ELUCIDATION OF COLOR REDUCTION INVOLVING PRECIPITATION OF NON-SUGARS IN SUGARCANE (*SACCHARUM* SP.) JUICE BY FOURIER-TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY

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ABSTRACT

This work aimed to evaluate the effect of hydrogen peroxide on the composition of sugarcane juice through mass spectrometry. The sugarcane juice was treated with different doses of hydrogen peroxide (1,000 and 50,000 ppm) and samples were collected during 2 h. The color reduction occurred only at a dose of 50,000 ppm and the peroxide promotes sedimentation of non-sugars in sugarcane juice under the conditions studied as there was increased relative abundance of sucrose within the ranges with increased reaction time.

PRACTICAL APPLICATIONS

In this paper, we studied the mechanism of color reduction of sugarcane juice as a new innovative technology to crystal sugar production as a substitute for sulfur use, which was related to health problems.

INTRODUCTION

There is growing concern about the use of sulphite in foods due to the difficulty in precisely quantifying its amount ingested daily and to the association of its consumption to risks to consumers' health (Machado *et al.* 2006). According to the Food and Agriculture Organization (FAO), 1 out of every 100 people has sensitivity to sulfite, and depending on the severity, consumers can suffer serious reactions of its ingestion, namely pain sensation in the throat, chest congestion cough, hypotension and contact dermatitis, and many times, these reactions are associated to the sulfite in foods. Asthma sufferers may have increased asthma attacks due to sulfite ingestion (Bush *et al.* 1986; Simon 1998). Thus, the world market claims for the consumption of white sugar free from residues of reduced sulfur compounds.

White crystal sugar features the presence of residual sulfite that comes from the clarification stage. Traditionally, the clarification stage of sugarcane juice consists of sulfur addition in a rotary oven, from which sulfur dioxide is produced and mixed to the juice in a sulfitation column reactor. After the clarification stage, the juice is submitted to the steps of acidity neutralization, heating and decantation, where the precipitation of calcium sulfite occurs (Marques *et al.* 2001).

As an alternative to this process, advanced oxidative processes (AOPs), such as the use of O_3/H_2O_2, O_3/UV, O_3/metals, have been developed in an attempt to increase the efficiency of the clarification process and to reduce the impact on the nutritional quality of foods. The AOPs are based on the action of highly oxidizing species, called hydroxyl radicals (•OH), generated in the reaction system, on organic compounds that can be partially or entirely mineralized (Environmental Protection Agency 1998).

Hydrogen peroxide (H_2O_2) is a transparent liquid, similar in appearance to water with characteristic odor, nonflammable, miscible in water and marketed as aqueous solution in concentrations of 20–60% (w/v) (Mattos *et al.* 2003).

Among the modifications caused by the use of ozone as a clarifying agent of sugarcane juice are significant reductions in the contents of reducing sugars, starch, amino acids and polyphenols, a decrease in ash content and viscosity, as well

as an increase in juice purity by removing non-sugars (Madsen *et al.* 1978; Mane *et al.* 1998, 2000).

However, the use of new technologies, such as ozone and hydrogen peroxide, may cause analytical problems in the tests commonly carried out at sugarcane mills using colorimetric methods to quantify color and sugars in sugarcane juice. As demonstrated in Alves *et al.* (2013), polarimetry and refractometry analyses may suffer variation in values of readings due to the presence of dextran and starch in the sugarcane juice and in standard solutions. In addition, these procedures require a longer time to prepare the samples, carry out the analyses and obtain the results.

Thus, this work aims to evaluate the effects of hydrogen peroxide on color reduction, as well as show the application of a little-used technique at sugarcane mills to assess the effect of clarification agent on the composition of sugarcane juice.

MATERIALS AND METHODS

Sugarcane Juice

The sugarcane juice was obtained from healthy plants of the SP 81-3250 variety, was subjected to juice extraction in a milling machine (Mausa Co., Piracicaba, São Paulo State, Brazil) and the juice was sieved through a 0.032-mm mesh. After sieving, the juice was filtered in a 2-µm-diameter pore qualitative filter.

Preliminary Study

We tested a wide range of hydrogen peroxide doses (control = original juice; 1,000; 5,000; 10,000; and 50,000 ppm) injected directly into the filtered sugarcane juice without heat treatment.

Peroxidation Kinetics

Samples of sugarcane juice were subjected to treatment at two concentrations, 1,000 and 50,000 ppm, of hydrogen peroxide solution at 35% w/w in water bath at 45C and constant circular shaking (100 rpm). Samples of 1 mL were collected at pre-established time intervals, which are control (time 0), 5, 10, 15, 20, 30, 40, 60, 90 and 120 min of reaction.

Fourier-Transform Ion Cyclotron Resonance (FT-ICR) Mass Spectrometry (MS)

About 2 µL of sugarcane juice was diluted in 1,000 µL of a solution of MeOH/H_2O (1/1) and 0.1% NH_4OH and then injected directly by infusion in an electrospray ionization source in a mass spectrometer Thermo LTQ-FT-Ultra (Thermo Scientific, Waltham, MA) operated in a negative mode. The conditions were as follows: voltage of capillary, 3.0 kV (negative-mode ion); voltage of cone of samples, 30 V; voltage of extraction cone, 3.0 V; source temperature, 100C; solvation temperature, 100C; and solvation flow, 400 mL/min of N_2. The experiments were obtained between 50 and 1,500 *m/z*.

RESULTS AND DISCUSSION

Preliminary studies on peroxidation of sugarcane juice show that color reduction (Fig. 1) is closely linked to an increase in the dose of hydrogen peroxide. As it was not possible to observe significant color changes of the juice at doses of up to 5,000 ppm, we decided to test at larger doses. The results, after 2 h of reaction, are shown in Fig. 1.

The analysis of the treatments showed the formation of precipitate at the bottom of the vials, which are higher in quantity as the hydrogen peroxide dose increases. The treatment with 1,000 ppm, after 2 h of reaction, showed that the sugarcane juice increased color, contrary to expectations for this clarification technology. Thus, there is an inverse correlation between the increased dose of peroxide and color reduction of sugarcane juice.

In Fig. 1, the formation of precipitates derived from the reaction of non-sugars with H_2O_2 is more remarkable, which has been reported in the literature as a precipitating agent in some reaction conditions such as those referred by Chunhong *et al.* (1993), Djogic *et al.* (2005) and Nikonov *et al.* (2010). Although in Fig. 2 it is not possible to notice the formation of precipitates, by analyzing the mass spectra,

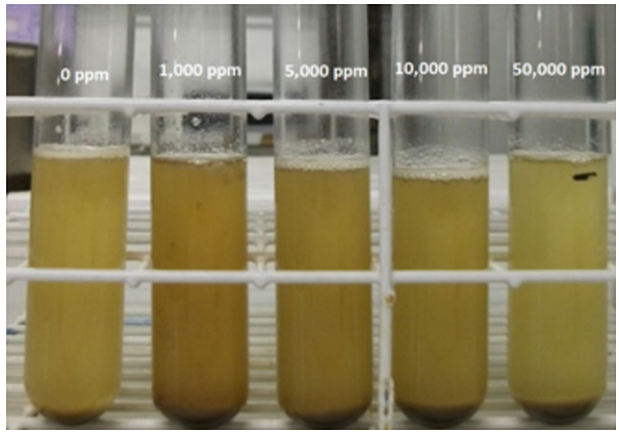

FIG. 1. ASPECT OF SUGARCANE JUICE SUBJECTED TO INCREASING DOSES OF PEROXIDATION (IN PPM) AT 35% (W/W) OF HYDROGEN PEROXIDE AT ROOM TEMPERATURE WITHOUT CONSTANT SHAKING In detail, the formation of waxy precipitate after 2 h of peroxidation reaction.

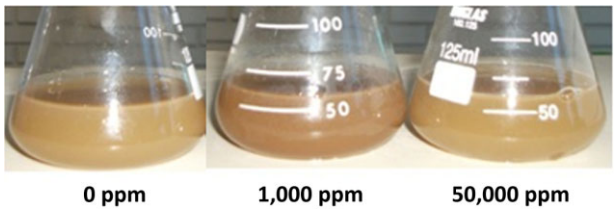

FIG. 2. ASPECT OF SUGARCANE JUICE SUBJECTED TO PEROXIDATION WITH TWO DOSES (1,000 AND 50,000 PPM) AT 35% (W/W) OF HYDROGEN PEROXIDE AT ROOM TEMPERATURE WITHOUT CONSTANT SHAKING

it is possible to conclude that there is a reduction of compounds in the spectra, with an increase of the relative abundance of molecular ions *m/z* 179 (glucose/fructose) and *m/z* 341 (sucrose), which confirms the hypothesis of the occurrence of precipitation process by H_2O_2 action on non-sugars present in sugarcane juice.

The kinetic analyses of the hydrogen peroxide doses at 1,000 and 50,000 ppm (Fig. 2) showed no degradation of sugars (sucrose; *m/z* 341 and glucose/fructose; *m/z* 179). Moreover, there is no formation of new compounds throughout the clarification process. These results oppose the traditional concepts that hydrogen peroxide has its use in the industry as a clarification agent (Mane *et al.* 1992). Davis (2001) affirms, in comparison with various processes used for color removal, that oxidative processes act on phenolic compounds, flavonoids (polyphenols found in sugarcane that are involved in enzymatic browning reactions) and color precursors. Shore *et al.* (1984) showed that the concentration of polyphenols decreased to less than 10% of juice treated with hydrogen peroxide when compared to the raw juice.

Mane *et al.* (1992) also showed that the use of hydrogen peroxide caused reduction of polyphenols and reducing sugars in the treatment of the sugarcane juice. Patel and Moodley (1991) and Madho and Davis (2001) conducted some preliminary studies on the use of hydrogen peroxide in sugarcane juice. The low discoloration capacity (about 16% of color reduction) associated with high doses required for the treatment of sugarcane juice showed a low economic viability of the process, according to the authors. According to the results obtained from FT-ICR MS, the treatments with hydrogen peroxide solutions did not provide effective decomposition of pigmental chemical compounds as no signals were observed within the ranges analyzed. In addition, the purification of sucrose (*m/z* 341) is characterized by the reduction of the relative abundance of other compounds within the ranges (Figs. 3–5) for the treatments with 1,000 ppm (Fig. 4) and with 50,000 ppm (Fig. 5) of hydrogen peroxide.

Figure 3 shows that the relative abundance of sucrose is smaller when compared to the relative abundance of fructose and glucose bonded with molecules of carbon, oxygen and hydrogen. There are also countless peaks with different masses illustrating the complex composition of sugarcane juice.

Based on the comparison of signals within the mass ranges of ions of greater relative abundance, we observed the presence of ions *m/z* 179 (glucose/fructose); *m/z* 207

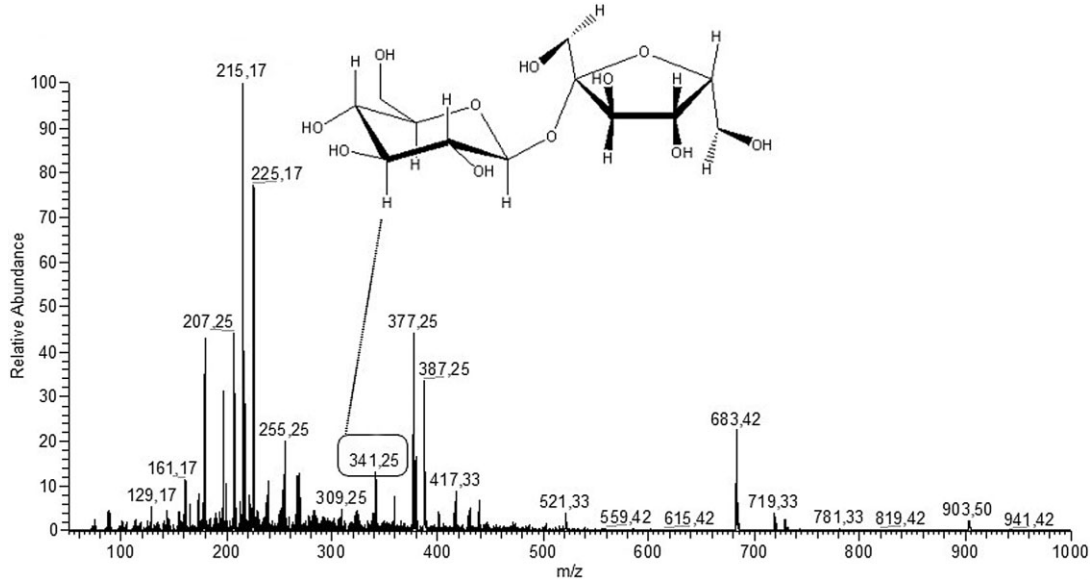

FIG. 3. MASS RANGES FOR SUGARCANE JUICE BEFORE SUBMISSION TO PEROXIDATION WITH A HYDROGEN PEROXIDE SOLUTION OF 35% (W/W) AT 45C, OBTAINED BY FT-ICR MS

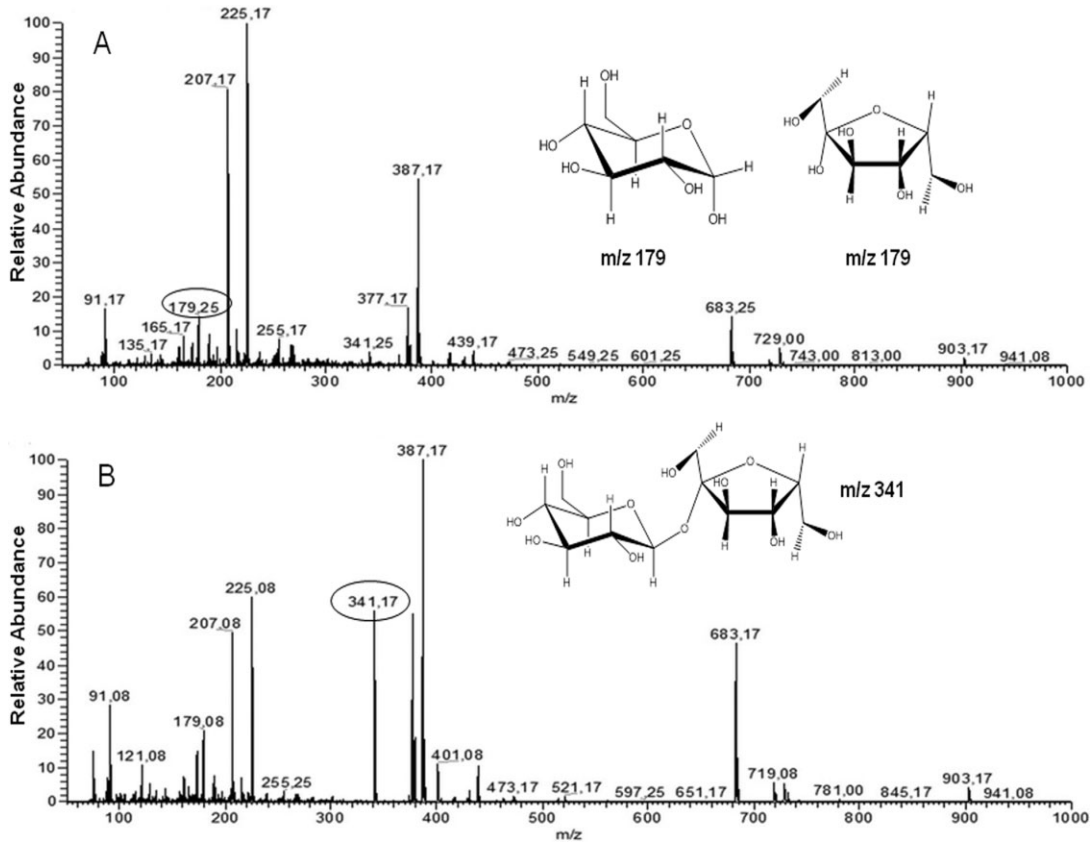

FIG. 4. MASS RANGES FOR SUGARCANE JUICE SUBJECTED TO PEROXIDATION WITH DOSE OF 1,000 PPM OF A SOLUTION OF HYDROGEN PEROXIDE OF 35% (W/W) AT 45C, OBTAINED BY FT-ICR MS, AT 5 MIN (A) AND 120 MIN (B)

(glucose/fructose + CO); *m/z* 225 (glucose/fructose + COOHH); *m/z* 341 (sucrose); *m/z* 377 (sucrose + Cl); *m/z* 387 (sucrose + COOHH); *m/z* 683 (sucrose dimer); *m/z* 719 (dimer + Cl); and *m/z* 729 (dimer + COOHH). The ions of sugars (sucrose, glucose and fructose) maintain the primary structures of the molecules during the reaction time, which occur only with the addition of carbonyl, Cl, among other substituents (i.e., COOHH).

The presence of molecular ions of sucrose dimer (*m/z* 683) as well as ions of *m/z* 207 (glucose/fructose + CO), *m/z* 225 (glucose/fructose + COOHH), *m/z* 377 (sucrose + Cl), *m/z* 387 (sucrose + COOHH), *m/z* 719 (dimer + Cl) and *m/z* 729 (dimer + COOHH) is the product of the ionization process, not representing compounds naturally present in sugarcane juice or products originated from hydrogen peroxide reaction with components of sugarcane juice. This fact has been reported by Yu *et al.* (1998) in their studies with atmospheric oxidation of hydrocarbons.

Figure 4 shows an increase in the relative abundance of sucrose along the time and the initial increase of glucose/fructose ions. However, at 120 min, these ions show a slight reduction of the relative abundance. Possibly, the amount of peroxide used was insufficient for a good sedimentation of the compounds in the sugarcane juice.

Figure 5 presents the mass ranges of the samples, in which the dosage of 50,000 ppm of hydrogen peroxide was carried out. We made analyses of the time and observed compound sedimentation as there was an increase in the relative abundance of sucrose and its ions, and no emergence of any compound, because no different mass was detected during the reaction.

Therefore, mass spectrometry showed that peroxidation, at the reaction conditions evaluated, was not able to significantly reduce sugarcane juice color. The observed color decrease is possibly caused by the sedimentation of some impurities of the juice, and, apparently, chemical reactions in the juice at doses of 50,000 ppm do not occur. Thus, hydrogen peroxide did not work as a clarifying agent under the conditions studied.

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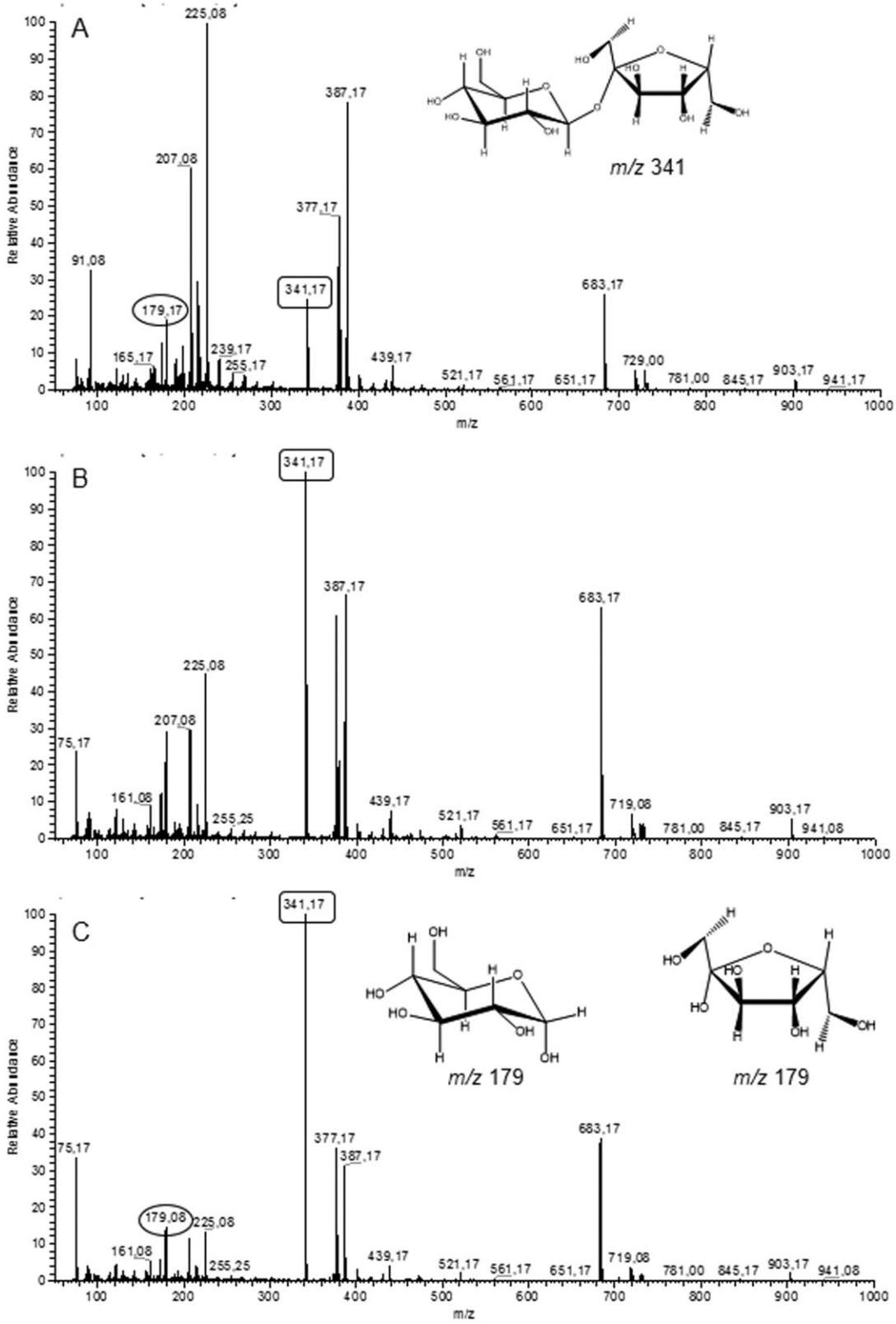

FIG. 5. MASS RANGES OF SUGARCANE JUICE SUBJECTED TO PEROXIDATION WITH A DOSE OF 50,000 PPM OF A SOLUTION OF HYDROGEN PEROXIDE OF 35% (W/W) AT 45C, OBTAINED BY FT-ICR MS, AT 5 MIN (A), 60 MIN (B) AND 120 MIN (C)

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REFERENCES

ALVES, F.V., AGUIAR, C.L. and SARMENTO, S.B.S. 2013. The effects of starch and dextran on technological analyses of sugarcane juice. Sugar J. *76*, 26–31.

BUSH, R.K., TAYLOR, S.L., HOLDEN, K., NORDLEE, J.A. and BUSSE, W.W. 1986. Prevalence of sensitivity to sulfiting agents in asthmatic patients. Am. J. Med. *81*, 816–820.

CHUNHONG, B., MEIYUAN, H. and ZHILAI, S. 1993. Kinetics of hydrogen peroxide-sulfur (IV) reaction in atmospheric precipitation. J. Environ. Sci. *5*, 351–366.

DAVIS, S.B. 2001. The chemistry of colour removal: A processing perspective. Proc. S. Afr. Sugar Technol. Assoc. *75*, 328–336.

DJOGIC, R., CUCULIC, V. and BRANICA, M. 2005. Precipitation of uranium (VI) peroxide (UO4) in sodium perchlorate solution. Croat. Chem. Acta *78*, 575–580.

ENVIRONMENTAL PROTECTION AGENCY. 1998. *Field Applications of In Situ Remediation Technologies: Chemical Oxidation*, Office of Solid Waste and Emergency Response, Technology Innovation Office, Washington, DC. 31 p.

MACHADO, R.M.D., TOLEDO, M.C.F. and VICENTE, E. 2006. Sulfitos em alimentos. Braz. J. Food Technol. *9*, 265–275.

MADHO, S. and DAVIS, S.B. 2001. Review of proven technologies available for the reduction of raw sugar colour. Proc. S. Afr. Sugar Technol. Assoc. *81*, 165–183.

MADSEN, R.F., KOTFOD-NIELSEN, W., WINSTROM-OLSEN, B. and NIELSEN, T.E. 1978. Formation of color compounds in production of sugar from sugar beet. Sugar Technol. Rev. *6*, 49–115.

MANE, J.D., PHADNIS, S.P. and JADHAV, S.J. 1992. Effects of hydrogen peroxide on cane juice constituents. Int. Sugar J. *94*, 322–324.

MANE, J.D., PACHPUTE, S.P. and PHADNIS, S.P. 1998. Effects of hydrogen peroxide treatment on cane syrup. Int. Sugar J. *100*, 210–212.

MANE, J.D., PHADNIS, S.P., JAMBHALE, D.B. and YEWALE, A.V. 2000. Mill scale evaluation of hydrogen peroxide as a processing aid: Quality improvement in plantation white sugar. Int. Sugar J. *102*, 530–533.

MARQUES, M.O., MARQUES, T.A. and TASSO JÚNIOR, L.C. 2001. *Sugar Technology – Sugarcane Production and Industry*, FUNEP, Jaboticabal. 170 p.

MATTOS, I.J., SHIRAISHI, K.A., BRAZ, A.D. and FERNANDES, J.R. 2003. Hydrogen peroxide – importance and determination. Quím. Nova *26*, 373–380.

NIKONOV, M.V., TANANAEV, I.G. and MYASOEDOV, B.F. 2010. Effect of hydrogen peroxide on precipitation of Pu(IV) in alkaline solutions. Radiochemistry *52*, 27–30.

PATEL, M.N. and MOODLEY, M. 1991. Decolourisation with hydrogen peroxide, ozone and other chemicals: some preliminary experimental work. SMRI Technical Report No. 1598, Sugar Milling Research Institute, Durban, South Africa.

SHORE, M., BROUGHTON, N.W., DUTTON, J.V. and SISSONS, A. 1984. Factors affecting white sugar colour. Sugar Technol. Rev. *12*, 1–99.

SIMON, R.A. 1998. Update on sulfite sensitivity. Allergy *53*, 78–79.

YU, J., FLAGAN, R.C. and SEINFELD, J.H. 1998. Identification of products containing –COOH, –OH, and –C=O in atmospheric oxidation of hydrocarbons. Environ. Sci. Technol. *32*, 2357–2370.