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Kinetic Study of the Activation of Banana Juice Enzymatic Browning by the Addition of Maltosyl-β-cyclodextrin

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In recent years, the use of cyclodextrins (CDs) as antibrowning agents in fruit juices has received growning attention. However, there has been no detailed study of the behavior of these molecules as substances, which can lead to the darkening of foods. In this paper, when the color of fresh banana juice was evaluated in the presence of different CDs, the evolution of several color parameters was the opposite of that observed in other fruit juices. Moreover, a kinetic model based on the complexation by CDs of the natural browning inhibitors present in banana is developed for the first time to clarify the enzymatic browning activation of banana juice. Finally, the apparent complexation constant between the natural polyphenoloxidase inhibitors present in banana juice and maltosyl-β-CD was calculated ($K_{ci} = 27.026 \pm 0.212$ mM^{-1}).

KEYWORDS: Cyclodextrin; browning; banana; juice; color

INTRODUCTION

In the last decade, the consumption of tropical fruit juices in Spain has increased as consumers look for new foods with different sensorial properties (*1, 2*). One of the most consumed tropical fruits in Spain is banana, with its enormous banana world production (*3, 4*), important nutritional value (*5, 6*), and widely appreciated flavor and aroma (*7, 8*) contributing to this popularity. However, the evolution of banana juice color during processing and storage is one of the principal inconveniences of this tropical juice, because color degradation impairs the sensory properties of the product and also lowers its nutritional value.

One of the main factors that can alter the color of banana juice and therefore limit its commercial shelf life, is browning because of the enzymatic activity of polyphenol oxidase (PPO) (monophenol dihydroxyphenylalanine: oxygen oxidoreductases, EC 1.14.18.1) (*9, 10*). The presence of PPO in banana fruit has been widely reported (*10–15*), and several authors in the last decade have focused on the use of postharvest chemical treatments to avoid enzymatic browning through the inhibition of PPO (*16–25*). Moreover, some authors have studied the possible interaction between the oxidation products of enzymatic browning (i.e., quinones) and other compounds present in the medium (*26–28*).

In recent decades, there has been growing interest in using antioxidants in food (*29*). However, although some of the most widely used antioxidants are very effective as antibrowning agents, they are synthetic products and tend to feed the growing concern about the potential health hazards of synthetic substances (*30*). Consumers are demanding a reduction in the overall use of chemicals in fresh products, and therefore, alternative methodologies need to be investigated (*31*). In recent years, the use of cyclodextrins (CDs) as natural antibrowning agents has been looked at for many foods (*32–36*). Recently, a paper concerning the use of CDs to slow the enzymatic browning of apple juice was published by our group and a kinetic model to clarify the controversy existing in the literature about the mechanism through which enzymatic browning is inhibited by CDs was proposed (*37*).

However, although these natural antioxidant agents can be used to prevent the enzymatic browning of fresh juice fruit, a detailed study of its effects on the sensorial properties of many juices must be developed. Indeed, many new antioxidant compounds proposed as antibrowning agents may well act as promotors of enzymatic browning depending upon such factors as the concentration, type of food to be protected, antioxidant source, etc. One example of this type of behavior concerns the

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Table 1. Effect of Different Additives on the Evolution of Total Color Difference (ΔE^*) of Several Fruit Juices from Different Sources Measured 30 min after Juicing

	total color difference (ΔE^*)			
additive	peach juice	apple juice	pear juice	banana juice
none	26.01 ± 1.76	42.65 ± 1.12	28.01 ± 0.62	17.01 ± 0.94
D-glucose (60 mM)	26.32 ± 1.45	41.98 ± 1.38	28.34 ± 1.31	17.10 ± 1.35
D-glucose (180 mM)	26.71 ± 1.56	42.24 ± 1.45	28.92 ± 1.87	16.95 ± 1.46
α-CD (10 mM)	20.43 ± 1.29	30.63 ± 1.38	24.11 ± 1.19	18.41 ± 0.82
α-CD (30 mM)	15.09 ± 0.84	25.73 ± 1.57	17.03 ± 0.81	20.97 ± 1.24

use of CDs. Although CDs are generally recognized as anti-browning agents, in a previous work, we studied the role of CDs as a modulator of latent banana pulp PPO and a browning activating effect of CDs was described (*38*). However, no kinetic model was proposed to explain the results, and the effect of the addition of CDs on CIELAB color parameters of banana juices was not studied. Moreover, further studies were required to compile a complete range of fruits or raw materials for which browning is activated or inhibited by CDs. In the present paper, we study the effect of CD on the browning of the most consumed fruit juices from different sources in Spain.

Bearing in mind that the activating effect of CD on banana enzymating browning has not been studied in detail, the effect of adding of CDs on CIELAB color parameters of banana juice has been evaluated. For this, several factors, which may affect the color evolution of banana juice, such as the type and concentration of CDs, have been studied. Moreover, to clarify the mechanism of banana juice enzymatic browning inhibition by CD, a kinetic model has been proposed. Finally, the apparent complexation constants for the complexes between the natural inhibitors of the enzymatic browning present in banana juice and different types of CD have been calculated.

MATERIAL AND METHODS

Materials. α- and *β*-CD were purchased from Sigma-Aldrich (Madrid, Spain) and used as received. Maltosyl-*β*-CD was kindly supplied by Ensuiko Sugar Refining Co. Ltd. (Japan). Anhydrous D-glucose was supplied by Prolabo (Fontenay-Sous-Bois, France).

Juice Preparation. Bananas (*Musa acuminata* subgr. Cavendish cv. Spanish Pequeña Enana), pears (*Pyrus communis* cv. Barlett), peaches (*Prunus persina* cv. Baby gold), grapes (*Vitis vinifera* cv. Dominga), and 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 fruit juices obtained were immediately collected and mixed in a beaker containing 25 mL of distilled water alone or containing enough α-CD, *β*-CD, maltosyl-*β*-CD (0–90 mM), or glucose (60 and 180 mM) to produce the final concentration of each compound indicated in each experiment.

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

The color parameters corresponding to the uniform CIELAB color space (*39*) 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, within the range of 0–100. The total color difference (ΔE^*), a single value which 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 for the determination of the degree of difference in comparison to a gray color with the same lightness for each hue and, therefore, is considered the quantitative attribute of "colorful". 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 to 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 the standard. This time corresponded to the first measurement, which was made 1 min after the fruits 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 fruit juice, i.e., 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

Effect of the Addition of Several Additives on the Evolution of Fruit Juice Color. The effect of different additives on the total color difference (ΔE^*) was measured in the different juices 30 min after juicing (**Table 1**). As expected, the addition of increasing concentrations of α-CD produced a reduction in the ΔE^* values of peach, apple, and pear juice with respect to the values obtained in the absence of any agent, as was demonstrated in previous works (*37*, *40*). However, the addition of the same concentrations of α-CD concentrations to banana juice increased the ΔE^* values compared to the control. Besides the fact that no study of banana juice color in the presence of CDs had been made previously, this observation made it necessary to carry out experiments to confirm that the colorimetric method developed in this paper is suitable for studying the effect of CDs on different fruit juices.

To study the possibility that inclusion complexes are formed between D-glucose (a molecule included in the CD structure) and the compounds responsible for enzymatic browning in fruit juices and to confirm that the effect of CDs on color evolution was not due to their glucidic nature, the effect of another sugar, in this case D-glucose, on the color of apple, peach, pear, and banana juices was studied. For this purpose, various amounts of D-glucose (60 and 180 mM), corresponding to 10 and 30 mM of α-CD with regard to the number of glucose units, were added to the reaction medium and the CIELAB parameters were measured. As shown in **Table 1**, the ΔE^* values of the different fruit juices were similar in the absence and presence of 60 and 180 mM D-glucose, although these values differed substantially from those obtained in the presence of 10 and 30 mM α-CD. These results are in good agreement with several studies that have demonstrated that D-glucose is unable to form inclusion complexes with guest molecules (*41*, *42*).

Several conclusions can be extrapolated from these data. First, CDs affect the evolution of the total color difference in the juices from many sources. Moreover, the ability of CDs to change the color parameters of these juices is not due to its glucidic nature but the formation of an inclusion complex, because no glucose/phenolic compound complexes

exist. Finally, colorimetric studies appear to be a satisfactory method for observing and characterizing phenolic compound–CD inclusion complexes.

Comparison of the Effect of CD on the Browning of Different Fruit Juices. To evaluate the effect of the addition of CDs on the enzymatic browning of fruit juices from different sources and to compile a range of fruit juices for which browning is activated or inhibited by CDs, as recommended in our previous work (*38*), a modified CD, in this case maltosyl-CD, was added to the reaction medium. To do this, the relative measurement between the light reflected and absorbed by the samples, i.e., the lightness (L^*) values of peach, pear, apple, grape, and banana juices, was measured in the absence and presence of 90 mM maltosyl-β-CD.

The extent to which the addition of maltosyl-β-CD inhibited enzymatic browning was expressed on a percent basis, i.e., the percent difference between the control and treatment ΔL values 1 h after juicing

$$\% \text{ variation} = \frac{\Delta L_{\text{control}} - \Delta L_{\text{treatment}}}{\Delta L_{\text{control}}} \times 100$$

where ΔL is the difference between the L^* value at time t with the units in minutes. As shown in **Figure 1**, the negative values of the percent variation of pear, peach, grape, and apple juices indicate that the addition of 90 mM maltosyl-β-CD as a browning inhibitor is effective to the extent calculated. However, the positive percent variation value obtained for banana juice indicates the activation of the enzymatic browning by the addition of maltosyl-β-CD.

Although several researchers are mostly interested in long-term browning control, Cheng and Crisosto (*43*) 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 banana juice enzymatic browning at the very beginning of the reaction (i.e., during the first hour). Moreover, the data presented in this paper show that the evolution of the total color difference (ΔE^*) in banana juice at 25 °C in the absence of CD was very significant in the first hour.

Because enzymatic browning, with a few exceptions (medlars, prunes, black raisins, dates, black figs, zapote, tea, coffee, and cocoa), is considered as product degradation, which lowers fruit quality both visually and with regard to taste and nutritional characteristics, several papers have studied how CDs slow enzymatic browning in several foods (*32*, *33*, *37*, *38*, *40*). However, although the mechanism of fresh apple juice enzymatic browning in the presence of maltosyl-β-CD was described by our group (*37*), this is the first time that an increase in a fruit juice L^* value because of the addition of CD has been demonstrated. For this reason and with the objective of clarifying the effect of CDs on banana juice, a detail study of the effect of different CDs on banana juice color was deemed necessary.

Effect of the CD Structure on the Browning of Banana Juice. To examine the variation of banana juice enzymatic browning by different types of CD, two types of natural CD generally recognized as safe (α-CD and β-CD) and one modified CD (maltosyl-β-CD) were used.

Figure 2 shows that the percent variation of banana juice enzymatic browning is dependent upon the CD type used. As can be seen, the optimum variation rate was produced by maltosyl-β-CD, followed by α-CD, while β-CD presented the lowest variation of banana juice enzymatic browning.

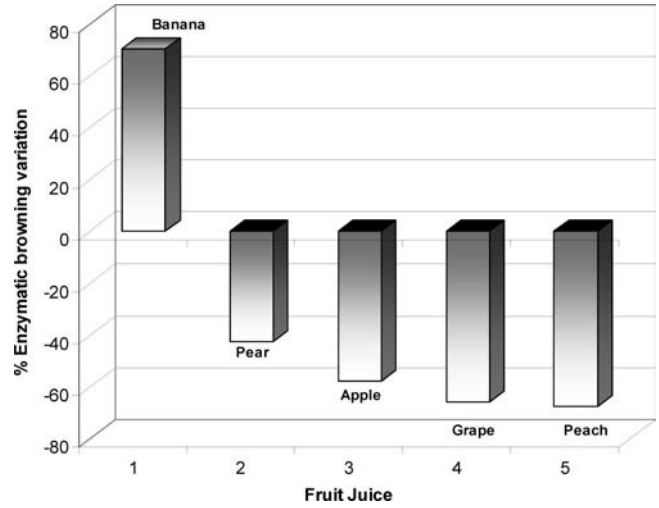


Figure 1. Percent variation of enzymatic browning in several juices in the presence of 90 mM maltosyl-β-CD. Each data point is the mean of three replicates.

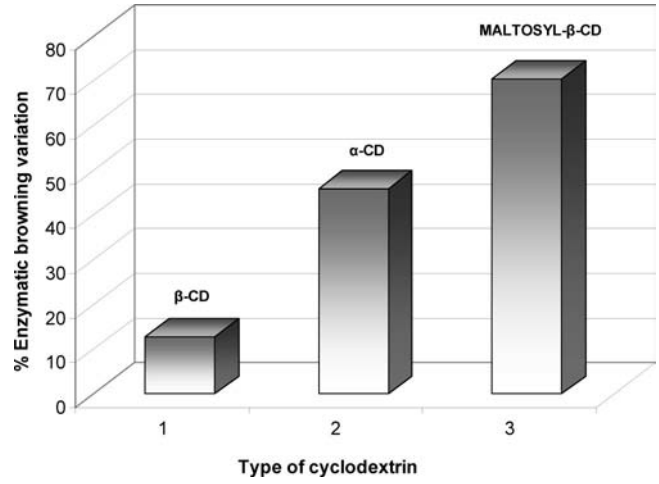


Figure 2. Percent variation of enzymatic browning in banana juice in the presence of different types of CDs at 90 mM. Each data point is the mean of three replicates.

These results are in good agreement with those obtained in previous studies, where maltosyl-β-CD presented the optimum structure to modify the enzymatic browning in both apple and grape juices (*37*, *40*). In recent years, the use of different functional groups (such as methyl, hydroxypropyl, maltosyl, acetyl, etc.) to modify natural CDs to complex many guest molecules has been improved because many different chemical moieties may be introduced into the CD molecule by the reaction with the hydroxyl groups lining the upper and lower ridges of the toroid (*41*). Moreover, the solubility of these modified CDs is generally higher than that of the respective underivatized CD.

In the case of β-CD, the low variation of banana juice enzymatic browning may have been be due to the lower concentration used (10 mM), because this is the least soluble of the three CDs used in this work. Although β-CD is the most used CD type to complex different guest molecules (*44*, *45*), its use in the food industry is limited by its poor solubility in aqueous medium (*46*). Moreover, the fact that β-CD presents lower enzymatic browning variation percent values than maltosyl-β-CD may also be due to the stronger interactions established between this type of modified CD and the guest molecules. In a comparison of the derivatives from the same natural CD (β derivatives), Veiga et al. (*47*) showed that the

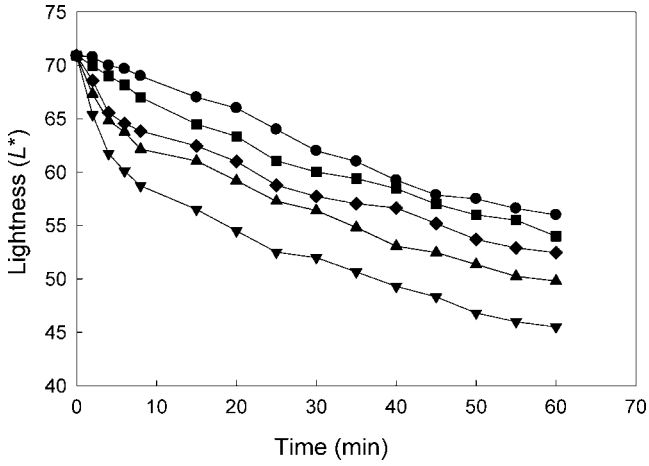


Figure 3. Effect of maltosyl-β-CD on the evolution of lightness (L^*) in banana juice at 25 °C in the absence of any CD (●) and in the presence of maltosyl-β-CD: 10 mM (■), 30 mM (◆), 60 mM (▲), and 90 mM (▼). Each data point is the mean of three replicates.

Table 2. Evolution of C^* and H^* Coordinates of Banana Juice in the Absence and Presence of Different Maltosyl-β-CD Concentrations

reaction time (min)	Hunter values	
	H^*	C^*
	without α-CD	
0	78.67 ± 3.21	34.98 ± 2.34
20	82.92 ± 3.45	24.35 ± 2.43
40	83.67 ± 3..62	17.78 ± 2.36
60	82.03 ± 3.73	12.05 ± 2.64
	10 mM Maltosyl-β-CD	
0	78.67 ± 3.21	34.98 ± 2.34
20	80.26 ± 3.91	23.65 ± 2.45
40	80.90 ± 3.85	18.24 ± 2.64
60	80.46 ± 3.79	15.51 ± 2.62
	30 mM Maltosyl-β-CD	
0	78.67 ± 3.21	34.98 ± 2.34
20	77.16 ± 3.39	21.59 ± 2.26
40	74.91 ± 3.23	15.39 ± 2.27
60	74.94 ± 3.63	13.36 ± 2.45
	60 mM Maltosyl-β-CD	
0	78.67 ± 3.21	34.98 ± 2.86
20	76.06 ± 3.46	21.72 ± 2.84
40	75.31 ± 3.85	19.34 ± 2.35
60	74.39 ± 3.60	15.60 ± 2.21
	90 mM Maltosyl-β-CD	
0	78.67 ± 3.21	34.98 ± 2.12
20	74.05 ± 3.78	23.87 ± 2.35
40	72.03 ± 3.32	19.4 ± 2.53
60	70.38 ± 3.46	16.59 ± 2.63

maltosyl derivative had a higher capacity to remove 1% of water, followed by the hydroxypropyl derivative and, finally, methyl and acetyl derivatives. These differences could be, in general, due to the chemical structure of the substitution group, which allows for the formation of a hydrogen bond with the hydration–water molecules, because the maltosyl substitution group gives rise to more intense bonding.

Finally, if we compare the results obtained for β-CD with those obtained for α-CD, both natural and generally recognized as safe, it seems that the optimum structure to activate banana juice enzymatic browning is that formed by six molecules of glucose, i.e., α-CD. This is probably due to the size of the CD cavity, with the inner diameter of the hydrophobic cavity being approximately 0.47–0.53 and 0.60–0.65 nm for α-CD and β-CD,

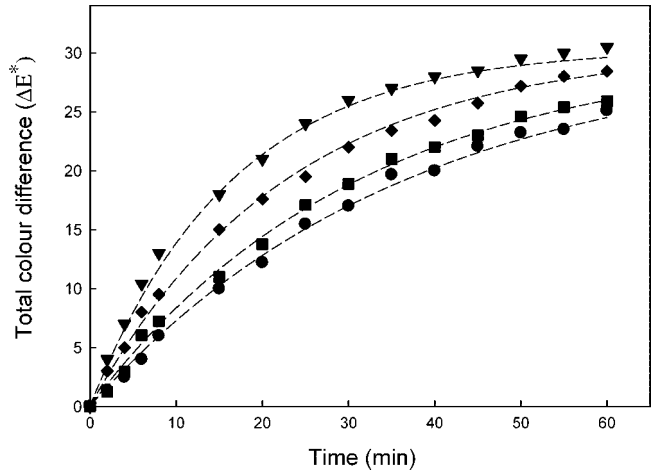


Figure 4. Effect of the maltosyl-β-CD concentration on the evolution of the total color difference (ΔE^*) in banana juice at 25 °C in the absence of maltosyl-β-CD (●) and in the presence of maltosyl-β-CD: 10 mM (■), 30 mM (◆), and 90 mM (▼). Each data point is the mean of three replicates.

respectively (*44*). However, the variation percent of α-CD was lower than that observed for maltosyl-β-CD (**Figure 2**).

For these reasons, we selected maltosyl-β-CD to study the effect of CD addition on the evolution of banana juice color parameters and to propose a kinetic model to explain the activation mechanism of banana juice enzymatic browning in the presence of CDs.

Effect of the Addition of Maltosyl-β-CD on Color Evolution of Banana Juice. Because there are no data in the literature concerning the effect of the addition of CDs on the color parameters of banana juice, the lightness (L^*), the angular coordinates (H^* and C^*), and the total color difference (ΔE^*) were considered to define the color of this fruit juice in the presence of these compounds.

In previous works, it was observed that the addition of antioxidant agents, such as CDs, to fruits juice, such as apple and grape, substantially slowed and diminished the decrease observed for lightness (L^*) in the absence of CDs (*37, 40*). In this paper and to clarify the effect of adding maltosyl-β-CD on the evolution of banana juice lightness (L^*) during the first minutes of processing, increasing concentrations of this CD were used (0–90 mM). As shown in **Figure 3**, L^* fell rapidly in the first 60 min without CD in the medium because the juice became darker. However and contrary to that observed for other fruit juices, when increasing concentrations of maltosyl-β-CD were used, the L^* value of the color scale fell ever more strongly.

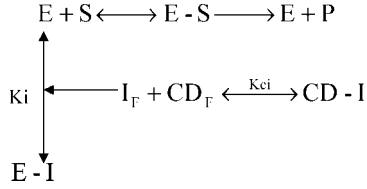
The behavior of the angular coordinate H^* for banana juice in the presence of CD was also the opposite of that published for apple and grape juices (*37, 40*) (**Table 2**). In the case of banana juice, when increasing concentrations of maltosyl-β-CD were used, the slight increase in H^* observed in the absence of any agent in the first hour after juicing was converted to a strong decay of the same parameter. Finally, the chroma value (C^*), a parameter that indicates the degree of saturation of color and is proportional to the strength of the color, behaved differently from the H^* angle and was very similar to that observed in the absence of any agent (**Table 2**).

Finally, the total color difference, ΔE^*, 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 maltosyl-β-CD (**Figure 4**). As can be seen, contrary to that observed for other fruit juices studied previously, the addition of maltosyl-β-CD did

Table 3. Effect of Adding CDs on the Browning of Several Fruit Juices and the Complexation of Their Main Polyphenolic Compounds

fruit juice	principal polyphenolic compounds	PPO substrates	reference	complexation by CDs	reference	effect of CD on browning
	chlorogenic acid	+	*45*	+	*50*	
	caffeic acid	+	*46*	+	*51*	
pear, apple, peach, grape	catechin	+	*47*	+	*52*	inhibitor
	epicatechin	+	*48*	+	*53*	
	quercetin	+	*49*	+	*54*	
banana	dopamine	+	*12*	–	*35*	activator

Scheme 1. CDs as Activating Agents of Juice Enzymatic Browning



not reduce the high variations observed in ΔE^* in the absence of CDs during the first 60 min. Indeed, when maltosyl-β-CD was added to the medium, these variations were higher than those observed for the control. Moreover, the degradation of initial color observed in the absence of CD was mostly due to dramatic depletions in both lightness (L^*) and the blue–yellow chromatism (b^*), whereas the greenness–redness (a^*) did not have the same weight in ΔE^*.

Bearing in mind that the results obtained for the evolution of color parameters of banana juice in the presence of CDs are opposite that observed for other fruits juices (*37*, *40*), the next step in this investigation was to look for a kinetic model to justify these differences.

Kinetic Model of the Banana Juice Color in the Presence and Absence of CD. To clarify the action mechanism of CDs as a compound that can produce a dual effect on enzymatic browning (as an activator or inhibitor depending upon the fruit source), a kinetic model is proposed.

As shown in **Figure 1**, the addition of CDs to apple, grape, pear, and peach juices inhibited enzymatic browning. The kinetic model explaining this behavior has been published by our group (*37*) and is based on the complexation of the different phenols present in these fruit juices by CDs, where by only molecules of substrate PPO may enter a CD molecule (stoichiometry of 1:1). In this way, their oxidation to *o*-quinones and subsequent polymerization to brown pigments are prevented. The main phenolic compounds present in these fruit juices that can be oxidized by PPO are shown in **Table 3** (*48–52*). As can be seen in this table, these compounds are of a hydrophobic nature and have been reported as guest molecules for different CDs (*53–57*). In the model described for the browning of these fruit juices in the presence of CDs, referring to 1 h after juicing, the velocity of the process is determined by a Michaelis–Menten equation, where the free concentration of the substrate is negligible with respect to the K_m.

However, this kinetic model is not suitable for clarifyng the activating effect of CDs on banana juice browning because, although banana fruit present a high concentration of phenols, the nature of these compounds is very different from that observed for the phenols present in the other fruits tested. Several papers published show how dopamine, a hydrophilic compound, is the main natural phenol present in banana (*58*, *59*). Moreover, the oxidation of dopamine by PPO leads to a reaction product (dopaminochrome: $\lambda_{max} = 480$ nm; $\epsilon = 3300$ M^{-1} cm^{-1}), which has a greater influence on the final color of banana juice than the oxidation of other phenols, with an ϵ value of about 1100 M^{-1} cm^{-1} (*12*, *59*). However, although the literature contains many studies showing how dopamine is oxidized by PPO, our group showed that the enzymatic activity of banana PPO using dopamine as the substrate was unaffected in the presence of CDs because this hydrophilic phenol does not form inclusion complexes (*38*).

Table 4. Values of the First and Second Apparent Kinetic Constants (k_{app} and k'_{app}, and Apparent Complexation Constant (K_{ci}) for the Interaction between Maltosyl-β-CD and Banana Juice

food	maltosyl-β-CD (mM)	k'_{app} (min^{-1})	k_{app} (min^{-1})	K_{ci} (mM^{-1})
banana juice	0	0.0274	0.098 ± 0.001	27.026 ± 0.212
	10	0.0323		
	30	0.0440		
	60	0.0540		
	90	0.0607		

For these reasons and to develop the kinetic model proposed in this paper to study the activating effect of CD on banana juice browning, two hypotheses were assumed: (i) CDs are able to complex the natural browning inhibitors present in banana; and (ii) the main substrate of PPO, i.e., dopamine, can not be complexed by CD, and the complexation constant between CDs and natural PPO substrates can be ignored.

The process can be expressed as indicated in **Scheme 1**, where E is the enzyme, S is the substrate, CD_F is the free cyclodextrin, I_F is the free PPO inhibitor, CD–I is the complex between PPO inhibitors and CDs, K_{ci} is the complexation constant between CDs and natural PPO inhibitors, and K_i is the inhibition constant.

The main objective of this kinetic model was to calculate the complexation constant between CDs and the natural PPO inhibitors present in banana juice using our experimental data. For this, we used the evolution of the total color difference (ΔE^*) data because this value takes into account the differences in L^*, a^*, and b^* between the samples and standards.

To evaluate the variations of ΔE^* when the CD concentration is increased and to calculate the apparent complexation constants between CDs and the mixture of inhibitors present in banana juice, a Michaelis–Menten defining equation for linear competitive inhibition must be obtained as a function of the only known parameter, that is, the total CD concentration.

Therefore, the velocity of ΔE^* evolution (v) can be expressed as

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

To develop the kinetic model proposed, we have assumed that the K_m values of some phenolic substrates of PPO from banana present higher values than the free concentrations of polyphenolic compounds in the juice elaborated from that fruit (*60*, *61*), which means that the concentration of substrate

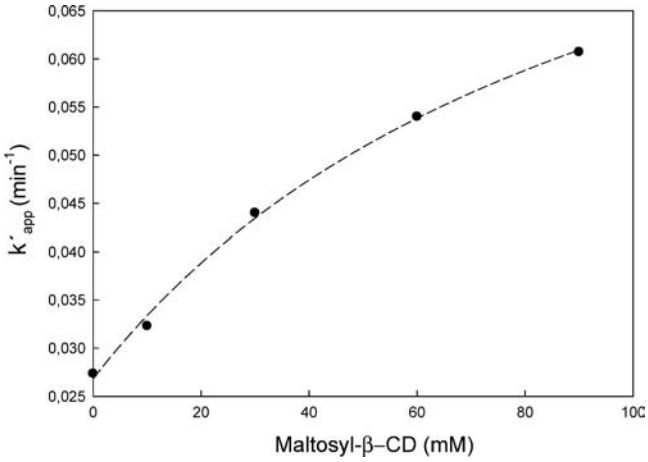


Figure 5. Effect of the maltosyl-β-CD concentration on k'_{app} in banana juice at 25 °C. Each data point is the mean of three replicates.

is negligible with respect to the K_m, i.e., $[S] \ll K_m$. In this case, the velocity of ΔE^* evolution can be expressed as

$$v = \frac{k_{app}[S]}{\left(1 + \frac{[I]_F}{K_i}\right)} \quad (2)$$

where k_{app} is a kinetic constant defined as

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

The next step was to calculate the $[I]_F$ to be substituted in eq 2 and, therefore, develop the velocity of ΔE^*.

For this, the K_{ci}, i.e., the complexation constant between CDs and PPO inhibitors present in banana juice, is defined as

$$K_{ci} = \frac{[I]_F[CD]_F}{[CD–I]} \quad (4)$$

The mass balance of the inhibitor and CDs is represented by

$$[I]_T = [CD–I] + [I]_F \quad (5)$$

$$[CD]_T = [CD–I] + [CD]_F \quad (6)$$

(where both subscripts T and F denote the total and free concentrations, respectively).

Taking into account eqs 4–6 and assuming that $[I]_T \ll [CD]_T$, then $[CD]_T \cong [CD]_F$ and the $[I]_F$ can be expressed as

$$[I]_F = \frac{K_{ci}[I]_T}{[CD]_T + K_{ci}} \quad (7)$$

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

$$v = \frac{k_{app}[S]}{\left(1 + \frac{\frac{K_{ci}[I]_T}{[CD]_T + K_{ci}}}{K_i}\right)} \quad (8)$$

The next step was to calculate the complexation constant between CDs and the PPO inhibitors present in banana juice, i.e., K_{ci}, using eq 8. To do this, a second apparent kinetic constant k'_{app}, dependent upon the $[CD]_T$, was defined as

$$k'_{app} = \frac{k_{app}K_i}{K_i + \frac{K_{ci}[I]_T}{K_{ci} + [CD]_T}} \quad (9)$$

To calculate the second apparent kinetic constant (k'_{app}) values for different maltosyl-β-CD concentrations, we used the model proposed by Soliva-Fortuny et al. (*62*, *63*) to study the influence of enzymatic browning on the color parameters of pears and apples in several conditions. The evolution of the total color difference (ΔE^*) experimental data were fitted (- - - in **Figure 4**) to the first-order fractional model described in eq 10 by nonlinear regression

$$\Delta E_f^* = \Delta E^* + \Delta E_f \cdot e^{-k'_{app}t} \quad (10)$$

where ΔE^* is the current value of the 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 second apparent kinetic constant.

The values obtained for k'_{app} at increasing concentrations of maltosyl-β-CD are shown in **Table 4**.

Finally, to calculate the complexation constant between CDs and natural PPO inhibitors (K_{ci}), k'_{app} was plotted versus the total CD concentration ($[CD]_T$) (**Figure 5**). The values of the first kinetic constant, k_{app}, and K_{ci} were determined using Sigma Plot (SPSS, Inc.), fitting the data by regression to a rational equation (eq 11) of the type

$$k'_{app} = \frac{k_{app}K_{ci} + k_{app}[CD]_T}{K_{ci} + \frac{K_{ci}[I]_T}{K_i} + [CD]_T} \quad (11)$$

The data are depicted in **Table 4**. The K_{ci} value obtained for the complexation of the natural inhibitors of PPO present in banana juice and maltosyl-β-CD were of the same order of magnitude as those described by our group for the complexation of the mixture of phenols present in apple juice and the same type of CD (*37*).

CONCLUSIONS

The results presented in this paper show that any antioxidant agent used to avoid enzymatic browning must be tested individually for each food because an opposite effect may be produced. Knowledge of the kinetic models of the enzymatic processes occurring in foods is essential to understand their macroscopic behavior, as in the case of organoleptic properties, such as color. The surprising observation that CDs chosen as natural antibrowning agents of fruit juice may behave as probrowning agents, depending upon by the fruit source, is explained in this investigation through the biochemical model reflecting the interaction between natural compounds present in banana juice and CDs. Therefore, the presence of hydrophobic or hydrophilic phenols in the fruit structure and the inability of CDs to complex dopamine mean that a recognized antibrowning agent, such as CDs, can be converted to a probrowning agent, leading to changes in color parameters not previously shown. Moreover, determination of the apparent complexation constant between the natural polyphenoloxidase inhibitors present in banana juice and CDs is of great importance to potential users of these food color modulators because, although browning food is usually associated in a loss of nutritional and sensorial quality, there is a restricted group of foods where darkening is preferable.

ABBREVATIONS USED

CD, cyclodextrin; PPO, polyphenol oxidase.

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