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Inhibition of browning by antibrowning agents and phenolic acids or cinnamic acid in the glucose–lysine model

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Abstract: The effects of antibrowning agents and phenolic acids or cinnamic acid on the inhibition of browning were investigated with a glucose–lysine model. Six antibrowning agents (cysteine, glutathione, sodium sulfite, pentasodium tripolyphosphate, citric acid and oxalic acid) and four phenolic acids (ferulic, hydroxybenzoic, syringic and vanillic acids) were used. In order to investigate the antibrowning capacity of these agents, model solutions containing glucose, lysine and an antibrowning agent were heated at 50 °C in the presence of $FeCl_2$, before being stored in nitrogen or air at 4 °C or 30 °C. Browning was accelerated to some degree during storage in air at 30 °C. In the case of storage at 4 °C, however, no browning was detected in nitrogen after four weeks. Citric acid was the most efficient antibrowning agent during storage in air at 30 °C and inhibited browning to 36% after four weeks. However, its antibrowning capacity was increased by 8–15% in the presence of any of the phenolic acids or cinnamic acid, essentially independently of concentration in the range 10 μM to 10 mM or the type of phenolic acid.

Keywords: inhibition of browning; antibrowning agent; phenolic acid; cinnamic acid; chelating activity

INTRODUCTION

The amino-carbonyl reaction has considerable influence on the color, flavor, functional properties and nutritional value of food. This reaction involves reducing sugars and amino acids or peptides, and is facilitated by cooking, thermal processing or storage.[1,2] The reaction products include a complex array of chemical intermediates and final brown pigments known collectively as melanoidins.[2,3] While this reaction is desirable to some degree, too much browning with a concomitant off-flavor decreases the food quality.

Because of its ε-amino group,[4] lysine is considered to be the most effective amino acid for the browning of most foods such as pasta,[5] milk[6] and soybean paste. In addition to the reactant,[7,8] metal ions,[9] temperature[2] and pH[8] also influence browning. Color deterioration can, therefore, be inhibited by controlling these factors. The temperature during storage, as well as that during processing, is especially crucial to the progress of the amino-carbonyl reaction.[10,11] Although storage at low temperature is an efficient way to inhibit browning,[11] browning cannot be completely inhibited, and the economics associated with keeping foods cold is a considerable problem. Alternative antibrowning methods are therefore required. An effective antibrowning agent that can preserve its antibrowning capacity at room temperature for a long time is one potential solution to this problem.

Sulfites and sulfur-containing amino acids or peptides appear to be particularly effective in inhibiting both enzymatic and nonenzymatic browning.[12–14] Citric acid,[15] oxalic acid[16] and phosphate[17] have been used to prevent enzymatic browning reactions by chelating copper from the active sites of polyphenol oxidases. Phenolic acids, such as chlorogenic acid and ferulic acid, are widely distributed in the plant kingdom. They are responsible for enzymatic browning[12] and act as antioxidants[18–21] by scavenging free radicals. They can complex weakly with metal ions,[20,21] and such ability is responsible for antioxidative their capacity. Cinnamic acid, an aromatic carboxylic acid, as well as its phenolic relatives such as coumaric, caffeic and ferulic acids, is present in many essential oils from plants and is known for its preservative[22] and flavoring actions.[23]

If browning of a lysine system model were effectively inhibited, a similar method might control browning of foodstuffs; however, few studies have been carried out on the inhibition of browning due to lysine. The objective of the present study was to investigate the inhibitory effect of six antibrowning agents (cysteine, glutathione, sodium sulfite, pentasodium

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tripolyphosphate, citric acid and oxalic acid) on browning of a glucose–lysine model system containing Fe(II). We also examine the additional effect on browning of phenolic acids (ferulic, hydroxybenzoic, syringic and vanillic acids) and of cinnamic acid to explore the development of new synergistic antibrowning agents.

MATERIALS AND METHODS

Materials

Glucose, lysine, citric acid, oxalic acid and cysteine were obtained from Wako Pure Chemicals (Osaka, Japan). Pentasodium tripolyphosphate, sodium sulfite, glutathione, iron(II) chloride ($FeCl_2 \cdot 4H_2O$), cinnamic acid, ferulic acid, hydroxybenzoic acid, syringic acid and vanillic acid were obtained from Sigma Chemical Co (St Louis, MO, USA), and distilled water (HPLC grade) was obtained from JT Baker (Phillipsburg, USA).

Preparation of the model solutions

Glucose (0.1 M) and lysine (0.1 M) were dissolved in a citrate-phosphate buffer (1 M, pH 6.50) in the presence of 0.2 mM $FeCl_2$, and an antibrowning agent was added. Six antibrowning agents were each used at different final concentrations: cysteine (2 and 5 mM), glutathione (2 and 5 mM), sodium sulfite (2 and 5 mM), pentasodium tripolyphosphate (2 and 5 mM), citric acid (5 and 50 mM) and oxalic acid (5 and 50 mM). Aliquots (15 ml) of each reaction solution were put into screw-capped tubes (25 × 200 mm) and heated in a block heater at 50 °C for 48 h. After heating, the tubes were immediately put into cool water to stop the reaction and then stored in a refrigerator until needed. A control was made up containing no antibrowning agent. Another group of model solutions containing a phenolic acid or cinnamic acid was also prepared. The phenolic acids (ferulic, hydroxybenzoic, syringic or vanillic acid) was added to a solution containing 50 mM citric acid to make up final concentrations of 10 μM and 10 mM, and model solutions were prepared by the procedure just described. In this case, the control contained no phenolic or cinnamic acid.

Methods

Storage experiment

We placed 10 ml of a model solution in a 30-ml vial, and stored it in nitrogen or air at 4 °C or 30 °C for four weeks. The model solution was flushed with nitrogen for 1 min, and the headspace similarly flushed for 30 s. A sample was taken weekly, and the antibrowning capacity during storage determined. The model solutions containing citric acid and phenolic acid were stored in air at 30 °C for four weeks.

Determination of the antibrowning capacity

The antibrowning capacity was determined as the optical density (OD) at 420 nm. The results were calculated by using the following equation:

$$\text{Antibrowning capacity (\%)} = 100 - [(\text{OD of model solution with an antibrowning agent/OD of control}) \times 100]$$

A model 8453 UV/VIS spectrophotometer (Hewlett-Packard, Germany) was used to monitor the OD.

Determination of the Fe(II)-chelating activity of phenolic acid

The Fe(II)-chelating activity was determined according to the method of Okada and Okada.[24] Ten milliliters of a 10 mM hexamine buffer containing 10 mM KCl and 3 mM $FeSO_4$ (pH 5.0) was added to 2 ml of a model solution, and 0.2 ml of 1 mM tetramethyl murexide (TMM) was then added. The model solutions containing citric acid and phenolic acid were used as samples after storing in air at 30 °C for four weeks. The OD was measured at 480 nm, since the TMM-free Fe(II) complex showed its maximum absorption at 480 nm. A lower value for the OD therefore indicates that the model solution had strong chelating activity. The results were calculated in the same way as the antibrowning capacity.

Statistical analysis

All experiments were conducted twice. Duncan's multiple-range test of the SPSS statistical package (version 10.0) was used to detect significance of differences ($p < 0.05$).

RESULTS AND DISCUSSION

Initial antibrowning capacity

The initial antibrowning capacity, which was determined immediately after preparing the model solution, is shown in Table 1. With the exception of pentasodium tripolyphosphate, browning was greatly inhibited at the higher concentration. The highest antibrowning capacity of the antibrowning agents was with sodium sulfite, 5 mM inhibiting browning by 82%. The next most effective were cysteine and glutathione, 5 mM inhibiting browning by 76 and 79%, respectively. Citric acid and oxalic acid at 50 mM both showed high antibrowning capacity of 56 and 51%, respectively, while the antibrowning capacity of 5 mM pentasodium tripolyphosphate was 29%. The following effects of thiol compounds in the antibrowning process are anticipated: suppression of free radical formation; strong neophilic reactivity with the amino, aldehyde or keto group and ability to dissipate free radicals; and reduction of carbonyl groups or reaction with carbonyl groups and double bonds in the browned products to form colorless materials.[13] However, the effects of sulfite are not understood, although it seems

Table 1. Initial antibrowning capacities of antibrowning agents in the glucose–lysine model solutions immediately after preparation[a]

Antibrowning agent	Antibrowning capacity (%)
2 mM cysteine	57.97 ± 3.87c
5 mM cysteine	79.61 ± 1.28ab
2 mM glutathione	48.00 ± 2.02e
5 mM glutathione	76.21 ± 1.26b
2 mM sodium sulfite	40.82 ± 5.54f
5 mM sodium sulfite	82.68 ± 2.27a
2 mM pentasodium tripolyphosphate	22.74 ± 0.52hi
5 mM pentasodium tripolyphosphate	28.98 ± 0.69g
5 mM citric acid	19.63 ± 0.20i
50 mM citric acid	56.02 ± 0.82cd
5 mM oxalic acid	25.61 ± 3.65gh
50 mM oxalic acid	51.78 ± 3.66de

[a] Each value is expressed as the mean ± standard deviation ($n = 2$). Means with different letters in the same column are significantly different by Duncan's multiple-range test ($p < 0.05$).

to have greater nucleophilic reactivity than thiol compounds.[13,14]

Considering their high antibrowning capacity, the subsequent experiments were performed with 5 mM sodium sulfite, cysteine, glutathione and pentasodium tripolyphosphate, and with 50 mM citric acid and oxalic acid.

Change in antibrowning capacity during storage

The antibrowning capacity of six of the antibrowning agents was maintained at the initial level in nitrogen at 4 °C, while that of cysteine decreased (Fig 1). However, storage in air over four weeks resulted in the antibrowning capacity of all except sodium sulfite and pentasodium tripolyphosphate decreasing to some degree. This result indicates oxygen is a factor in browning, even at low temperature.

Figure 2 shows storage at 30 °C, the antibrowning capacity of all antibrowning agents decreasing greatly with storage time. The decrease in antibrowning capacity of the sulfur compounds was more significant than that of citric acid, oxalic acid and pentasodium tripolyphosphate. We conclude from this that the sulfur compounds only exhibited their high antibrowning capacity immediately after the treatment or during limited-term storage at room temperature. This result is in accordance with those of Friedman and Molnar-Perl[13] and Molnar-Perl and Friedman.[14] Storage in nitrogen only appears to have been effective in the short term.

Citric acid showed the highest antibrowning capacity after four weeks being 40% in nitrogen and 36% in air. This was due to the chelating effect of the carboxyl group, citric acid and oxalic acids, both known to be good chelators.[15,16] The chelating activity of citric acid, having three carboxyl groups, seems to have been stronger than that of oxalic acid, having two. No antibrowning effect was apparent from the decrease in pH value due to the addition of organic acids. It had been anticipated for the non-enzymatic browning reaction that organic

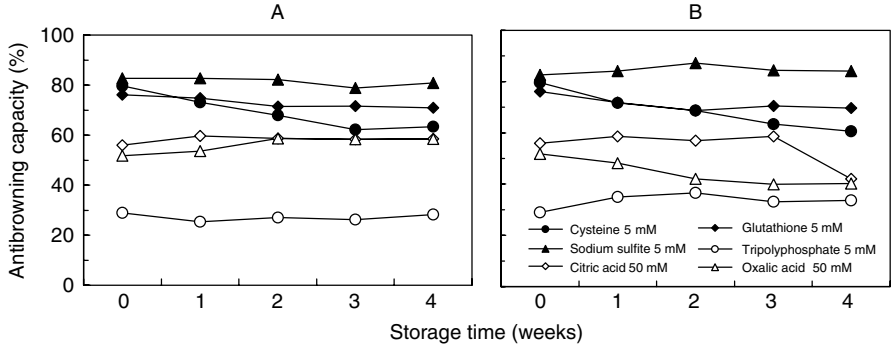

Figure 1. Change in antibrowning capacity of the glucose–lysine model solutions during storage in nitrogen or air at 4 °C for four weeks. Data represent the means of two determinations. A; in nitrogen, B; in air.

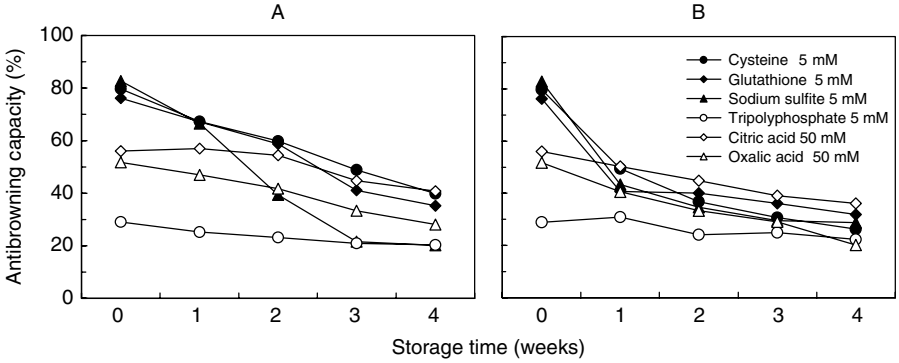

Figure 2. Change in antibrowning capacity of the glucose–lysine model solutions during storage in nitrogen or air at 30 °C for four weeks. Data represent the means of two determinations. A; in nitrogen, B; in air.

Table 2. pH value of the glucose–lysine model solutions before and after four weeks of storage in nitrogen or air at 4 °C or 30 °C[a]

		After 4 weeks of storage			
		4 °C		30 °C	
Antibrowning agent	Before storage	In nitrogen	In air	In nitrogen	In air
5 mM cysteine	6.47 ± 0.01a	6.43 ± 0.02a	6.46 ± 0.01a	6.43 ± 0.02a	6.44 ± 0.03a
5 mM glutathine	6.46 ± 0.01a	6.43 ± 0.01a	6.44 ± 0.00a	6.42 ± 0.03a	6.43 ± 0.04a
5 mM sodium sulfite	6.49 ± 0.00a	6.45 ± 0.02a	6.46 ± 0.02a	6.43 ± 0.07a	6.46 ± 0.03a
5 mM pentasodium tripolyphosphate	6.48 ± 0.01a	6.44 ± 0.03a	6.46 ± 0.03a	6.42 ± 0.01a	6.42 ± 0.03a
50 mM citric acid	6.03 ± 0.01c	5.99 ± 0.07c	6.02 ± 0.03c	5.97 ± 0.03c	6.03 ± 0.04c
50 mM oxalic acid	6.16 ± 0.00b	6.13 ± 0.03b	6.15 ± 0.02b	6.13 ± 0.03b	6.16 ± 0.03b
Control	6.48 ± 0.00a	6.46 ± 0.00a	6.46 ± 0.02a	6.43 ± 0.04a	6.44 ± 0.00a

[a] Each value is expressed as the mean ± standard deviation ($n = 2$).
Means with different letters in the same column are significantly different by Duncan's multiple-range test ($p < 0.05$).

acids could be used as an antibrowning agent, since the amino-carbonyl reaction is inhibited under acidic conditions.[25] However, there was no further decrease in pH value during the storage period, and the antibrowning capacity of oxalic acid was not as high as that of citric acid, despite the similar pH values of these two acids (Table 2).

The foregoing observations suggest that browning ascribable to lysine is unlikely to be easily controlled in foods during long-term storage at room temperature.

Antibrowning effect of phenolic acids and cinnamic acid

In order to increase the antibrowning capacity, we needed to search for another antibrowning agent or synergist. Natella *et al*[20] and Kweon *et al*[21] have stated that the strong antioxidative activity of phenolic acids is attributable to complex formation of metal ions with the phenolic acids,[9] which implies that metal ions cannot influence browning in the presence of phenolic acids, although metal ions generally enhance browning. On the basis of this hypothesis, we investigated the effect of phenolic acids and cinnamic acid on the inhibition of browning.

The initial antibrowning capacity of model solutions containing 50 mM citric acid in the presence or absence of phenolic acids or cinnamic acid was not significantly different in the range 55–60% (Table 3). However, after storage in air at 30 °C for four weeks, the antibrowning capacity of the control had decreased to 36%, while it was higher, at 43–50%, in the presence of phenolic acids or cinnamic acid. The type of phenolic acid had little effect nor had its concentration between 10 μM and 10 mM. This suggests that any of the phenolic acids or cinnamic acid would be effective in enhancing the effect of an antibrowning agent such as citric acid, even at the lowest concentration. The initial antibrowning capacity of each model solution containing a phenolic acid in the absence of citric acid was not high, being in the range 12–21% and 23–35% for 5 mM and 50 mM solutions (Table 4). The conclusion can thus be drawn that phenolic acids or cinnamic acid work synergistically with citric acid. Although phenolic acids are contained in a such everyday foods, such as cereals, fruits, vegetables and beverages,[26,27] they are not approved food additives. Further studies should be performed in order to use these compounds for inhibiting browning. However, cinnamic acid will be a useful synergist for antibrowning since it is a flavoring agent permitted for direct addition to foods.[23]

Table 3. Effect of phenolic acids and cinnamic acid on the antibrowning capacity of the glucose–lysine model solutions containing 50 mM citric acid before and after storage in air at 30 °C for four weeks[a]

	Antibrowning capacity (%)	
Acid	Before storage	After 4 weeks of storage
10 mM ferulic acid	59.29 ± 1.63a	44.81 ± 1.72a
10 mM hydroxybenzoic acid	57.20 ± 2.32a	45.26 ± 0.36a
10 mM syringic acid	57.53 ± 1.45a	42.95 ± 6.35a
10 mM vanillic acid	59.82 ± 2.71a	46.41 ± 0.54a
10 mM cinnamic acid	57.02 ± 3.36a	45.26 ± 0.36a
10 μM ferulic acid	58.63 ± 0.66a	49.47 ± 1.39a
10 μM hydroxybenzoic acid	55.67 ± 1.50a	46.08 ± 0.87a
10 μM syringic acid	58.39 ± 2.34a	49.49 ± 1.37a
10 μM vanillic acid	54.85 ± 0.66a	47.43 ± 0.65a
10 μM cinnamic acid	56.62 ± 5.51a	47.97 ± 2.87a
Control	56.02 ± 0.82a	36.09 ± 4.41b

[a] Each value is expressed as the mean ± standard deviation ($n = 2$).
Means with different letters in the same column are significantly different by Duncan's multiple-range test ($p < 0.05$).

Table 4. Antibrowning capacity of phenolic acids and cinnamic acid in the glucose–lysine model solutions immediately after preparation[a]

	Antibrowning capacity (%)	
Acid	5 mM	50 mM
Ferulic acid	21.06 ± 1.50a	35.00 ± 2.35a
Hydroxybenzoic acid	20.54 ± 0.77a	34.08 ± 2.00a
Syringic acid	12.00 ± 0.47c	22.83 ± 2.60c
Vanillic acid	15.00 ± 2.35bc	25.33 ± 1.89c
Cinnamic acid	16.95 ± 0.56b	28.33 ± 2.35bc

[a] Each value is expressed as the mean ± standard deviation ($n = 2$).
Means with different letters in the same column are significantly different by Duncan's multiple-range test ($p < 0.05$).

Fe(II)-chelating activities of phenolic acids and cinnamic acid

To investigate the relationship between antibrowning and Fe(II)-chelating activity, we determined the ability of the model solution to form an iron complex by using the chelating agent, TMM. As shown in Table 5, after storage in air at 30 °C for four weeks, the chelating activities of the model solutions in the presence of phenolic acids or cinnamic acid were more than 30%, the control value being 15%. This increased antibrowning capacity can be explained by greater chelating activity due to the addition of phenolic acid or cinnamic acid. The chelating activity was not strongly dependent on the type of phenolic acid nor on its concentration in the 10 μM to 10 mM range.

It has been reported that phenolic hydroxy groups are at the site where a complex is formed with a metal ion, and that only phenolics, such as protocatechuic acid or caffeic acid, with *ortho*-dihydroxy groups form copper–phenolic acid complexes.[20] However, it is thought that the carboxyl group, as well as these hydroxy groups may also be able to bind metal ions.[15,16] The fact that, even in the presence of a phenolic acid with aromatic substitution other than a dihydroxy group or a phenolic acid without any hydroxy group, such as cinnamic acid, the model solutions showed mild Fe(II)-chelating activity increases the possibility that the carboxyl group contributes to iron complex formation. Further work is therefore needed to investigate this possibility of using such other phenolic acids as chlorogenic acid having more carboxyl groups. It was not possible to use protocatechuic acid or caffeic acid in this study since they formed a deposit or would not dissolve in the preliminary experimental conditions.

CONCLUSIONS

Browning of the glucose–lysine model was difficult to inhibit during long-term storage at either 4 °C or 30 °C. Although citric acid appeared to be the most efficient agent tested after storage at 30 °C for four weeks, irrespective of excluding air, its antibrowning capacity was not particularly high. However, the capacity could be increased in the presence of 10 μM phenolic acid or cinnamic acid. Our results reveal that phenolic acids and cinnamic acid were useful for inhibiting browning. Considering its current legal acceptability, cinnamic acid can be used as a synergist. Further work should be focused on investigating the chelating activity of other phenolic acids having one or more carboxyl groups.

Table 5. Chelating activities of phenolic acids and cinnamic acid in the glucose–lysine model solutions containing 50 mM citric acid after storage in air at 30 °C for four weeks[a]

Acid	Chelating activity (%)
10 mM ferulic acid	30.49 ± 5.65a
10 mM hydroxybenzoic acid	34.11 ± 7.51a
10 mM syringic acid	33.49 ± 1.57a
10 mM vanillic acid	33.44 ± 3.10a
10 mM cinnamic acid	29.66 ± 3.58a
10 μM ferulic acid	35.62 ± 6.04a
10 μM hydroxybenzoic acid	35.71 ± 4.50a
10 μM syringic acid	32.97 ± 0.18a
10 μM vanillic acid	37.98 ± 6.42a
10 μM cinnamic acid	33.49 ± 9.37a
Control	15.98 ± 3.60b

[a] Each value is expressed as the mean ± standard deviation ($n = 2$). Means with different letters in the same column are significantly different by Duncan's multiple-range test ($p < 0.05$).

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