M 12. PHENOLICS IN SUGAR CANE JUICE: POTENTIAL DEGRADATION BY HYDROGEN PEROXIDE AND FENTON'S REAGENT

By

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Abstract

THE PRESENCE of colour in raw sugar plays a key role in the marketing strategy of the Australian raw sugar industry. Some sugars are relatively difficult to decolourise during refining and develop colour during storage. A new approach that might result in efficient and cost-effective colour removal during the sugar manufacturing process is the use of an advanced oxidation process (AOP), known as Fenton oxidation, that is, catalytic production of hydroxyl radicals from the decomposition of hydrogen peroxide using ferrous iron. As a first step towards developing this technology, this study determined the composition of colour precursors present in the juice of cane harvested by three different methods. The methods were harvesting cane after burning, harvesting the whole crop with half of the trash extracted and harvesting the whole crop with no trash extracted. The study also investigated the degradation at pH 3, 4 and 5 of a phenolic compound, caffeic acid (3,4–dihydroxycinnamic acid), which is present in sugar cane juice, using both hydrogen peroxide and Fenton's reagent. The results show that juice expressed from whole crop cane has significantly higher colour than juices expressed from burnt cane. However, the concentrations of phenolic acids were lower in the juices expressed from whole crop cane. The main phenolic acids present in these juices were *p*-coumaric, vanillic, 2,3–dihydroxybenzoic, gallic and 3,4–dihydroxybenzoic acids. The degradation of caffeic acid significantly improved using Fenton's reagent in comparison to hydrogen peroxide alone. The Fenton oxidation was optimum at pH 5 when up to ~86 % of caffeic acid degraded within 5 min.

Introduction

One of the most important parameters in sugar quality is colour. Australian raw sugars are considered to be of high quality. This plays a key role in the marketing strategy of the Australian raw sugar industry. However, some raw sugars produced both in Australia and overseas are relatively difficult to decolourise and can develop colour during storage.

The costs of refining are directly proportional to the amount of colouring matter in raw sugar, possibly decreasing the market value of raw sugar. In sugar refining, colour removal comes at a major cost, hence the sugar refineries require raw sugars that are easy to decolourise and have low impurity loading

The formation of colour in raw sugar is a common problem in both sugar cane and sugar beet industries (Paton, 1992). The colorants that are difficult to decolourise are mainly hydrophobic and they cover a wide range of molecular weights. They exhibit an anionic behaviour at high pH levels. Their behaviour and reactivity at various stages of the sugar manufacturing process are extremely complex. They can participate in a number of reactions which lead to the formation of polymeric substances, highly coloured compounds and iron metal complexes. A reduction of colour in sugar or a cheap and effective method of removal in processing would lead to lower refining costs. There are few known simple processes in the raw sugar manufacturing process, apart from the crystallisation process, that can effectively and economically reduce colour. The options that are in current use in Australia for colour removal in raw sugar include double purging (washing) of sugar crystals and modification of crystallisation boiling schemes. These treatment procedures involve rejection of colour during or after the crystallisation process but are less effective with highly coloured raw sugars.

Phenolic compounds are well known to be colour precursors leading to the formation of coloured compounds with iron and copper and to oxidise to high molecular weight coloured polymers. They relate to the tendency of sugar to darken in storage and are not removed during clarification.

On the basis of the colour profile across the sugar manufacturing stage, to reduce colour in raw sugar, colour removal strategies should be targeted at mixed juice and/or juices during the evaporation stage. Chemical additives such as hydrogen peroxide (H_2O_2), ozone and coagulants have been used to decolourise sugar process streams (Madsen, 2006; Mane *et al.*, 1998; Mane *et al.*, 1992; Mane *et al.*, 2000; Moodley *et al.*, 1999; Patel and Moodley, 1991; Saska, 2007). Their use has not been widely implemented due to limited benefits.

A recent study by Pala and Erden (2005) has demonstrated the potential of the Fenton oxidation process to decolourise molasses. Madsen and Day (2010) demonstrated the removal of phenolic and other colourants from raw juice using endogenous proteins as well as ferric iron (Fe^{3+}) as an oxidative catalyst via cold liming. The treatment produced clarified juice with up to 70 % lower colour than juice produced by hot liming.

This study builds on these works by examining the degradation of a model phenolic compound, caffeic acid with ferrous iron (Fe^{2+}) and H_2O_2. The project also determined the composition of phenolic compounds present in burnt sugar cane juices and juices expressed from whole crop cane with half of the trash extracted and whole crop cane with trash not extracted.

Decolourisation using advanced oxidation processes

The use of oxidative decolourants to decolourise sugar process streams and/or raw sugars has received increasing interest in recent times. They are strongly oxidising chemicals which include H_2O_2, ozone and hypochlorite. Chlorinated compounds are not recommended because of toxicological concerns surrounding the production of unwanted by-products in juice (Davis, 2001).

Hydrogen peroxide

The reason for the difference between the action of oxidative chemicals and other decolourisation techniques lies in the reaction mechanism pathway. Oxidants destroy colour by cleaving unsaturated bonds (i.e. conjugated species) (Riffer, 2000).

Ferrous iron salts (Fenton's reagent)

Advanced oxidation processes (AOPs) are based on the generation of hydroxyl radicals ($^{\bullet}OH$). In these processes, the oxidation strength of H_2O_2 is enhanced by combining with acids, transition metal salts or UV-light to form $^{\bullet}OH$ and hydroxyl ions (Dwyer *et al.*, 2008).

An example of this is the activation of H_2O_2 using Fe^{2+}, typically referred to as the Fenton reaction. The Fenton reaction involves the generation of $^{\bullet}OH$ through the catalytic decomposition of H_2O_2 using Fe^{2+} as the catalyst under acidic conditions

The use of Fenton's reagent is more attractive in comparison to other AOPs for various reasons:

- reagents are relatively cheap and available commercially
- hydrogen peroxide decomposes to H_2O and O_2 spontaneously
- iron is present in sugar process streams
- equipment requirements and operating costs are minimal
- complete oxidative destruction of colourants to harmless compounds (viz. H_2O and CO_2) is achieved (Neyens and Baeyens, 2003).

The use of Fenton's reagent and H_2O_2 for the oxidative degradation of caffeic acid (Figure 1) in aqueous solution was investigated.

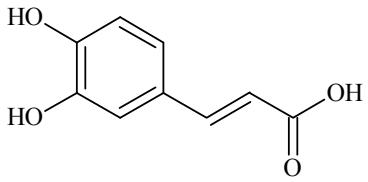

Fig. 1–Molecular structure of caffeic acid ($C_9H_8O_4$).

Materials and methods

Reagents and solvents

Analytical grade phenolic acids were purchased commercially from Sigma-Aldrich (St. Louis, MO, USA). Analytical grade hydrogen peroxide (30 % w/v) and ferrous sulfate heptahydrate were obtained from Ajax Finechem (Seven Hills, NSW, Australia). Solvents obtained from Merck (Darmstadt, Germany) were HPLC grade.

Juice sampling

First expressed juice (FEJ) from burnt cane was obtained from the processing lines at Condong Mill, NSW, Australia. The FEJ of whole crop cane was obtained by harvesting whole crop in the field and expressing juice with a laboratory hammer mill. Both FEJs were obtained during the crushing season in 2009. Primary juices (PJs) from burnt cane and 50 % whole crop cane were obtained from Condong Mill during the crushing season in 2010. All juices were stored at –22 °C. In total, four juices (2 FEJs and 2 PJs) were analysed in this study. The following analyses of the four juice samples are unrelated and not comparable.

The results obtained provide an insight on the levels of colour and phenolic compounds present in each juice type.

Colour analyses of sugar cane juices

Colour of each juice sample was determined at pH 7 by measuring the absorbance at 420 nm in 1 cm cells. Samples were diluted to an appropriate absorbance range and membrane filtered (0.45 μm) before adjusting the pH using 0.1 M and 0.01 M NaOH solutions respectively. The colour was calculated as:

Colour = 1000 x A420 cell length (cm) x sucrose concentration (g/mL)

Readings were performed in triplicate for each juice sample and reported as an average of the three readings. The precision of the experimental results is expressed as the relative standard deviation (% RSD) by calculating:

%RSD = Standard deviation of triplicate readings/ average of triplicate readings x100

The impurity of juice samples is expressed as the ratio of impurity to water (I/W) content as:

The brix of the samples was measured at ambient temperature using a Bellingham + Stanley RFM 342 refractometer accurate to ± 0.01 °Bx.

Extraction of phenolic acids from sugar cane juices

Juices were treated via alkaline hydrolysis. The individual mixtures were neutralised and extracted with diethyl ether (3 × 20 mL). The individual dried residues were weighed and dissolved in water (10 mL). The solutions were membrane filtered (0.45 μm) prior to evaluation by high-performance liquid chromatography.

High-Performance Liquid Chromatography (HPLC) conditions and analysis

The HPLC method used was similar to a previously reported method for the determination of phenolic acids in apple and pear juices (Schieber *et al.*, 2001). The assignment of eluted peaks was confirmed by spiking the juices with known phenolic acids and 5–hydroxymethylfurfural (HMF) and by comparing retention times to a standard mixture of nine phenolic acids and HMF. A set of five standard solutions was prepared for each compound and injected to generate a five-point calibration curve separately. The calibration curves were linear with a $R^2 \geq 0.9985$. The peak areas of the target compounds were within the linear range of the calibration curve. The relative standard deviation (% RSD) for triplicate injections of each standard for a set of five standard solutions was less than 5 %.

Degradation efficiency of caffeic acid using H_2O_2 and Fenton's reagent

Caffeic acid solution (10 000 ppm) was prepared by dissolving caffeic acid in a degassed solvent consisting of absolute ethanol and 0.056 μS/cm high purity water (1:1, v/v). Aqueous Fe^{2+} solution (50 000 ppm) was prepared by dissolving solid $FeSO_4.7H_2O$ in high purity water. An H_2O_2 solution was prepared from stock analytical grade H_2O_2 and high

purity water. The solution (5000 ppm) was standardised by iodometric titration. In order to establish the stoichiometry that exists between caffeic acid (200 ppm), Fe^{2+} (200 ppm) and H_2O_2 (100 and 400 ppm), the materials were used to prepare a series of solutions according to the sample matrix given in Table 1.

Table 1–Volumes of reagents used in the degradation reaction studies.

Sample*	Water (µL)	Caffeic acid (µL)	Fe^{2+} (µL)	H_2O_2 (µL)	[H_2O_2] (ppm)	Total (µL)
Blank A	49 000	1000	0	0	0	50 000
Exp. #1	48 000	1000	0	1000	100	50 000
Exp. #2	45 000	1000	0	4000	400	50 000
Blank B	48 880	1000	200	1000	0	50 000
Exp. #3	47 800	1000	200	1000	100	50 000
Exp. #4	44 800	1000	200	4000	400	50 000

*Blank solutions A and B are for H_2O_2 and Fenton's reagent reactions respectively.

The procedure for the addition of the Fenton's reagent can be described as follows: (a) adjusting the pH to 3, 4 or 5 of the caffeic acid solution (b) addition of $FeSO_4$ solution (c) addition of H_2O_2 and (d) the reaction allowed to run for up to 30 min at ambient temperature with agitation. The procedure for the addition of H_2O_2 without Fe^{2+} was identical with the exception that the reaction was allowed to run for up to 60 min. The pH was measured using a Radiometer Analytical MeterLab PHM 220 pH-meter. Aliquots (1 mL) were taken at 5 min intervals, diluted 10-fold and analysed by UV-visible spectrometry. Absorbance measurements were conducted on a GBC Cintra 40 double beam UV-visible spectrometer for the wavelengths ranging between 190 nm and 800 nm.

Results and discussion

Colour analyses of juices

Colour is conventionally measured at pH 7. Flavonoids and phenolic compounds are pH sensitive and their colour profile increases greatly from minimal colour in untreated mixed juice and FEJ (at pH 4–5) up to near-maximum colour at pH 9 (Paton, 1992). Therefore, colour measured at the ideal pH of 7 or higher would provide satisfactory measurement of the presence of flavonoids and phenolic compounds. The colour of Condong Mill juices is presented in Table 2.

Table 2–Colour of various sugar cane juices recorded at pH 7. *

	50% whole crop PJ	Burnt cane PJ	Whole crop, FEJ	Burnt cane, FEJ
Colour (IU on dry solids)	152 000	84 800	69 600	53 500
C/I ratio^	58 300	27 400	40 900	9 070

*% RSD was less than 0.7 %.
^C/I = Colour (on dry solids)-to-impurity ratio.

Analysis of phenolic acids

The phenolic compounds separated from cane juice are shown in Figure 2. Baseline separation was achieved for all identified components. The *m*– and *o*– isomers of coumaric acid were not detected in any of the analysed cane juice extracts. The elution order of the phenolic compounds was consistent with previous studies under different HPLC conditions

with the exception of 2,3–dihydroxybenozic acid and chlorogenic acid (Curtin and Paton, 1980).

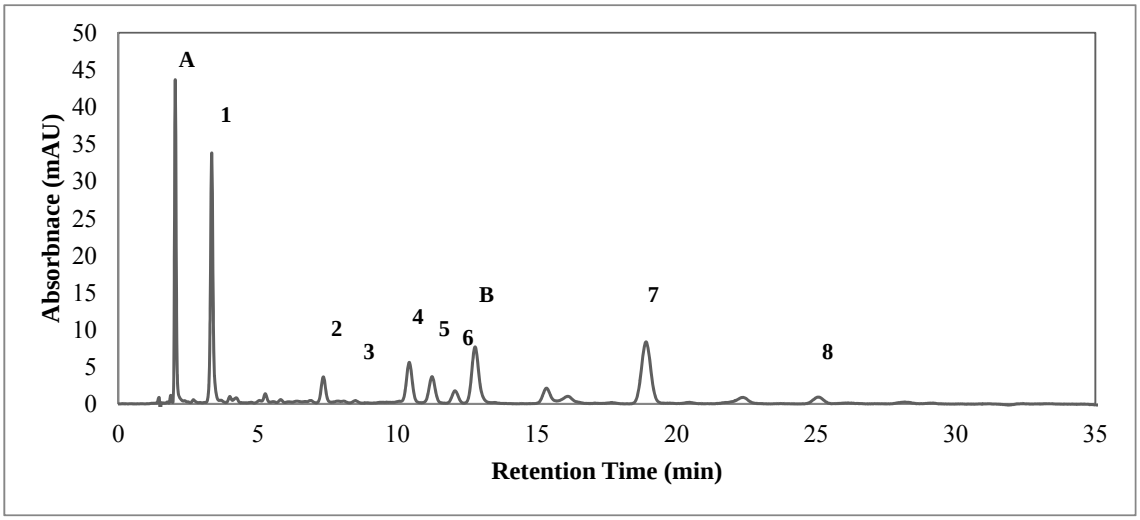

Fig. 2–Separation of a typical mixture of phenolic compounds in the FEJ of burnt cane by HPLC with UV detection at 280 nm. A = gallic acid (tentative) 1 = HMF;
2 = 4–hydroxybenzoic acid; 3 = chlorogenic acid; 4 = vanillic acid; 5 = caffeic acid;
6 = 2,3–dihydroxybenozic acid; B = 3,4–dihydroxybenzoic acid (tentative);
7 = *p*–coumaric acid; 8 = ferulic acid.

The concentrations of each compound varied with the juice type (Table 3). The concentrations of phenolic acids in whole crop juices were substantially lower than burnt cane juices. This is probably due to the valorisation of lignin (thermal degradation of lignin and biomass to produce profitable products) during cane burning.

Higher amounts of HMF were identified in both PJ and FEJ of burnt cane juices compared to whole crop juices (Table 3). This may be because of the dehydration of sugars (particularly reducing sugars) to HMF (Huber *et al.*, 2006) as a result of high temperatures generated during burning of cane prior to harvesting. To our understanding, the quantification of HMF in Australian PJ and FEJ using this method has not been described in the literature. Caffeic acid concentrations in each juice sample were relatively lower than other phenolic acids.

Higher concentrations of phenolics are present in PJs compared to FEJs (Table 3). This is probably due to the decomposition of certain flavonoids followed by oxidation of the intermediate products and further degradation of lignin products at relatively higher processing temperatures of PJ.

Table 3–HMF and phenolic compounds isolated from various sugar cane juice samples (ppm on dry solids). *

Compound	50% whole crop PJ	Burnt cane PJ	Whole crop FEJ	Burnt cane FEJ
HMF	330	6550	261	1380
4–Hydroxybenzoic acid	7030	18 800	322	5300
Chlorogenic acid	4710	44 400	358	23 000
Vanillic acid	13 900	27 800	592	9350
Caffeic acid	11 300	24 300	633	9480
2,3–Dihydroxybenzoic acid	11 700	24 900	634	9020
p–Coumaric acid	14 000	95 900	995	23 600
Ferulic acid	7130	13 700	477	7310

*% RSD < 5 %

The concentrations of phenolic acids are considerably higher than those previously described by Curtin and Paton (1980). Table 4 shows a comparison of the phenolic acid composition for juices from Table 3 in terms of ppm on juice, to those of Curtin and Paton (1980). The differences between the two sets of data may be related to differences in the cane varieties or to differences in the analytical procedures used for phenolic acid analysis.

Table 4–HMF and phenolic compounds isolated from various sugar cane juice samples (ppm on juice). *

Compound	50% whole crop PJ	Burnt cane PJ	Whole crop FEJ	Burnt cane FEJ	Burnt cane PJ^
HMF	1.5	2.6	0.2	3.3	–
4–Hydroxybenzoic acid	5.8	7.6	4.9	4.1	0.2
Chlorogenic acid	25.3	17.9	3.3	4.6	–
Vanillic acid	10.3	11.2	9.8	7.6	0.3
Caffeic acid	10.4	9.8	7.9	8.1	15.0
2,3–Dihydroxybenzoic acid	9.9	10.0	8.2	8.1	0.1
p–Coumaric acid	26.0	38.7	9.8	12.7	0.6
Ferulic acid	8.0	5.5	5.0	6.1	0.3
3,4–Dihydroxybenzoic acid (tentative)	nq	nq	nq	nq	60.0
Gallic acid (tentative)	nq	nq	nq	nq	-
Sinapinic acid	–	–	–	–	0.1

*% RSD < 5 %.
^Cane juice data based from (Curtin and Paton, 1980); nq, not quantified

As evident from Tables 2, 3 and 4, the juices expressed from whole crop contain higher colour but lower phenolics than the juices expressed from burnt cane. It is therefore deduced that that the whole crop juices contain a higher proportion of cane pigments.

Degradation of caffeic acid by H_2O_2 and Fenton's reagent

The average concentration of caffeic acid in the juices was ~10 ppm, although a caffeic acid concentration of 200 ppm was chosen for the degradation studies in order to account for other phenolics and colour precursors (amines and amino acids) present in cane juice.

Figure 3 shows the degradation of caffeic acid with a 100 ppm dosage of H_2O_2 at pH 3 at 0, 30 and 60 min. Two isosbestic points at 292 nm and 320 nm are attributable to the deprotonated caffeate anion ($C_9H_7O_4^-$) and the caffeic acid molecule ($C_9H_8O_4$) respectively

(Cornard *et al.*, 2006). These points were present in reaction mixtures containing caffeic acid and Fenton's reagent at pH 3, 4 and 5.

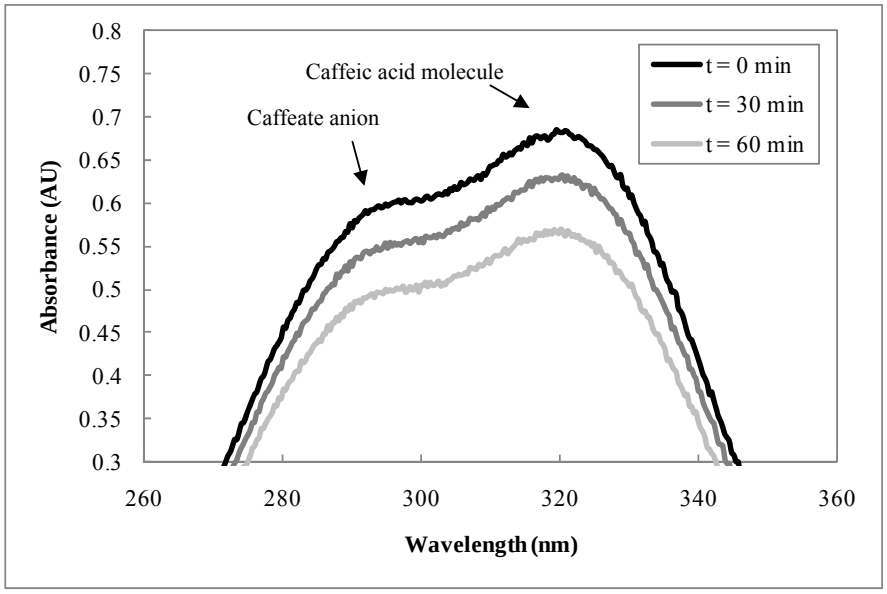

Fig. 3–Absorption spectra of caffeic acid after the addition of 100 ppm H_2O_2 at pH 3.

At pH 3 and 60 min, 16.5 % caffeic acid was degraded. At pH 4 and 5, there was no observable caffeic acid degradation. At a higher H_2O_2 dosage of 400 ppm, no reduction in absorbance was noticeable at either isosbestic points even at pH 3. There appeared to be some degradation occurring at lower wavelengths but this was not conclusive. It is speculated that with the addition of 400 ppm of H_2O_2 after initial reactions between the hydroxyl radicals and caffeic acid, there were subsequent recombination reactions.

The degradation of caffeic acid with the Fenton's reagent monitored at 320 nm is shown in Figure 4. The reaction was virtually complete within 5 min. Within 30 min, 85.8 % of caffeic acid was destroyed upon addition of 200 ppm Fe^{2+} and 400 ppm H_2O_2 at pH 5, in which most of the degradation took place within 5 min. At lower pH, the degradation of caffeic acid was less with 61.7 % and 66.4 % degradation for pH 3 and 4 respectively. The degradation of the deprotonated caffeate anion was also observed. The degradation ratio of the neutral and anionic forms was 1:1, hence the Fenton's reagent is capable of attacking both forms of caffeic acid. A similar trend was observed when a lower dosage of H_2O_2 was used with less than 70 % degradation observed within the studied pH region.

The degradation trends were similar for both 100 ppm and 400 ppm H_2O_2 dosages with the latter having a larger decrease in absorbance. A faster degradation rate was observed at pH 5 for both H_2O_2 dosages despite a higher initial absorbance. The higher initial absorbance is attributable to the chelating ability of ferrous iron on caffeic acid to produce coloured complexes (Smith, 1983).

No other prominent peaks were observed across the spectral wavelength range during the course of both experiments. This suggests that the degradation products formed from the use of Fenton's reagent are colourless compounds or low molecular weight compounds with a weak or no UV-chromophore.

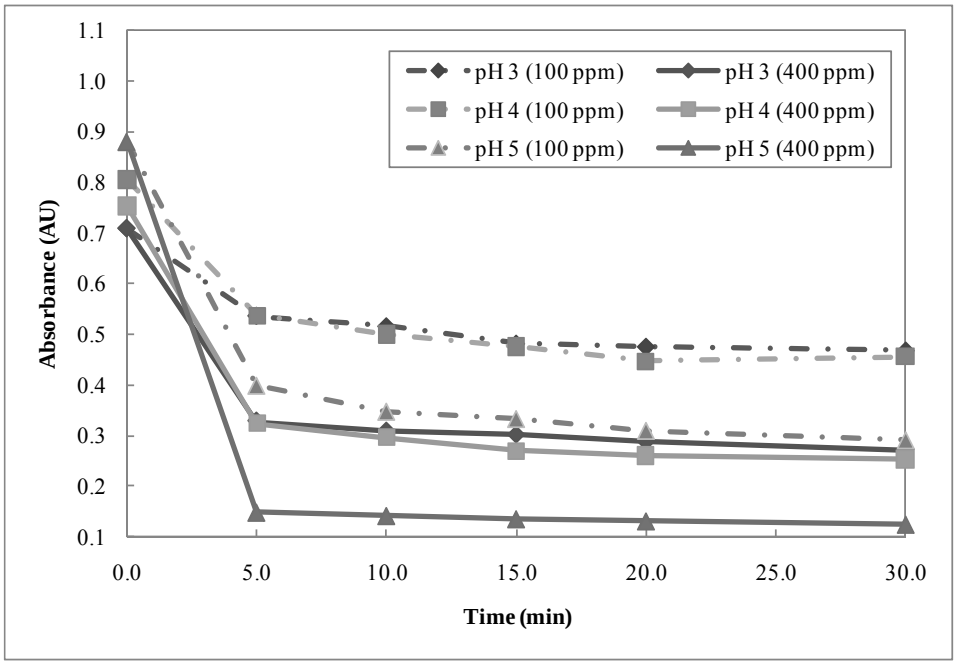

Fig. 4–Typical degradation of caffeic acid (at 320 nm) using Fenton's reagent at different pH values.

Conclusions

Phenolic acids present in different juices types were determined by HPLC analysis. The concentrations of identified phenolic acids were substantially higher in burnt cane juices than in whole crop juices, though the overall juice colour (measured at 420 nm at pH 7) was higher in the latter juices. The Fenton's reagent is an effective oxidant for the degradation of caffeic acid compared to H_2O_2.

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