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Inhibitory effect of caramelisation products on enzymic browning

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Caramelisation products (CP) were prepared by heating a sucrose solution (1.47 M) at 200°C under various conditions to study the inhibitory activity of CP on enzymic browning. Inhibition by fractionated CP was measured by enzymic kinetic analysis of polyphenol oxidase (PPO) and *o*-dihydroxyphenols, such as 4-methylcatechol (4-MC), catechol, caffeic acid and DL-3,4-dihydroxyphenylalanine (DL-DOPA). Colour intensity of CP produced at pH 4 was higher than that produced at pH 6 and pH 8, and increased with heating time. The reducing power of CP and their inhibitory effect on enzymic browning increased with prolonged heating and with increased amounts of CP. CP heated at pH 4 and pH 6 for 90 min gave a relative inhibitory activity on enzymic browning of 85.8% and 72.2%, respectively. One of two CP fractions obtained by Biogel P6 column chromatography had an inhibitory effect on enzymic browning, with the fraction having a molecular weight of 1000–3000 showing the highest activity. Based on the double reciprocal plot of the PPO and 4-MC system, the active fraction of CP appeared to be a competitive inhibitor and was most effective on inhibition of catechol browning, followed by caffeic acid and DL-DOPA. © 1997 Elsevier Science Ltd

INTRODUCTION

Caramelisation, a non-enzymatic sugar browning reaction, is a common reaction in food processing, which generates a brown colour and a caramel-like flavour. It involves the heat-induced decomposition of sugars, normally monosaccharides. They undergo initial enolisation, known as the Lobry de Bruyn–A. van Eckenstein rearrangement, and progress to subsequent complex reactions, such as dehydration, dicarboxylic cleaving and aldol condensation (Kroh, 1994). The reaction generally releases H^+, thus the pH of the solution decreases with time. Caramelisation products (CP) vary in chemical and physical properties and constituents depending on temperature, pH and duration of heating (del Buera *et al.*, 1987; Shallenberger & Birch, 1975). CP consist of volatile and non-volatile fractions of low and high molecular weights (Tomasik *et al.*, 1989).

CP from glucose have been reported to have an antioxidant activity which consists mainly of colourless intermediates, such as reductones and dehydroreductones, produced in the earlier stages of the caramelisation reaction (Rhee & Kim, 1975). However, Kirigaya *et al.* (1968) suggested that high molecular weight and coloured pigments may play an important role. CP from glucose also showed an inhibitory effect on polyphenol oxidase (PPO), which is largely responsible for the enzymic browning reactions in fruits and vegetables. Recently, Pitotti *et al.* (1995) have reported that the antibrowning effect of some CP is, in part, related to their reducing power. The objectives of this study were to investigate the effect of CP produced from sucrose under various conditions on enzymic browning, and to find a relationship between some properties of CP and their browning activity.

MATERIALS AND METHODS

Materials

Mushroom tyrosinase (2260 units mg^{-1}) and *o*-dihydroxyphenols, 4-methylcatechol (4-MC), catechol, caffeic acid and DL-dihydroxyphenylalanine (DL-DOPA) were obtained from Sigma Chemical Co. (St Louis, MO). Sucrose was purchased from Fisher Scientific Co. (Fair Lawn, NJ). All other chemicals were of analytical grade.

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Caramelisation products

Five grams of sucrose (1.47 M) were dissolved in 10 ml of McIlvaine buffer (0.2 M citrate and 0.1 M phosphate) at pH 4.0, 6.0 and 8.0. Caramelisation of each sample was carried out by heating at 200°C on a hot plate in an open beaker for up to 90 min. The temperature was monitored with a digital thermometer (Omega HH 82; Omega Engineering, Stamford, CT). Samples of CP prepared at various pH values and heated for different times were dissolved in water to give a final volume of 50 ml. The pH of CP solutions initially at pH 4.0, 6.0 and 8.0 and heated for 90 min were 3.6, 4.2, and 6.2, respectively, after heating.

Ultrafiltration

Samples of CP were fractionated with Diaflo ultrafiltration membranes (Amicon Division, W. R. Grace and Co., Danvers, MA) under a pressure of 50 psi. Three fractions (molecular weight (MW) below 1000, MW 1000–3000 and MW above 3000) were compared as to their colour intensity and inhibitory effects on enzymic browning.

Measurement of reducing power

Reducing capacity of CP was determined by the methods of Crowe *et al.* (1948) and Yen & Tsai (1993). After dilution (10 or 100 times) with distilled water, 5 ml of CP and partially fractionated CP by ultrafiltration were each mixed with 5 ml of potassium phosphate buffer (0.2 M, pH 7.4) and 5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min and then cooled immediately to room temperature in tap water. Then 5 ml of the reaction mixtures were transferred to test tubes and 1 ml of fresh ferric chloride solution (0.1%) was added. After mixing thoroughly, each tube was held for exactly 10 min and then the absorbance at 660 nm was determined using a Hewlett-Packard 8452A spectrophotometer.

Inhibitory effect of CP on enzymic browning

The inhibitory effect of CP on enzymic browning was measured by the following method: varying amounts of CP solution (0–200 µl) were added to flasks containing 8 ml of 4-MC (1 mM) and adequate volumes of McIlvaine buffer (pH 5) to make a final volume of 10 ml. This substrate–CP solution (2.5 ml) was measured into a cuvette of a Hewlett-Packard 8452A spectrophotometer equipped with a diode-array detector and a Series 300 computer. The absorbance was balanced to zero and 0.5 ml of PPO (tyrosinase, 1 mg per 10 ml) was added. Changes in absorbance at 410 nm were recorded at 20°C. PPO activity was expressed as the change in absorbance at 410 nm per min. The relative percent browning inhibitory activity was calculated by subtracting residual activity measured in the presence of CP from 100% (the absence of CP).

Measurement of colour intensity

Colour intensity of CP was determined by measuring the absorbance at 294 nm and 420 nm as described by Lerci *et al.* (1990).

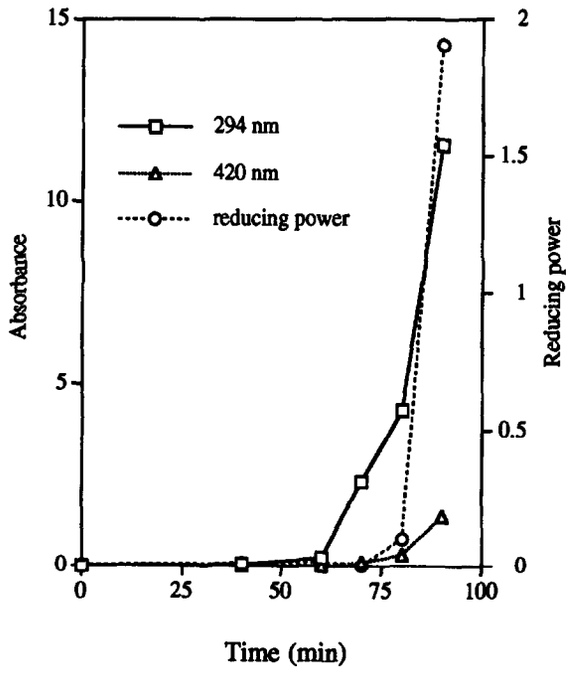

Fig. 1. Changes in colour intensity and reducing power of CP prepared at pH 4 with different heating times. Absorbance was measured after 1000-fold dilution.

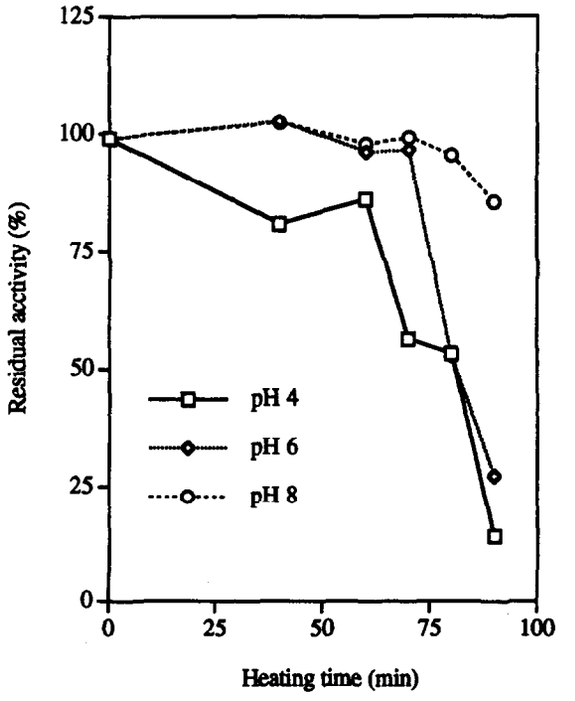

Fig. 2. Inhibitory effect of CP produced at different pHs on enzymic browning.

Inhibition kinetics

To determine the inhibition mode of CP on enzymic browning, different volumes (0–200 μl) of CP were used with 4-MC. Browning inhibitory effects on other *o*-dihydroxyphenols such as catechol, caffeic acid and DL-DOPA were also determined. The rate of oxidation on different substrates was measured at the following wavelengths according to Kahn *et al.* (1993): catechol, 400 nm; caffeic acid, 470 nm; DL-DOPA, 475 nm. Results were expressed as percent inhibition.

Gel filtration

Samples of CP prepared at pH 4 for 90 min were applied to a Biogel P6 (Bio-Rad, Richmond, CA) column (1.5 cm×50 cm), equilibrated with K_2HPO_4 buffer (0.01 M, pH 7.0). Elution was performed using the same buffer at a flow rate of 30 ml h^{-1}. Fractions of 3 ml were collected and the colour intensity and inhibitory effect on enzymic browning were measured.

RESULTS AND DISCUSSION

Colour formation and reducing power of CP

All CP samples produced at 200°C for 90 min at various pHs showed maximum absorption at 280 nm. Colour intensity of CP obtained at pH 4 was higher than that at pH 6 and pH 8 at the different wavelengths (data not shown). Spectrophotometric measurements at 278, 280 and 294 nm for pyrazine compounds and 420 nm for brown pigment are most commonly used for the Maillard reaction (Lerci *et al.*, 1990). Figure 1 presents the absorbances at 294 nm and 420 nm and the reducing power of CP at 200°C, pH 4, at different time periods. The initial colourless sucrose solution changed to a light yellow after 60 min of heating at pH 4. The colour intensity and reducing power of CP increased with prolonged heating. Pitotti *et al.* (1995) reported that, when sucrose was heated at 90°C, some hydrolysis preceded the caramelisation. Colour development in a sucrose solution seems to be different from monosaccharides. This may be attributed to the acid-catalysed hydrolysis of sucrose. Thus, caramelisation at low pH produced two effects regarding colour development: fructose, produced by sucrose hydrolysis, facilitated rapid colour development, and, at the same time, a low pH reduced the caramelisation reaction (del Buera *et al.*, 1987).

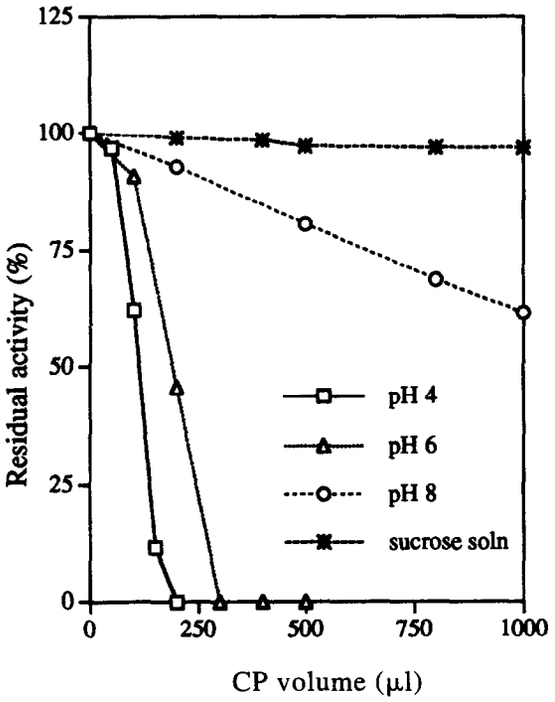

Fig. 3. Inhibitory effect of different amounts of CP on enzymic browning. CP were prepared at various pHs for 90 min.

Table 1. Yield, colour intensity, reducing power and inhibitory effect on enzymic browning of three fractions by ultrafiltration

Fraction	Yield (%)	Colour intensity (420 nm)	Reducing power (660 nm)	Inhibitory effect (%)
Unfractionated CP	100[a]	20.8	0.89	45.8
MW below 1000	71.6	12.4	0.21	43.7
MW 1000–3000	18.3	42.1	1.52	76.8
MW above 3000	9.9	11.4	0.20	14.8

[a]50 ml of CP prepared at pH 4 for 90 min at 200°C were fractionated by ultrafiltration. After lyophilization, each fraction was dissolved in water and the colour intensity and inhibitory effect on enzymic browning were measured.

Inhibitory effect of CP on enzymic browning

The inhibitory effect of CP (200 μl) produced at different pHs on enzymic browning is shown in Fig. 2. A progresive decrease in residual activity during heating time indicated a constant increase in inhibitory capacity of CP with the heating time. Under the conditions of this experiment, CP heated at pH 4 and pH 6 for 90 min inhibited enzymic browning by 85.8% and 72.7%, respectively. However, CP prepared at pH 8 showed a minimal effect. Acidic conditions during the caramelisation reaction appeared to produce more inhibitory compounds than an alkaline environment. Figure 3

Table 2. Relative inhibitory effect of CP on different *o*-diphenols

CP volume (μl)	4-MC	Catechol	Caffeic acid	DL-DOPA
0	0[a]	0	0	0
50	18.0	21.8	26.5	13.1
100	38.0	40.0	37.7	17.0
200	72.8	100.0	57.5	33.0

[a]Inhibitory effect of CP on enzymic browning was measured by adding different amounts of CP to 8 ml of 1 mM phenolics and 0.5 ml of tyrosinase as described, and expressed as percent inhibition.

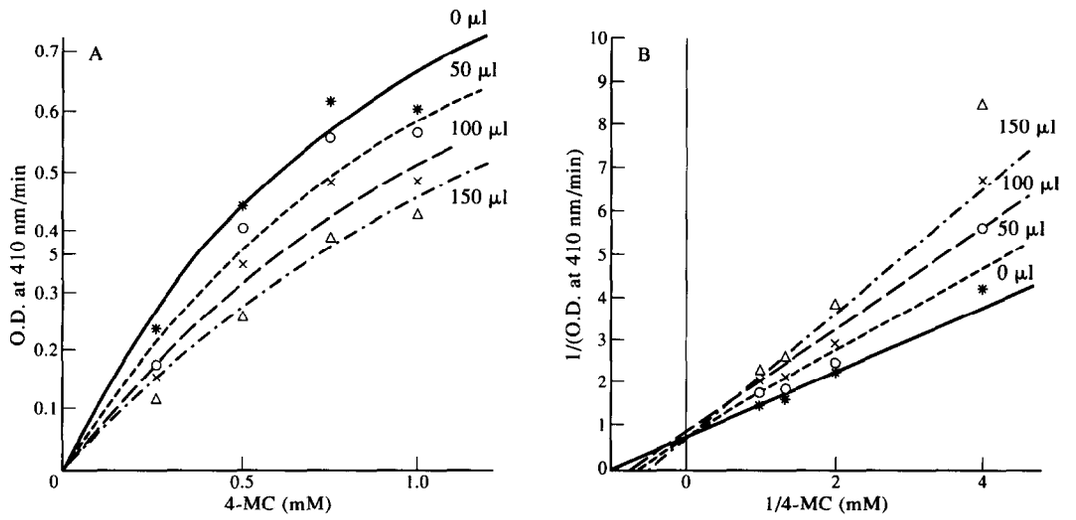

Fig. 4. Inhibitory kinetics of CP on enzymic browning. (A) Effect of CP concentration on the enzymic browning rate of 4-MC. (B) Reciprocal plot of CP inhibition on 4-MC browning reaction.

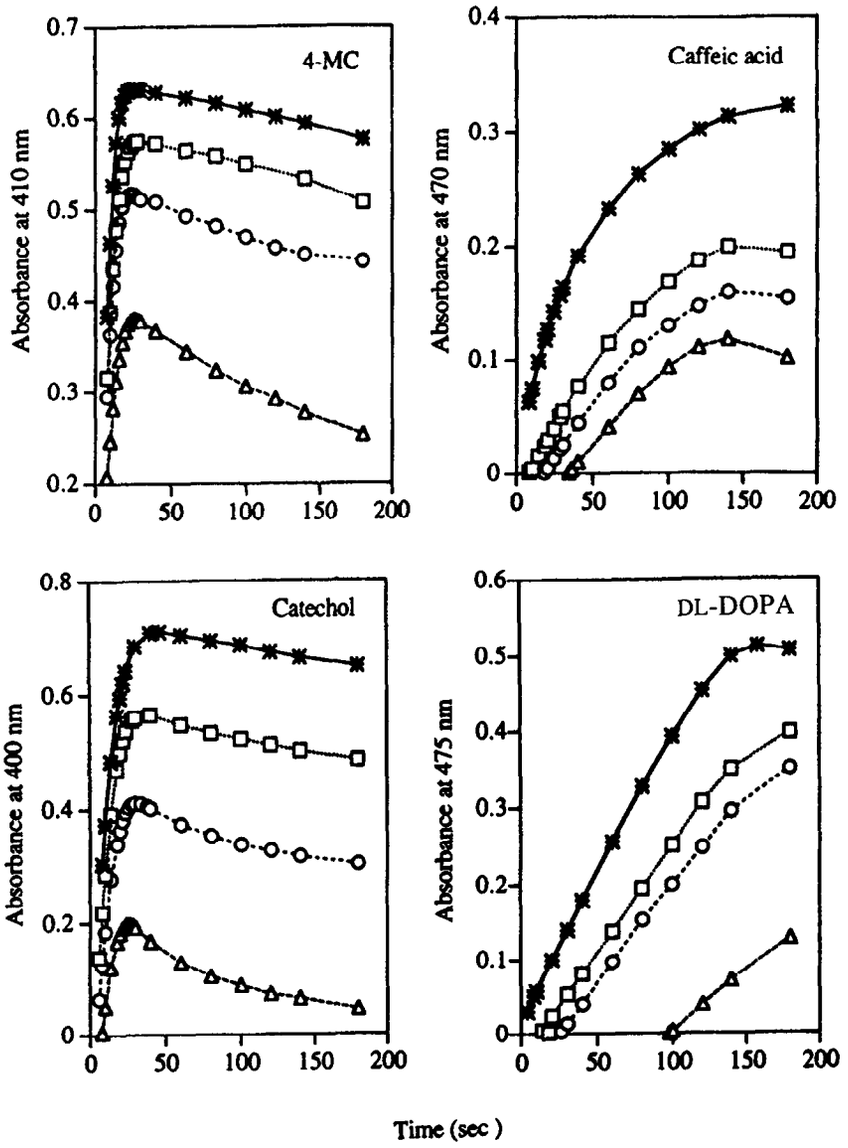

Fig. 5. Inhibitory characteristics of different amounts of CP on enzymic browning of different *o*-dihydroxyphenols. *, 0 μl; □, 50 μl; ○, 100 μl; △, 200 μl.

shows the inhibitory effect on enzymic browning of different amounts of CP produced at various pHs when heated for 90 min. The unheated sucrose solution exhibited minimal inhibition, while CP prepared at pH 4 needed only 200 μl to inhibit browning completely. These results also indicated that the inhibitory effect of CP on enzymic browning directly correlated with its colour intensity and reducing power. It appeared that the active inhibitory compounds were formed at the later stage of caramelisation when colour development was at a maximum.

Characteristics of fractionated CP

Some chemical properties of CP fractionated with membrane filters into different molecular weights (MW below 1000, MW 1000–3000, and MW above 3000) are shown in Table 1. Among the three fractions, the fraction of MW below 1000 was the largest (71.6%) and the fraction of MW above 3000 was the smallest (9.9%). Each fraction exhibited a different degree of colour intensity, reducing power and inhibitory effects on browning. The fraction of MW between 1000–3000, which showed the highest colour intensity, exhibited the greatest inhibition (76.8%) on enzymic browning and the highest reducing power.

Inhibition kinetics of fractionated CP

The initial velocity of the PPO-catalysed 4-MC oxidation reaction decreased with increased CP concentration, as shown in Fig. 4(A). Based on the double reciprocal plot of the PPO and 4-MC reaction rate, CP appeared to be a competitive inhibitor (Fig. 4(B)). The inhibitory effect of partially fractionated CP was also compared using other *o*-dihydroxyphenols such as catechol, caffeic acid and DL-DOPA (Table 2). Addition of 200 µl of CP to the PPO and substrate system used in this experiment inhibited catechol browning completely and decreased the caffeic acid reaction rate to 57% and DL-DOPA to 33%. Figure 5 shows that the kinetics of the reaction depended on the nature of the four substrates. With 4-MC and catechol, the initial velocity of the reaction decreased with increasing CP concentrations. However, a lag phase was observed with the caffeic acid and DL-DOPA systems, the duration of which depended on the amount of CP present in the medium. The lag phase observed in this study may be related to the reducing power of CP. This is consistent with the findings of Pitotti *et al.* (1995), who reported that the antibrowning effect of some CP is, in part, related to the reducing power of their oxygen-scavenging properties. Nicoli *et al.* (1991) reported that Maillard reaction products showed two different effects on PPO: an inhibition of the enzyme activity and the appearance of an initial lag phase, due to the chelating properties of PPO on Cu^{2+} and the reducing properties of the Maillard reaction products.

Biogel P6 column chromatography was used to separate CP into two peaks (data not shown). Only the second peak (fractions 23–29) showed an inhibitory effect on enzymic browning. Separation by Sephadex G15 was not successful.

In conclusion, CP produced from sucrose at 200°C at pH 4 showed a strong inhibitory effect on enzymic browning. The molecular weight of the active compounds in CP are in the range of 1000–3000. The active compounds appeared to be competitive inhibitors. The presence of a lag phase in the inhibitory studies of CP with caffeic acid and DL-DOPA may be due to the reducing power of the active compounds.

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