Color reduction of raw sugar syrup using hydrogen peroxide

Redução de cor em xarope de açúcar bruto usando peróxido de hidrogênio

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Abstract
A commercial H2O2 solution (35%, v/v) was evaluated as a clarifying agent for raw, type VHP (very high polarization) sugar syrup, using an experimental design applying artificial neural networks (ANN). Fifteen experimental runs were carried out and the samples were taken at the following time intervals: 0, 5, 10, 15, 20, 25, 30, 40, 50, 60, 75 and 90 min. The treatments were carried out using an experimental design consisting of three variables: H2O2 (X1: 0; 202.4; 500; 797.6 and 1.000 mg L−1); pH (X2: 3.32, 5, 7.5, 10 and 11.68) and temperature (X3: 16.4, 30, 50, 70 and 83.6°C). The theoretical and measured values fitted the analysis by artificial neural networks (ANN) well. A reduction in colour (ICUMSA method) was observed between 60 and 75 min, except for treatments #11 (pH= 11.68; 50°C and 500 mg H2O2 L−1), #13 (pH= 7.5; 83.6°C and 500 mg H2O2 L−1) and #15 (pH= 7.5; 50°C and 1.000 mg H2O2 L−1), which showed a colour reduction after 30 min. In the treatments at pH 3.32 or 11.68, temperature of 83.6°C and H2O2 dose of 1.000 mg L−1, an average sucrose degradation of 55% was observed. The best colour reduction result was obtained with treatment #9 (pH 7.5, 50 °C and 500 mg H2O2 L−1), although sucrose degradation of 26% was observed.

Keywords: Clarification; Oxidation; Sucrose; Reduction; Kinetics; Degradation.
1 Introduction

Hydrogen peroxide (H$_2$O$_2$) has a standard reduction potential ($E^0$) of 1.77 V, higher than that of other oxidants such as chlorine ($E^0$ = 1.36 V), chlorine dioxide ($E^0$ = 1.50 V), molecular oxygen ($E^0$ = 1.23 V) and potassium permanganate (E = 1.67 V). In a reaction medium, H$_2$O$_2$ can be converted to hydroxyl radicals (•OH), a highly reactive species ($E^0$ = 2.80 V) (Üzer et al., 2017; Mattos et al., 2013), making it of interest in the treatment of drinking water and industrial effluents (Guo et al., 2017; Russo et al., 2017; Spasiano et al., 2016). Stout et al. (2017), Jervis et al. (2015) and Fairbanks (2009) reported that hydrogen peroxide has been used in the bleaching of different types of food. Since 1979, H$_2$O$_2$ has been recognized as a GRAS (Generally Recognized as Safe) product by the Food and Drug Administration (FDA, 2015).

Studies on the use of H$_2$O$_2$ in sugarcane juice or sugar beet clarification have been carried out (Mane et al., 2000; Sartori et al., 2015a; Sartori et al., 2015b; Mandro et al., 2015), and the action of H$_2$O$_2$ on compounds such as melanoids, melanins, caramels, polyphenols, starch and amino acids, which are present during the processes used to obtain sugar, reported. H$_2$O$_2$ can also act during the purification/clarification step of Very High Polarization (VHP) or Very Very High Polarization (VVHP) raw sugar syrup during sugar refining (Mandro et al., 2017). Nowadays, the sugar refining process consists of the dissolution of crystal sugar in hot water, and its passage through ion exchange resins and activated carbon to remove the impurities (Crema, 2012). The high costs are related to the regeneration pof the resins, due to a progressive loss of ion exchange capacity (Rodrigues, 1998; Baccar et al., 2009).

Thus, by way of an analysis in artificial neural networks, this study aimed to evaluate the clarification of sugar syrup (VHP type) using H$_2$O$_2$ as the clarification agent.

2 Material and methods

2.1 Sugar and reagents

VHP type crystal sugar (Very High Polarization) was obtained in Piracicaba – SP, Brazil. The sugar samples were characterized, and the values obtained are presented below: pH = 6.56 (in aqueous solution at 60° Brix), humidity = 0.026%, polarization (or sucrose content by polarimetry) = 97.21 °Z, ash = 0.66% and ICUMSA colour = 417.58 IU. Hydrogen peroxide (commercial solution at 35% (w/w)); Catalase (freeze-dried powder, 2000–5000 units/mg protein; Sigma-Aldrich); HCl 0.1 mol L$^{-1}$; NaOH 0.1 mol L$^{-1}$; sucrose ($\geq$ 99.5%; Sigma-Aldrich) and acetonitrile (Tedia Co., HPLC grade).

2.2 Sugar syrup preparation

The sugar syrup was prepared by dilution in ultrapure water (18 MΩ cm) up to a final concentration of 66 Brix (% soluble solids). Each treatment used 400 mL of 66 Brix syrup, whose concentration was measured in a refractometer Mod. RFM-712 (Bellingham+Stanley Co., UK).
2.3 Treatment with hydrogen peroxide

The conical flasks (500 mL) were maintained under different reaction conditions depending on the experimental design: temperature (16.4 to 83.6 °C); pH (3.32 to 11.68) and H$_2$O$_2$ doses (between 0 and 1000 mg L$^{-1}$) (Table 1). Each experimental run was monitored for 90 min, and samples were collected at the following time intervals 0, 5, 10, 15, 20, 25, 30, 40, 50, 60, 75 and 90 min. Colour reduction was analysed by the ICUMSA method and the sucrose contents by HPLC. The results were expressed as the average ± standard deviation of three analytical replicates. Bovine liver catalase (freeze-dried powder, 2000 – 5000 U/mg protein; Sigma-Aldrich) was added at the end of each treatment to disrupt the oxidation reactions of the hydrogen peroxide.

Table 1. Initial conditions of the 15 treatments used in the peroxidation treatments.

| Runs # | Initial °Brix | Initial pH | Initial temperature (°C) | H$_2$O$_2$ (mg mL$^{-1}$) |
|--------|---------------|------------|--------------------------|--------------------------|
| 1      | 62.6          | 5.0        | 30                       | 202.4                    |
| 2      | 62.6          | 10.0       | 30                       | 202.4                    |
| 3      | 62.6          | 5.0        | 70                       | 202.4                    |
| 4      | 62.6          | 10.0       | 70                       | 202.4                    |
| 5      | 62.6          | 5.0        | 30                       | 797.6                    |
| 6      | 62.6          | 10.0       | 30                       | 797.6                    |
| 7      | 65.5          | 5.0        | 70                       | 797.6                    |
| 8      | 65.5          | 10.0       | 70                       | 797.6                    |
| 9      | 66.2          | 7.5        | 50                       | 500                      |
| 10     | 66.2          | 3.32       | 50                       | 500                      |
| 11     | 66.2          | 11.68      | 50                       | 500                      |
| 12     | 66.2          | 7.5        | 16.4                     | 500                      |
| 13     | 66.2          | 7.5        | 83.6                     | 500                      |
| 14     | 66.2          | 7.5        | 50                       | 0                        |
| 15     | 66.2          | 7.5        | 50                       | 1.000                    |

2.4 ICUMSA colour analysis of the sugar syrups

The colour analyses of the samples treated with hydrogen peroxide were carried out using method GS1/3-7 (International Commission for Uniform Methods of Sugar Analysis, 2011). Initially, the sample Brix was adjusted to 30 Brix using a bench refractometer and they were then vacuum filtered through a PTFE 0.45 μm pore membrane (Millipore Co., Brazil). The pH was then adjusted to 7±0.05 with HCl 0.1 mol L$^{-1}$ or NaOH 0.1 mol L$^{-1}$ and the absorbance measured in a UV-Mini 1240 spectrophotometer (Shimadzu Co., Japan) at 420 nm. The ICUMSA colour of the samples was expressed as the result obtained from the Expression (1):

\[
\text{ICUMSA colour (420 nm)} = \frac{\text{ABS}}{b \times c} \times 1000
\]

Where: ABS: absorbance at 420 (nm); b: Cell optical path (cm); c: Sucrose concentration (g mL$^{-1}$) in solution in Brix at 20 °C.
2.5 HPLC-ELSD analysis of sucrose contents

Sucrose was determined by HPLC equipped with a low temperature evaporative light scattering detector (ELSD-LT). The mobile phase consisted of acetonitrile and water (85:15, v/v) previously filtered through a 0.45 µm pore diameter PTFE membrane. The samples were passed through a Kromasil 100 NH₂-50 (250 mm × 46 mm, 5 µm) column, maintained at 30 °C, under the following analytical conditions: flow rate of 1.0 mL min⁻¹, detector temperature of 35 °C and nitrogen as the nebulizer gas at a pressure of 350 kPa. The sucrose concentrations (injection volume = 5 µL) were determined in triplicate by comparison with a calibration curve for sucrose of from 0.1 to 0.5 g L⁻¹ (Sigma-Aldrich, HPLC grade).

2.6 Data analysis of the sugar syrup treated by hydrogen peroxide

An artificial neural network (ANN) was structured for the empirical prediction of the results. In the input layer, the values added were divided between the dependent (ICUMSA colour responses and sucrose contents of the sugar syrup samples) and controllable independent values (temperature (°C); pH; hydrogen peroxide doses (mg mL⁻¹) and Brix). The validation test of the networks was carried out using 4 to 10 neurons. The software used for data preparation, adjustment and simulation of the neural networks was developed at CESQ/DEQ-EPU/EPUSP (Nascimento et al., 2000) and the relative importance of the variables was assessed using the Holdback Input Randomization Method (HIPR) proposed by Kemp et al. (2007). This method is based on a comparison of the errors caused in each output of the model by random disturbances imposed on each of the input variables.

3 Results and discussion

The reductions of the ICUMSA colour and sucrose contents of the sugar syrup samples were analysed with an ideal number of neurons for each treatment by way of the Learning-set (LS) and Test-set (TS). The values obtained for the angles and determination coefficients of the Learning-set (LS) and Test-set (TS) from 4 to 10 neurons can be seen in Figures 1a and 1b, respectively. For the ICUMSA colour and sucrose, the value closest to 1 for the Learning-set (LS) and Test-set (TS) coefficients was 8 neurons. Thereby, a representation of 8 neurons was chosen for a better fit of the artificial neural networks (ANN).
The simulated values were similar to those obtained in the experiment. The artificial neural networks obtained good adjustment and were able to predict the results of the ICUMSA colour (Figure 2). Treatments #9 (pH = 7.5; 50 °C and 500 mg\textsubscript{H\textsubscript{2}O\textsubscript{2}} L\textsuperscript{-1}) and #12 (pH = 7.5; 16.4 °C and 500 mg\textsubscript{H\textsubscript{2}O\textsubscript{2}} L\textsuperscript{-1}) presented greater similarity between the real and simulated values (Figure 2). Treatment #9 showed a greater reduction in ICUMSA colour (82% reduction) (Table 2), reaching its peak between 60 and 75 min. On the other hand, treatment #14 (pH = 7.5; 50 °C; 0 mg\textsubscript{H\textsubscript{2}O\textsubscript{2}} L\textsuperscript{-1}) showed the lowest reduction in ICUMSA colour, obviously due to the absence of the clarifying agent.
Table 2. Experimental test conditions with variations in pH, temperature and hydrogen peroxide and the concentrations of ICUMSA colour (IU) and sucrose (mg L⁻¹) at 30 min intervals.

| Runs # | Brix | Initial pH | Initial temperature °C | H₂O₂ mg L⁻¹ | t₀ = 0* Colour | t₁ = 30* Colour | t₂ = 60* Colour | t₃ = 90* Colour | Colour | Sucrose | Colour | Sucrose |
|-------|------|------------|-------------------------|-------------|---------------|---------------|---------------|---------------|--------|---------|--------|---------|
| 1     | 62.6 | 5.0        | 30                      | 202.4       | 408.5         | 520.2         | 308.1         | 507.7         | 325.0  | 535.1   | 325.7  | 545.8   |
| 2     | 62.6 | 10.0       | 30                      | 202.4       | 511.3         | 578.5         | 288.4         | 515.4         | 331.5  | 524.3   | 264.7  | 540.3   |
| 3     | 62.6 | 5.0        | 70                      | 202.4       | 425.0         | 750.7         | 320.1         | 616.9         | 32.7   | 543.9   | 262.5  | 618.2   |
| 4     | 62.6 | 10.0       | 70                      | 202.4       | 435.1         | 534.7         | 251.1         | 543.8         | 87.0   | 676.4   | 95.5   | 588.9   |
| 5     | 62.6 | 5.0        | 30                      | 797.6       | 412.7         | 769.7         | 234.9         | 470.4         | 101.6  | 548.5   | 171.6  | 537.2   |
| 6     | 62.6 | 10.0       | 30                      | 797.6       | 465.0         | 877.9         | 335.6         | 579.0         | 291.1  | 437.4   | 193.9  | 525.3   |
| 7     | 65.5 | 5.0        | 70                      | 797.6       | 500.2         | 631.2         | 288.9         | 354.5         | 259.2  | 438.1   | 264.3  | 350.6   |
| 8     | 65.5 | 10.0       | 70                      | 797.6       | 434.9         | 645.2         | 236.9         | 339.3         | 178.4  | 351.9   | 225.0  | 381.8   |
| 9     | 66.2 | 7.5        | 50                      | 500         | 850.4         | 341.6         | 256.0         | 290.6         | 207.7  | 223.3   | 157.3  | 229.2   |
| 10    | 66.2 | 3.32       | 50                      | 500         | 477.8         | 597.1         | 277.2         | 214.7         | 166.8  | 224.7   | 309.0  | 219.1   |
| 11    | 66.2 | 11.68      | 50                      | 500         | 464.2         | 830.3         | 155.9         | 516.7         | 267.9  | 354.6   | 290.5  | 382.4   |
| 12    | 66.2 | 7.5        | 16.4                    | 500         | 941.5         | 418.9         | 484.2         | 221.7         | 539.4  | 189.4   | 516.9  | 219.5   |
| 13    | 66.2 | 7.5        | 83.6                    | 500         | 526.5         | 431.1         | 183.9         | 284.1         | 48.1   | 247.9   | 124.5  | 237.3   |
| 14    | 66.2 | 7.5        | 50                      | 0           | 444.6         | 362.0         | 447.3         | 333.6         | 452.9  | 540.7   | 425.7  | 378.4   |
| 15    | 66.2 | 7.5        | 50                      | 1000        | 454.6         | 335.7         | 228.9         | 187.8         | 221.7  | 156.9   | 194.3  | 157.5   |

*Real values obtained after clarification treatments with hydrogen peroxide.
Treatments #11 (pH = 11.68; 50°C and 500 mgH₂O₂ L⁻¹), #13 (pH = 7.5; 83.6°C and 500 mgH₂O₂ L⁻¹) and #15 (pH = 7.5; 50°C and 1,000 mgH₂O₂ L⁻¹) showed greater reductions in ICUMSA colour up to 30 min.

According to Lange et al. (2006), the pH of the reaction is important for H₂O₂ stability, because the speed and efficiency of oxidation are affected since H₂O₂ rapidly decomposes producing oxygen and water in alkaline pH values.

When H₂O₂ is in excess, it captures the OH⁺ radicals (H₂O₂+OH → OH⁺₂ + H₂O), which reduces oxidation efficiency (Araujo et al., 2006; Teran, 2014).

**Figure 2.** Profiles of the ICUMSA colour reduction treatments #9 (pH= 7.5; 50 °C and 500 mgH₂O₂ L⁻¹), #11 (pH= 11.68; 50 °C and 500 mgH₂O₂ L⁻¹), #12 (pH= 7.5; 16.4 °C and 500 mgH₂O₂ L⁻¹), #13 (pH=7.5; 83.6 °C and 500 mgH₂O₂ L⁻¹), #14 (pH= 7.5; 50 °C; 0 mgH₂O₂ L⁻¹) and #15 (pH= 7.5, 50 °C and 1,000 mgH₂O₂ L⁻¹) in sugar syrup treated with H₂O₂ as compared to the values simulated by the ANN.
The percentage of the mean square error of each variable for the reduction in ICUMSA colour (Figure 3), showed a higher significance degree for the initial Brix (42.63% of the total value) followed by temperature (21.19%), pH (21.35%) and H₂O₂ (14.82%). During the reaction time, there was a reduction in the Brix values, which was directly related to the reduction in the ICUMSA colour values. Sartori et al. (2017) reported that the reduction in the ICUMSA colour in sugarcane juice by H₂O₂ is associated with the precipitation of impurities from the sugarcane juice, such as proteins and phospholipids.

Figure 3. Relative importance of the neural network variables in the reduction of the ICUMSA colour.

Treatments #7 (pH = 5.0; 70 °C and 797.6 mgH₂O₂ L⁻¹), #8 (pH = 10.0; 70 °C and 797.6 mgH₂O₂ L⁻¹), #10 (pH = 3.32; 50 °C and 500 mgH₂O₂ L⁻¹), #11 (pH = 11.68; 50 °C and 500 mgH₂O₂ L⁻¹), #13 (pH = 7.5 and 83.6 °C and 500 mgH₂O₂ L⁻¹) and #15 (pH = 7.5; 50 °C and 1.000 mgH₂O₂ L⁻¹) presented sucrose degradation of around 55% (Figure 4). At extreme pH values (acidic or basic) and high temperatures, sucrose is hydrolysed to glucose and fructose (Amani et al., 2017). Treatments #10, #11 and #13 involved high temperatures and extreme pH values. According to Eggleston & Vercellotti (2000), the rate of sucrose degradation can be influenced by the concentration of ions (H⁺ and ‘OH), reaction temperature, presence of salts and the sucrose and monosaccharide concentrations. For Favero (2017), sucrose degradation occurs intensely at high temperatures and Mandro et al. (2017) found that at temperatures above 30°C, sucrose was more severely degraded by hydrogen peroxide.

The constant speed for each sugar syrup treatment required to cause sucrose degradation was calculated in 15 min reactions of sugar syrup with H₂O₂.

Treatment #10 (pH = 3.32; 50 °C and 500 mgH₂O₂ L⁻¹) presented the highest rate of sucrose degradation (Figure 4). On the other hand, treatment #4 (pH = 10.0; 70 °C and 202.4 mgH₂O₂ L⁻¹) presented the lowest degradation rate, although the pH of 10.0 and temperature of 70°C represent conditions that are normally unfavourable for the stability of the sucrose structure.
Figure 4. a) Profiles of sucrose degradation in treatments #7 (pH = 5.0; 70 °C and 797.6 mgH₂O₂ L⁻¹), #8 (pH = 10.0; 70 °C and 797.6 mgH₂O₂ L⁻¹), #10 (pH = 3.32; 50 °C and 500 mgH₂O₂ L⁻¹), #11 (pH = 11.68; 50 °C and 500 mgH₂O₂ L⁻¹), #13 (pH = 7.5; 83.6 °C and 500 mgH₂O₂ L⁻¹) and #15 (pH = 7.5 and 50 °C and 1.000 mgH₂O₂ L⁻¹) in sugar syrup.
according to the values simulated by the ANN. b) Kinetics of sucrose degradation reactions in \( \text{H}_2\text{O}_2 \) treatments, highlighting the phase with greater decomposition.

Of the treatments, #9 (Figure 5) presented the best colour × sucrose relationship, because it showed greater colour reduction (about 82%) with lower sucrose degradation (about 33%). In all the treatments analysed, the results were similar when comparing the theoretical and experimental values, and the best fits were observed for treatments #8 (pH= 10.0; 70 °C and 797.6 mg\( \text{H}_2\text{O}_2 \) L\(^{-1}\)) and #9 (pH= 7.5; 50 °C and 500 mg\( \text{H}_2\text{O}_2 \) L\(^{-1}\)).

The calculation of the relative importance of the variables showed that the factor with the greatest effect on sucrose degradation was the initial Brix, with a percentage of 54.38% (Figure 6). For Sartori et al. (2017), in studies with sugarcane juice, sucrose was the most abundant soluble component in sugarcane juice and thus, any changes in Brix values affect the sucrose concentration. Hydrogen peroxide represented 17.50% followed by pH with 15.31% and temperature with 12.79%.

Figure 5. a) Profiles of sucrose degradation in treatments #8 (pH= 10.0; 70 °C and 797.6 mg\( \text{H}_2\text{O}_2 \) L\(^{-1}\)) and #9 (pH= 7.5; 50 °C and 500 mg\( \text{H}_2\text{O}_2 \) L\(^{-1}\)) in sugar syrup according to values simulated by the ANN. b) Kinetics of the sucrose degradation reactions in the \( \text{H}_2\text{O}_2 \) treatments, highlighting the phase with greater decomposition.
Conclusion

In all cases an ICUMSA colour reduction occurred in the sugar syrup after treatment; however, it was more pronounced under conditions of pH = 7.5; 50 °C and 500 mgH2O2 L⁻¹. The initial Brix was indicated as the variable with the greatest influence on ICUMSA colour reduction and sucrose degradation. The profile of sucrose degradation up to 90 min showed that the first 15 min were the most critical for degradation. The models presented a good fit, with the simulated values very close to the experimental values.

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