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Use of Cyclodextrins as Secondary Antioxidants to Improve the Color of Fresh Pear Juice

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In this paper, the color of fresh pear juice was evaluated for the first time in the presence of some natural and modified cyclodextrins (CDs) and the effect of these compounds as browning inhibitors was determined using the color space CIELAB system. Furthermore, because contradictory claims have been published concerning the mechanism by which enzymatic browning is inhibited by CDs, a kinetic model in the presence of CDs is proposed and the corresponding apparent complexation constants between the mixtures of diphenols present in pear juice and α-, β-, and maltosyl-β-CD have been calculated. Moreover, pear juice color was studied in the presence of different polyphenoloxidase inhibitors. Finally, we show that maltosyl-β-CD enhances the ability of ascorbic acid to prevent enzymatic browning because of the protection that maltosyl-β-CD offers against ascorbic acid oxidation. In this respect, maltosyl-β-CD seems to act as a "secondary antioxidant", reducing pear juice browning and enhancing the naturally occurring antioxidant capacity of pear juice.

KEYWORDS: Cyclodextrin; browning; pear juice; color; ascorbic acid; antioxidant

INTRODUCTION

Color is a sensory property with a strong influence on food acceptance as it contributes decisively to the initial perception of a food's condition, ripeness, degree of processing, and other characteristics (*1*). One of the main factors that can alter the color of fruit juice and so limit its commercial shelf life is browning since the organoleptic and nutritional properties of foods may be strongly altered if this undesirable reaction is not controlled. Therefore, the control of the browning during the processing stages of food has always been a challenge for food researchers (*2*−*4*).

The degree of browning depends on the presence of oxygen, reducing substances, metallic ions, pH, temperature, and the activity of different oxidizing enzymes (*2*−*4*). One of the main factors that must be controlled is the enzymatic activity of polyphenol oxidase (PPO) (monophenol dihydroxyphenylalanine: oxygen oxidoreductases, EC 1.14.18.1) (*5*). The presence of this enzyme in pears has been reported by several authors in the past decade, and much research has focused on the use of postharvest chemical treatments to avoid enzymatic browning (*6*−*13*). However, many of these treatments present serious disadvantages for use in the food industry because they can have negative effects on the sensorial properties of the products (*8*). Moreover, some chemical treatments have been associated with severe allergy-like reactions in certain populations, for which reason the Food and Drug Administration has restricted their use to only a few applications to inhibit the browning of foods (*4*, *14*−*17*).

Therefore, alternative methodologies are being investigated to extend the shelf life of foods, including fresh juice fruit. In recent years, there has been growing interest in finding new natural antioxidants for use in food, although another approach would be to look for substances that would help preserve the natural antioxidant capacity of a particular food (*18*, *19*). In this approach, the use or development of new natural secondary antioxidants is a new and challenging field. In this paper, we propose two new strategies to inhibit the enzymatic browning of pear juice: (i) using new antibrowning agents to slow down the challenge to pear juice color produced by enzymatic browning and (ii) maintaining the capacity of ascorbic acid (AA), the main primary antioxidant widely used in the food industry, through the use of secondary antioxidants.

For these purposes, we have used cyclodextrins (CDs), which are naturally occurring cyclic oligosaccharides derived from starch with six, seven, or eight glucose residues linked by α(1 → 4) glycosidic bonds in a cylindrically shaped cavity with a hydrophobic internal surface and a hydrophilic outer surface, designated α-, β-, and γ-CDs, respectively (*20*−*22*). Although the use of CDs has been proposed in recent decades to control the enzymatic browning of different foods caused by PPO (*23*), this is the first paper to analyze the effect of different CDs on pear juice. Moreover, many of the works that study the effect of CDs in enzymatic browning on different foods do so after long storage periods. However, Cheng and Crisosto (*24*) showed that 83% of the browning measured at the end of long incubation times in some foods had occurred during the first hour, which is why we have studied the behavior of pear juice enzymatic browning at the very beginning of the reaction (i.e., during the first hour). Furthermore, some contradictory claims have been

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Table 1. Effect of Different Additives on the Evolution of Total Color Difference (ΔE^*) and Hue Angle (H^*) in Pear Juice 15 min after Juicing

color parameter	no additive	D-glucose		α-CD	
		60 mM	180 mM	10 mM	30 mM
ΔE^*	28.01 ± 0.62	28.34 ± 1.31	28.92 ± 1.87	24.11 ± 1.19	17.03 ± 0.81
H^*	66.57 ± 1.45	66.21 ± 1.82	66.07 ± 1.25	69.82 ± 1.11	76.66 ± 1.32

published concerning the mechanism by which enzymatic browning is inhibited by CDs (*18*, *25*−*29*).

Bearing in mind that there is no information on the inhibition of pear juice enzymatic browning by CDs, the purpose of this work was to examine the potential use of CDs as agents in the control and preservation of natural color to preserve the quality of this food because its organoleptic and nutritional properties are strongly altered by browning.

MATERIALS AND METHODS

Materials. Biochemicals were purchased from Fluka (Madrid, Spain) and used without further purification. Maltosyl-β-CD was kindly supplied by Ensuiko Sugar Refining Co. Ltd. (Japan). AA, α- and β-CD, and the inhibitors L-cysteine and metabisulfite were purchased from Sigma-Aldrich (Madrid, Spain) and were used as received. Anhydrous D-glucose was supplied by Prolabo (Fontenay-Sous-Bois, France).

Juice Preparation. Pears (*Pyrus communis* cv. Barlett) 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 pear juice obtained was immediately collected and mixed in a beaker containing 25 mL of distilled water alone or α-CD (10−90 mM), β-CD (3−10 mM), maltosyl-β-CD (10−90 mM), D-glucose (60, 180 mM), AA (2.28 mM), L-cysteine (1 mM), or metadisulfite (1 mM).

Color Evolution Assessment. Color determinations were made, at 25 °C, using a ColorFlex version 1.72 colorimeter (Hunterlab, Reston, United States) certified by ISO 9001 with a D75 light source and the observer at 10°.

The color parameters corresponding to the uniform CIELAB color space (*30*) were obtained directly from the apparatus. Within this uniform space, two color coordinates, a^* and b^*, as well as a psychometric index of lightness, L^*, are defined. While a^* takes positive values for reddish colors and negative values for the greenish ones, b^* takes positive values for yellowish colors and negative values for the bluish ones. L^* is an approximate measurement of luminosity, which is the property according to which each color can be considered a member on the gray scale, between black and white, taking values within the range of 0−100. The total color difference (ΔE^*), a single value that takes into account the differences between L^*, a^*, and b^* of the sample and standard, was also studied. Chroma (C^*) is the attribute that allows the determination of the degree of difference in comparison with a gray color with the same lightness for each hue and so is considered the quantitative attribute of "colorfull". Hue angle (H^*) is the attribute according to which colors have been traditionally defined as reddish, greenish, etc. This is the attribute that allows a color to be distinguished with reference to a gray color with the same lightness. This attribute is related with the differences in absorbance at different wavelengths and is considered the qualitative attribute of color.

For all of the experiments, the previously described mixtures were used in the color evolution assays, using the measurements at time 0 as standards. This time corresponded to the first measurement, which was made 1 min after the pears had been juiced, and the chemical agents dissolved in the juice. All of the measurements were made at different times during the first hour after the materials had been dissolved in the pear juice, that is, just when the enzymatic browning was beginning.

Three readings were obtained for each replicate to obtain uniform color measurements: hue (H^*), total color difference (ΔE^*), and chroma (C^*), which were calculated using equations:

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

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

$$C^* = [(a^*)^2 + (b^*)^2]^{1/2}$$

RESULTS AND DISCUSSION

Validation of the Colorimetric Method To Characterize the Inhibition of Pear Juice Enzymatic Browning by CDs. Because the use of CDs to slow down pear juice enzymatic browning has not been described and to evaluate the ability of the colorimetric method presented in this paper to study the complexation by CDs of the mixture of phenolic compounds present in fresh pear juice, the effect of the addition of different additives on the evolution of pear juice color was studied.

Several studies have demonstrated that D-glucose (a molecule included in the CDs structure) is unable to form inclusion complexes with guest molecules (*31*−*33*). However, to confirm that the CD's effect on color evolution was not due to their glucidic nature, the effect of another sugar, in this case glucose, on pear juice color was studied. For this purpose, different amounts of D-glucose (60 and 180 mM), corresponding to 10 and 30 mM α-CD in the number of glucose units, were added to the reaction medium and the CIELAB parameters were measured.

As shown in **Table 1**, the evolution of both pear juice total color difference (ΔE^*) and Hue angle (H^*) 30 min after juicing was very similar in the absence and presence of 60 and 180 mM D-glucose, while all of the values were substantially different from those obtained in the presence of 10 and 30 mM α-CD.

These data confirm that the colorimetric study presented in this paper is a correct method for characterizing phenolic compound−CD inclusion complexes. Moreover, the absence of changes in the color parameters caused by the addition D-glucose to the juice (**Table 1**) is due to the fact that no glucose/phenolic compound complexes exist. Finally, the differences in the way that the color parameters evolved following the addition of D-glucose or CDs confirmed that the ability of CDs to slow down the enzymatic browning was not due to their glucidic nature.

Effect of Adding α-CD on Evolution of Pear Juice Color. Three types of CDs (two natural, α-CD and β-CD, and one modified, maltosyl-β-CD) were used to study the evolution of the color parameters of pear juice. In all cases, both the scalar (L^*, a^*, and b^*) and the angular coordinates (H^* and C^*) were evaluated to define the color of pear juice completely in the absence and presence of each type of CDs. To evaluate the behavior of pear juice enzymatic browning after the addition of CDs, increasing concentrations of α-CD (0−90 mM) were used.

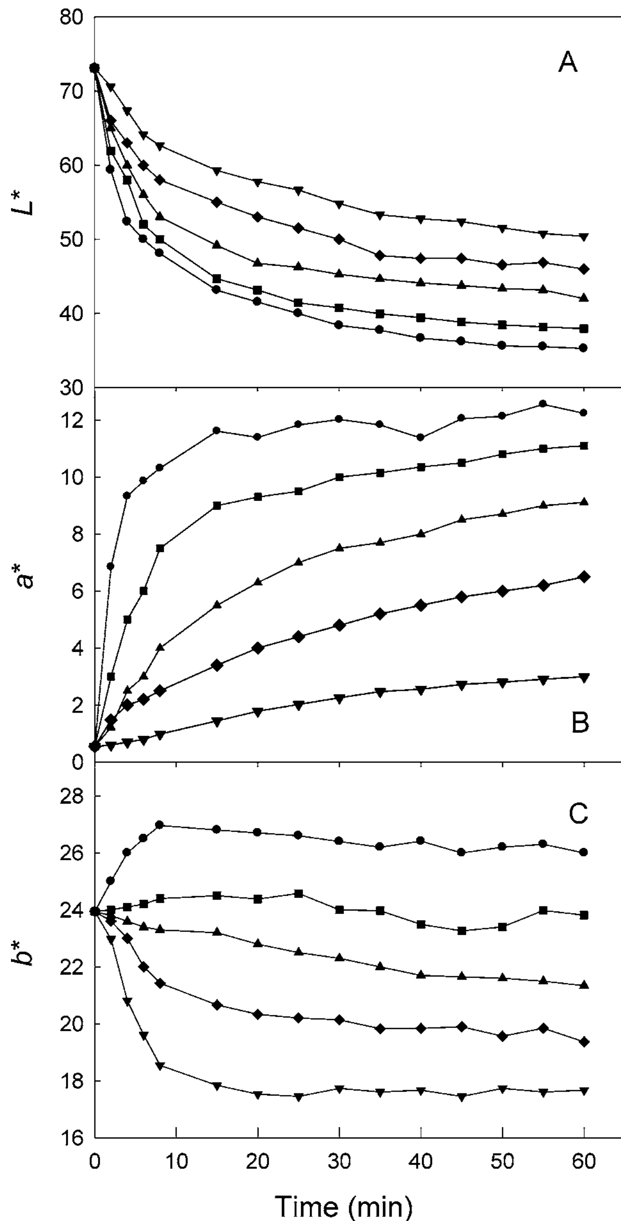


Figure 1. Effect of α-CD concentration on the evolution of L^*, a^*, and b^* in pear juice at 25 °C in the absence of any CD (●) and in the presence of α-CD: 10 (■), 30 (▲), 60 (◆) and 90 mM (▼). Each data point is the mean of three replicates.

First, lightness (L^*) was tested in the first hour after juicing. In the absence of any agent, L^* showed a significant and rapid decrease in the first 60 min as the juice became darker (**Figure 1A**). However, the different concentrations of α-CD delayed this decay in the L^* value although not even the higher concentration of α-CD tested (90 mM) was able to eliminate it totally. With regard to the other scalar parameters tested, **Figure 1B** shows the evolution of a^* in the absence and presence of 0, 10, 30, 60, and 90 mM α-CD. Without CDs in the medium, a^* increased sharply during the first 10 min and then less so, leading the pear juice to become redder during the reaction time. However, the different concentrations of α-CD led to significantly lower values of a^*, the initial green color still remaining in the presence of 90 mM α-CD. **Figure 1C** shows the rapid increase of b^* toward yellow colors in the absence of CDs, changes that were very similar to that observed for the thermal degradation of color in pear puree by Ibarz et al. (*34*). However,

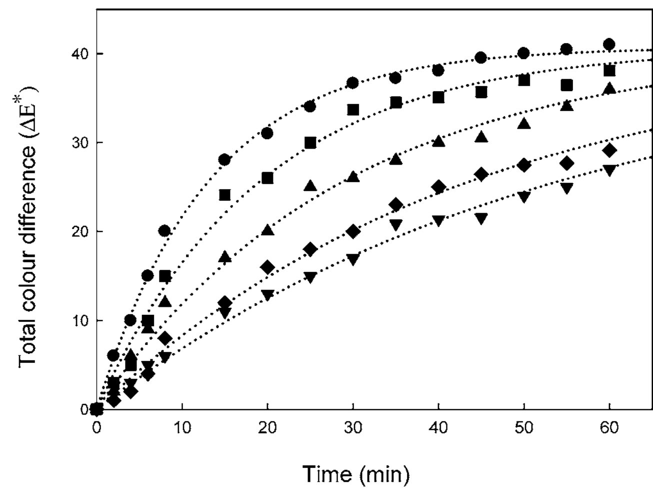


Figure 2. Effect of α-CD concentration on the evolution of total color difference (ΔE^*) in pear juice at 25 °C in the absence of any CD (●) and in the presence of α-CD: 10 (■), 30 (▲), 60 (◆), and 90 mM (▼). Each data point is the mean of three replicates.

in the presence of all of the concentrations of α-CD used, this increase in b^* slowed down.

Finally, the total color difference (ΔE^*) of pear juice, a colorimetric parameter extensively used to characterize the variation of colors in foods during processing, was studied in the presence of increasing concentrations (0−90 mM) of α-CD, as is shown in **Figure 2**. As can be observed, in the presence of α-CD, the ΔE^* of pear juice color during storage was strongly dependent on the CD concentration and the addition of α-CD resulted in lower variations in ΔE^* during the 60 min measured. Moreover, the evolution of this parameter in the presence of α-CD was more linear than with no CDs. The degradation of initial color observed in the absence of CDs was mostly due to a sharp depletion in both lightness (L^*) and the blue-yellow chromatism (b^*), whereas greenness-redness (a^*) did not have the same weight.

Kinetic Model of Pear Juice Color Evolution in the Presence and Absence of CDs. The mechanism through which CDs act on fruit juices has been the subject of controversy, as mentioned in the introduction section (*18*, *25*−*29*). Bearing in mind that there is no information on the effect of CDs on pear enzymatic browning and to clarify the CDs action mechanism on pear juice, this paper describes a kinetic model of the browning in the presence of CDs.

Because pear juice browning in the first hour after juicing is produced enzymatically, the velocity of the process is determined by a Michaelis−Menten equation. Because the only known parameter in the system is the total CD concentration, a velocity equation as a function of this parameter is proposed to evaluate the variations in total color (ΔE^*) with increasing CD concentrations. The velocity of ΔE^* evolution (v) can be expressed as:

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

where $[S]_F$ is the free concentration of substrate.

To develop the kinetic model proposed, two hypotheses have been assumed as follows: The first considers that the K_m values of some phenolic substrates of PPO from fruits present higher values than the free concentrations of polyphenolic compounds in the juices elaborated from those fruits (*35*, *36*); hence, it is assumed that the free concentration of substrate is negligible

with respect to the K_m, that is, $[S]_F <<< K_m$. So, the velocity of ΔE^* evolution can be expressed as:

$$v = k[S]_F \tag{2}$$

where k is a specific kinetic constant defined as:

$$k = \frac{V_{max}}{K_m} \tag{3}$$

The second assumption is based on the fact that only one molecule of substrate PPO can enter a CD molecule (stoichiometry 1:1), as has been demonstrated in several papers published by our group (*18*, *25*). Moreover, in these research studies, we demonstrated that PPO is only able to work with free substrate and not with the complex formed between CD and PPO substrates. Then, the process can be expressed as:

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

where CD−S is the complex between PPO substrates and CDs, CD_F is the free CD, 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:

$$K_c = \frac{[S]_F[CD]_F}{[CD{-}S]} \tag{4}$$

The mass balance of the substrate and CDs is represented by

$$[S]_T = [CD{-}S] + [S]_F \tag{5}$$

$$[CD]_T = [CD{-}S] + [CD]_F \tag{6}$$

where both subscript T and F denote total and free concentration, respectively.

Taking into account eqs 4−6 and assuming that $[S]_T <<< [CD]_T$, then $[CD]_T \cong [CD]_F$.

From this, $[S]_F$ can be expressed as:

$$[S]_F = \frac{K_c[S]_T}{[CD]_T + K_c} \tag{7}$$

Substituting eq 7 into eq 2, the velocity of ΔE^* evolution can be expressed as:

$$v = \frac{kK_c[S]_T}{[CD]_T + K_c} \tag{8}$$

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:

$$k_{app} = \frac{kK_c}{[CD]_T + K_c} \tag{9}$$

This k_{app} was calculated using the first-order fractional model described in eq 10.

$$e^{-k_{app}t} = \frac{(\Delta E_f^* - \Delta E^*)}{(\Delta E_f^*)} \tag{10}$$

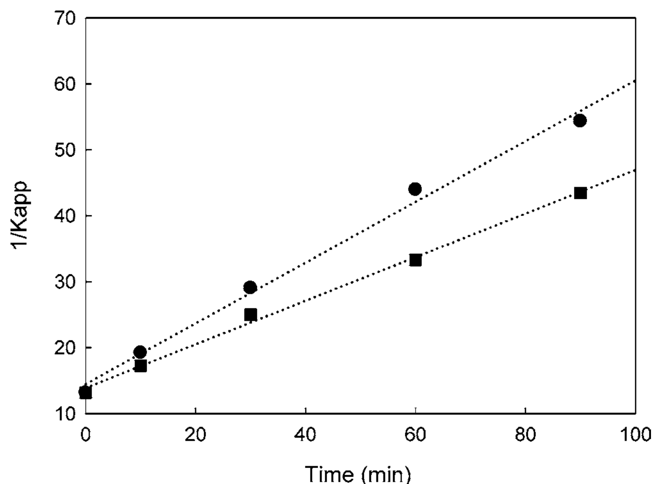


Figure 3. Lineweaver–Burk of the effect of the α-CD (●) and maltosyl-β-CD (■) concentration on k_{app} of the evolution of total color difference (ΔE^*) at 25 °C. Each data point is the mean of three replicates.

Table 2. Values of the Kinetic Constant (k) and Apparent Complexation Constant (K_c) for the Interaction between Different Types of CDs and Two Fruit Juices

fruit juice	type of CD	k (min^{-1})	K_c (mM^{-1})
pear juice	α-CD	0.069 ± 0.01	31.42 ± 1.83
	maltosyl-β-CD	0.071 ± 0.01	42.02 ± 2.43
apple juice[a]	maltosyl-β-CD	0.270 ± 0.01	4.09 ± 0.12

[a] Data obtained from our previous work (*19*).

where ΔE^* is the current value of total color difference, ΔE_f^* is the nonzero value of the parameter upon prolonged storage, t is the storage time, and k_{app} is the apparent specific kinetic constant.

To examine whether the two assumptions of our mathematical model were corrected and to calculate the apparent specific kinetic constant (k_{app}) for α-CD, ΔE^* data were fitted (dotted line) to eq 10 by nonlinear regression, as shown in **Figure 2**. The values obtained for k_{app} at increasing concentrations of α-CD were 0.0759 (in the absence of α-CD), 0.0520 (in the presence of 10 mM α-CD), 0.0344 (in the presence of 30 mM), 0.0227 (in the presence of 60 mM), and 0.0227 min^{-1} (in the presence of 90 mM). The fitted data confirm that the two assumptions made for the mathematical model were corrected as follows: (i) The free concentration of substrate is negligible with respect to the K_m, and (ii) only one molecule of substrate PPO may enter a CD molecule (stoichiometry 1:1).

To calculate the kinetic constant, k, and the apparent complexation constant, K_c, the reciprocal of k_{app} was plotted vs the total CD concentration ([CD]T) (**Figure 3**). Fitting the data by linear regression using Sigma Plot (SPSS Inc.), the values of k and K_c were determined (**Table 2**).

Effect of Adding β-CD on the Evolution of Pear Juice Color. One of the main disadvantages of β-CD for use as a browning inhibitor in the food industry is its poor solubility in aqueous media. For this reason, a lower concentration of this CD was employed to test the effect of β-CD on pear juice browning.

No significant differences in the evolution of the color scalar parameters L^*, a^*, and b^* of pear juice were observed in the absence, and in the presence of increasing concentrations of β-CD (0−10 mM), differences were observed (**Figure 4**). As shown, the strong decay of L^* observed in the absence of any additive was not reduced by the addition of this type of CD.

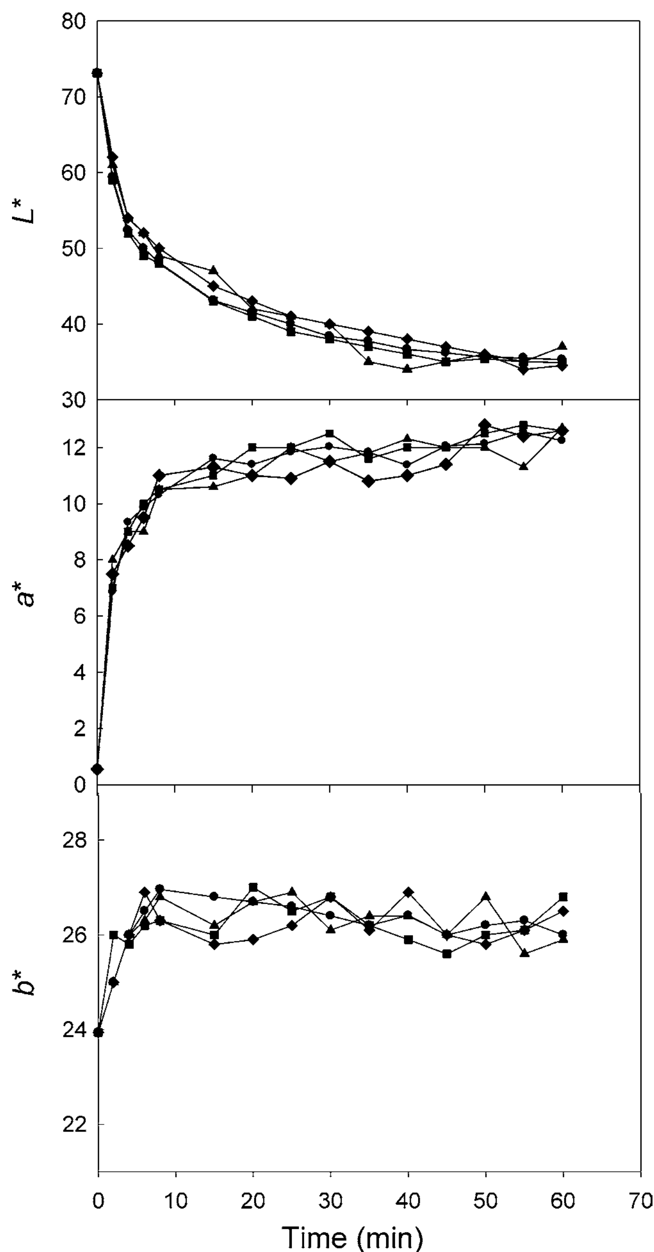


Figure 4. Effect of β-CD concentration on the evolution of L^*, a^*, and b^* values color parameters in pear juice at 25 °C in the absence of β-CD (●) and in the presence of β-CD: 3 (■), 5 (▲), and 10 mM (◆). Each data point is the mean of three replicates.

Moreover, the behavior of the other scalar coordinates (a^* and b^*) at all of the concentrations of β-CD used was very similar to the control. For the same reason, the effect of β-CD on the total color difference (ΔE^*) and the angular coordinates (H^* and C^*) of pear juice was not significant at any of the concentrations assayed.

Although the FDA has conceded GRAS status to both β-CD and α-CD, the data obtained in this paper show that only α-CD presents the optimum structure to inhibit pear juice enzymatic browning. There may be two reasons for this: First, the lower concentration of β-CD is used because of its poor solubility in aqueous medium, and second, the inner diameter of the hydrophobic cavity of α-CD (0.47−0.53 nm, corresponding to a structure formed by six molecules of glucose) leads to a more favorable interaction between the CDs and the mixture of phenols present in pear juice than when β-CD is used.

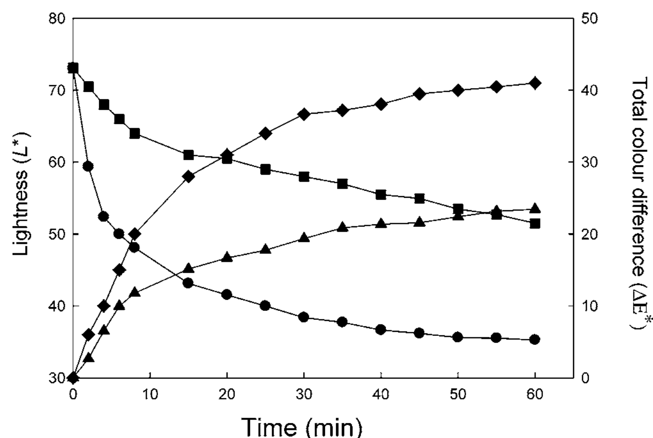


Figure 5. Effect of maltosyl-β-CD concentration on the evolution of lightness (L^*) [in the absence (●) and in the presence (■) of 90 mM maltosyl-β-CD] and total color difference (ΔE^*) [in the absence (▲) and in the presence (◆) of 90 mM maltosyl-β-CD] in pear juice at 25 °C. Each data point is the mean of three replicates.

Effect of Adding Maltosyl-β-CD on the Evolution of Pear Juice Color. In recent years, the use of modified CDs (for example, methyl, hydroxypropyl, carboxymethyl, acetyl, etc.) to complex different guest molecules has been improved because many different chemical moieties have been introduced into the CD molecule by reaction with the hydroxyl groups lining the upper and lower ridges of the toroid (*37−39*). Because β-CD was not able to inhibit the enzymatic browning of pear juice, we used a derivatized β-CD, here maltosyl-β-CD, which is more soluble than underivatized β-CD, to ascertain whether by modifying the nature of the CD it could be used to maintain the initial color of pear juice during the first hour of processing.

When the effect of maltosyl-β-CD on lightness (L^*) was evaluated, increasing concentrations of this more soluble derivative (up to 90 mM) led to increasing inhibition of browning during the required 60 min at 25 °C (**Figure 5**) and produced a greater reduction in the L^* parameter than when α-CD was used.

Moreover, the effect of maltosyl-β-CD on total color evolution in pear juice (ΔE^*) was very similar to the effect described above for α-CD. The ΔE^* values increased rapidly during the first 10 min in the absence of maltosyl-β-CD, as shown in **Figure 5**, but in the presence of all of the maltosyl-β-CD concentrations used, the increase in total color difference was significantly lower.

Effect of the CDs Structure on Apparent Complexation Constants. The molecular characterization of the complexed phenols present in pear juice and the different CDs is essential to understand the nature of the complexation between the phenols and the different host CDs studied in this paper. For this reason, the apparent complexation constants between the mixture of phenolic compounds responsible for enzymatic browning and the two types of CDs were calculated.

Because of the poor solubility of β-CD and because no changes were determined in either the scalar or the angular parameters of pear juice in the absence and presence of β-CD, the apparent complexation constant for this type of CD could not be calculated.

However, the presence of the substitution group, as maltosyl, in the β-CD structure positively affected the ability of the CDs to inhibit pear juice enzymatic browning, as was shown in the previous section. To determine the apparent specific kinetic constant (k_{app}) values, data for the total color evolution of pear juice in the absence and presence of increasing maltosyl-β-CD

Table 3. Evolution of L^* and a^* Coordinates of Pear Juice in the Absence and Presence of Different PPO Inhibitors

reaction time (min)	Hunter values L^*	Hunter values a^*
	without inhibitor	
0	73.10 ± 3.21	0.55 ± 0.02
60	35.26 ± 2.53	12.23 ± 1.46
	90 mM maltosyl-β-CD	
60	51.50 ± 2.83	2.31 ± 0.07
	2.28 mM AA	
60	35.30 ± 2.48	12.21 ± 1.45
	1 mM L-cysteine	
60	55.81 ± 2.11	0.23 ± 0.01
	1 mM metabisulfite	
60	60.82 ± 2.01	0.05 ± 0.01

concentrations were fitted to eq 9. The k_{app} value was 0.0759 (in the absence of maltosyl-β-CD), 0.0580 (in the presence of 10 mM maltosyl-β-CD), 0.040 (in the presence of 30 mM maltosyl-β-CD), 0.030 (in the presence of 60 mM maltosyl-β-CD), and 0.0230 min^{-1} (in the presence of 90 mM maltosyl-β-CD). To calculate the values of both the kinetic constant, k, and the apparent complexation constant, K_c, the reciprocal of k_{app} was fitted vs the total maltosyl-β-CD concentration (**Figure 3**); the values obtained can be seen in **Table 2**.

As can be seen in this table, maltosyl-β-CD presented higher K_c values than its parent β-CD and α-CD, indicating that the mixture of phenolic compounds responsible for the enzymatic browning of pear juice interacts more strongly with this CD. The differences observed when a modified CD was used may have been due to the chemical structure of the substitution group, which allowed the formation of a hydrogen bond with the hydration water molecules, because of the maltosyl substitution group giving rise to more intense bonding. In a study comparing derivatives from the same natural CD (β-derivatives), the maltosyl derivative showed a higher value of energy to remove 1% of water, followed by hydroxypropyl derivative and, finally, methyl and acetyl derivatives (*37*). Moreover, the better affinity of CDs derivaties for guest molecules was demonstrated by our group in several papers (*38*, *39*). Moreover, the apparent complexation constant obtained here for pear juice is higher than that found by López-Nicolás et al. (*19*) for the complexation of diphenols present in apple juice and maltosyl-β-CD. For these reasons, maltosyl-β-CD was considered the best CD to continue our investigation.

Effect of Different Inhibitors on the Evolution of Pear Juice Color. To further characterize the evolution of pear juice enzymatic browning, a detailed study of its inhibition was carried out. Food organoleptic properties, such as color, are closely connected to the phenolic composition and PPO activity. For this reason, the color was studied in pear juice in the absence and presence of different PPO inhibitors, following the evolution of lightness (L^*) pears liquefied in the absence and in the presence of several inhibitors, such as AA, metabisulfite, L-cysteine, and maltosyl-β-CD.

Of the inhibitors used, the reducing agents metabisulfite and L-cysteine appeared to be more effective inhibitors of L^* than AA or maltosyl-β-CD (**Table 3**). Moreover, L^* was only reduced for 30 min when AA was used because, after this time, the AA was oxidized by *o*-quinones (*o*-Qs) to dehydroascorbic acid (DHAA) and the lightness strongly decayed to reach the levels observed in the absence of PPO inhibitor.

Moreover, as is shown in **Table 3**, a significant difference was observed in the greenness-redness (a^*) parameter depending on whether the pears were liquefied in the absence or presence of inhibitors. In the absence of any agent, a^* increased rapidly in the first 20 min toward red colors. However, in the presence of 90 mM maltosyl-β-CD and 2.28 mM AA, this increase was slowed down; however, when metabisulfite or L-cysteine was added to the pear juice, the changes in a^* values were totally eliminated. These results clearly show that the inhibition of pear PPO by reducing agents or by substrate sequestering agents has an antibrowning effect on the color of pear juice. The results also indicate that enzymatic browning by PPO is the main process involved in pear juice browning at room temperature.

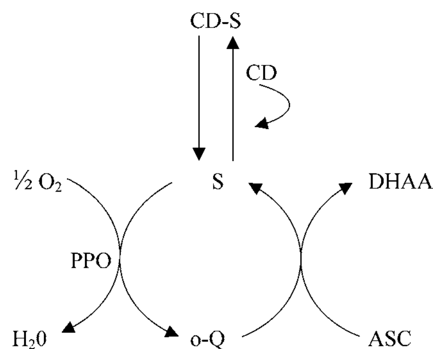
The different results obtained with the different PPO inhibitors could be due to the specific action mechanisms operating with each reducing agent used. Inhibition by thiol compounds may be due to an addition reaction with the quinones to form stable colorless products and/or a binding to the active center of the enzyme, as in the case of metabisulfite (*40*, *41*). Ascorbate acts as an antioxidant rather than as an enzyme inhibitor because it reduces the initial quinone formed by the enzyme to the original diphenol before it undergoes a secondary reaction that leads to browning (*42*). Finally, as has been demonstrated in a previous section, CDs are able to complex the PPO substrates, thereby preventing their oxidation to quinones and subsequent polymerization to brown pigments.

Use of Maltosyl-β-CD as a Secondary Antioxidant. As shown in the previous section, the synthetic antioxidants, metabisulfite and L-cysteine, had a higher antibrowning effect on pear juice than those from natural sources, such as AA or CDs. However, there is a growing interest in natural antioxidants for use in food, although another strategy would be to look for "preservers" of the natural antioxidant capacity of a particular food. In this approach, the development and use of new natural secondary antioxidants is a fresh and challenging task as shown by our group for the oxidation of phenols by lipoxygenase (*18*) or PPO (*19*), in which CDs act as secondary antioxidants in synergism with AA.

In this paper, we have seen how maltosyl-β-CD can enhance the ability of AA to prevent the enzymatic browning due to the protective effect of maltosyl-β-CD against AA oxidation. Maltosyl-β-CD seems to act as a ‘‘secondary antioxidant”, reducing pear juice browning and enhancing the naturally occurring antioxidant capacity of the food. This capacity of maltosyl-β-CD to function as a secondary antioxidant in pear 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 food can be kept (*18*, *19*). AA is the best known chemical agent for reducing the browning reaction (*3*, *43*, *44*). However, once the added AA has been completely oxidized to DHAA, *o*-Qs accumulate and undergo browning. 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 (*3*).

In the model proposed in this paper, CDs can enhance the ability of AA to prevent enzymatic browning due to the protection that they offer AA against oxidation by *o*-Qs (**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*-Qs. 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*-Qs and, hence, the oxidation of AA, because only free substrates, in equilibrium with CD-bound phenol (CD−S), are oxidized by

Scheme 1. Use of Maltosyl-β-CD as a Secondary Antioxidant on the Browning of Pear Juice[a]



[a] S, free PPO substrate; CD–S, complex between PPO substrates and CD.

PPO in the presence of O_2 to *o*-Qs (*18*, *19*). In this way, the reaction is slowed down and the shelf life of the food is prolonged.

To confirm this hypothesis, the evolution of pear juice color in the absence and presence of 2.28 mM AA and 90 mM maltosyl-β-CD was studied (**Figure 6A**). As was previously seen in **Table 3**, pear juice lightness (L^*) fell significantly when 90 mM maltosyl-β-CD was added to the medium. Moreover, when 2.28 mM AA was added, the rapid decay in L^* observed in the absence of any reagent was drastically reduced at the first 30 min. After this time, L^* quickly decayed to reach the same values as observed in the absence of any agent. Furthermore, in **Figure 6A**, we can see when both 2.28 mM AA and 90 mM maltosyl-β-CD were added simultaneously to pear juice, the decrease in lightness (L^*) was less than in the absence of either of them. Moreover, in the presence of both agents, the pronounced decay observed in L^* values after the first 30 min was eliminated and the initial lightness of pear juice was almost totally maintained. This behavior is probably due to the preservation of the antioxidant capacity of AA by maltosyl-β-CD. To corroborate these results, the evolution of total color difference (ΔE^*) in the presence and absence of these enzymatic browning inhibitors was tested. As shown in **Figure 6B**, the presence of AA in the medium reduced the change in this parameter more effectively than maltosyl-β-CD but only during the first 30 min of the reaction time. After this time, the total color difference increased sharply to reach the maximum variation observed in absence of any regent. However, in the presence of 2.28 mM AA plus 90 mM maltosyl-β-CD, the changes in ΔE^* were lower than when these reagents were added independently. Moreover, the inhibition rate of ΔE^* was maintained after the first 30 min. These results for L^* and ΔE^* confirm the hypothesis described in **Scheme 1**, that is, the hydrophilic nature of AA precludes its inclusion in maltosyl-β-CD, and so the apparent CD-mediated protection of AA in pear juice is probably due to the complexation of the mixture of phenols present in pear juice into the hydrophobic cavity of the maltosyl-β-CD. These data are in good agreement with the results previously obtained by our group (*18*, *19*) and with those presented by different authors using CDs combined with other antioxidant agents (*45*−*47*).

Synergistic Effect of AA and Maltosyl-β-CD on Pear Juice Browning. Finally, the mechanism of pear juice enzymatic browning inhibition in the presence of both AA plus maltosyl-β-CD was studied. Some of the antioxidants used in PPO inhibition, such as BHA and BHT (generally termed "primary antioxidants"), are used in various combinations with synergistic effects; that is, the combined effect of the two antioxidants is greater than the sum of the individual effects obtained when used alone (*48*). This synergistic effect should not be confused with the synergism that occurs when a primary antioxidant is added along with acid chelators or sequestrants such as citric, tartaric, oxalic, malic, phytic, or succinic acid (*49*, *50*), which are known as "secondary antioxidants" (*51*).

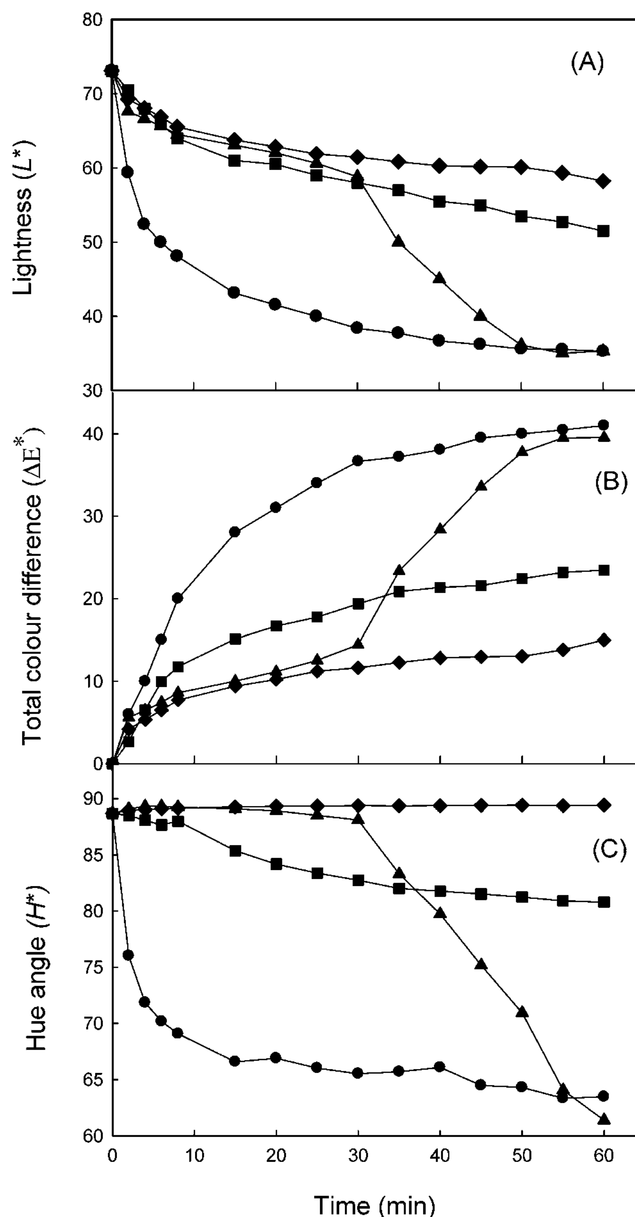


Figure 6. Evolution of lightness (L^*) (**A**), total color difference (ΔE^*) (**B**), and Hue angle (H^*) (**C**) in the absence of any agent (●) and in the presence of 2.28 mM AA (▲), 90 mM maltosyl-β-CD (■), and 90 mM maltosyl-β-CD plus 2.28 mM AA (◆). Each data point is the mean of three replicates.

To ascertain whether the combined action of AA and maltosyl-β-CD corresponded to a synergistic or synergism effect, the evolution of Hue angle (H^*) was studied. As is shown in **Figure 6C**, the evolution of this parameter presented a lag time whose duration depended on the enzymatic browning inhibitor used. In the presence of AA, the Hue angle (H^*) values of pear juice enzymatic browning presented a lag time of 30 min, during which time the pear juice is protected by AA against enzymatic browning. However, after 30 min, AA is completely oxidized to DHAA and *o*-Qs can accumulate and undergo browning. Moreover, the existence of a lag time in the presence

of maltosyl-β-CD (8 min) was due to the protective effect of the maltosyl-β-CD on the AA occurring naturally in pears (i.e., prior to juicing). Finally, **Figure 6C** shows that the lag time in the presence of 90 mM maltosyl-β-CD plus 2.28 mM AA is not observed in the first 60 min of the reaction time. This fact confirms that the lag time in the presence of both enzymatic browning inhibitors is longer that the sum of the lags observed in the presence of 90 mM maltosyl-β-CD alone (8 min) or 2.28 mM AA alone (30 min). The absence of a lag time when the two pear juice enzymatic browning inhibitors, AA and maltosyl-β-CD, added to the reaction medium confirms the protection of AA by CDs. These results show that the combination of both AA and maltosyl-β-CD had a synergic effect; that is, the combined effect of both was greater than the sum of the two single treatments.

ABBREVIATIONS USED

CDs, cyclodextrins; PPO, polyphenol oxidase; AA, ascorbic acid; DHAA, dehydroascorbic acid; *o*-Q, *ortho*-quinone.

LITERATURE CITED

(1) Alos, E.; Cercos, M.; Rodrigo, M. J.; Zacarias, L.; Talon, M. Regulation of color break in citrus fruits. Changes in pigment profiling and gene expression induced by gibberellins and nitrate, two ripening retardants. *J. Agric. Food Chem.* **2006**, *54*, 4888−4895.

(2) Sapers, G. M.; Hicks, K. B.; Miller, R. L. Antibrowning agents. In *Food Additives*, 2nd ed.; Branen, A. L., Davidson, P. M., Salminen, S., Thorngate, J. H., Eds.; Marcel Dekker Inc.: New York, 2001; p 543.

(3) Walker, J. R. L. Enzymatic browning in foods. Its chemistry and control. *Food Technol.* **1977**, *12*, 19−25.

(4) Sapers, G. M. Browning of foods. Control by sulfites, antioxidants and other means. *Food Technol.* **1993**, *47*, 75−84.

(5) Sánchez-Ferrer, A.; Rodríguez-López, J. N.; García-Cánovas, F.; García-Carmona, F. Tyrosinase: A comprehensive review of its mechanism. *Biochim. Biophys. Acta* **1995**, *1247*, 1−11.

(6) Sapers, G. M.; Miller, R. L. Browning inhibition in fresh-cut pears. *J. Food Sci*. **1988**, *63*, 342−346.

(7) Dong, X.; Wrolstad, R. E.; Sugar, D. Extending shelf life of fresh-cut pears. *J. Food Sci*. **2000**, *65*, 181−186.

(8) Abreu, M.; Beirao-da-Costa, S.; Gonzalves, E. M.; Beirao-da-Costa, M. L.; Moldao-Martins, M. Use of mild heat pre-treatments for quality retention of freshcut 'Rocha' pear. *Postharvest Biol. Technol.* **2003**, *30*, 153−160.

(9) Kim, M.; Kim, C. Y.; Park, I. Prevention of enzymatic browning of pear by onion extract. *Food Chem.* **2005**, *89*, 181−184.

(10) Rosen, J. C.; Kader, A. A. Postharvest physiology and quality maintenance of sliced pear and strawberry fruits. *J. Food Sci.* **1989**, *54*, 656−659.

(11) Gorny, J. R.; Gil, M. I.; Kader, A. A. Postharvest physiology and quality maintenance of fresh-cut pears. *Acta Hortic.* **1998**, *464*, 231−236.

(12) Pittia, P.; Nicoli, M. C.; Comi, G.; Massini, R. Shelf-life extension of fresh-like ready-to-use pear cubes. *J. Sci. Food Agric.* **1999**, *79*, 955−960.

(13) Sapers, G. M.; Hicks, K. B. Quality factors of fruits and vegetables: Chemistry and technology. In *Inhibition of Enzymatic Browning in Fruits and Vegetables*; Jen, J. J., Ed.; American Chemical Society: Washington, DC, 1989; p 29.

(14) Joslyn, M. A.; Braverman, J. B. S. The chemistry and technology of the pre-treatment and preservation of fruit and vegetables products with sulphur dioxide and sulfites. *Adv. Food Res.* **1954**, *5*, 97−160.

(15) Grice, H. C. Food antioxidants: International perspectives, proceedings sponsored by the International Life Sciences. Institute-Nutrition Foundation, Antioxidant Technical Committee. *Food Chem. Toxicol.* **1986**, *24*, 819−825.

(16) Shahidi, F.; Janitha, P. K.; Wanasundara, P. D. Phenolic antioxidants. *Crit. Rev. Food Sci. Nutr.* **1992**, *32*, 67−75.

(17) Jones, J. M. *Food Additives in Food Safety*; Eagan Press: St. Paul, MN, 1992; p 203.

(18) Nuñez-Delicado, E.; Sanchez-Ferrer, A.; Garcia-Carmona, F. Cyclodextrins as secondary antioxidants: Synergism with ascorbic acid. *J. Agric. Food Chem.* **1997**, *45*, 2830−2835.

(19) López-Nicolás, J. M.; Núñez-Delicado, E.; Sánchez-Ferrer, A.; García-Carmona, F. Kinetic model of apple juice enzymatic browning in the presence of cyclodextrins: The use of maltosyl-β-cyclodextrin as secondary antioxidant. *Food Chem.* **2007**, *101*, 1164−1171.

(20) Saenger, W. Cyclodextrin inclusion compounds in research and industry. *Angew. Chem. Int. Ed. Engl.* **1980**, *19*, 344−362.

(21) López-Nicolás, J. M.; Bru, R.; García-Carmona, F. Kinetic characteristics of the enzymatic conversion in presence of cyclodextrins: study of the oxidation of polyunsaturated fatty acids by lipoxygenase. *Biochim. Biophys. Acta* **1997**, *1347*, 140−150.

(22) López-Nicolas, J. M.; Bru, R.; Garcia-Carmona, F. Enzymatic oxidation of linoleic acid by lipoxygenase forming inclusion complexes with cyclodextrins as starch model molecules. *J. Agric. Food Chem.* **1997**, *45*, 1144−1148.

(23) Hicks, K. B.; Sapers, G. M.; Seib, P. A. Process for preserving raw fruit and vegetables juices using cyclodextrins and compositions thereof. U.S. Patent, 293, 4975, 1990.

(24) Cheng, G. W.; Crisosto, C. H. Browning potential, phenolic composition, and polyphenoloxidase activity of buffer extracts of peach and nectarine skin tissue. *J. Am. Soc. Horts.* **1995**, *120*, 835−838.

(25) Sojo, M. M.; Núñez-Delicado, E.; García-Carmona, F.; Sánchez-Ferrer, A. Cyclodextrins as activator and inhibitor of banana pulp polyphenol oxidase. *J. Agric. Food Chem.* **1999**, *47*, 518−523.

(26) Pilizota, V.; Subaric, D. Control of enzymatic browning of foods. *Food Technol. Biotechnol.* **1998**, *36*, 219−227.

(27) Gacche, R. N.; Zore, G. B.; Ghole, V. S. Kinetics of inhibition of polyphenol oxidase mediated browning in apple juice by beta-cyclodextrin and L-ascorbate-2-triphosphate. *J. Enzyme Inhib. Med. Chem.* **2003**, *18*, 1−5.

(28) Gacche, R. N.; Warangkar, S. C.; Ghole, V. S. Glutathione and cinnamic acid: Natural dietary components used in preventing the process of browning by inhibition of polyphenol oxidase in apple juice. *J. Enzyme Inhib. Med. Chem.* **2004**, *19*, 175−179.

(29) Álvarez-Parrilla, E.; de la Rosa, L. A.; Rodrigo-García, J.; Escobedo-González, R.; Mercado-Mercado, G.; Moyers-Montoya, E.; Vázquez-Flores, A.; González-Aguilar, G. A. Dual effect of β-cyclodextrin (β-CD) on the inhibition of apple polyphenol oxidase by 4-hexylresorcinol (HR) and methyl jasmonate (MJ). *Food Chem.* **2007**, *101*, 1346−1356.

(30) Meléndez-Martínez, A. J.; Vicario, I. M.; Heredia, F. J. Application of tristimulus colorimetry to estimate the carotenoids content in ultrafrozen orange juices. *J. Agric. Food Chem.* **2003**, *51*, 7266−7270.

(31) López-Nicolás, J. M.; Núñez-Delicado, E.; Pérez-López, A. J.; Carbonell Barrachina, A.; Cuadra-Crespo, P. Determination of stoichiometric coefficients and apparent formation constants for β-cyclodextrin complexes of *trans*-resveratrol using reversed-phase liquid chromatography. *J. Chromatogr. A* **2006**, *1135*, 158−165.

(32) Fujimura, K.; Ueda, T.; Kitagawa, M.; Takayanagi, H.; Ando, T. Reversed-phase retention behavior of aromatic compounds involving. beta.-cyclodextrin inclusion complex formation in the mobile phase. *Anal. Chem.* **1986**, *58*, 2668−2674.

(33) Clarot, I.; Cledat, D.; Battu, S.; Cardot, P. J. P. Chromatographic study of terpene derivatives on porous graphitic carbon stationary phase with β-cyclodextrin as mobile phase modifier *J. Chromatogr. A* **2000**, *903*, 67−76.

(34) Ibarz, A.; Pagán, J.; Garza, S. Kinetic models for colour changes in pear puree during heating at relatively high temperatures. *J. Food Eng.* **1999**, *39*, 415−422.

(35) Fayad, N.; Marchal, L.; Billaud, C.; Nicolas, J. Comparison of β-cyclodextrin effect on polyphenol oxidation catalysed by purified polyphenol oxidase from different sources. *J. Agric. Food Chem.* **1997**, *45*, 2442−2446.
(36) Van der Sluis, A. A.; Dekker, M.; Skrede, G.; Jongen, W. M. F. Activity and concentration of polyphenolic antioxidants in apple juice. Effect of existing production methods. *J. Agric. Food Chem.* **2002**, *50*, 7211−7219.
(37) Veiga, M. D.; Merino, M.; Fernandez, D.; Lozano, R. Characterization of some cyclodextrin derivatives by thermal analysis. *J. Therm. Anal. Calorim.* **2002**, *68*, 511−516.
(38) López-Nicolás, J. M.; Brú, R.; Sánchez-Ferrer, A.; García-Carmona, F. Use of "soluble lipids" for biochemical processes: Linoleic acid:cyclodextrin inclusion complexes in aqueous solutions. *Biochem. J.* **1995**, *308*, 151−154.
(39) Bru, R., López-Nicolás, J. M.; García-Carmona, F. Aggregation of polyunsaturated fatty acids in the presence of cyclodextrins. *Colloids Surf.* **1995**, *97*, 263−269.
(40) Valero, E.; Varón, R.; García-Carmona, F. A kinetic study of irreversible enzyme inhibition by an inhibitor that is rendered unstable by enzymic catalysis. The inhibition of polyphenol oxidase by L-cysteine. *Biochem. J.* **1991**, *277*, 869−874.
(41) Valero, E.; Varón, R.; García-Carmona, F. Kinetic study of effect of metabisulfite on polyphenol oxidase. *J. Agric. Food Chem.* **1992**, *40*, 904−908.
(42) Whitaker, J. R. Polyphenol oxidase. In *Food Chemistry*; Fennema, O. R., Ed.; Dekker: New York, 1996; pp 492−494.
(43) Martinez, M. V.; Whitaker, J. R. The biochemistry and control of enzymatic browning. *Trends Food Sci. Technol.* **1995**, *6*, 195−200.
(44) Bauernfeind, J. C.; Pinkert, D. M. Food processing with added ascorbic acid. *Adv. Food Res.* **1970**, *18*, 219−315.
(45) Hicks, K. B.; Haines, R. M.; Tong, C. B. S.; Sapers, G. M.; El-Atawy, Y.; Irwin, P. L.; Seib, P. A. Inhibition of enzymatic browning in fresh fruit and vegetable juices by soluble and insoluble forms of beta-cyclodextrin alone or in combination with phosphates. *J. Agric. Food Chem.* **1996**, *44*, 2591−2594.
(46) Sapers, G. M.; Hicks, K. B. Inhibition of enzymatic browning in fruits and vegetables. In *Quality Factors of Fruits and Vegetables: Chemistry and Technology*; Jen, J. J., Ed.; American Chemical Society: Washington, DC, 1989; p 29.
(47) 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.; Ei-Atawy, Y. S. Control of enzymatic browning in apple with ascorbic acid derivatives, polyphenol oxidase inhibitors, and complexing agents. *J. Food Sci.* **1989**, *54*, 997−1002.
(48) Sherwin, E. R. Antioxidants for food fats and oils. *J. Am. Oil Chem. Soc.* **1972**, *49*, 468−472.
(49) St. Angelo, A. J.; Vercellotti, J. R.; Dupuy, H. P.; Spanier, A. M. Assessment of beef flavor quality: A multidisciplinary approach. *Food Technol.* **1988**, *42*, 133−145.
(50) St. Angelo, A. J.; Crippen, K. L.; Dupuy, H. P.; James, C., Jr. Chemical and sensory studies of antioxidant-treated beef. *Food Sci.* **1990**, *55*, 1501−1510.
(51) St. Angelo, A. J. Lipid oxidation in foods. *Crit. Rev. Food Sci. Nutr.* **1996**, *36*, 175−224.

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