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ORIGINAL ARTICLE

Reaction's mechanism of fresh apple juice enzymatic browning in the presence of maltosyl-β-cyclodextrin

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Abstract Polyphenol oxidase causes enzymatic browning by catalyzing natural phenolic compounds to quinones. Subsequent nonenzymatic oxidative polymerization reactions form brown, black, or red pigments (melanins). Many methods can be shown experimentally to diminish or prevent browning but are not all applicable for food use. In this work, the colour of fresh apple juice was evaluated in the presence of maltosyl-β-CD, compound that complex polyphenol oxidases substrates. The effectiveness of CD as browning inhibitor was determined by the difference between the colours observed in the CD-treated sample and the controls, using the colour space CIE- L, a*, b* system. We have kinetically modelled apple juice enzymatic browning in the absence and presence of maltosyl-β-CD. The complexation constant between the mixtures of diphenols present in apple juice and maltosyl-β-CD was calculated ($K_c = 4.09\ mM^{-1}$).

Keywords Apple juice · Browning · Colour · Cyclodextrin · Kinetic model

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Abbrevations

CD	cyclodextrin
PPO	polyphenol oxidase
G_2-β-CD	maltosyl-β-CD

Introduction

Sulfites are highly effective in controlling enzymatic browning in fruits and vegetables. However, because of adverse health effects, the Food and Drug Administration banned their use in the fruit and vegetable industry [1]. To meet the needs of the food industry for alternatives to sulfites, several methods for controlling enzymatic browning are proposed. In recent years, compounds that bind or complex polyphenoloxidase (PPO) (monophenol, dihydroxy-L-phenylalanine: oxygen oxidoreductase, E.C. 1.14.18.1) substrates, such as cyclodextrins (CDs), have been used for their potential value as browning inhibitors [2]. The use of CDs has been proposed for the control of enzymatic browning in apple products by several authors [3, 4]. However, very few have studied the behaviour of apple juice enzymatic browning at the very beginning of the reaction, as we do in this paper. Moreover, the action mechanism of CDs on apple juice has been the subject of controversy. This work mainly focuses on the effect of CDs on the enzymatic browning of apple juice. We propose a kinetic model to explain the slow down of the juice apple browning in presence of CDs.

Experimental

Reagents

Biochemicals were purchased from Fluka (Madrid, Spain). Maltosyl-β-CD (G_2-β-CD) was kindly supplied by Ensuiko Sugar Refining CO. LTD (Japan).

Juice preparation and treatments

Apples (*Malus domestica* cv. Fuji) were purchased from local supermarkets and stored at 4 °C until needed. They were peeled, cored and sliced prior to juicing in a Moulinex Y36 blender. The apple juice obtained was immediately collected and mixed in a beaker containing 25 ml of distilled water alone or containing enough G_2-β-CD to produce the final concentration of each compound indicated in each experiment.

Colour evolution assessment

The apple juice became darker and the initial green colour turned brownish as storage progressed. The CIE coordinates, L^* (Lightness), a^* (red–green) and b^* (yellow–blue), of the apple juice, were determined using a ColorFlex version 1.72 colorimeter (Hunterlab, Reston, USA) certified by ISO 9001 with a D75 light source and the observer at 10 °. The previously described mixtures were used in the colour evolution assays, using the measurements at time 0 as standard. This 0 time corresponded to the first measurement, which was made one minute after the apples had been juiced and the materials dissolved in the juice.

Results and discussion

As is shown in Fig. 1, the total colour difference (ΔE^*) of the apple juice increased rapidly during the first ten minutes in the absence of any reagent. After this time, the increase slowed significantly until 60 min. The addition of G_2-β-CD slowed the apple juice enzymatic browning, its presence (60 mM) resulting in lower variations in total colour difference (ΔE^*) during the 60 min measured than when this CD was absent. Moreover, the evolution of this parameter when G_2-β-CD was added to the medium was more linear than observed previously with no CD. Figure 2 shows the evolution of the L*, a* and b* coordinates in the absence and presence of 30, 60 and 90 mM G_2-β-CD. As can be seen, increasing concentrations of G_2-β-CD (0-90 mM) led to significantly lower values of a* and higher of L* and b*. These findings agree with those

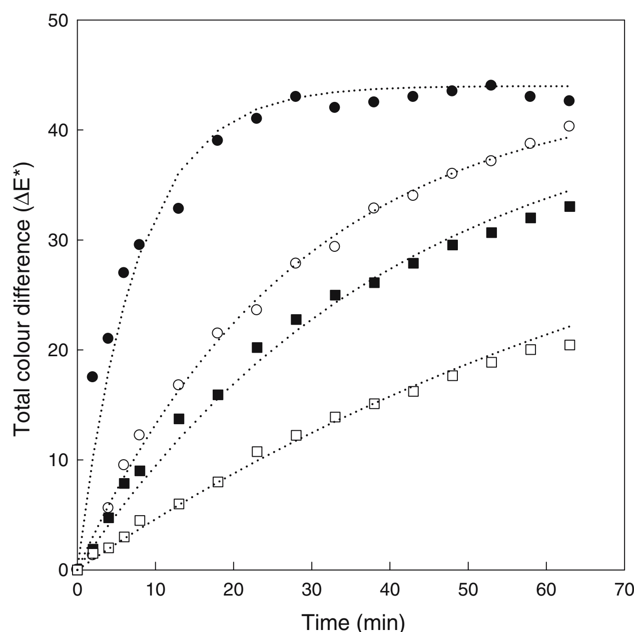

Fig. 1 Effect of G_2-β-CD concentration on the evolution of total colour difference (ΔE^*) at 25 °C in the absence of G_2-β-CD (●) and in the presence of G_2-β-CD: 30 mM (○), 60 mM (■) and 90 mM (□). Each data point is the mean of three replicates

presented by some authors [5], who measured changes in lightness (L*) and red-green (a*) in apple juice in the absence and presence of CDs. However, in contrast with the data presented by other authors [6] for apple juice, the blue-yellow chromatism (b*) was also affected in our system in both the absence and presence of CDs.

To help to understand the action of CDs on apple juice enzymatic browning, a kinetic model of the browning in the presence of CDs is proposed. To explain the evolution of the total colour (ΔE^*) when CD concentration is increased, a velocity equation is proposed as a function of the only known parameter, i.e., total CD concentration. For this purpose, v can be expressed as:

$$v = \frac{V_{max}[S]_F}{K_m + [S]_F} \qquad (1)$$

where v is the velocity of ΔE^* evolution and $[S]_F$ is the free concentration of substrate. Several authors have calculated the K_m values of some phenolic substrates of apple PPO [7]. These values are higher than the free concentrations of polyphenolic compounds in apple juice [8]. So, we can assume that the free concentration of substrate is negligible with respect to the K_m, i.e. $[S]_F \lll K_m$, and then, the velocity of ΔE^* evolution can be expressed as:

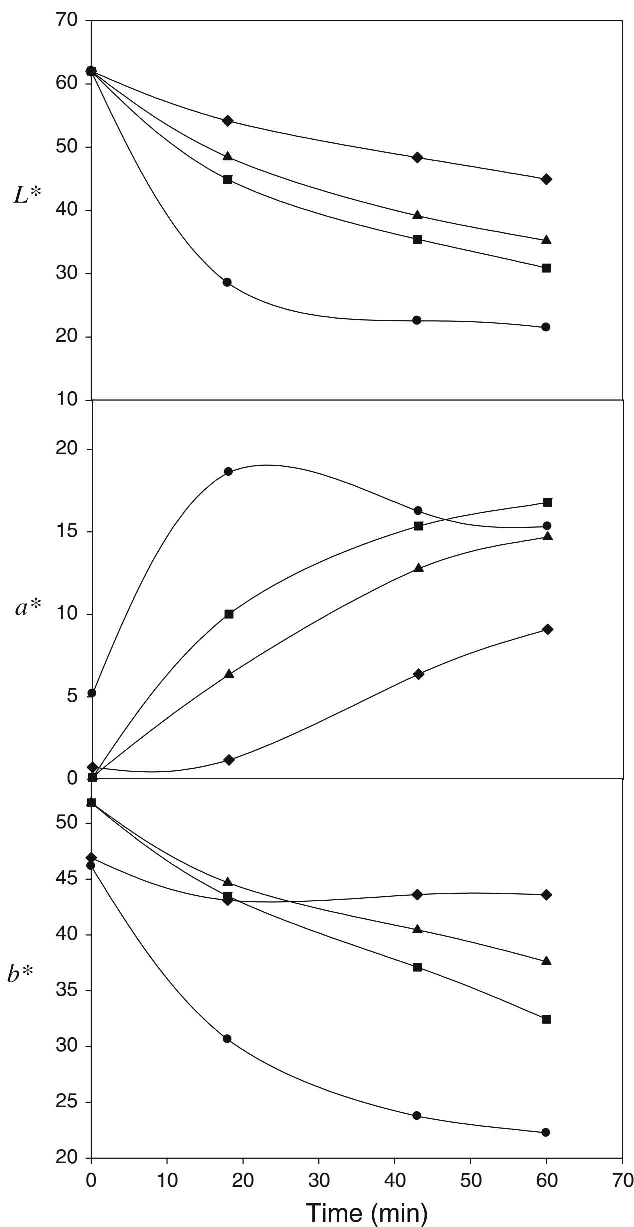

Fig. 2 Evolution of L*, a*, b* and H* coordinates of apple juice 25 °C in the absence of G_2-β-CD (•) and in the presence of G_2-β-CD: 30 mM (■), 60 mM (▲) and 90 mM (◆). Each data point is the mean of three replicates

$$v = \frac{V_{max}}{K_m}[S]_F \quad (2)$$

A specific kinetic constant,k, was defined as:

$$k = \frac{V_{max}}{K_m} \quad (3)$$

and substituted in Eq. 1 to give:

$$v = k[S]_F \quad (4)$$

Taking into account that several research studies have demonstrated that PPO is only able to work with free substrate and not with the complex between CD and PPO substrates [9]; and that only one molecule of substrate PPO can enter into a CD molecule (stoichiometry 1:1) [10], the equilibrium can be expressed as:

$$\text{CD-S} \overset{Kc}{\longleftrightarrow} \text{CD} + S_F \overset{k}{\longrightarrow} P$$

where CD–S is the complex between PPO substrates and cyclodextrins, CD_F is the free cyclodextrin, S_F is the free PPO substrate concentration, k is the specific kinetic constant of transformation of free substrate in product and K_c is the complexation constant defined as:

$$K_c = \frac{[S]_F[CD]_F}{[CD\text{-}S]} \quad (5)$$

Taking into account the mass balance (where both subscript T and F denote total and free concentration, respectively)

$$[S]_T = [CD\text{-}S] + [S]_F \quad (6)$$

and

$$[CD]_T = [CD\text{-}S] + [CD]_F \quad (7)$$

and also Eq. 5, and assuming that $[S]_T \lll [CD]_T$, then $[CD]_T \cong [CD]_F$ From this, $[S]_F$ can be expressed as:

$$[S]_F = \frac{K_c[S]_T}{[CD]_T + K_c} \quad (8)$$

and substituted in Eq. 4 to give:

$$v = \frac{k\ K_c[S]_T}{[CD]_T + K} \quad (9)$$

To determine k and K_c, an apparent specific kinetic constant k_{app} was calculated. This k_{app} was dependent on the$[CD]_T$ and was defined as:

$$k_{app} = \frac{k\ K_c}{[CD]_T + K_c} \quad (10)$$

To calculate k_{app} a first-order fractional model was used (Eq. 11).

$$e^{-k_{app}t} = \frac{(\Delta E_f * - \Delta E*)}{(\Delta E_f*)} \quad (11)$$

where ΔE* is the current value of total colour difference, ΔE_f* is the non-zero value of the parameter upon

prolonged storage, t is the storage time and k_{app} is the apparent specific kinetic constant. Total colour difference (ΔE^*) data were fitted (dotted line) to equation 11 by non-linear regression procedures of the Sigma Plot (SPSS Inc.). The values obtained for k_{app} at increasing concentrations of G_2-β-CD were 0.1325 min^{-1} (in the absence of G_2-β-CD), 0.0357 min^{-1} (in the presence of 30 mM G_2-β-CD), 0.0192 min^{-1} (in the presence of 60 mM G_2-β-CD) and 0.0111 min^{-1} (in the presence of 90 mM G_2-β-CD). When several k_{app} were calculated at the different [CD]T used, both the kinetic constant, k, and the complexation constant, K_c, were determined by $1/k_{app}$ *vs* [CD]T plot. Fitting the data by linear regression using Sigma Plot (SPSS Inc), values of 0.27 $min^{-1}$and 4.09 mM^{-1} were obtained for k and K_c, respectively.

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