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Kinetic model of apple juice enzymatic browning in the presence of cyclodextrins: The use of maltosyl-β-cyclodextrin as secondary antioxidant

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Abstract

Enzymatic browning reactions limit the commercial shelf life of apple juice, so that colour preservation during storage is one of the main objectives of fruit processors. In this paper, the colour of fresh apple juice was evaluated in the presence of different types of cyclodextrins (CDs) (α-CD, β-CD, γ-CD and maltosyl-β-CD), compounds that bind or complex polyphenol oxidase substrates. The effectiveness of CDs as browning inhibitors was determined as the difference between the colours observed in the CD-treated sample and the controls, using the colour space CIE-L^*, a^*, b^* system. Although the effect of CDs on apple juice enzymatic browning has been studied, the action mechanism involved remains a subject of controversy. In this work, 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 ($K_c = 4.09\ mM^{-1}$). Different concentrations of maltosyl-β-CD modified the evolution of lightness (L^*), total colour (ΔE^*) and hue angle (H^*) because of the higher affinity constant it shows for the compounds responsible for apple juice browning than do α-CD, β-CD and γ-CD. Moreover, in this paper we show that maltosyl-β-CD can enhance the ability of ascorbic acid to prevent the enzymatic browning due to the protective effect of maltosyl-β-CD against ascorbic acid oxidation. Hence, maltosyl-β-CD seems to act as a ''secondary antioxidant'', reducing apple juice browning and enhancing the naturally occurring antioxidant capacity of a food.

Keywords: Browning; Cyclodextrin; Colour; Polyphenol oxidase; Ascorbic acid

1. Introduction

The browning of foods during processing and storage decreases their sensory qualities due to associated changes in the colour and flavour, besides increased softening and altered nutritional properties (Martínez & Whitaker, 1995). Therefore, its control is essential for preserving the quality of a food. The degree of browning depends on the presence of oxygen, reducing substances, metallic ions, pH, temperature, and the activity of different oxidizing enzymes, especially polyphenol oxidase (PPO) (monophenol dihydroxyphenylalanine: oxygen oxidoreductases, EC 1.14.18.1). This copper-containing enzyme catalyzes two different reactions in the presence of molecular oxygen: the hydroxylation of monophenols to *o*-diphenols (monophenolase activity) and the oxidation of *o*-diphenols to *o*-quinones (diphenolase activity) (Mayer & Harel, 1979; Sánchez-Ferrer, Rodríguez-López, García-Cánovas, & García-Carmona, 1995; Vamos-Vigyazo, 1981).

Abbreviations: CD, cyclodextrin; PPO, polyphenol oxidase; AA, ascorbic acid; G_2-b-CD, maltosyl-b-CD; DHAA, dehydroascorbic acid.

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To date, several methods have been used to assess the inhibition of PPO activity in fruits and vegetables because the phenolic composition is closely related to food properties, such colour. Although sulfites have been successfully used as antibrowning agents (Joslyn & Braverman, 1954), they have been associated with severe allergy-like reactions in certain populations and, as a result, the Food and Drug Administration (FDA) has limited their use to only a few applications (Sapers, 1993). Because thermal treatment is not suitable for inhibiting this sort of reaction, several methods have been tried to lessen PPO activity. The addition of ascorbic acid (AA), the best and earliest known alternative to sulfites, or chemical agents, the exclusion of oxygen, refrigeration and various non-thermal treatments are among the most effective for preventing browning in fruits (Sapers, Hicks, & Miller, 2001).

In recent years, compounds that bind or complex PPO substrates, such as cyclodextrins (CDs), have been used for their potential value as browning inhibitors (Fayad, Marchal, Billaud, & Nicolas, 1997; Hicks, Sapers, & Seib, 1990).

CDs are a group of structurally related natural products formed during the bacterial digestion of starch (Haga, Harata, Nakamura, & Yamane, 1997). These cyclic oligosaccharides consist of α (1 → 4) linked α-D-glucopyranose units and contain a somewhat lipophilic central cavity and a hydrophilic outer surface. The natural α-, β-, and γ-CDs consist of six, seven and eight glucopyranose units, respectively. Substitution of any of the hydrogen bond-forming hydroxyl groups results in a dramatic improvement of their aqueous solubility (Saenger, 1980). The most important functional property of CDs is their ability to form inclusion complexes with a wide range of organic guest molecules, including PPO substrates (Irwin et al., 1994).

The use of CDs has been proposed for the control of enzymatic browning in apple products by several authors (Billaud, Regaudie, Fayad, Richard-Forget, & Nicolas, 1995; Gacche, Zore, & Ghole, 2003, 2004; Hicks et al., 1996; Irwin et al., 1994; Ozoglu & Bayindirli, 2004; Pilizota & Subaric, 1998).

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. Some research works have studied the complexation of PPO substrates by CDs, by means of which their oxidation to *o*-quinones and subsequent polymerisation to brown pigments is prevented (Billaud et al., 1995; Hicks et al., 1996; Irwin et al., 1994; Laveda, Núñez-Delicado, García-Carmona, & Sánchez-Ferrer, 2000; Núñez-Delicado, Sánchez-Ferrer, & García-Carmona, 1997; Ozoglu & Bayindirli, 2004; Sojo, Núñez-Delicado, García-Carmona, & Sánchez-Ferrer, 1999).

However, other studies have described CDs as inhibitors which react with the copper-containing prosthetic group of PPO (Pilizota & Subaric, 1998). Finally, other authors have shown that CDs act as non-competitive inhibitors of PPO (Gacche et al., 2003, Gacche, Warangkar, & Ghole, 2004). In other words, the mechanism by which CDs inhibit enzymatic browning needs further studies.

This work mainly focusses on the effect of CDs on the enzymatic browning of apple juice. We propose a kinetic model to explain the slow-down of apple juice browning in the presence of CDs. Several factors which may affect the colour evolution of apple juice, such as type and concentration of CDs, were studied. Moreover, the presence in the medium of another browning inhibitor, such as AA, was also evaluated. There is a growing interest in finding new natural antioxidants for use in food, although another approach might be to maintain the natural antioxidant capacity of a particular food (Núñez-Delicado et al., 1997). In this approach, the development and use of new natural secondary antioxidants is a fresh and challenging task. In this paper, we show that natural CDs can be used to enhance the naturally occurring antioxidant capacity of a food, thus acting as secondary antioxidants.

2. Materials and methods

2.1. Reagents

Biochemicals were purchased from Fluka (Madrid, Spain) and used without further purification. AA was from Sigma (Madrid, Spain). α-CD, β-CD, γ-CD and maltosyl-β-CD (G_2-β-CD) were kindly supplied by Ensuiko Sugar Refining CO. LTD (Japan).

2.2. 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 α-CD, β-CD, γ-CD, G_2-β-CD (13–90 mM) and/or AA (2.28 mM) to produce the final concentration of each compound indicated in each experiment.

2.3. Colour evolution assessment

The apple juice became darker and the initial green colour turned brownish as storage progressed. Because apple juice is not a transparent liquid, its browning was determined by measuring CIELAB coordinates.

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 1 min after the apples had been juiced and the materials dissolved in the juice. All the measurements were made at different times during the first hour after the materials had been dissolved in the apple juice, i.e., just when the enzymatic browning was beginning.

The total colour difference (ΔE^*), a single value which takes into account the differences between L^*, a^* and b^*, of the sample and standard, and the evolution of hue angle (H^*) were also studied.

Three readings were obtained for each replicate to obtain uniform colour measurements. Hue (H^*) and total colour difference (ΔE^*) were calculated using equations:

$$H^* = \tan^{-1}\left(\frac{b^*}{a^*}\right)$$

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

3. Results and discussion

3.1. Effect of the type of cyclodextrins on colour evolution of apple juice

In order to study the behaviour of CD on apple juice browning and to select the optimal type of CD, the inhibition of PPO activity by α-CD, β-CD, γ-CD and G_2-β-CD, a natural modified CD, was examined. A concentration of 13 mM was used for all CD types because this was close to the limit solubility of β-CD (the least soluble of all the CDs used). As shown in Fig. 1, G_2-β-CD was the only type of CD which reduced the decay in lightness (L^*), the parameter which better described the degree of browning in apple juice (Hicks et al., 1996), probably because G_2-β-CD shows a higher affinity constant for the phenolic compounds responsible for apple juice browning than do the other CDs. The data reported in this paper agree with those presented by several authors, who mention that a modified CD, such as G_2-β-CD, can show inclusion behaviour (with several compounds) different from β-CD, stabilizing the inclusion complexes (Acarturk, Imai, Saito, Ishikawa, & Otagiri, 1993; López-Nicolás, Brú, Sánchez-Ferrer, & García-Carmona, 1995). Therefore, the use of G_2-β-CD to complex the substrates of apple PPO can strongly inhibit enzymatic browning as shown in Fig. 1. For these reasons we chose G_2-β-CD as the optimal CD to continue the study.

3.2. Effect of G_2-β-CD on the total colour difference and hue angle evolution of apple juice

As shown in Fig. 2, the total colour difference (ΔE^*) increased rapidly during the first 10 min in the absence of any reagent. After this time, the increase slowed significantly until 60 min. The hue angle (H^*) decreased dramatically at the start of enzymatic browning and then remained practically constant (Fig. 2, inset). The behaviours observed for both ΔE^* and H^* parameters were similar to that demonstrated by several authors over longer times (Hicks et al., 1996; Soliva-Fortuny, Grigelmo-Miguel, Odriozola-Serrano, Gorinstein, & Martín-Belloso, 2001, 2002).

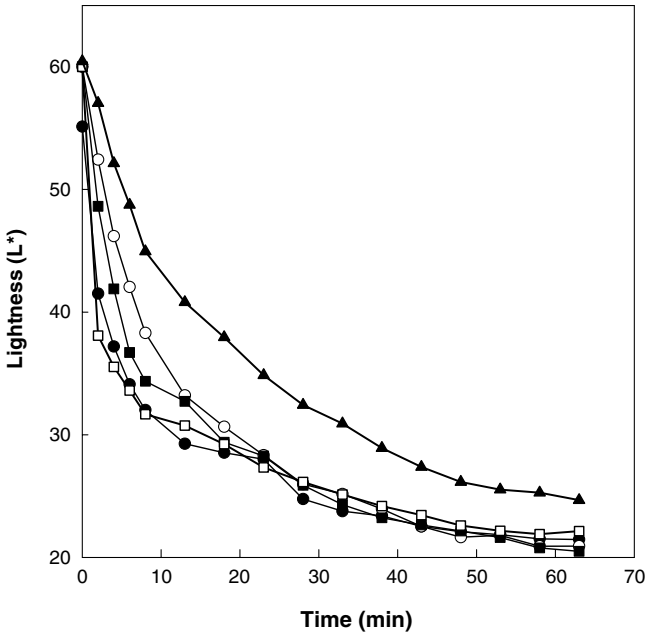


Fig. 1. Effect of different types of CD on the evolution of lightness (L^*) in apple juice at 25 °C in the absence of any CD (●) and in the presence of 13 mM of: α-CD (■), β-CD (○), γ-CD (□) and G_2-β-CD (▲). Each data point is the mean of 3 replicates.

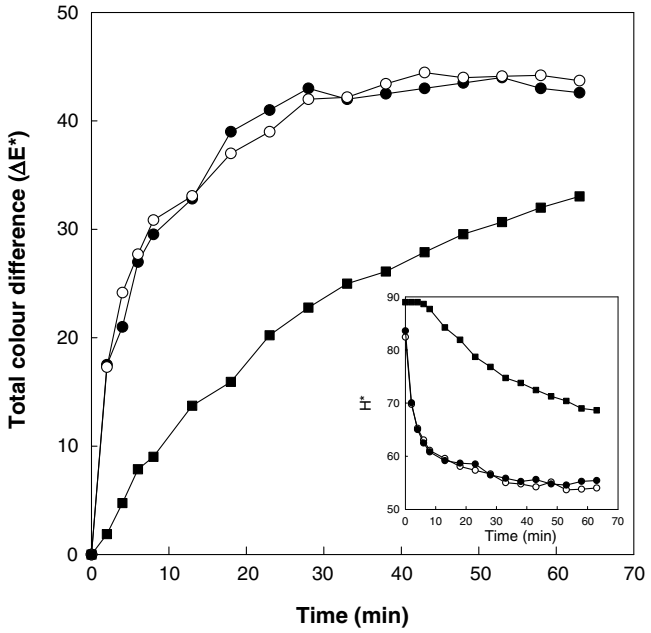


Fig. 2. Evolution of total colour difference (ΔE^*) in apple juice at 25 °C in the absence of any agent (●) and in the presence of: 0.23 M sucrose (○) or 60 mM G_2-β-CD (■). Inset: Evolution of hue angle (H^*) in apple juice at 25 °C in the absence of any agent (●) and in the presence of: 0.23 M sucrose (○) or 60 mM G_2-β-CD (■). Each data point is the mean of 3 replicates.

As can be observed in Fig. 2, the addition of G_2-β-CD slowed the apple juice enzymatic browning, its presence (60 mM) resulting in lower variations in both total colour difference (ΔE^*) and hue angle (H^*) during the 60 min measured than when this CD was absent. Moreover, the evolution of these parameters when G_2-β-CD was added to the medium was more linear than that observed previously with no CD.

Different studies have described CDs as non-competitive inhibitors of PPO (Gacche et al., 2003, 2004). However, several papers published by our group have demonstrated the ability of CDs to form inclusion complexes with PPO substrates (Laveda et al., 2000; Núñez-Delicado et al., 1997; Sojo et al., 1999), thereby preventing their oxidation to *o*-quinones and subsequent polymerization to brown pigments. Other authors have suggested that the CDs are able to react with the copper contained in the prosthetic group of PPO and thus, inhibit the enzymatic browning process (Pilizota & Subaric, 1998). However, the fact that CDs have been described by our group as activators and inhibitors of banana pulp PPO ruled out the latter possibility (Sojo et al., 1999).

Finally, in order to confirm that the slowing-down effect on enzymatic browning caused by G_2-β-CD was not due to their glucidic nature, the effect of another sugar, sucrose, on the colour of apple juice was studied. As can be seen in Fig. 2, the evolutions of both the total colour difference (ΔE^*) and the hue angle (H^*) in apple juice during the first 60 min were similar in the absence and presence of 0.23 M sucrose, but both values were far that those obtained in the presence of 60 mM G_2-β-CD. These results confirmed that the ability of CDs to slow down the enzymatic browning was not due to their glucidic nature. The differences observed in the value of hue angle (H^*) at time 0 were due to the fact that the measurements were taken only 1 min after the apples were juiced and the materials dissolved, as was described in the Section 2.

3.3. Effect of the concentration of G_2-β-CD on colour evolution of apple juice

To evaluate the effect of CD concentration on the colour evolution of apple juice, increasing concentrations of G_2-β-CD (0–90 mM) were used. As shown in Fig. 3, the total colour difference (ΔE^*) increased rapidly during the first 10 min in the absence of G_2-β-CD, after which the increase was significantly lower and even a plateau was reached after 30 min of reaction time. The total colour difference (ΔE^*) for the 60 min was less when increasing concentrations of G_2-β-CD were used (Fig. 3). Moreover, the evolution of this parameter was more linear at higher concentrations of G_2-β-CD. Table 1 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 values of L^* and b^*. These findings agree with those presented by Hicks et al. (1996), 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 several authors for apple juice (Soliva-Fortuny et al., 2001, Soliva-Fortuny, Biosca-Biosca, Grigelmo, & Martín-Belloso, 2002), the blue–yellow chromatism (b^*) was also affected in our system in both the absence and presence of CDs. Moreover, the hue angle (H^*) decreased rapidly in the absence of G_2-β-CD (Table 1). However, the presence of increasing concentrations of G_2-β-CD affected the values of this parameter less throughout the reaction time. Thus it could be concluded that the reduction of apple juice colour during storage is strongly dependent on CD concentrations.

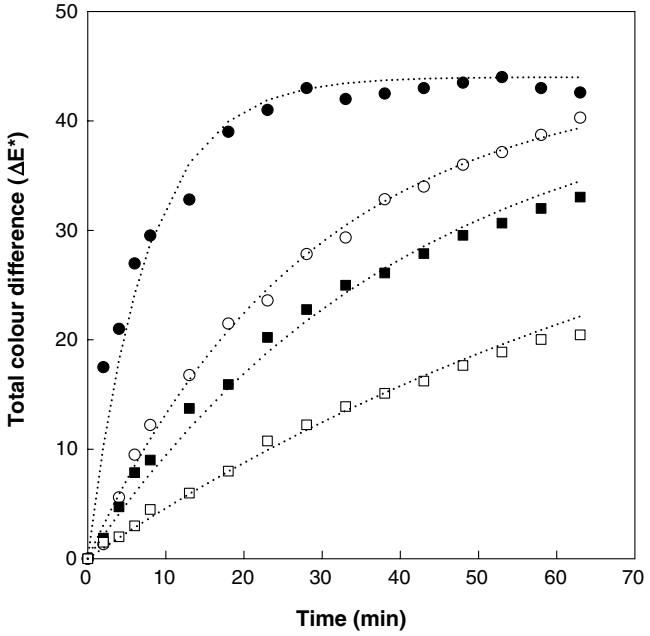


Fig. 3. Effect of G_2-β-CD concentration on the evolution of total colour difference (ΔE^*) at 25 °C in the absence of G_2-β-CD (●) and in the presence of G_2-β-CD: 30 mM (○), 60 mM (■) and 90 mM (□). Each data point is the mean of 3 replicates.

3.4. Kinetic model of apple juice colour evolution in the presence and absence of G_2-β-CD

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:

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

where v is the velocity of ΔE^* evolution and $[S]_F$ is the free concentration of substrate.

Table 1
Evolution of L^*, a^*, b^* and H^* coordinates of apple juice in the absence and in the presence of different G_2-β-CD concentrations

Reaction time (min)	Hunter values			
	L^*	a^*	b^*	H^*
Without G_2-β-CD				
0	62.02 ± 3.24	5.19 ± 0.34	46.15 ± 2.33	83.58 ± 5.67
18	28.53 ± 1.15	18.62 ± 0.65	30.62 ± 2.51	58.69 ± 3.14
43	22.53 ± 1.85	16.25 ± 0.93	23.75 ± 1.91	55.61 ± 4.67
60	21.46 ± 1.54	15.32 ± 1.05	22.24 ± 1.13	55.43 ± 4.21
G_2-β-CD 30 mM				
0	62.02 ± 3.24	0.11 ± 0.01	51.84 ± 3.24	89.05 ± 5.24
18	44.89 ± 3.41	10.02 ± 0.52	43.48 ± 2.39	77.02 ± 6.32
43	35.44 ± 1.94	15.36 ± 1.24	37.10 ± 3.03	67.50 ± 5.15
60	30.89 ± 2.29	16.79 ± 0.83	32.45 ± 2.67	62.64 ± 3.41
G_2-β-CD 60 mM				
0	62.02 ± 3.24	0.15 ± 0.01	51.84 ± 3.24	89.05 ± 5.24
18	48.43 ± 3.21	6.35 ± 0.14	44.68 ± 2.15	81.91 ± 7.53
43	39.16 ± 3.05	12.78 ± 0.83	40.45 ± 3.97	72.46 ± 6.85
60	35.23 ± 3.41	14.70 ± 1.12	37.60 ± 2.85	68.64 ± 5.31
G_2-β-CD 90 mM				
0	62.02 ± 3.24	0.74 ± 0.05	46.91 ± 2.15	89.05 ± 5.24
18	54.17 ± 3.47	1.18 ± 0.17	43.10 ± 4.01	88.43 ± 6.19
43	48.36 ± 3.21	6.38 ± 0.49	43.61 ± 3.91	81.67 ± 6.91
60	44.94 ± 3.51	9.09 ± 0.85	43.59 ± 3.82	78.22 ± 7.12

Fayad et al. (1997) calculated the K_m values of some phenolic substrates of apple PPO. These values are higher than the free concentrations of polyphenolic compounds in apple juice (Van der Sluis, Dekker, Skrede, & Jongen, 2002). So, we can assume that the free concentration of substrate is negligible with respect to the K_m, i.e. $[S]_F \ll K_m$, and then, the velocity of ΔE^* evolution can be expressed as:

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

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 (Laveda et al., 2000; Núñez-Delicado et al., 1997; Sojo et al., 1999); and that only one molecule of substrate PPO can enter into a CD molecule (stoichiometry 1:1) (Irwin et al., 1994; Laveda et al., 2000; Núñez-Delicado et al., 1997; Sojo et al., 1999), the equilibrium can be expressed as:

$$CD - S \overset{K_c}{\leftrightarrow} CD_F + S_F \overset{k}{\rightarrow} P$$

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:

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

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

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

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

and also Eq. (5), and assuming that $[S]_T \ll [CD]_T$, 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:

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

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:

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

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 Eq. (11) by non-linear regression procedures of the Sigma Plot (SPSS Inc.) as shown in Fig. 3. 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). The fitted data confirm that the assumptions of the mathematical model that the free concentration of substrate is negligible with respect to the K_m, and that only one molecule of substrate PPO may enter a CD molecule (stoichiometry 1:1), were correct.

When several k_{app} values were calculated at the different [CD]T used, both the kinetic constant, k, and the complexation constant, K_c, were determined by a $1/k_{app}$ vs. [CD]T plot (Fig. 4). 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.

3.5. Effect of G_2-β-CD as a secondary antioxidant

AA is the best known chemical agent for reducing the browning reaction (Bauernfeind & Pinkert, 1970; Sapers, 1993; Sapers et al., 2001; Walker, 1977). It acts as an oxygen scavenger for the removal of molecular oxygen in the enzymatic browning reaction and so PPO inhibition by AA has been attributed to the reduction of enzymatically formed *o*-quinones to their precursor diphenols (Bauernfeind & Pinkert, 1970). However, once the added AA has been completely oxidized to dehydroascorbic acid (DHAA), *o*-quinones accumulate and undergo browning. Additionally, DHAA itself can cause non-enzymatic browning (Bauernfeind & Pinkert, 1970). More stable forms of AA derivatives, such as erythorbic acid, 2- and 3-phosphate derivatives, phosphinate esters, and ascorbyl-6-fatty acid esters, have been developed to overcome these problems but the results have not been very satisfactory (Walker, 1977).

In the study described in this paper, the capacity of CDs to function as secondary antioxidants in apple juice was evaluated using the model described by our group to increase the half-life of natural antioxidants in food and therefore prolong the time that the food can be kept (Núñez-Delicado et al., 1997). CDs can enhance the ability of AA to prevent enzymatic browning due to the protection they offer AA against oxidation by *o*-quinones (Scheme 1). In the absence of CDs, the total concentration of PPO substrate is available to be oxidized by PPO in the presence of O_2 to *o*-quinones. However, in the presence of CDs, AA is protected, due to the complexation of PPO substrates in the hydrophobic cavity of CDs. CDs slow down the production of *o*-quinones and, hence, the oxidation of AA, because only free substrates, in equilibrium with cyclodextrin-bound phenol (CD-S), are oxidized by PPO in the presence of O_2 to *o*-quinones (Núñez-Delicado et al., 1997). In this way, the reaction is slowed down and the shelf life of the food is prolonged.

To confirm this hypothesis, apple juice browning in the absence and presence of 2.28 mM AA and 90 mM G_2-β-CD was studied. Fig. 5A shows that the values of total colour difference (ΔE^*) were not reduced when 2.28 mM AA (filled squares) was added to the medium compared with what was observed in the absence of the reagent (filled triangles). However, ΔE^* fell significantly when 60 mM G_2-β-CD (filled circles) was added to the medium, as was previously demonstrated in Fig. 3. Moreover, when 2.28 mM AA was added in the presence of 60 mM G_2-β-CD, the parameter (ΔE^*) was reduced more drastically (open circles). These results confirm the hypothesis described in Scheme 1.

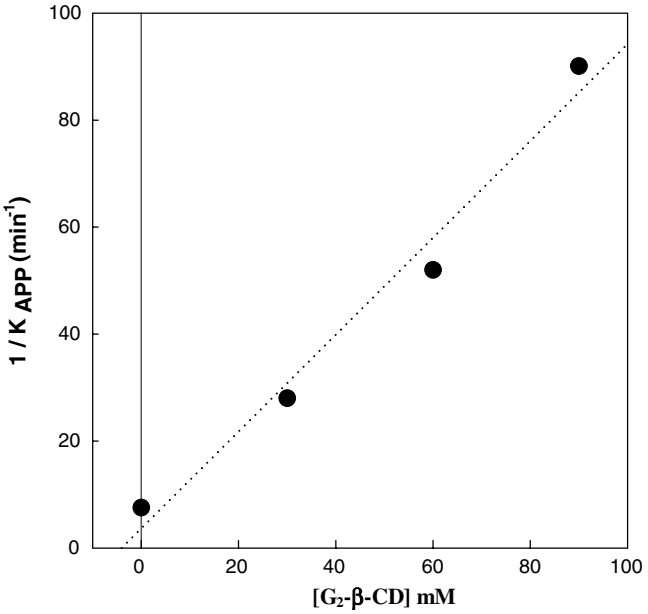
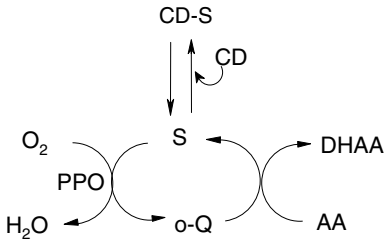


Fig. 4. Lineweaver–Burk plot of the effect of the G_2-β-CD concentration on k_{app} of the evolution of total colour difference (ΔE^*) at 25 °C. Each data point is the mean of 3 replicates.



Scheme 1. Use of G_2-β-CD as secondary antioxidant on the browning of apple juice. PPO: polyphenol oxidase, CD: cyclodextrin, S: free PPO substrate, CD-S: complex between PPO substrates and CD, *o*-Q: *ortho*-quinone, AA: ascorbic acid, DHAA: dehydroascorbic acid.

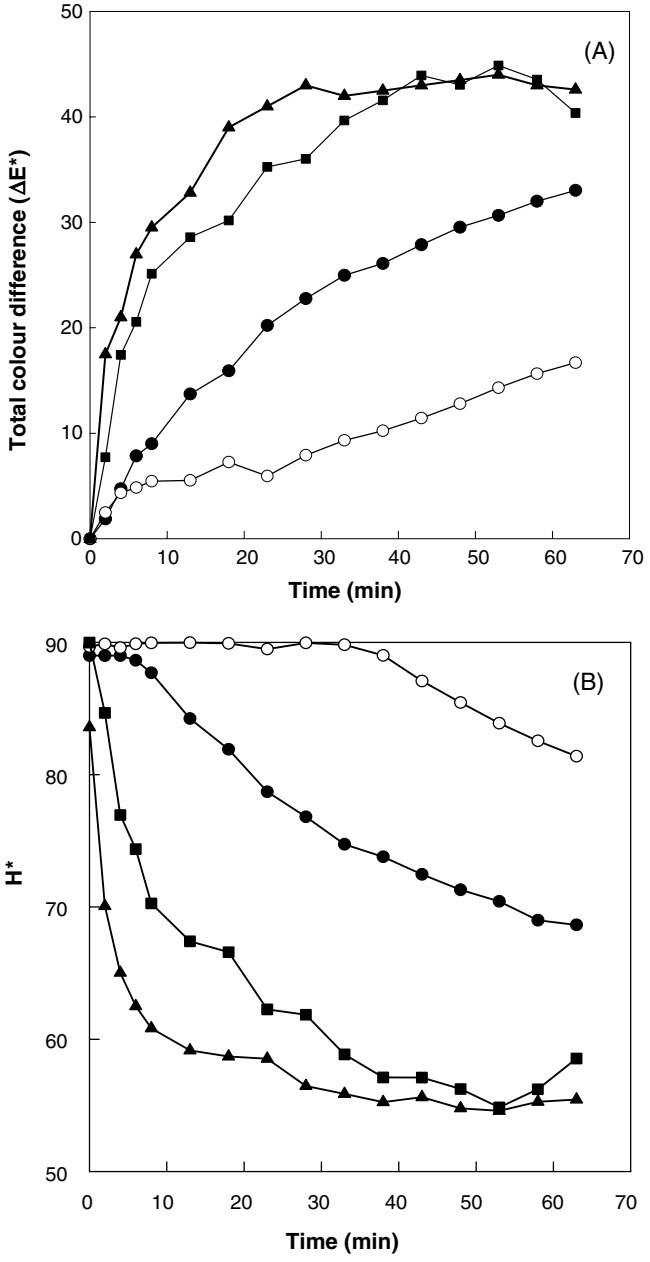


Fig. 5. Synergic effect of ascorbic acid and G_2-β-CD on apple juice browning. (A) Evolution of the total colour difference (ΔE^*) in apple juice in the absence of any agent (▲) and in the presence of 2.28 mM AA (■), 60 mM G_2-β-CD (●) and 60 mM G_2-β-CD plus 2.28 mM AA (○). (B) Evolution of angle hue (H^*) in the absence of any agent (▲) and in the presence of 2.28 mM AA (■), 60 mM G_2-β-CD (●) and 60 mM G_2-β-CD plus 2.28 mM AA (○). Each data point is the mean of 3 replicates.

Moreover, as shown in Fig. 5B, when 2.28 mM AA (filled squares) or 60 mM G_2-β-CD (filled circles) were included in our system, the decrease in hue angle (H^*) was less than that in the absence of either them (filled triangles). However, in the presence of 2.28 mM AA plus 60 mM G_2-β-CD (open circles), the hue angle (H^*) decreased less significantly, due to the preservation of the antioxidant capacity of AA by G_2-β-CD. These results also confirm the hypothesis described in Scheme 1 and are in agreement with those presented by different authors using CDs combined with other antioxidant agents (Hicks et al., 1996; Sapers & Hicks, 1989; Sapers et al., 1989). Fig. 5B also shows that the combination of both AA and G_2-β-CD had a synergic effect; that is, the combined effect of both was greater than the sum of the two single treatments. The lag time observed in the evolution of H^* represents the time that AA is protected by CD. By the time, the lag period finishes, the AA has been completely oxidized to DHAA and *o*-quinones can accumulate and undergo browning. As is shown in Fig. 5B, the lag time in the presence of 60 mM G_2-β-CD plus 2.28 mM AA is longer (32 min) that the sum of the lags observed in the presence of 60 mM G_2-β-CD alone (6 min) or 2.28 mM AA alone (no lag).

In Fig. 6, the effect of G_2-β-CD concentration on the lag time in the evolution of hue angle of apple juice (H^*) in the absence and presence of 2.28 mM AA was plotted. The existence of a lag time in the presence of G_2-β-CD was due to the protective effect of the G_2-β-CD on the AA occurring naturally in apples (i.e. prior to juicing). This lag period increased with G_2-β-CD concentration (Fig. 6, open circles). Moreover, the addition of 2.28 mM AA to the reaction medium led to a longer lag period with respect to that obtained in the absence of added AA (Fig. 6, filled circles). These results demonstrate that the higher the concentration of G_2-β-CD added to apple juice, the longer was the effect of AA, corroborating the secondary antioxidant effect of natural cyclic oligosaccharides.

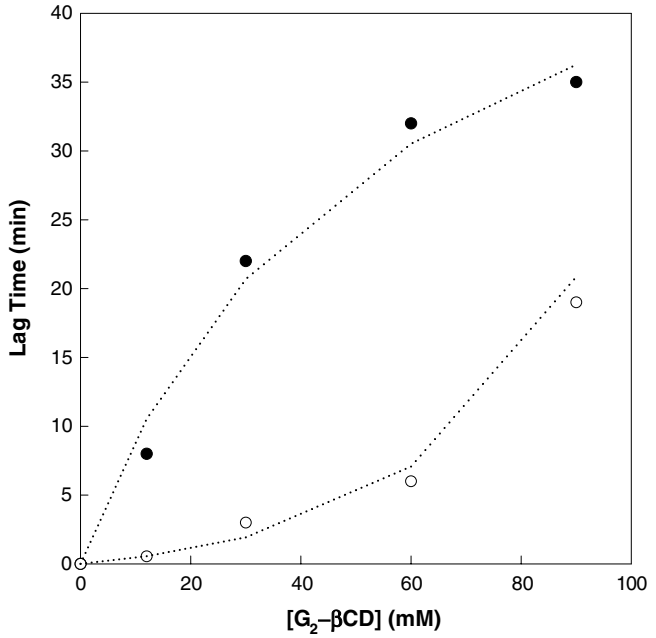


Fig. 6. Effect of G_2-β-CD concentration on the lag time of the evolution of hue angle of apple juice (H^*) in the absence (○) and the presence (●) of 2.28 mM AA. Each data point is the mean of 3 replicates.

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