

# Precipitation of nonsugars as a model of color reduction in sugarcane juice (*Saccharum* spp.) submitted to the hydrogen peroxide clarification of the crystal sugar process

Nathália Torres Corrêa Magri<sup>1</sup>  | Juliana Aparecida de Souza Sartori<sup>1</sup>  |  
José Luiz Paz Jara<sup>2</sup> | Marcos Nogueira Eberlin<sup>2,3</sup> | Claudio Lima de Aguiar<sup>1</sup> 

<sup>1</sup>Hugot Sugar Technology Laboratory, Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba, Brazil

<sup>2</sup>Thomson Mass Spectrometry Laboratory, Institute of Chemistry, University of Campinas, Campinas, Brazil

<sup>3</sup>School of Engineering, Mackenzie Presbyterian University São Paulo, São Paulo, Brazil

## Correspondence

Claudio Lima de Aguiar, Hugot Sugar Technology Laboratory, Luiz de Queiroz College of Agriculture, University of São Paulo, Pádua Dias Avenue, 11, Piracicaba, SP, Brazil.  
Email: claguair@usp.br

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## Abstract

Proteins, waxes, lipids, minerals, colloids, and pigments are known as nonsugars in sugarcane and their precipitation has been associated with ICUMSA color reduction. This work aimed to evaluate the effect of three hydrogen peroxide doses (1,000; 5,000; and 10,000 mg/L), after 1 hr, on the ICUMSA color, turbidity, and total soluble solids (Brix) and by gas chromatography-mass spectrometry the composition of the chemical compounds presents in precipitates formed by the action of hydrogen peroxide and in the sugarcane wax was identified. Reductions occurred in ICUMSA color for the three doses (39.7%, 48.3%, and 60.7%, respectively) and in the Brix (63.9%, 65.2%, and 67.1%, respectively). For turbidity, the dosage of 1,000 mg/L showed an increase (3.7%), while the others reduced (7.3% and 13.3%, respectively). By gas chromatography-mass spectrometry, 32 compounds were identified, 10 of them were present in all the samples and the major class components were hydrocarbons and fatty acids.

## Practical applications

In this work, we studied the potential application of hydrogen peroxide as an alternative of the sulfitation process, which it has been associated with health disturbs.

## 1 | INTRODUCTION

The conventional clarification process of sugarcane juice, using sulfite and liming, for the white crystal sugar production is based on burning sulfur (powder, 95% purity) and reaction of gas produced with juice water ( $S_{(s)} + O_{2(atm)} + \text{heat} \rightarrow SO_{2(gas)} + H_2O + \text{heat} \rightarrow H_2SO_3 \rightarrow H^+ + HSO_3^- \rightarrow 2 H^+ + SO_3^{2-}$ ). The use of sulfite ( $SO_3^{2-}$ ) as the clarifying agent reduces the sugarcane juice color (Gharib-Bibalan, Keramat, & Hamdami, 2017; Rein, 2007; Sartori, Ribeiro, et al., 2017). However, studies show that one in 100 people in the world is sensitive to sulfite. In addition, anyone can develop sensitivity to sulfite throughout life (Grotheer, Marshall, & Simonne, 2005; Lester, 1995; Papazian, 1998). Timbo, Koehler, Wolyniak, and Klontz (2004) state that 1% to 10% of people with asthma worldwide are sensitive to sulfite. Mane, Phadnis, and Jadhav (1992) report that

sulfite is corrosive to equipment and polluting, when released into the atmosphere.

The sugarcane juice is composed of soluble solids in the form of organic nonsugars, such as protein, starch, gums, and waxes and in other components such as colorants, color precursors, etc. (Saha, Balakrishnan, & Ulbricht, 2006). Pigments that originate from sugarcane are chlorophylls, anthocyanins, and polyphenols (Godshall & Grimm, 1994; Harborne, 1984; Paton, 1992) during the process, such as compounds from Maillard reactions and caramelization (Hayman, 2014). Flavonoids, polyphenols, and organic acids that contribute to the dark brown color of the juice are usually reported to be responsible for the color in the sugarcane juice (Campiol, Magri, Sartori, Ogando, & de Aguiar, 2019; Laksameethanasana, Somla, Janprem, & Phochuen, 2012; Lima et al., 2016; Mandro et al., 2015; Sartori, Angolini, Eberlin, & de Aguiar, 2017; Sartori, Magri, & de Aguiar, 2015; Sartori, Ribeiro,

et al., 2017; Silva, Sartori, & de Aguiar, 2015). Chandra, Bharagava, and Rai (2008) reported that melanoidins might be discolored by the action of reactive oxygen species (ROS), such as hydrogen peroxide. Our research group has studied some alternatives for sulfite ( $\text{SO}_3^{2-}$ ) in the sugarcane juice treatment. Sartori Angolini, et al. (2017) and Silva et al. (2015) reported that ozone ( $\text{O}_3$ ) reduced the ICUMSA color in the raw cane juice, while Lima et al. (2016) reported a reduction in the ICUMSA color with electron beam (20 kGy) by 49% and gamma radiation (20 kGy) by 30%. Some alternatives have been studied to replace sulfite in the food industry, especially in the white sugar production.

Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) that has a high reduction potential (1.77 V) is highly employed in bleaching processes in the textile industries and in paper and cellulose. In a reaction medium, the molecule  $\text{H}_2\text{O}_2$  is converted to hydroxyl radicals ( $\cdot\text{OH}$ ) which also have a high reduction potential (2.80 V), being inferior only to fluorine (3.0 V) and reacts with most organic compounds (Kong, Watts, & Choi, 1998; Mattos, Shiraishi, Braz, & Fernandes, 2003). The radicals  $\cdot\text{OH}$ , due to its high reduction potential, can promote the degradation of several pollutants in reduced times (Mattos et al., 2003). The decomposition of  $\text{H}_2\text{O}_2$  releases molecular oxygen and heat. When present in dilute solutions, the heat ends up being absorbed by the water present in the medium, whereas, if the solution is more concentrated, the heat increases the temperature of the medium and accelerates the rate of the decomposition of the reagent. In combination,  $\text{H}_2\text{O}_2$  can be used in flocculation and precipitation procedures (Mattos et al., 2003).

Hydrogen peroxide has been used as an alternative to sulfitation of sugarcane juice. According to Mane, Phadnis, Jambhale, and Yewale (2000), the reduction of ICUMSA color in the sugarcane juice with hydrogen peroxide promotes the color stabilization of stored sugar for long periods. However, the ICUMSA color reduction of sugarcane juice with  $\text{H}_2\text{O}_2$  is associated with turbidity reduction, that is, there is a precipitation of nonsugars including pigments, not necessarily a color reduction by the degradation of sugarcane juice pigments according to Sartori, Magri, et al. (2015), Sartori, Galaverna, et al. (2015), Sartori, Ribeiro, et al. (2017), Mandro et al. (2015), Mandro, Magri, Sartori, and de Aguiar (2017), and Campiol et al. (2019).

The precipitates formed during the treatment with  $\text{H}_2\text{O}_2$  may be associated with the reduction of color in the sugarcane juice. This work aimed to evaluate the quality of the sugarcane juice treated with  $\text{H}_2\text{O}_2$ , through the analyzes of turbidity, °Brix and ICUMSA

color identify the compounds present in the precipitates of sugarcane juice and in the sugarcane peel extract using gas chromatography-mass spectrometry (GCMS).

## 2 | MATERIAL AND METHODS

### 2.1 | Plant material and preparation of sugarcane juice

Healthy and mature sugarcane plants were collected at the municipality of Iracemópolis, São Paulo State, Brazil (lat 22°34'50" S, long 47°31'07" W and altitude 608 m). The plants were cleaned and milled for juice extraction. The juice was filtered (200 mesh) to remove insoluble impurities (soil, sand, fibers, etc.) and stored in polypropylene flasks at  $-18 \pm 2^\circ\text{C}$  until analyses.

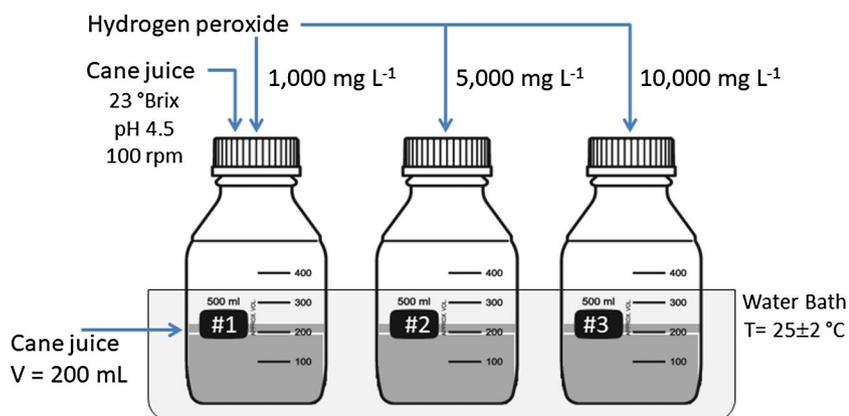
### 2.2 | Treatment of sugarcane juice with hydrogen peroxide

In three borosilicate flasks (total volume = 500 ml; Figure 1), 200 ml of sugarcane juice at a pH 4.5 and 23 °Brix was packed separately at three different doses of commercial solution of hydrogen peroxide at 35% (w/w), that is, 1,000; 5,000; and 10,000 mg/L. Sugarcane juice with hydrogen peroxide was kept under constant stirring at 100 rpm for 1 hr at room temperature. Immediately after the time of treatment, 50  $\mu\text{l}$  of 0.1 g/L catalase solution (bovine liver, 2,000 – 5,000  $\text{mg}^{-1}$  protein units; Sigma-Aldrich) was added to stop the action of hydrogen peroxide.

The samples were centrifuged at 3,000 g for 10 min and the supernatant and precipitate from each sample were stored separately at  $-18^\circ\text{C}$  until analysis in precipitates by gas chromatography coupled to mass spectrometry (GCMS) and color turbidity and Brix in the liquid fraction.

### 2.3 | Waxes from the sugarcane peel and precipitates from the juice treated with hydrogen peroxide

For the analysis of compounds in the sugarcane peel, sugarcane stalks were scraped on the surface and then hexanes were added to the solid material at 1:50 ratio (g solid/mL hexane). Likewise, the same amount of hexane was added to the precipitates followed



**FIGURE 1** Schematic illustration of the sugarcane juice treatment by hydrogen peroxide at different doses

by centrifugation at 3,300 g at 5°C. Residual hexane was removed under heating for 10 min at 105°C in spencer stove and samples were stored after drying until analysis.

## 2.4 | Characterization of sugarcane juice

Fractions of sugarcane juice supernatant were analyzed, in triplicate, for the ICUMSA color according to the official method G.S. 2/3-9 (ICUMSA, 2011), turbidity (Copersucar, 2001), Brix by ABNT NBR 16223 (Consecana, 2015) before ( $t = 0$  min) and after the treatment ( $t = 60$  min) with hydrogen peroxide.

The ICUMSA color was determined by the preliminary dilution of samples up to 1.25 °Brix (i.e., % of total soluble solids in the sugarcane juice). Samples were filtered through a membrane  $\phi = 0.45 \mu\text{m}$  under vacuum (Sartorius Stedim Biotech, Germany). Then, the pH was corrected to  $7.0 \pm 0.05$ . The spectrophotometric analysis was carried out at 420 nm using a UV-Mini 1,240 spectrophotometer (Shimadzu Co., Japan) and the ICUMSA color was expressed by:

$$\text{Icumsa color (420nm)} = \left[ \frac{\text{ABS} \times 1000}{\left( \text{density} \times \left( \frac{\text{Brix}}{100} \right) \right)} \right]$$

$$\text{Density} = 1 + \left( \left( \text{Brix0} \times \frac{200 + \text{Brixc}}{5400} \right) \times \left( \frac{\text{Brixc}}{\text{Brix0}} \right) \right)$$

where ABS: sample absorbance read at 420 nm; Brix: total soluble solids of the diluted sample ( $=1.25$ , if the value is slightly different, it must be indicated) of sugarcane juice; Brix0: reading of soluble solids in the original sample; BrixC: reading of soluble solids in the diluted sample with pH adjusted to  $7.0 \pm 0.05$ .

Turbidity in the sugarcane juice was determined using a TB-1000 digital turbidimeter (Tecnonon Co., Brazil) and soluble solid contents (Brix) were determined using a digital refractometer (Bellingham-Stanley Ltd., UK). The equipment were calibrated prior to the analysis following the manufacturer's instructions.

## 2.5 | Statistical analysis

Statistical analysis was performed using the software R (R Core Team, 2015). The results were analyzed by the  $F$  test of the analysis of variance and the averages of turbidity, °Brix, and ICUMSA color were compared with the Tukey multiple comparison test. Significance was defined at  $p < 0.05$ .

## 2.6 | Analysis of precipitates and peel waxes by GCMS

Hexanic extracts from juice precipitates and waxes from the sugarcane peel were prepared according to Metcalfe, Schmitz, and Pelka (1966). Approximately, 150 mg of sample was mixed with 4.0 ml of NaOH in 0.50 mol/L of methanol and heated in a water bath at 100°C for 5 min. Then, 5.0 ml of 12%  $\text{BF}_3$  in methanol was added and

the mixture was heated at 60°C for 2 min. After cooling the sample to room temperature, 5.0 ml of a saturated NaCl was slowly added to the samples, in a separatory funnel. Hexane of 20.0 ml was added to the mixture and the funnel was vigorously stirred for 1 min and kept for the phase separation. The organic phase was filtered and packed in a volumetric flask. The solvent was evaporated at 60°C in the water bath and the residual solvent was removed through the nitrogen flow at room temperature. Methyl esters were solubilized using *n*-heptane before the injection in gas chromatography coupled with mass spectrometer. The analyses were performed on a GC-MS Agilent system, composed of a gas chromatography (7890A) coupled to a quadrupole mass spectrometer (MSD 5975C) operating in the electronic ionization mode (EI) (70 eV), as described by Bataglion et al. (2015).

The column was an Agilent J&W DB-23 (60 m  $\times$  0.25 mm I.D., 0.15  $\mu\text{m}$  film thickness), specific for the separation of fatty acid methyl esters (FAMES). Helium was the entrainment gas with 1 ml/min flow. Temperatures of the injector, the GCMS interface, and the MSD source were 275, 310, and 230°C, respectively. To obtain the optimum oven temperature, the following sequence was used: initial temperature 150°C was held for 2 min, increased by 5°C/min to 300°C, and held for 10 min. In order to protect the ion multiplier from the MS saturation, the collection of solvent ions was delayed by 5 min. MS was operated in the full scan mode with an  $m/z$  range from 50 to 550. Aliquots of 1  $\mu\text{l}$  of the samples were injected in the splitless injection mode. The compounds were identified with aid of NIST and Willey libraries attached to the GCMS equipment.

## 3 | RESULTS AND DISCUSSION

In this work,  $\text{H}_2\text{O}_2$  was studied at three doses (1,000; 5,000; and 10,000 mg/L) and all the doses were responsible for reducing the Brix values (63.9%, 65.2%, and 67.1%, respectively) and the ICUMSA color of the juices (39.7%, 48.3%, and 60.7%, respectively) (Table 1). Both reductions can be justified by the formation of precipitates (supplementary material) which are formed by the action of hydrogen peroxide. The formation of precipitates by the action of hydrogen peroxide had already been reported in the literature to uranium (Cahill & Burkhart, 1990; Djogic, Cuculić, & Branica, 2005; Hopkins & Golding, 1987; Kim et al., 2011; Mojica-Rodríguez et al., 2015; Shabbir & Tame, 1974), and PU(IV) (Nikonov, Tananaev, & Myasoedov, 2010). Nikonov et al. (2010) reported that soon after the addition of excess  $\text{H}_2\text{O}_2$  a change occurred in the coloration of the solution until discoloration and the formation of a green precipitate occurred.

Previously for the sugarcane juice precipitation, our group studied doses, between 1,000 and 10,000 mg/L of hydrogen peroxide that were able to reduce the sugarcane juice color (Sartori, Galaverna, et al., 2015). This work defines a milestone in the study of the sulfur-free treatment of sugarcane juice, showing that there is no color reduction due to the degradation of pigments (Mane et al., 1992), but there is a decantation of nonsugars, including sugarcane

**TABLE 1** Characterization of sugarcane juice treated with 1,000, 5,000, and 10,000 mg/L of hydrogen peroxide

Variables	Doses of hydrogen peroxide					
	1,000 mg/L		5,000 mg/L		10,000 mg/L	
	0 min	60 min	0 min	60 min	0 min	60 min
Turbidity <sup>†</sup>	1,000 ± 0 <sup>a</sup>	1,037.3 ± 1.15 <sup>b</sup>	1,000 ± 0 <sup>a</sup>	927 ± 3 <sup>b</sup>	1,000 ± 0 <sup>a</sup>	867.3 ± 2.89 <sup>b</sup>
Brix	15.8 ± 0 <sup>a</sup>	15.7 ± 0 <sup>b</sup>	15.8 ± 0 <sup>a</sup>	15.5 ± 0 <sup>b</sup>	15.8 ± 0 <sup>a</sup>	15.2 ± 0 <sup>b</sup>
ICUMSA color <sup>‡</sup>	15,155.4 ± 50.2 <sup>a</sup>	9,142.7 ± 0 <sup>b</sup>	14,968.4 ± 912 <sup>a</sup>	7,740.4 ± 93.7 <sup>b</sup>	16,627.5 ± 100.5 <sup>a</sup>	6,537.3 ± 301.5 <sup>b</sup>

Note: Averages followed by the same letter in the row within the same treatment do not differ significantly from each other. The Tukey's test was analyzed at 5% for each of the treatments (1,000; 5,000; and 10,000 mg/L) separately.

<sup>†</sup>Turbidity was expressed by nephelometric turbidity units (NTUs) directly from the equipment.

<sup>‡</sup>Color was expressed by ICUMSA units (IUs) at 420 nm.

pigments, from the reaction with hydrogen peroxide, thus promoting the formation of a precipitate in the juice.

Since there was a reduction in the number of soluble solids and ICUMSA color of the juice, it was expected that there would be the same tendency in juice turbidity. The turbidity was reduced only in the treatment between 5,000 and 10,000 mg/L (7.3% and 13.3%, respectively), while in the treatment with 1,000 mg/L H<sub>2</sub>O<sub>2</sub> had an increase (3.7%) after 60 min (Table 1). The turbidity of a solution is the reduction in its transparency due to the presence of the suspended material (Caldas, 2005), i.e., the greater the amount of suspended material the higher the turbidity index of a sample. In case of treatment with 1,000 mg/L the low concentration of hydrogen peroxide used may not have been sufficient to precipitate all the suspended material. The reduction of color and soluble solids is directly linked to the increase in the doses of hydrogen peroxide used, as reported by Sartori, Galaverna, et al. (2015), since higher the dose of hydrogen peroxide used, the greater the reduction occurs in color, soluble solids, and turbidity of the juice.

The use of hydrogen peroxide in the treatment of sugarcane juice has already been reported by other authors. Mbanjwa, Deppa, and Pillay (2010) showed that regardless of the H<sub>2</sub>O<sub>2</sub> concentration used on the crystal sugar (15; 30; 65; 165; and 330 mg/L) there was a color reduction in crystal sugar when this method was tested in sugar refineries. Sartori, Galaverna, et al. (2015) evaluated five different doses of H<sub>2</sub>O<sub>2</sub> (0; 1,000; 5,000; 10,000; and 50,000 mg/L) and reported that doses up to 5,000 mg/L did not show significant changes in ICUMSA color values after 2 hr. Saska (2007) obtained a reduction between 20% and 30% with 100 to 500 mg/L of H<sub>2</sub>O<sub>2</sub>, respectively, while Mandro et al. (2015) reported that the action of H<sub>2</sub>O<sub>2</sub> in the sugarcane juice reduced the ICUMSA color by 16.1% and rutin degradation (pigment in sugarcane juice). Mane et al. (1992) also reported a color reduction of 40% and polyphenol contents in the sugarcane juice. According to the authors, phenolics participate in color-forming reactions in juice throughout the crystal sugar process.

In this present work, we have identified the compounds present in precipitates formed by the action of hydrogen peroxide and the compounds presents in the sugarcane peel. The nature of the compounds from the sugarcane wax also had been studied in the literature

(Antolín et al., 2004; Bravo et al., 2002; Delange, Antolín, Canavaciolo, Medina, & Granja, 2002; Inarkar & Lele, 2012; Qi et al., 2017). The GC-MS analysis of the precipitate and wax sample revealed the presence of the 32 compounds, with 10 of them been observed in precipitation samples and sugarcane peel (Table 2). The major groups identified in samples were hydrocarbons and fatty acids.

The sugarcane wax is found in stalks near the knots and on plant leaves (Singh et al., 2015; Vieira, 2003) and approximately 40% of all the material is extracted for juice during the milling of canes (Nazato et al., 2012). According to Parish, Li, and Bell (2008), wax acts as a protection mechanism, preventing the water loss and protecting against the microorganisms. In sugarcanes, the wax is a dark yellow resinous material (Inarkar & Lele, 2012), consisting of 45% waxes, 35% greases, and 20% resins (Frutuoso, 1989; Gandra, 2006). The amount of wax varies from 0.1% to 0.3% (w/w) according to varieties (Taylor, 2000). However, few studies have reported the influence of wax on the sugarcane juice color.

Hexadecanoic acid methyl ester, 9-octadecenoic acid methyl ester, 9,12-octadecadienoic acid methyl ester (fatty acids), and methyl stearate (ester) were found in all precipitation juice samples and in the hexanic extract of the sugarcane peel (Figure 2). Hexadecanoic acid methyl ester (or palmitic acid; C<sub>16</sub>H<sub>32</sub>O<sub>2</sub>; MW: 256.4241 g/mol), 9-octadecenoic acid, and methyl ester (C<sub>19</sub>H<sub>36</sub>O<sub>2</sub>; MW: 296.4879 g/mol) (Figure 3) were the compounds identified with the highest concentration in the wax samples from the peel and in all samples of the sugarcane juice treated with H<sub>2</sub>O<sub>2</sub>.

Inarkar and Lele (2012) were found to contain five main groups of compounds, such as alkanes (28.8%), ester (66.3%), aldehydes (0.1%), alcohol (0.2%), and fatty acids (4.6%). In the samples of sugarcane wax, alkanes were one of the main components identified, namely isobutane, triptane, and heneicosane, among others with C<sub>4</sub> to C<sub>21</sub> chains. Among the compounds in the class of fatty acids, propanoic acid (C<sub>3</sub>H<sub>6</sub>O<sub>2</sub>; MW: 74.07854 g/mol) was observed at higher concentrations. The hexadecanoic acid accounts for 0.75% of the total composition. Whyte and Hengeveld (1950) evaluated the composition of wax from the filter cake and observed that the acid profile could be divided into two major fractions, fatty acids, in particular, linoleic acid (C<sub>18</sub>H<sub>32</sub>O<sub>2</sub>) with 36.1% and palmitic acid (C<sub>16</sub>H<sub>32</sub>O<sub>2</sub>) with 25% and heptane (18.5%). Gutierrez and Silva (1993) found large amounts

**TABLE 2** Identification of compounds from precipitates after the treatment of sugarcane juice by hydrogen peroxide and extracted from sugarcane peel waxes

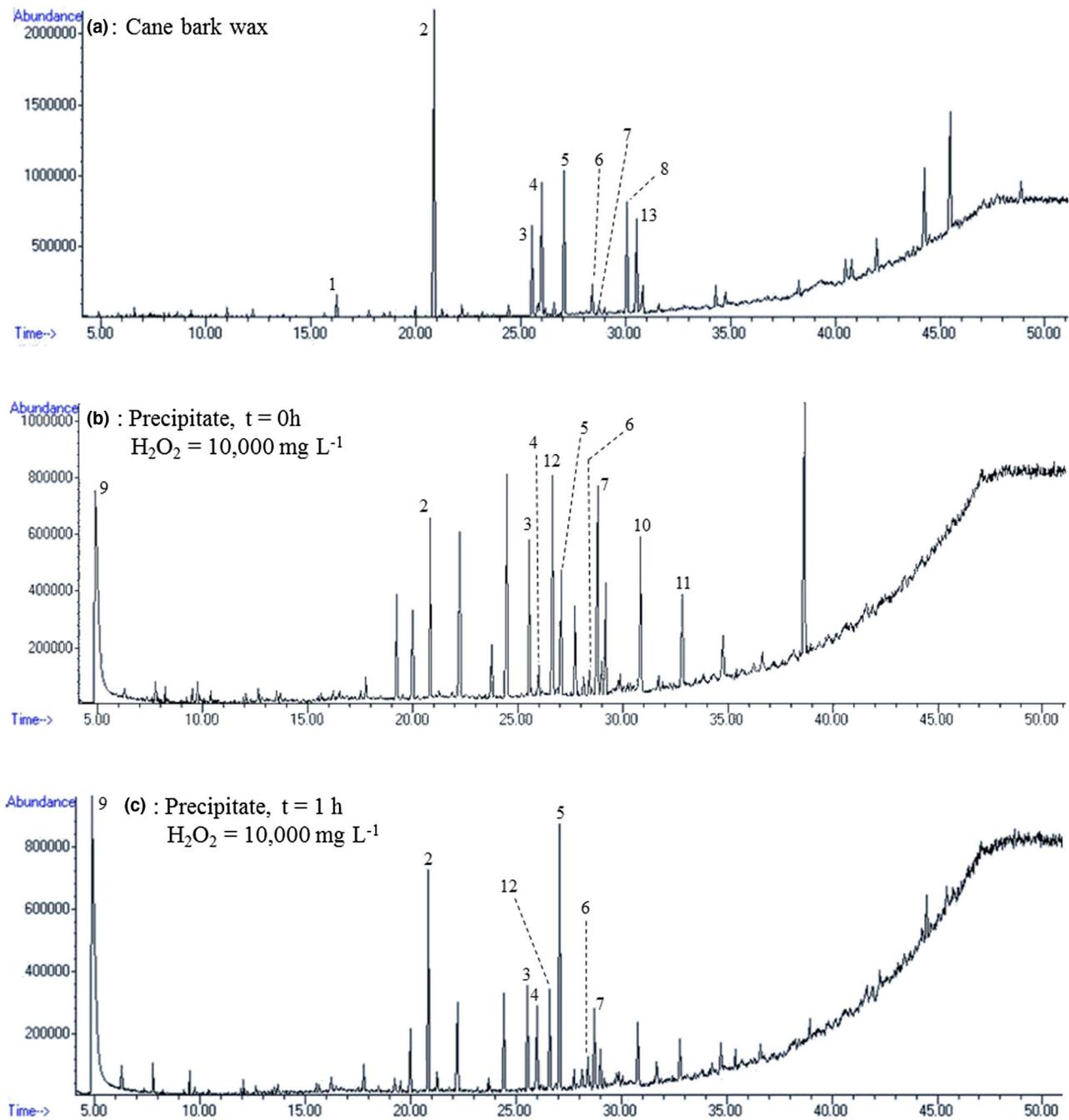
Identified compounds	RT (min)	m/z	Cane wax	Sugarcane juice treated with H <sub>2</sub> O <sub>2</sub>					
				1,000 mg/L		5,000 mg/L		10,000 mg/L	
				0 min	60 min	0 min	60 min	0 min	60 min
Octadecane	9.75	71.10	A	A	A	P	A	A	A
Heneicosane	12.66	71.05	P	A	A	A	P	P	A
Methyl tetradecanoate	16.22	74.10	P	A	A	A	A	A	A
Triacotane	16.47	71.10	A	A	A	A	P	P	P
Phenol, 2,4-bis (1,1-dimethylethyl)-	19.21	191.2	A	P	P	P	P	P	A
Phenol, 2,5-bis(1,1-di- methylethyl)-	19.25	191.2	A	A	A	A	A	A	A
Docosane	19.99	57.10	A	A	A	A	A	A	P
Hexadecanoic acid, methyl ester	20.82	74.10	P	P	P	P	P	P	P
Hexacosane	22.23	57.10	P	A	A	A	A	P	A
2-methyloctacosane	24.42	57.10	P	A	P	P	A	A	A
Tetracosane	24.46	57.10	A	A	A	A	A	P	P
Methyl stearate	25.52	74.10	P	P	P	P	P	P	P
Cis-13-octadecenoic acid, methyl ester	25.80	55.05	P	A	A	A	A	A	A
9-octadecenoic acid, methyl ester, (E)-	25.96	55.10	P	P	P	P	P	P	P
9,12-octadecadienoic acid (Z,Z)-methyl ester	27.04	67.10	P	P	P	P	P	P	P
Methyl 8,11,14-heptadec- atrienoate	28.39	79.10	A	A	A	P	A	A	A
9,12,15-octadecatri- enoic acid, methyl ester, (Z,Z,Z)-	28.39	79.10	P	P	P	A	P	P	A
benzenepropanoic acid, 3,5-bis(1,1-dimethyl- ethyl)-4-hydroxy-, methyl ester	29.15	277.2	A	A	A	P	P	P	A
Dodecane, 2-methyl-	29.52	57.05	A	A	A	A	A	A	A
Butyl 9,12,15-octadec- atrienoate	28.41	79.10	A	A	A	A	A	A	P

(Continues)

TABLE 2 (Continued)

Identified compounds	RT (min)	m/z	Cane wax	Sugarcane juice treated with H <sub>2</sub> O <sub>2</sub>									
				1,000 mg/L			5,000 mg/L			10,000 mg/L			
				0 min	60 min	0 min	60 min	0 min	60 min	0 min	60 min		
Hexacosane	28.78	57.10	P	A	A	A	A	A	A	P	P	P	P
Tetracosane	32.79	57.10	A	A	A	A	A	A	A	P	P	P	P
Octacosane	32.83	57.10	A	A	A	A	A	A	A	P	P	P	A
Methyl 18-methylnonadecanoate	30.06	74.10	P	A	A	A	A	A	A	A	A	A	A
cis-11-eicosenoic acid, methyl ester	30.52	55.10	P	A	A	A	A	A	A	A	A	A	A
6,11-eicosadienoic acid, methyl ester	31.57	67.00	P	A	A	A	A	A	A	A	A	A	A
Methyl 20-methyl-heneicosanoate	34.30	74.10	P	A	A	A	A	A	A	A	A	A	A
Hentriacontane	34.72	57.10	A	A	A	A	A	A	A	A	A	A	A
Hexacosane	34.76	57.10	A	A	A	A	A	A	A	P	P	P	P
Tetracosanoic acid, methyl ester	38.26	74.00	P	A	A	A	A	A	A	A	A	A	A
Hexacosanoic acid, methyl ester	41.98	74.05	P	A	A	A	A	A	A	P	P	P	A
Octacosanoic acid, methyl ester	45.47	74.05	P	A	A	A	A	A	A	A	A	A	A

Note: Where P represents the compound in the sample and A represents the absence in the same sample.

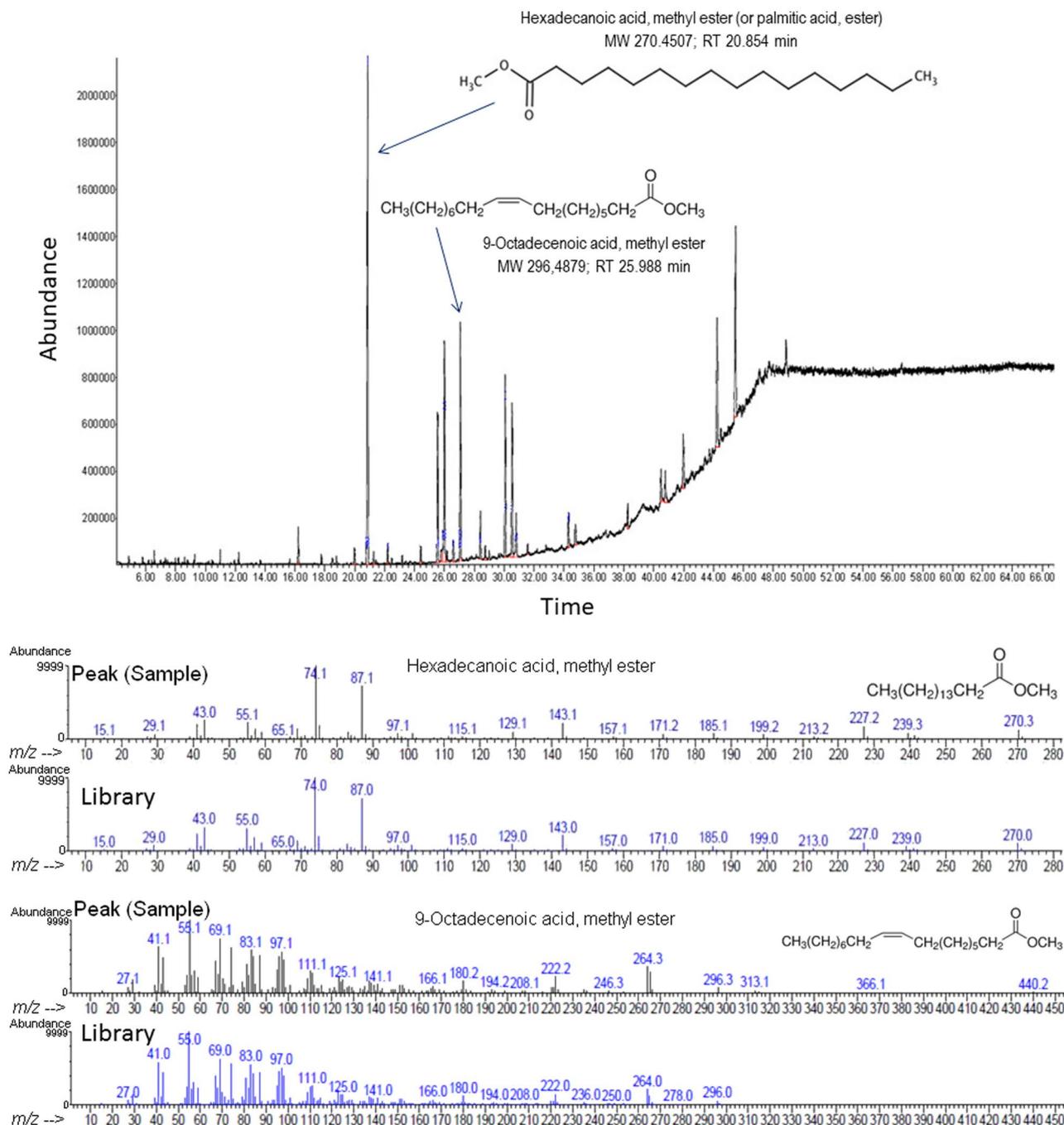


**FIGURE 2** GC-MS chromatogram of (a) hexanic fraction from the cane peel wax; (b) precipitate from the sugarcane juice without the treatment by  $\text{H}_2\text{O}_2$ ; and (c) precipitate from the sugarcane juice treated with  $\text{H}_2\text{O}_2$  (10,000 mg/L; 1 hr). 1. Methyl tetradecanoate (or methyl myristate); 2. hexadecanoic acid, methyl ester (or methyl palmitate); 3. methyl stearate; 4. 9-octadecenoic acid, methyl ester; 5. 9,12-octadecadienoic acid, methyl ester; 6. 9,12,15-octadecatrienoic acid, methyl ester; 7. hexacosane; 8. methyl 18-methylnonadecanoate; 9. acetic acid; 10. heptacosane; 11. octacosane; 12. pentacosane; 13. cis-11-eicosanoic acid, methyl ester

of linoleic acid (42% of total) followed by palmitic, oleic, and linolenic acids in sugarcane molasses. Fatty acids of the sugarcane peel were present in different products and/or by-products in the production of crystal sugar. Qi et al. (2017) observed the presence of fatty acids, fatty aldehydes, hydrocarbons, esters, fatty acids derivatives, and lignin derivatives in the crude sugarcane wax, between them the authors identified the presence of hexadecanoic acid methyl ester; 9-octadecenoic acid methyl ester; and 9,12-octadecadienoic acid methyl ester

(fatty acids). Řezanka and Sigler (2006) identified long-chain fatty acids in the sugarcane wax.

It was also observed that with the increase of  $\text{H}_2\text{O}_2$ , the areas of hexadecanoic acid methyl ester; 9-octadecenoic acid methyl ester; 9,12-octadecadienoic acid methyl ester; and methyl stearate (Table 3) which were identified in all precipitated fraction and wax increased. The results corroborate with that observed by Sartori, Galaverna, et al. (2015).



**FIGURE 3** Hexadecanoic acid, methyl ester, 9-octadecenoic acid, and methyl ester identified in all samples of precipitate from the sugarcane juice treated with  $H_2O_2$  and hexanic extract of waxes from the sugarcane peel

**TABLE 3** Areas of compounds observed in the precipitates of sugarcane juice subjected to peroxidation with doses of 1,000 5,000, and 10,000 ppm of hydrogen peroxide for 60 min

Compounds	R.T (min)	m/z	1,000	5,000	10,000
			60 min	60 min	60 min
Hexadecanoic acid, methyl ester	20,82	74,10	1,378,424	1,862,656	2,261,145
methyl stearate	25,52	74,10	247,131	211,436	1,085,524
9-octadecenoic acid, methyl ester, (E)-	25,96	55,10	232,633	287,151	892,188
9,12-octadecadienoic acid (Z,Z)-methyl ester	27,04	67,10	1,284,266	1,736,698	2,987,106

Note: Where A represents the absence in the same sample.

## 4 | CONCLUSION

The precipitating action of the hydrogen peroxide promoted the reduction of the color ICUMSA and the soluble solids in the sugarcane juice in all the dosages. In relation to the turbidity, it had an increase with a dosage of 1,000 mg/L. The higher concentration of H<sub>2</sub>O<sub>2</sub> resulted in higher reductions of °Brix, color ICUMSA, and turbidity (67.1%, 60.7%, and 13.3%, respectively). These reductions may have occurred due to the sedimentation of the organic nonsugars present in the juice by the action of hydrogen peroxide, since the hydrogen peroxide decomposes into hydroxyl molecules that react with most of the organic compounds. The main groups identified in precipitates and sugarcane wax were hydrocarbons and fatty acids, and some of these compounds were present in both cane wax and in precipitated samples, showing that many compounds present in the bark become the part of the juice due to the extraction process of grinding the cane.

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## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

## ORCID

Nathália Torres Corrêa Magri  <https://orcid.org/0000-0003-3926-9720>

Juliana Aparecida de Souza Sartori  <https://orcid.org/0000-0002-9995-8440>

Claudio Lima Aguiar  <https://orcid.org/0000-0002-0086-9449>

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