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Criegee mechanism as a safe pathway of color reduction in sugarcane juice by ozonation

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ABSTRACT

The production of crystal sugar is based on sugarcane juice clarification through sulphitation, that is, heat treatment with sulfur dioxide. The use of ozonation as an alternative to sulphitation aims to eliminate the disadvantageous presence of residual sulfite in crystal sugar. Both treatments are used to reduce color of sugarcane juice. The objective of this work was to evaluate two process parameters (temperature and pH) to reduce gallic acid, a low molecular weight pigment (MW 170 g mol^{-1}) widely found in sugarcane. Gallic acid was used as a model compound in sucrose solutions. The results showed that degradation of gallic acid was favored from pH 7.0 to 7.82 and temperature values between 50 and 70 °C. The reaction mechanism was proposed for gallic acid degradation by ozone based on Criegee mechanism. Ozonation was an efficient method to reduce the potential low molecular weight pigment present in the sugarcane.

1. Introduction

Few studies have been conducted aimed at understanding formation and decomposition of compounds. This has taken an important role in the sugar industry, mainly in terms of removal of phenolics by oxidation techniques, providing color reduction and reducing refining costs (Nguyen, Zhang, & Doherty, 2015).

There are at least four mechanisms that contribute to color formation during crystal sugar production at mills, namely: (a) melanoidins formed during reactions of the reducing sugars-amino acids by the Maillard reaction; (b) thermal degradation and condensation reaction of sugar by caramelization; (c) alkaline degradation and condensation reactions of reducing sugar; (d) oxidative reaction of phenolic compounds. The first three are non-enzymatic reactions, while the oxidative reaction of phenolic compounds to chemically reactive quinones is an enzymatic reaction and occurs prior to the milling process, when sugarcane is milled to extract juice (Kort, 1979). Compounds that most affect juice color are those naturally found in sugarcane, that is, phenolic compounds and flavonoids, responsible for 60–75% of juice color (Clarke & Legendre, 1999; Bucheli & Robinson, 1994). The total proportion of color attributed to enzymatic browning reactions depend on maturation and pH of juice. The color from enzymatic browning reactions in mature stalks accounts for more than half of coloring (Goodacre & Coombs, 1978; Qudsieh et al., 2002). Among the phenolic acids in the sugarcane juice, caffeic (2.26 ± 0.06 mg L^{-1}), gallic (1.15 ± 0.02 mg L^{-1}) and ferulic acid (1.13 ± 0.02 mg L^{-1}) are found in greater amounts (Zhao, Zhu, Yu, Fu, & Zeng, 2008).

Sulphitation, a traditional method of sugarcane juice clarification, aims to remove impurity that increases turbidity and color through direct contact of hot juice with sulfur dioxide gas (SO_2) resulting from sulfur combustion in absorption columns (Vilela, D'Avila, & Menato, 2008). SO_2 acts in the conversion of color compound into colorless, preventing the color formation by oxidation and inhibiting the development of browning reaction between sugars and amino acids (Payne, 2010). The presence of residual sulfite in crystal sugar above the technical and health specifications is a disadvantage of sulphitation. Adverse effects on human health have been linked to sulfite intake, such as nausea, gastric irritation, hives and bronchospasm in sensitive asthmatics (Machado, Toledo, & Vicente, 2006).

On the other hand, oxidative decolorants, which include any strongly oxidizing chemicals, have been studied and used at mills as sulphitation substitutes. These oxidative decolorants, such as ozone (Silva, Sartori, & Aguiar, 2015) and hydrogen peroxide (Sartori et al., 2015), produce active free radical in solution that

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attack susceptible functional groups (Davis, 2001). Moodley, Davis, and Adendorff (1999) proved to be possible ozone application in the bleaching process of refined sugar at large scale in sugar refineries in South Africa. Ozone was capable of destroying colored compounds and color precursors in sugar solutions. In Brazil, the ozone technology has been experimentally used in sugarcane juice treatment at mills in the states of Paraíba, Bahia, Rio de Janeiro and Mato Grosso do Sul (GASIL, 2016).

The objective of this study was to evaluate, in details, the best operating conditions of pH and temperature to optimize the degradation of phenolic compounds using ozonation, thereby promoting a better understanding of degradation kinetics of color precursor present in sugarcane juice by ozone. A model system consisting of a phenolic low molecular weight acid (gallic acid) and sucrose solution (13.5% w v^{-1}) was used to eliminate interference from other sugarcane juice components on the results.

2. Materials and methods

An aqueous solution of sucrose (13.5% w v^{-1}) added to 1000 mg L^{-1} of gallic acid (⩾99%; HPLC grade; Sigma-Aldrich, São Paulo, Brazil) were ozonized in stainless steel AISI 316 reactors of 6 L. A useful volume of 2 L of mixed solution of sucrose and gallic acid (1 g L^{-1}) was used in each treatment. Gallic acid was chosen as a model phenolic compound since it is one of the phenolic acids present in greater quantities in the sugarcane juice (Zhao et al., 2008). The effect of ozone application combined with different pH (X_1) and temperature (X_2) was studied through the Central Composite Rotatable Design (CCRD) 2^2, containing three central and four axial points. The values of pH ranged from 2.18 to 7.82 and temperature values ranged 30–70 °C (The study design is available in Supplementary material – Table 1).

The dose rate used during the ozonation was 3.82 mg O_3 - min^{-1} with a total time reaction of 4 h. The statistical analysis and generated surface were made from degradation rate of gallic acid after 240 min (4 h), using the Statistic version 12 software (STATSOFT, 2013). Degradation rate of gallic acid was calculated using this following equation: *Gallic acid* = (C_n/C_0), where C_n is concentration of gallic acid in *n* time and C_0 is the initial concentration of gallic acid.

Samples were collected at 5, 10, 20, 30, 40, 50, 60, 90, 120, 180 and 240 min, aiming to monitor the kinetics of gallic acid degradation. One sample was taken prior the beginning of the process as a sample-control system. The sucrose content (g L^{-1}) was also analyzed. The final volume collected did not exceed 10% of the initial volume.

2.1. Determining total phenolics

The total phenolic compounds were determined by Folin-Ciocalteu method, where sample aliquots (1.0 mL) were added to 0.5 mL of Folin-Ciocalteu reagent previously diluted 1:10 (v v^{-1}) with deionized water and 2.5 mL of 20% solution of sodium carbonate (Na_2CO_3). The absorbance reading was carried out at 725 nm in UV–Visible Mini1240 spectrophotometer (Shimadzu Co., Japan) after a 45-min reaction at room temperature in the dark. The total phenolics were expressed as mg mL^{-1} of gallic acid, according to the modified methodology described by Julkunen-Tiitto (1985).

2.2. Sugar analysis by ion chromatography

Sucrose concentration was determined by ion chromatography, according to the methodology described by IC Application Note (2016). The sugar was determined using 930 Compact IC Flex ion chromatography (Metrohm AG, Switzerland) with an amperometric detector. The eluent was 100 mM of sodium hydroxide. The samples were diluted and filtered previously using 0.45 μm membrane (Millipore). The column used was Metrosep Carb 1 (150/4.0) at 35 °C and flow 1.0 mL min^{-1}, with 12 min total run time. The analyses were performed in triplicate after injection of 0.25 μL of each sample. Before the quantitative analysis, the standard solution of sucrose (⩾99.5%, Sigma-Aldrich, São Paulo, Brazil) was made to prepare the calibration curve at concentration between 0.1 and 0.5 g L^{-1}.

2.3. Gallic acid analysis by Quadrupole time of Flight (Q-ToF) mass spectrometry

The identity of gallic acid was confirmed by ESI-MS. The ESI-MS analysis was performed using an HPLC (Hewlett Packard, Agilent Technologies 1290 series), without column, coupled to a Q-ToF iFunnel 6550 mass spectrometer. A solvent system of acetonitrile (ACN; ⩾99.9%, Sigma-Aldrich, Brazil) and ultrapure H_2O (1:1) was used at a flow rate of 0.6 mL min^{-1}. Ten μL of samples were diluted in 1 mL of ACN and then 1 μL was injected. The analysis of the mass spectra for the first order (MS) and for the remaining multistage experiments was performed under the following conditions: gas temp at 290 °C; drying gas flow at 14 L min^{-1}; nebulizer at 45 psi; sheath gas temp at 350 °C; sheath gas flow 12 L min^{-1}; VCap 3000; nozzle voltage 320 V; fragmentor 100 V; OCT 1 RFVpp 750 V; and collision energy 35 V. Agilent MassHunter Qualitative Analysis software version B.07.00 was used to acquire and process the data.

2.4. Ozonolysis pathways of gallic acid degradation by Q-ToF/MS

Aqueous solutions of gallic acid (100 mg L^{-1}) (⩾99%; HPLC grade; Sigma-Aldrich, São Paulo, Brazil) were ozonized in glass microreactor of 145 mL. Trials were performed using different conditions of pH (3.0–7.0), temperature (40–80 °C) and ozone dose (0.58–3.82 mg min^{-1}). Samples were collected at 5, 10, 20 and 30 min, aiming to monitor the kinetics of gallic acid degradation. They were analyzed according to the methodology to Q-ToF/MS described above.

3. Results and discussion

The definition of better operational conditions in industrial process is key to the success of the productive chain of various products of commercial interest.

Based on Pareto chart (Fig. 1a), the factors pH (L), temperature (Q) and the pH × temperature interaction were significant at a confidence interval of 95%. These three significant parameters were kept in the model for the construction of the analysis of variance (ANOVA) adjusted (ANOVA table is available in Supplementary material – Table 2). The factors pH (Q) and temperature (L) were not significant because the standardized effect of estimated values for these variables did not exceed the significance line and they could be removed without compromising the prediction.

In ozonation, the exact nature of the radical depends on the pH solution particularly. In neutral or acidic media, ozone acts via an oxygen radical. On the other hand, the hydroxyl radical predominates in alkaline media (Davis, 2001). Therefore, the pH influence in ozonation, which was confirmed by the results obtained in Fig. 1a.

The regression model generated was significant ($p \leqslant 0.05$), since $F_{calculated}$ (0.95; 3.7) = 16.55 was greater than $F_{tabulated}$ (0.95; 3.7) = 4.35 and predictive, whereas $F_{calculated}$ was three times greater than $F_{tabulated}$ (according to theoretical data from