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# Inhibition of enzymic browning in cloudy apple juice with selected antibrowning agents

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**Abstract**

Golden Delicious apple juice was subjected to enzymic browning in the presence of the selected antibrowning agents: ascorbic acid, isoascorbic acid, L-cysteine, sorbic acid, benzoic acid, cinnamic acid and β-cyclodextrin. The relative effectiveness of these antibrowning agents for inhibition of enzymic browning in apple juice was determined in terms of colour and enzyme activity measurements with respect to time for approximately one day storage period at $25 \pm 1$ °C. The most effective agents were determined as L-cysteine, cinnamic acid and ascorbic acid. Response surface methodology was used to evaluate the potency of the L-cysteine, ascorbic acid and cinnamic acid combination for the control of enzymic browning. The ascorbic acid, L-cysteine and cinnamic acid combination provided better results than the individual compounds. The optimum combination was determined as 0.49 mM ascorbic acid, 0.42 mM L-cysteine and 0.05 mM cinnamic acid in the cloudy apple juice stored for 2 h at $25 \pm 1$ °C. 

*Keywords:* Apple juice; Enzymic browning; Inhibition; Polyphenol oxidase; Response surface methodology

## 1. Introduction

Enzymic browning starts with the initial enzymic oxidation of phenols to quinones by the enzyme polyphenol oxidase in the presence of oxygen. Then these quinones are subjected to further reactions, enzymically catalyzed or not, leading to the formation of pigments.

Cloudy apple juice has increasing market value due to its sensory and nutritional qualities. Although a typical amber-like hue is commercially desirable in clarified apple juice, both apple puree and cloudy juice are expected to have the yellowish colour which characterizes the fresh product (Lozano, Drudis-Biscari, & Ibarz-Ribas, 1994). The food industry has given increasing attention to minimally processed products. This might be achieved in raw juices by membrane filtration, ultra high pressure treatment and preservation by freezing. The control of enzymic browning has great importance just at the start of these processes. One approach for the prevention of enzymic browning of fruit juices has been the use of antibrowning agents.

The most widespread agents used for control of browning are sulfiting agents. Due to adverse health effects, several studies have been devoted to the non-sulfite antibrowning agents such as reducing agents (ascorbic acid and analogs, glutathione, L-cysteine), enzyme inhibitors (aromatic carboxylic acids, substituted resorcinols, anions, peptides), chelating agents (phosphates, EDTA, organic acids), acidulants (citric acid, phosphoric acid), complexing agents (cyclodextrins) and enzymes (Labuza, Lillemo, & Taoukis, 1992; Lambrecht, 1995; Martinez & Whitaker, 1995; McEvily, Iyengar, & Otwell, 1992; Molnar-Perl & Friedman, 1990a, 1990b; Monsalve-Gonzalez, Barbosa-Canovas, Cavalieri, McEvily, & Iyengar, 1993; Moon, Son, & Lee, 1999; Nicolas, Richard-Forget, Goupy, Amiot, & Aubert, 1994; Vamos-Vigyazo, 1995; Walker, 1995). According to the literature, enzymic browning control by agents can be classified into three categories, depending on whether they mainly affect reaction products, substrates or polyphenol oxidase. *o*-Quinones are reactive primary products and they can be either reduced

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back to *o*-diphenols or trapped as colourless addition compounds. However, secondary products resulting mainly from the oxidative polymerization of *o*-quinones often give highly coloured compounds that become less reactive as the browning reaction proceeds. Therefore, many of the compounds that act on *o*-quinones were investigated. Ascorbic acid is most frequently used for browning control of food products. Among sulfur-containing agents, L-cysteine is an effective compound to prevent enzymic browning. Aromatic carboxylic acids are inhibitors of polyphenol oxidase due to their structural similarities with the phenolic substrates and are reported to be effective antibrowning agents for providing long-term inhibition (McEvily et al., 1992; Nicolas et al., 1994; Sapers et al., 1989).

These differences in the mechanism of inhibition may allow the use of combinations of antibrowning agents that may result in enhancement of inhibition. Most combinations of antibrowning agents cited in the literature or commercially available are ascorbic acid-based compositions (Pizzocarno, Torreggiani, & Gilardi, 1993). Mixtures of ascorbic and cyclodextrins were reported to be effective in the inhibition of apple juice browning (Hicks, Sapers, & Seib, 1990). It was found that cinnamate and benzoate enhanced the effectiveness of ascorbic acid and ascorbic acid derivatives as browning inhibitors in apple juice. Combinations of ascorbic acid with an acidic polyphosphate (ascorbic acid-2-phosphate and -triphosphate) were found highly to be effective in apple juice (Sapers et al., 1989).

Golden Delicious apple juice is browned more gradually and the enzyme activity was directly related more to the phenolic concentration in Golden apples. This cultivar is grown commercially in several countries and is also a stable cultivar to heat treatments (Amiot, Tacchini, Aubert, & Nicolas, 1992; Coseteng & Lee, 1987; Kim, Smith, & Lee, 1993; Sapers & Douglas, 1987).

In this research, colour measurements and enzyme activity (EA) were used to compare the effectiveness of a series of compounds for the inhibition of browning in the apple juice. Antibrowning agents tested include ascorbic acid, isoascorbic acid, L-cysteine, β-cyclodextrin, benzoic acid, cinnamic acid and sorbic acid. Finally, the effect of mixtures of ascorbic acid, L-cysteine and cinnamic acid was studied by using response surface methodology for the cloudy apple juice.

## 2. Materials and methods

*Materials*: Golden Delicious apples were obtained at commercial maturity and stored at 4 °C. Antibrowning agents tested included ascorbic acid (Merck), benzoic acid (Merck), sorbic acid (Merck), isoascorbic acid (Sigma), β-cyclodextrin (Sigma), L-cysteine (Sigma) and cinnamic acid (Sigma).

*Anti-browning treatments*: Apples were peeled and juiced with an ordinary domestic food processor. Samples of the juice (25 $cm^3$) were poured into beakers containing antibrowning agents and stirred with a magnetic stirrer for 10 s. The concentrations of the antibrowning agents in apple juice were adjusted to 0.3, 1 and 1.8 mM. After 60 s from juicing, the first measurements were done and the samples were analyzed with different time intervals at a temperature of 25 ± 1 °C. The experiments were duplicated. Absorbance at 420 nm, colour, and EA were determined during the experiments and all measurements were duplicated.

*Absorbance measurements*: Absorbance values at 420 nm ($A_{420}$) were recorded with an UV spectrophotometer (Shimadzu UV-1202) after centrifugation at 5000 rpm (Nüve NF 615) for 10 min.

*Colour measurement*: $L$ (lightness), $a$ (red to green colour dimension) and $b$ (yellow to blue colour dimension) values of the cloudy apple juice samples were measured with a Shimadzu UV-2100, UV–Visible Spectrophotometer (illuminant C, colour system Lab.). The instrument was calibrated using the standard white reflector plate ($L = 98.25$, $a = 0.007$, $b = 1.111$). Normalized $L$ values were calculated from the following equation

$$\text{Normalized } L = (L - L_{\min})/(L_{\max} - L_{\min}). \quad (1)$$

*Polyphenol oxidase activity*: 0.5 $cm^3$ of sample was mixed with 1 $cm^3$ catechol solution (0.2 M) and 2 $cm^3$ McIlvane buffer solution (pH 6.5) and then absorbance values at every 10 s were recorded at 420 nm. The EA was calculated on the basis of the slope of the linear portion of the curve plotted of $A_{420}$ against time. One unit of EA was defined as 0.001 $\Delta A_{420}/\min$ under the assay conditions.

*Estimation of inhibition*: The browning inhibition was calculated from $A_{420}$ or $L$ values of the antibrowning agent added samples and the corresponding controls as follows:

$$\text{Inhibition } (\%) = (\Delta L_{\text{control}} - \Delta L_{\text{treatment}}) \times 100/L_{\text{control}}, \quad (2)$$

$$\text{Inhibition } (\%) = (\Delta A_{420,\text{control}} - \Delta A_{420,\text{treatment}}) \times 100/\Delta A_{420,\text{control}}, \quad (3)$$

where $\Delta$ in Eqs. (2) and (3) shows the change in $L$ (or $A_{420}$) between time $t$ and the initial time $t_0$.

Total colour change ($\Delta E$) was also used to evaluate browning potential

$$\Delta E = [(L_t - L_{t_0})^2 + (a_t - a_{t_0})^2 + (b_t - b_{t_0})^2]^{0.5}, \quad (4)$$

where $t$ and $t_0$ correspond to any time during the experiments and the initial time, respectively.

The effect of antibrowning agents on polyphenol oxidase activity (EA) were determined as

$$\text{Inhibition } (\%) = (\text{EA}_{\text{control}} - \text{EA}_{\text{treatment}}) \times 100/\text{EA}_{\text{control}}. \quad (5)$$

*Response surface methodology*: The combination effect of ascorbic acid, L-cysteine and cinnamic acid on enzymic browning in the cloudy apple juice stored for 2 h at $25 \pm 1$ °C was studied by response surface methodology. For this purpose, central composite design was chosen (Myers, 1971; Thompson, 1982). The experimental design for three factors in terms of coded and uncoded levels are given in Table 1. The second-order polynomial model was used for responses ($y_n$)

$$y_n = \beta_\theta + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_i X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j, \quad (6)$$

where the factors $X$ represent the code of concentrations of antibrowning agents and the parameters are constant coefficients.

The coefficients of the response functions were calculated by linear regression enter method of SPSS software program. A suitable combination of the antibrowning agents was determined by Mathematica software program.

## 3. Results and discussion

During the experiments, Hunter Lab values for the cloudy apple juice and $A_{420}$ values for the centrifuged apple juice were measured and $\Delta E$ values were calculated from Hunter Lab values. The initial $L$, $a$ and $b$ values of the cloudy apple juice were $41.06 \pm 4.11$, $6.89 \pm 1.44$ and $24.82 \pm 2.25$, respectively. During the extent of browning, the change of $L$ values were more significant than the changes of $a$ and $b$ values (data not shown here). The normalized $L$ values (Eq. (1)) of the untreated cloudy apple juice with respect to the storage time at $25 \pm 1$ °C are given in Fig. 1. This figure shows the results of the five independent experiments that growing times and conditions were not same for the apples. The variations in $L$ values with respect to these five experiments were not significant ($p < 0.05$), so that the length of time that browning is inhibited by the antibrowning agents may not be significantly dependent on the growing and storage conditions of the Golden Delicious apples. The change of polyphenol oxidase activity in the same apple juice with respect to time was represented in Fig. 2. The enzyme activity decreased with respect to time due to enzymic browning reaction inactivation of the enzyme which slows down *o*-dihydroxyl phenol oxidation and may be due to other natural environmental effects.

The effect of the antibrowning agents at different concentrations on the enzymic browning based on $L$

Table 1
Experimental design and data[a]

| Exp no. | Coded (uncoded[b]) variable | | | Inhibition of enzymatic browning (%) based on | | Total colour change $\Delta E$ | Inhibition of enzyme activity (%) |
|---|---|---|---|---|---|---|---|
| | $X_1$ (ascorbic acid) | $X_2$ (cysteine) | $X_3$ (cinnamic acid) | $L$ | $A_{420}$ | | |
| 1 | 1 (0.48) | 1 (0.48) | 1 (0.48) | 104.2 | 132.0 | 0.6 | 62.6 |
| 2 | 1 (0.48) | 1 (0.48) | −1 (0.12) | 103.4 | 113.5 | 1.4 | 47.6 |
| 3 | 1 (0.48) | −1 (0.12) | 1 (0.48) | 91.5 | 106.0 | 2.8 | 56.8 |
| 4 | −1 (0.12) | 1 (0.48) | 1 (0.48) | 94.4 | 118.3 | 2.8 | 41.1 |
| 5 | −1 (0.12) | 1 (0.48) | −1 (0.12) | 81.5 | 103.9 | 2.9 | 37.8 |
| 6 | −1 (0.12) | −1 (0.12) | 1 (0.48) | 67.7 | 93.8 | 4.2 | 23.7 |
| 7 | 1 (0.48) | −1 (0.12) | −1 (0.12) | 77.9 | 95.2 | 3.3 | 20.5 |
| 8 | −1 (0.12) | −1 (0.12) | −1 (0.12) | 64.0 | 65.2 | 4.8 | 11.4 |
| 9 | 1.68 (0.6) | 0 (0.3) | 0 (0.3) | 90.8 | 119.4 | 1.7 | 43.7 |
| 10 | −1.68 (0) | 0 (0.3) | 0 (0.3) | 77.9 | 89.4 | 3.9 | 36.9 |
| 11 | 0 (0.3) | 1.68 (0.6) | 0 (0.3) | 103.1 | 111.6 | 1.8 | 38.7 |
| 12 | 0 (0.3) | −1.68 (0) | 0 (0.3) | 67.3 | 90.9 | 4.9 | 32.1 |
| 13 | 0 (0.3) | 0 (0.3) | 1.68 (0.6) | 88.3 | 111.5 | 2.5 | 42.9 |
| 14 | 0 (0.3) | 0 (0.3) | −1.68 (0) | 66.1 | 84.6 | 4.6 | 26.0 |
| 15 | 0 (0.3) | 0 (0.3) | 0 (0.3) | 80.3 | 100.3 | 2.9 | 24.3 |
| 16 | 0 (0.3) | 0 (0.3) | 0 (0.3) | 90.3 | 114.3 | 2.4 | 33.1 |
| 17 | 0 (0.3) | 0 (0.3) | 0 (0.3) | 85.1 | 107.9 | 2.7 | 27.2 |
| 18 | 0 (0.3) | 0 (0.3) | 0 (0.3) | 81.0 | 109.5 | 2.8 | 36.6 |
| 19 | 0 (0.3) | 0 (0.3) | 0 (0.3) | 87.9 | 116.2 | 2.7 | 34.4 |
| 20 | 0 (0.3) | 0 (0.3) | 0 (0.3) | 79.6 | 96.9 | 2.9 | 27.4 |

[a] Experimental runs were performed in random order.
[b] Concentration of antibrowning agents in apple juice (mM).

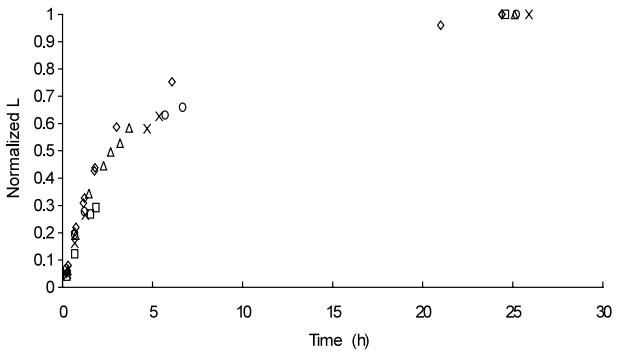


Fig. 1. Change of normalized $L$ values with respect to time in the cloudy Golden Delicious apple juice. The results are from five independent experiments.

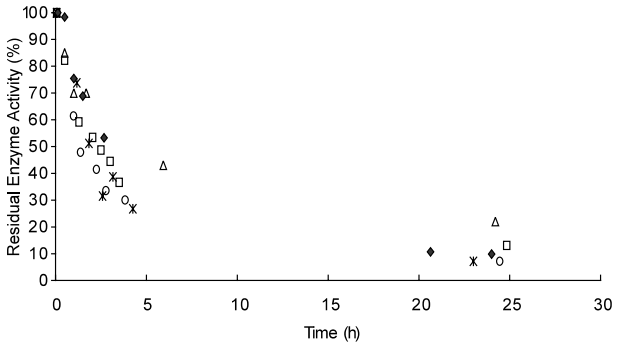


Fig. 2. Polyphenol oxidase activity in Golden Delicious apple juice. The results are from five independent experiments.

measurements (Eq. (2)) can be seen in Fig. 3. The concentrations of antibrowning agents in the apple juice were selected as 0.3, 1 and 1.8 mM according to the preliminary works by considering all agents tested. The extent of inhibition exceeds 100% in some cases such as in the case of L-cysteine. This is a well-recognized phenomenon in such studies and it was due to a combination of standard errors and associated sample bleaching by the treatment.

Ascorbic acid was more effective than its cheaper isomer isoascorbic acid at all concentrations studied for preventing colour change in the cloudy apple juice. These acids at the concentrations tested did not effectively lessen browning in the apple juice if compared with L-cysteine. The effect of these reducing agents can be considered temporary because these compounds are oxidized irreversibly by reaction with pigment intermediates, endogenous enzymes and metals such as copper. The effectiveness of ascorbic acid and isoascorbic acid at 1.8 mM concentration lasted for about 4 and 3 h, respectively.

L-cysteine prevents browning by reacting with *o*-quinones to produce stable, colourless products. L-cysteine was found to form a product with catechol and this product inhibited the EA (Dudley & Hotchkiss, 1989; McEvily et al., 1992). According to the results obtained (Fig. 3), L-cysteine appears to be an effective inhibitor at 1–1.8 mM concentrations, but such high concentrations produced undesirable odour and a bleaching effect. L-cysteine at lower concentrations induced colour development. While L-cysteine is at low critical amounts, the *o*-quinones formed in excess can cooxidaze the L-cysteine–quinone addition compound, leading to a phenol regeneration with a deep colour formation according to Richard-Forget, Goupy, Nicolas, Lacombe, and Pavia (1991, 1992). L-cysteine at lower concentrations than 1 mM may be used together with different types of antibrowning reagent to control enzymic browning and this combination may be more suitable in terms of odour and bleaching effect.

To control the enzymic browning, aromatic carboxylic acids (benzoic and cinnamic acids) affecting the active site for phenolic substrates were also tested. The tested aromatic carboxylic acids were inhibitors, due to their structural similarities with phenolic substrates. Cinnamic acid was the most effective one. It was reported that cinnamic acid added to Granny Smith apple juice at concentrations greater than approximately 1 mM was very effective to prevent browning (Sapers et al., 1989). Experimental results showed that small amounts of cinnamic acid sufficient to control enzymic browning, since the enzymic browning was directly related to phenolic concentration in Golden Delicious apples.

Sorbic acid has no structural similarity with phenolic substrates and appears to have inhibited browning more effectively than benzoic acid. When a carboxyl group was present, either directly bound to benzene cycle (benzoic series) or to the conjugated double bonds (cinnamic series or sorbic acid), it can form a complex with the copper at the active site that is reported in the study of Janovitz-Klapp, Richard, Goupy, and Nicolas (1990).

β-cyclodextrin was not effective in the apple juice at the studied 0.3–1.8 mM concentration ranges (Fig. 3). Higher concentrations are required to inhibit the browning. β-cyclodextrin inhibits browning by binding substrate in its hydrophobic core. The gradual browning of apple juice at the concentrations tested indicated that complex formation might not go to completion. Increasing the concentration can increase the effectiveness but this may cause the loss of flavour or colour compounds present in low concentrations, due to the lack of specificity of inclusion complex formation.

Among the tested antibrowning reagents, the most effective ones were L-cysteine and cinnamic acid. Ascorbic acid can also be considered as an effective compound at higher concentrations for approximately 5 h. The inhibition based on the enzyme activities (Eq. (5)) with respect to time during the treatments with these effective antibrowning agents are given in Fig. 4. The

inactivation was biphasic for L-cysteine and ascorbic acid, there was an initial slow rate of inactivation and then a fast rate of inactivation that decreased with time. The effect of cinnamic acid increased with time. The cinnamic acid was seen more effective in apple juice ($pH = 4.3 \pm 0.1$) than the assay mixture ($pH = 6.5$). Inhibition by the cinnamic acids is pH dependent, increasing as the pH decreased. Also the type of inhibition observed is dependent on the substrate being assayed and also the amount of substrate. The assay solution contains higher amount of catechol than the apple juice.

In the second part of the study, the effect of the combinations of L-cysteine, ascorbic acid and cinnamic acid on the enzymic browning reaction in the cloudy apple juice were evaluated by response surface methodology at the end of 2 h storage period. The response surface methodology with an experimental design can be easily applied in order to find such a formulations. The results for each of the responses are given in Table 1. There are differences between per cent inhibition values based on measurements of $L$ (Eq. (2)) and $A_{420}$ (Eq. (3)). Initial $A_{420}$ value was obtained as $0.482 \pm 0.101$ for the juices tested. Values were dependent on methods and optical conditions of measurement and on physical state of the samples examined. Absorbance measurements were done at a single wavelength and evaluated only the soluble pigments. Also as the reaction proceeds, polymerization occurs and solubility of a large part of the brown pigments decreases and these insoluble entities may be eliminated during centrifugation steps. A significant correlation was obtained between total colour change ($\Delta E$, Eq. (4)) and the inhibition of browning based on $L$ measurements ($p < 0.05$).

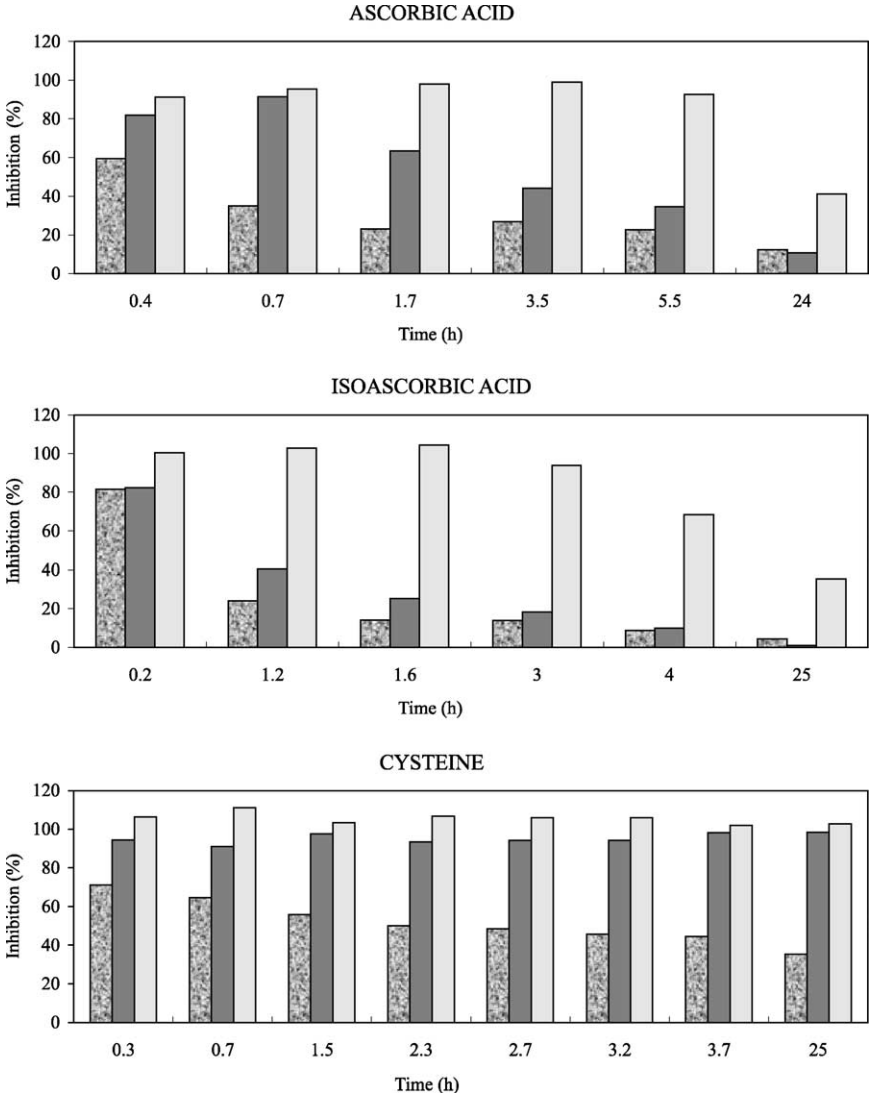


Fig. 3. Inhibition of enzymatic browning in Golden Delicious apple juice with different antibrowning agents ((▒) 0.3 mM, (■) 1 mM, (□) 1.8 mM).

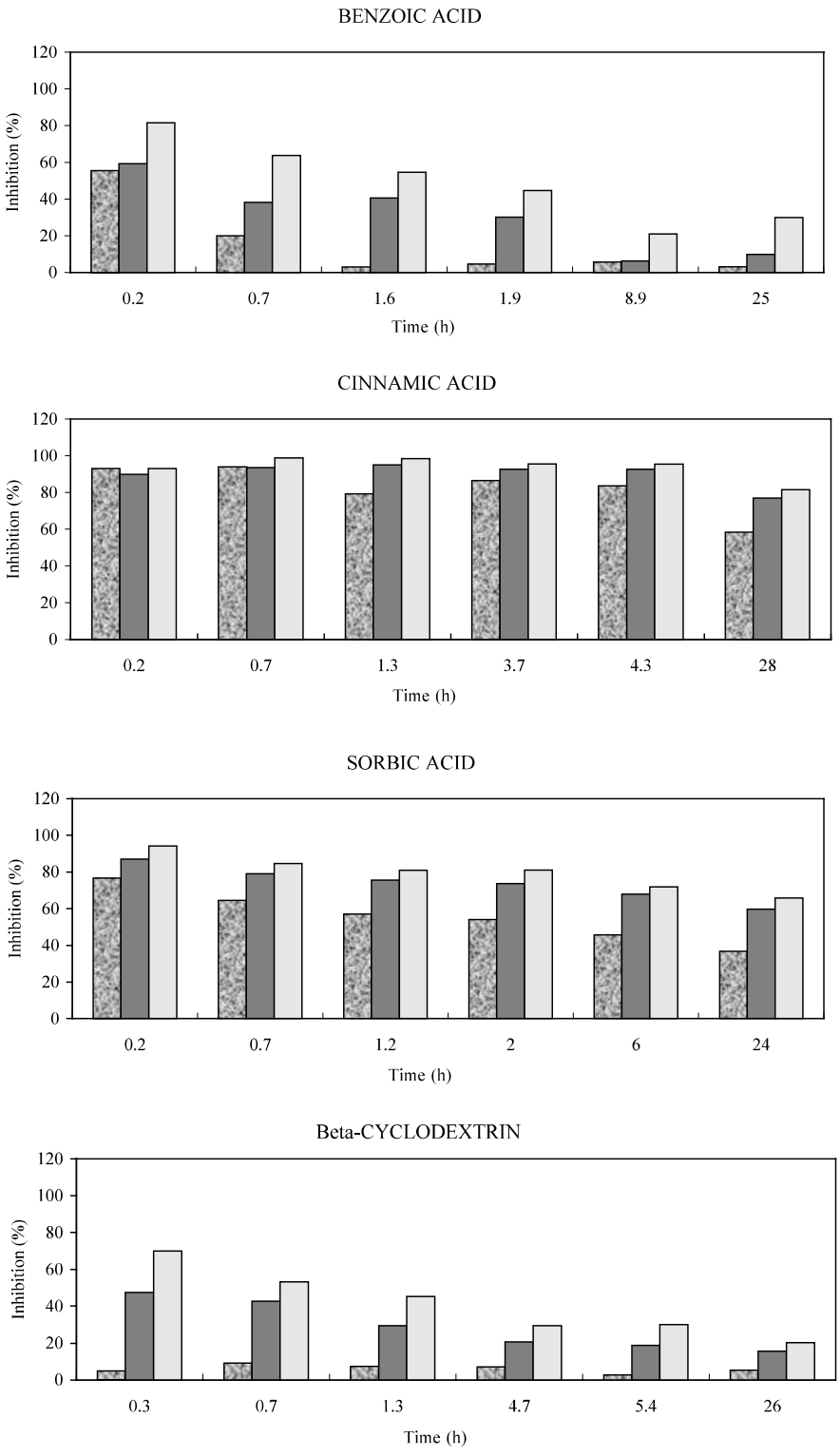


Fig. 3 (*continued*)

A response surface prediction was fitted for each response. Table 2 shows the regression coefficients obtained by the fitting of experimental data to second-order response models (Eq. (6)). $b_0$ is the value of the fitted response at the centre of the design, $b_1$, $b_2$ and $b_3$ are linear, $b_{11}$, $b_{22}$, $b_{33}$ are quadratic and $b_{13}$, $b_{23}$ and $b_{12}$ are cross product regression terms. Linear regression terms were found to be significant according to the *t*-test. The positive slopes indicated that inhibition increases with increasing amounts of antibrowning re-

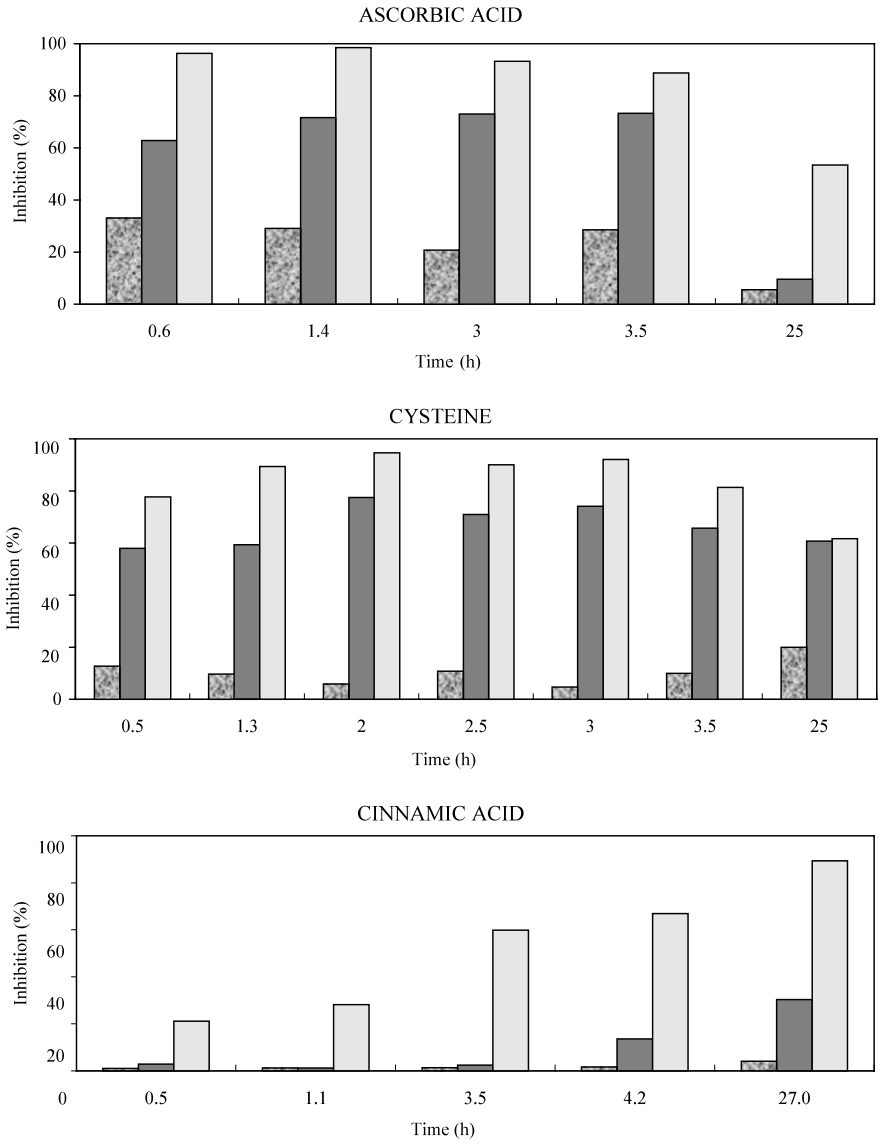


Fig. 4. Inhibition of polyphenol oxidase activity in Golden Delicious apple juice with ascorbic acid, cysteine and cinnamic acid ((▒) 0.3 mM, (■) 1 mM, (□) 1.8 mM).

Table 2
Model constants and regression analysis

| Constants | Inhibition of enzymatic browning (%) based on | | Total colour change $\Delta E$ | Inhibition of enzyme activity (%) |
|---|---|---|---|---|
| | $L$ | $A_{420}$ | | |
| $b_0$ | 83.91 | 107.30 | 2.74 | 30.50 |
| $b_1$ | 6.68 | 8.49 | −0.74 | 6.22 |
| $b_2$ | 10.43 | 10.42 | −0.92 | 6.43 |
| $b_3$ | 5.01 | 8.62 | −0.41 | 6.98 |
| $b_{11}$ | 0.85 | −0.51 | −0.06 | 3.58 |
| $b_{22}$ | 1.13 | −1.61 | 0.13 | 1.86 |
| $b_{33}$ | −1.70 | −2.76 | 0.19 | 1.51 |
| $b_{12}$ | −0.76 | −2.35 | −0.10 | −1.37 |
| $b_{13}$ | −0.28 | −1.71 | −0.08 | 4.47 |
| $b_{23}$ | −0.45 | −0.82 | 0.03 | −3.78 |
| $R^2$ | 0.89 | 0.87 | 0.94 | 0.81 |
| $F$ | 9.42 | 7.42 | 18.95 | 4.69 |
| Signif. $F$ | 0.0008 | 0.0021 | 0.0001 | 0.0120 |

agents. L-cysteine was found to be the most effective antibrowning reagent in the combination in terms of colour measurements. For the EA, the effectiveness of cinnamic acid is slightly higher than the others. For total colour difference ($\Delta E$), L-cysteine was again found to be the most effective compound. Generally, the effect appeared to be additive rather than synergistic. Fig. 5 represents some examples for response surfaces for $\Delta E$ change. Then a numerically feasible combination of factors for limiting response values (constraints) was determined simultaneously by using Newton's Method. In the application of the method, the inhibition data based on $L$, $A_{420}$ and $\Delta E$ measurements were taken into consideration and a formulation of the antibrowning reagents were found as 0.49 mM ascorbic acid ($X_1 = 1.09$), 0.42 mM L-cysteine ($X_2 = 0.72$) and 0.05 mM

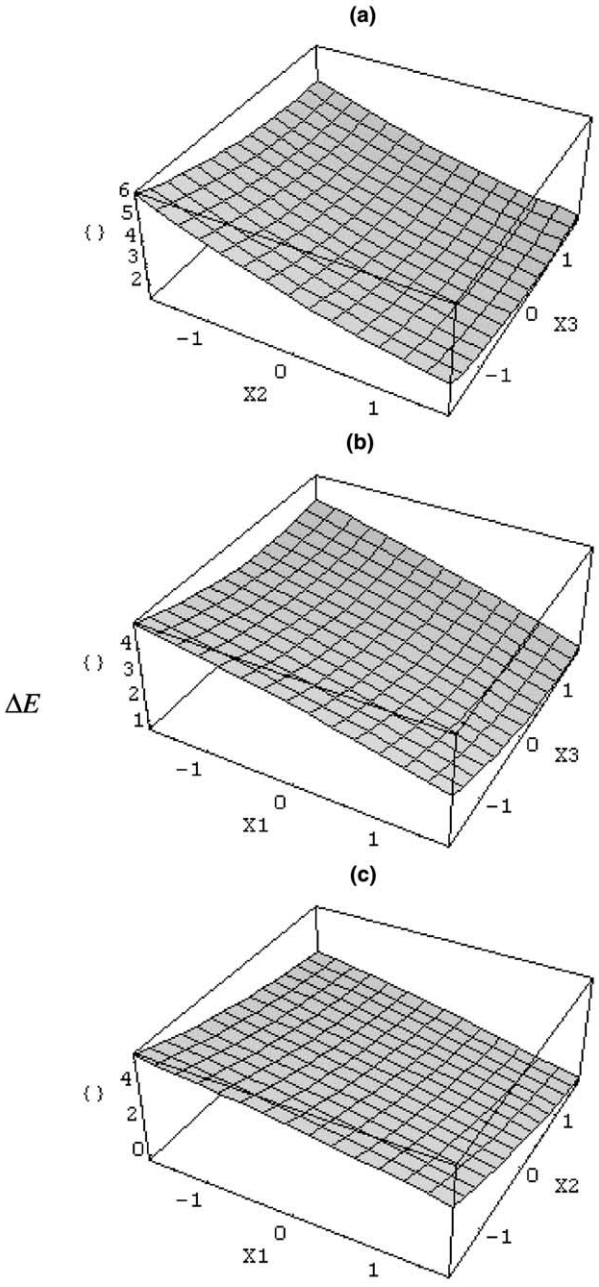


Fig. 5. Response surface examples for total colour change ($\Delta E$) at $X_1 = 0$ (a), $X_2 = 0$ (b), $X_3 = 0$ (c).

cinnamic acid ($X_3 = -1.42$). The calculated inhibition values were 99% and 106% in terms of $L$ and $A_{420}$ measurements, where the change of $\Delta E$ was 2.2. The inhibition of EA for this formulation was determined as 36%. These inhibition results show that the main effect of the combination of ascorbic acid, L-cysteine and cinnamic acid was on substrates or products of browning reaction.

Cloudy apple juice has increasing markets due to its superior sensory and nutritional qualities. The ascorbic acid, L-cysteine and cinnamic acid combination worked better than the individual compounds in the cloudy apple juice. The development of a combination mixture, which is more effective than the individual compounds, may have some technological or commercial benefits.

## References

Amiot, M. J., Tacchini, M., Aubert, S., & Nicolas, J. (1992). Phenolic composition and browning susceptibility of various apple cultivars at maturity. *Journal of Food Science, 57*(4), 958–962.

Coseteng, M. Y., & Lee, C. Y. (1987). Changes in apple polyphenol oxidase and polyphenol concentrations in relation to degree of browning. *Journal of Food Science, 52*(4), 985–989.

Dudley, E. D., & Hotchkiss, J. H. (1989). Cysteine as an inhibitor of polyphenol oxidase. *Journal of Food Biochemistry, 13*, 65–75.

Hicks, K. B., Sapers, G. M. & Seib, P. A. (1990). Process for preserving raw fruit and vegetable juices using cyclodextrin and compositions. US Patent 4,975,293.

Janovitz-Klapp, A. H., Richard, F. C., Goupy, P. M., & Nicolas, J. J. (1990). Inhibition studies on apple polyphenol oxadase. *Journal of Agricultural Food Chemistry, 38*, 926–931.

Kim, D. M., Smith, N. L., & Lee, C. Y. (1993). Apple cultivar variations in response to heat treatment and minimal processing. *Journal of Food Science, 58*(5), 1111–1124.

Labuza, T. P., Lillemo, J. H., & Taoukis, P. S. (1992). Inhibition of polyphenol oxidase by proteolytic enzymes. *Fruit Processing, 2*, 9–13.

Lambrecht, H. S. (1995). Sulfite substitutes for the prevention of enzymatic browning in foods. In: C. Y. Lee, & J. R. Whitaker (Eds.), *ACS Symposium Series 600*, *Enzymatic browning and its prevention* (pp. 313–323). Washington, DC: American Chemical Society.

Lozano, J. E., Drudis-Biscari, R., & Ibarz-Ribas, A. (1994). Enzymatic browning in apple pulps. *Journal of Food Science, 59*(3), 564–567.

Martinez, M. V., & Whitaker, J. R. (1995). The biochemistry and control of enzymatic browning. *Trends in Food Science and Technology, 6*, 195–200.

McEvily, A. J., Iyengar, R., & Otwell, W. S. (1992). Inhibition of enzymatic browning in foods and beverages. *CRC Critical Reviews in Food Science and Nutrition, 32*(3), 253–273.

Molnar-Perl, I., & Friedman, M. (1990a). Inhibition of browning by sulfur amino acids. 2. Fruit juices and protein-containing foods. *Journal of Agricultural Food Chemistry, 38*, 1648–1651.

Molnar-Perl, I., & Friedman, M. (1990b). Inhibition of browning by sulfur amino acids. Part 3. Apples and potatoes. *Journal of Agricultural and Food Chemistry, 38*, 1652–1656.

Monsalve-Gonzalez, A., Barbosa-Canovas, G. V., Cavalieri, R. P., McEvily, A. J., & Iyengar, R. (1993). Control of browning during storage of apple slices preserved by combined methods. 4-Hexylresorcinol as anti-browning agent. *Journal of Food Science, 58*(4), 797–800.

Moon, K. D., Son, S., & Lee, C. Y. (1999). Inhibitory effects of various antibrowning agents on apple slices. *IFT Annual Meeting 1999. Book of abstracts, Vol. 79D-45* (p. 227).

Myers, R. H. (1971). *Response surface methodology*. Boston: Allyn & Bacon.

Nicolas, J. J., Richard-Forget, F. C., Goupy, P. M., Amiot, M. J., & Aubert, S. Y. (1994). Enzymatic browning reactions in apple and apple products. *CRC Critical Reviews in Food Science and Nutrition, 34*(2), 104–157.

Pizzocarno, F., Torreggiani, D., & Gilardi, G. (1993). Inhibition of apple polyphenol oxidase by ascorbic acid, citric acid and sodium chloride. *Journal of Food Processing and Preservation, 17*, 21–30.

Richard-Forget, F. C., Goupy, P. M., Nicolas, J. J., Lacombe, J. M., & Pavia, A. A. (1991). Cysteine as an inhibitor of enzymatic browning. 1. Isolation and characterization of addition compounds formed during oxidation of phenolics by apple polyphenol oxidase. *Journal of Agricultural Food Chemistry, 39*, 841–847.

Richard-Forget, F. C., Goupy, P. M., & Nicolas, J. J. (1992). Cysteine as an inhibitor of enzymatic browning. 2. Kinetic studies. *Journal of Agricultural Food Chemistry, 40*(1), 2108–2113.

Sapers, G. M., & Douglas, F. W, Jr. (1987). Measurement of enzymatic browning at cut surfaces and in juice of raw apple and pear fruits. *Journal of Food Science, 52*(5), 1258–1262, see also p. 1285.

Sapers, G. M., Hicks, K. B., Phillips, J. G., Garzarella, L., Pondish, D. L., Matulaitis, R. M., McCormack, T. J., Sondey, S. M., Seib, P. A., & El-Atawy, Y. S. (1989). Control of enzymatic browning in apple with ascorbic acid derivatives, polyphenol oxidase inhibitors, and complexing agents. *Journal of Food Science, 54*(4), 997–1002.

Thompson, D. (1982). Response surface experimentation. *Journal of Food Processing and Preservation, 6*, 155–188.

Vamos-Vigyazo, L. (1995). Prevention of enzymatic browning in fruits and vegetables. In: C. Y. Lee, & J. R. Whitaker (Eds.), *ACS Symposium Series* (600). *Enzymatic browning and its prevention* (pp. 49–62). Washington, DC: American Chemical Society.

Walker, J. R. L. (1995). Enzymatic browning in fruits: its biochemistry and control. In: C. Y. Lee, & J. R. Whitaker (Eds.), *ACS Symposium Series* (600). *Enzymatic browning and its prevention* (pp. 8–22). Washington, DC: American Chemical Society.