00</td>
<td>0.63 ± 0.00</td>
</tr>
<tr>
<td>Obs: V1, SP801842; V2, SP813250; V3, RB855486; nd, Not detected. A Data are expressed in mg/100 g as means ± SD from triplicate. b Values with different superscript letters within the same columns are significantly different (p &lt; 0.05; one-way ANOVA and Newman Keuls test).</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
samples were diluted to the concentration of 100 μM of chlorogenic acid, based on estimates by HPLC analyses (Tables 1 and 2).

### 2.6.1. Radical scavenging activity (RSA)

The method determines the activity of substances at scavenging free radicals (Brand-Williams, Cuvelier, & Berset, 1995). Analyses were carried out using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method (Brand-Williams et al., 1995; Laskar, Sk, Roy, & Begum, 2010). Solutions of samples in methanol were individually added to 0.1 mM DPPH in methanol. The mixture was incubated in the dark at 25°C for 30 min (Duarte-Almeida, Santos, Genovese, & Lajolo, 2006). Scavenging capacity was determined by monitoring the absorbance at 517 nm. Lower absorbance indicates higher free radical scavenging activities. The RSA percent was calculated as follows:

$$\% \text{RSA} = \frac{a_{\text{control}} - a_{\text{sample}}}{a_{\text{control}}} \times 100$$

where $a_{\text{control}}$ is the absorbance of the control (methanol plus DPPH), and $a_{\text{sample}}$ is the absorbance of the sample plus DPPH. A Trolox 80 μM methanol solution was used as reference for comparison of relative RSA efficiency of the samples analysed. All assays were run in five replicates.

### 2.6.2. β-Carotene/linoleic acid method (β-CLAM)

The method evaluates the antioxidant activity at inhibiting the discoloration of β-carotene caused by free radicals yielded during peroxidation of linoleic acid (Yanishilieva & Marinova, 1995). The procedure used was based on Duarte-Almeida et al. (2006). Reactant solutions were prepared mixing 25 μl of 2 mg/ml chloroform solution of β-carotene with 200 mg Tween 40 and 0.5 ml chloroform. Chloroform was then completely evaporated under nitrogen flow, and 25 ml distilled water saturated with oxygen was added to the mixture. The absorbance was adjusted with water to 0.6. For the oxidation reaction, 10 μl of the solutions of concentrated extracts were mixed with 250 μl of the β-carotene/linoleic acid solution in a microplate. The samples were submitted to autoxidation at 45°C for 120 min. The absorbance at 470 nm was measured at time zero and at 15 min intervals using a microplate spectrophotometer (Benchmark Plus, Bio-Rad). Methanol was used as control and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as reference antioxidant. All assays were run in five replicates. Antioxidant activity of the sample was calculated as follows:

$$\text{Antioxidant Activity} = \frac{A_{0} - A_{t}}{A_{0}} \times 100$$

where $A_{0}$ is the absorbance at time zero and $A_{t}$ is the absorbance at time $t$. Values of antioxidant activity were compared using one-way ANOVA, and differences between means were considered significant at the 5% level.

### Table 2

Phenolic compounds content of the sugarcane products obtained by HPLC-DAD.

<table>
<thead>
<tr>
<th>Flavonoids</th>
<th>Luteolin</th>
<th>Tricin</th>
<th>Caffeic</th>
<th>Chlorogenic</th>
<th>Coumaric</th>
<th>Ferulic</th>
<th>Total flavonoids</th>
<th>Total cinnamic acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apigenin</td>
<td>1.73 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.27 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.59 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>nd</td>
<td>nd</td>
<td>0.90 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.28 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.60 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>RJm</td>
<td>nd</td>
<td>nd</td>
<td>1.87 ± 0.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>nd</td>
<td>0.51 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>nd</td>
<td>nd</td>
<td>1.87 ± 0.48&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SYm</td>
<td>2.31 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.05 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.15 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>nd</td>
<td>2.93 ± 0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.55 ± 1.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.41 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.51 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SYa</td>
<td>nd</td>
<td>nd</td>
<td>3.53 ± 0.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>nd</td>
<td>0.77 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>nd</td>
<td>nd</td>
<td>3.53 ± 0.22&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MOm</td>
<td>16.82 ± 0.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.35 ± 0.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.23 ± 1.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>nd</td>
<td>24.29 ± 1.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.43 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.88 ± 1.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.40 ± 0.50&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MOa</td>
<td>nd</td>
<td>nd</td>
<td>26.16 ± 2.38&lt;sup&gt;f&lt;/sup&gt;</td>
<td>nd</td>
<td>3.29 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>nd</td>
<td>nd</td>
<td>26.16 ± 2.38&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>VHPm</td>
<td>0.71 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.42 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.31 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>nd</td>
<td>0.57 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.38 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.13 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.43 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>VHPa</td>
<td>nd</td>
<td>nd</td>
<td>0.46 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.46 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Obs: RJ, raw juice; SY, syrup; MO, molasses; VHP, VHP sugar; nd, Not detected.

<sup>A</sup> Data are expressed in mg/100 g as means ± SD from triplicate.

<sup>a,b,c,d,e,f</sup> Values with different superscript letters within the same columns are significantly different ($p < 0.05$; one-way ANOVA and Newman Keuls test).
lated as percent inhibition of oxidation versus control, using the equation

\[
\% \text{Inhibition} = 100 \times \left(1 - \frac{a_{\text{sample}}}{a_{\text{control}}} \right)
\]

where \(a_{\text{sample}}\) and \(a_{\text{control}}\) are the absorbances of sample and control at \(t = 0\) min and \(t = 120\) min, respectively.

2.7. Statistical analysis

Results are expressed as means ± standard deviation. The statistical analyses were carried out using Statistica version 5.0 (StatSoft Inc., Tulsa, OK). Comparison of means of triplicate determinations, using a significance level \(p < 0.05\), was performed by one way analysis of variance (ANOVA). When significant differences were detected, Newman-Keuls post hoc test was used to determine the degree of statistical significance of the differences.

3. Results and discussion

3.1. Phenolic profiles of sugarcane culms and products

In the present paper, flavonoids, irrespective of being aglycones or glycosides, are referred to by the respective aglycon. Thus, all apigenin derivatives are designated “apigenin”. Although comprising several isomers, all chlorogenic acids are designated “chlorogenic acid”. Derivatives of caffeic and ferulic acids, conjugated or not, are designated “caffeic” and “ferulic acid”, respectively. The same applies to methoxyxycumaric acid derivatives, irrespective of being the \(o\)-, \(m\)- or \(p\)-isomer and being conjugated or not.

Most phenylpropanoids and flavones were obtained in the solid phase by methanol elution. Tricin was eluted mostly with methanol and methanol/ammonium hydroxide. Fig. 1 contains chromatograms of sugarcane products containing chlorogenic acid at 100 \(\mu\)M concentration.

An increase of total solids implies higher contents of phenolic substances: SY has more phenolic substances than RJ (\(p = 0.0017\)) and MO has the highest values. At the latter phase, chlorogenic acid becomes the predominant phenylpropanoid (Table 2). An inverse relationship between browning and chlorogenic acid, some neutral phenolic compounds and luteolin derivatives contents was previously observed (Paton & Duong, 1992). This finding suggests that these compounds participate in the browning process, undergoing enzymatic and oxidative reactions (Bucheli & Robinson, 1995). Chlorogenic acid still predominates among phenylpropanoids in VHP (Table 2). Results of Table 1 disagree with the data of Payet, Cheong, and Smadja (2005), who reported neither chlorogenic nor flavones in their analyses of seven samples of brown sugar.

Predominance of tricin is maintained throughout the sugar processing (Table 2). Tricin derivatives are relatively rare in nature (Cai, Steward, & Gescher, 2005; Harborne & Hall, 1964; Hudson, Dinh, Kokubun, Simmonds, & Gescher, 2000) and is probably related to resistance of plants to several illnesses (Harborne & Hall, 1964; McGhie, 1993).

3.2. Antioxidant activity

3.2.1. RSA

Some eluates from sugarcane products presented activity higher than Trolox 80 \(\mu\)M (Table 3). SYa had the highest activity (57.8% of DPPH reduction), 74% over Trolox. Other products stood out, such as MOa (45.2%) and RJb (38.4%) (Table 3), both with values higher than Trolox (\(p < 0.01\)). Activities of eluates RJm, SYm, VHPm and VHPm were not distinct from Trolox. Except for VHP, \(a\) eluates had RSA higher than \(m\) eluates from the same product (Table 3) (\(p < 0.05\)). Eluates of \(a\) of RJ, MO and SY were 35%, 89% and 80% higher, respectively, than the \(m\) counterparts. No significant difference was observed between the activities of VHPm and VHPm eluates.

3.2.2. \(\beta\)-Carotene/linoleic acid method (\(\beta\)-CLAM)

Except for RJ, antioxidant activity of eluates and Trolox varied in the range 72–85% (Table 3). Contrary to results of RSA, differences between \(a\) and \(m\) eluates were not the rule. Activities of eluates RJ and RJm were significantly distinct; however, higher activity corresponded to the \(m\) eluate (\(a\) eluates were more active by RSA, Table 3). Activities of VHP eluates were low by RSA, but by \(\beta\)-CLAM were the highest, being the only samples exceeding Trolox activity (Table 3).

Antioxidant activity depends on many factors, such as contents of phenolic compounds and structural details (Moure et al., 2001).
Phenolic substances with o-dihydroxy systems tend to be highly active (Cuyckens & Clayes, 2004; Hopia & Heinonen, 1999).

Often no correlation is observed between results by RSA and β-CLAM (Tsao, Yang, Xie, Sockovie, & Khanizadeh, 2005). RAS is more selective toward hydrophilic compounds, while β-CLAM reflects contents of lipophilic substances (Sahreen, Khan, & Khan, 2010; Xu, Tang, Liu, Li, & Dai, 2010). For these reasons, conclusions based only on one method may lead to underestimation of antioxidant activities. Hence it is prudent to evaluate antioxidant activities at least by two different methods (Tsao et al., 2005; Verhagen et al., 2003).

4. Conclusions

Sugarcane products are appreciated as food in social-economically disadvantaged areas of Brazil and other countries. Results of the present work indicate that consumption of these products should be encouraged. Some of them, such as “rapadura”, are cheap sources of antioxidant substances, vitamins (A, C and D) and minerals (Fe, Ca, P, K, Mg) (Bernal, Guzman, & Jimenez, 2004). These facts lend support to programs, now in course, aiming to stimulate or reintroduce the use of “rapadura” as complement food for children in elementary school.

Acknowledgements

The authors thank CNPq (Conselho Nacional para o Desenvolvimento Científico e Tecnológico) and FAPESP (Fundação de Amparo à Pesquisa de São Paulo) for financial support.

References