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Use of Natural and Modified Cyclodextrins as Inhibiting Agents of Peach Juice Enzymatic Browning

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Although cyclodextrins (CDs) have been successfully used as antibrowning agents in different fruit juices, no research has studied the effect of these compounds on enzymatic browning in peach juice. In this paper, the color of fresh peach juice was evaluated in the presence of two types of natural (α-CD and β-CD) and a modified (maltosyl-β-CD) CD, and the effectiveness of these compounds as browning inhibitors was determined using the color space CIELAB system. Moreover, to clarify the mechanism by which CDs inhibit peach juice enzymatic browning, the process was kinetically modeled in the absence and presence of CDs using a colorimetric method; the apparent complexation constants between the mixtures of diphenols present in peach juice and some types of CD were calculated. The results show that the highest affinity constant was presented by α-CD ($K_c = 18.31$ mM^{-1}) followed by maltosyl-β-CD ($K_c = 11.17$ mM^{-1}), whereas β-CD was incapable of inhibiting peach juice enzymatic browning.

KEYWORDS: Cyclodextrin; browning; peach; juice; color; polyphenol oxidase

INTRODUCTION

The color preservation of fruit juice during processing and storage is one of the main objectives of fruit processors because changes in the structure of fruit products modify the color and final appearance of the product. One of the main factors that can alter the color of fruit juice and so limit its commercial shelf life is browning (*1*, *2*). Therefore, browning needs to be controlled during the processing stages of this food if its quality is to be preserved, because the organoleptic and nutritional properties may be strongly altered. For these reasons, and due to the importance of appearance as a quality parameter, the prevention of these undesirable reactions has always been a challenge for food scientists (*3*, *4*).

Peach discoloration is a growing problem for the Spanish fruit industry, especially in the peach cultivars 'Baby Gold', 'Sudanell', 'Carson', and 'Calanda' because of their high browning potential, as has been mentioned by several researchers (*5*). The degree of browning depends on the presence of oxygen, reducing substances, metallic ions, pH, temperature, and the activity of different oxidizing enzymes (*1*−*4*). One of the main factors that must be controlled because of its effect on food browning is the activity of polyphenol oxidase (PPO) (monophenol dihydroxyphenylalanine:oxygen oxidoreductases, EC 1.14.18.1) (*6*). This enzyme has been characterized for peach, in which its activity has been related with the phenolic composition and browning potential of the fresh fruit (*7*−*11*). In recent decades, several methods have been used to prevent PPO activity in foods (*1*−*4*, *12*, *13*). However, because sulfites have been associated with severe allergy-like reactions in certain populations, the U.S. Food and Drug Administration (FDA) has restricted their use to only a few applications to inhibit the browning of foods (*3*, *14*). Moreover, heat treatments are not suitable for inhibiting this sort of reaction, and so several other methods have been tried to reduce PPO activity, including the addition of ascorbic acid or chemical agents, the exclusion of oxygen, refrigeration and various nonthermal treatments (*12*, *15*, *16*).

In this respect, there has been a renewal of interest in naturally occurring antibrowning agents. Among the most promising of such agents are 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 (*17*−*19*). The hydrophobic cavity is able to form inclusion complexes with a wide range of organic guest molecules, including PPO substrates (*20*).

The use of CDs has been proposed in recent decades to control the enzymatic browning of different foods caused by PPO (*21*−*24*), and indeed, their use in preventing browning

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Table 1. Effect of Different Additives on the Evolution of Total Color Difference (ΔE^*) and Hue Angle (H^*) in Peach Juice at 30 min after Juicing

color parameter	no additive	60 mM D-glucose	180 mM D-glucose	10 mM α-CD	30 mM α-CD
ΔE^*	26.01 ± 1.76	26.32 ± 1.45	26.7 ± 1.56	20.43 ± 1.29	15.09 ± 0.84
H^*	76.46 ± 1.32	76.39 ± 1.47	76.51 ± 1.05	78.47 ± 1.21	81.06 ± 1.81

has been patented (*25*). However, the use of CDs for the control of enzymatic browning in peach products has not been described to date, and this is the first paper to analyze the effect of different CDs on peach juice. Moreover, many of the works that study the effect of CDs on enzymatic browning on different foods do so after long time periods. However, Cheng and Crisosto (*10*) showed that 83% of peach browning measured at the end of long incubation times occurred during the first hour, which is why we have studied the behavior of peach juice enzymatic browning at the very beginning of the reaction (in the first hour).

Furthermore, some contradictory claims have been published concerning the mechanism by which enzymatic browning is inhibited by CDs. There are four hypotheses in this respect: (1) CDs are able to complex PPO substrates, by means of which their oxidation to *o*-quinones and subsequent polymerization to brown pigments is prevented (*26*, *27*); (2) CDs are able to react with the copper-containing prosthetic group of PPO, thus acting as inhibitors of the enzyme (*4*); (3) CDs are able to act as noncompetitive inhibitors of PPO (*28*, *29*); (4) CDs are able to act by two different mechanisms, that is, (i) the complexation of PPO substrates and (ii) by interacting with some hydrophobic amino acids from the enzyme (*30*).

Bearing in mind that there is no information on the inhibition of peach juice enzymatic browning by CD, we proposed this work is 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. Different factors that may affect the color evolution of peach juice, such as the type and concentration of CDs, have been studied. Moreover, to clarify the mechanism of inhibition of peach juice enzymatic browning by CD, a kinetic model to explain the inhibition of the peach juice browning in the presence of CDs has been proposed. Finally, the apparent complexation constants for the complexes between the mixture of phenolic compounds responsible for enzymatic browning and different types of CDs have been calculated.

MATERIALS AND METHODS

Materials. α- and *β*-CDs 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. Peaches (*Prunus persina* cv. Baby Gold) were purchased from local supermarkets and stored at 4 °C until needed. They were peeled, destoned, and sliced prior to juicing in a Moulinex Y36 blender. The peach juice obtained was immediately collected and mixed in a beaker containing 25 mL of distilled water alone or containing enough α-CD, *β*-CD, maltosyl-*β*-CD (10−90 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 ISO 9001 with a D75 light source and the observer at 10°.

The color parameters corresponding to the uniform color space CIELAB (*31*) were obtained directly from the apparatus. Within the uniform space CIELAB, two color coordinates, a^* and b^*, as well as a psychometric index of lightness, L^*, are defined. In this system, a^* takes positive values for reddish colors and negative values for greenish ones, whereas b^* takes positive values for yellowish colors and negative values for bluish ones. L^* is an approximate measurement of luminosity, which is the property according to which each color can be considered as equivalent to a member of 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 to be determined in comparison with a gray color with the same lightness for each hue, so it is considered to be the quantitative attribute of colorfulness. Hue angle (H^*) is the attribute according to which colors have been traditionally defined as reddish, greenish, etc. It 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 to be the qualitative attribute of color.

For all experiments, the previously described mixtures containing peach juice were used in the color 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 peaches had been juiced and the materials 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 peach 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^*) were calculated using the 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 Addition of Different Additives on the evolution of peach juice color. Although the use of CDs to study the inhibition of enzymatic browning has been described for several foods, the fact that these molecules have not been used to avoid these undesirable reactions in peach juice made it necessary for us to carry out some preliminary experiments before conducting the research.

To study the posibility that inclusion complexes are formed between D-glucose (molecules included in CD structure) and the compounds responsible for enzymatic browning in peach juice 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 glucose, on the color of peach juice 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.

The effects of different additives on the evolution of both peach juice total color difference (ΔE^*) and hue angle (H^*) 30 min after juicing can be observed in **Table 1**. The ΔE^* and H^* values of peach juice were similar in the absence and presence of 60 and 180 mM D-glucose, but all of the values were substantially different from those obtained in the presence of 10 and 30 mM α-CD.

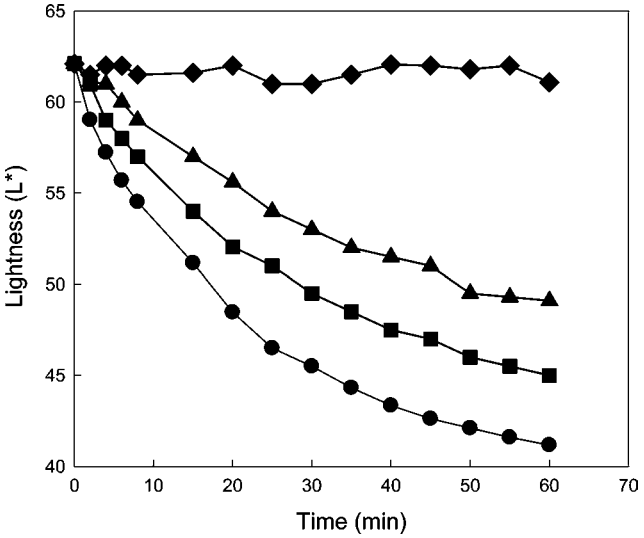

Figure 1. Effect of α-CD concentration on the evolution of lightness (*L**) in peach juice at 25 °C in the absence of CD (●) and in the presence of α-CD: 10 mM (■); 30 mM (▲); 60 mM (◆). Each data point is the mean of three replicates.

These results indicate that (1) colorimetric studies appear to be a satisfactory method for observing and characterizing phenolic compound−CD inclusion complexes, (2) the variation in color parameters caused by the addition of CD to the juice is due to the formation of an inclusion complex because no glucose/phenolic compound complexes exist, and (3) the ability of CDs to slow the enzymatic browning was not due to its glucidic nature.

Effect of Addition of α-CD on Color Evolution of Peach Juice. To examine the evolution of color parameters of peach juice by different types of CD, two types of natural CDs generally recognized as safe (α-CD and *β*-CD) and a modified CD (maltosyl-*β*-CD) were used. To define the color of peach juice completely in the absence and presence of each type of CD, the scalar coordinates (*L**, *a**, *b**) and the angular coordinates (*H** and *C**) were considered.

To determine whether the addition of α-CD could inhibit the degradation of peach juice color in the first minutes of processing, increasing concentrations of this CD were used (0−60 mM).

To clarify the effect of α-CD on peach juice enzymatic browning, the behavior of the lightness (*L**), the relative measurement between the light reflected and absorbed by the samples, was analyzed in the absence and presence of this type of CD. For this purpose, the peach juice *L** values obtained at different α-CD concentrations in the first hour after juicing were fitted versus time. As shown in **Figure 1**, *L** presented a significant and rapid decrease in the first 60 min without CD in the medium because the juice became darker. However, when increasing concentrations of α-CD were used, the decay of the *L** value of the color scale was strongly reduced. Therefore, when a 60 mM concentration of α-CD was employed, the evolution of *L** was totally eliminated and the peach juice did not darken.

Moreover, in **Table 2** we can observe the other color parameters of peach juice determined in the absence and presence of α-CD. For the scalar parameters, *a** was the variable with the lowest values in the first 60 min, whereas the contribution of *b** to color evolution was similar to that observed for *L** (**Figure 1**) and higher than *a**. With regard to the angular parameters, *H** showed higher weight than *C**, which presented

Table 2. Evolution of *a**, *b**, *C**, and *H** Coordinates of Peach Juice in the Absence and in the Presence of Different α-CD Concentrations

reaction time (min)	Hunter values			
	*a**	*b**	*H**	*C**
		Without α-CD		
0	6.51 ± 0.31	54.03 ± 2.12	83.12 ± 2.25	54.42 ± 2.31
20	7.91 ± 0.42	37.9 ± 1.98	78.22 ± 3.01	38.71 ± 1.65
40	8.62 ± 0.41	32.2 ± 1.05	75.02 ± 2.54	33.35 ± 1.76
60	9.17 ± 0.57	30.23 ± 1.08	73.12 ± 2.25	31.59 ± 1.82
		10 mM α-CD		
0	6.51 ± 0.31	54.03 ± 2.12	83.12 ± 2.25	54.42 ± 2.31
20	7.39 ± 0.43	42.10 ± 3.24	80.14 ± 6.42	42.62 ± 3.52
40	8.01 ± 0.65	35.02 ± 3.14	77.12 ± 6.65	35.90 ± 3.03
60	8.8 ± 0.68	33.4 ± 2.98	75.23 ± 6.34	34.53 ± 2.94
		30 mM α-CD		
0	6.51 ± 0.31	54.03 ± 2.12	83.12 ± 2.25	54.42 ± 2.31
20	6.45 ± 0.53	45.2 ± 3.76	81.84 ± 7.01	45.45 ± 3.65
40	6.70 ± 0.43	39.5 ± 2.98	80.37 ± 6.72	40.06 ± 3.01
60	6.50 ± 0.46	37.05 ± 2.98	80.03 ± 6.54	37.56 ± 2.12
		60 mM α-CD		
0	6.51 ± 0.31	54.03 ± 2.12	83.12 ± 2.25	54.42 ± 2.31
20	4.56 ± 0.32	47.00 ± 3.41	84.44 ± 6.54	47.22 ± 3.45
40	3.97 ± 0.21	42.05 ± 3.59	84.58 ± 6.78	42.18 ± 3.65
60	3.52 ± 0.25	39.3 ± 2.87	84.87 ± 6.54	39.15 ± 2.98

color values similar to *L**. **Table 2** also shows the evolution of both *a** and *b** coordinates in the absence and presence of 0, 10, 30, and 60 mM α-CD. As can be seen, in the absence of α-CD peach juice also loses its yellowness and becomes more red during the reaction time. This change was manifested by a decrease of *b** value and an increase of the *a** value, behavior very similar to that observed for the thermal degradation of color in peach puree by Ávila and Silva (*32*). However, increasing concentrations of α-CD led to significantly lower values of *a**, thus maintaining the initial green color. On the contrary, the *b** values were also affected in our system in both the absence and presence of CDs, and the behavior was very similar to that observed for *L** but different from that observed for *a**. As shown in **Table 2**, in the absence of CDs the initial *b** value decayed rapidly to bluish colors. However, in the presence of increasing α-CD concentrations, this rapid decay in *b** slowed and the loss of the initial yellowish colors in peach juice was lower than in the absence of α-CD.

Considering the angular coordinates (*H** and *C**), the behavior of the hue angle was very similar to that described for lightness (*L**). When α-CD concentrations were used, the changes in *H** were reduced and a 60 mM concentration of α-CD led to no variation in this parameter with respect to that obtained without any additive. Finally, the chroma value (*C**), a parameter that indicates the degree of saturation of color and is proportional to the strength of the color, changed differently compared with the variations observed for the other color parameters. As was observed for *L** and *H**, the rapid decay of the *C** value of peach juice was slowed when increasing α-CD concentrations were added (**Table 2**). Not even the highest α-CD concentration used maintained the initial *C** value.

Another color parameter studied was the total color difference, Δ*E**, which is a colorimetric parameter extensively used to characterize the variation of colors in foods during processing. The evolution of total color difference in peach juice with increasing concentrations (0−60 mM) of α-CD is shown in **Figure 2**. 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

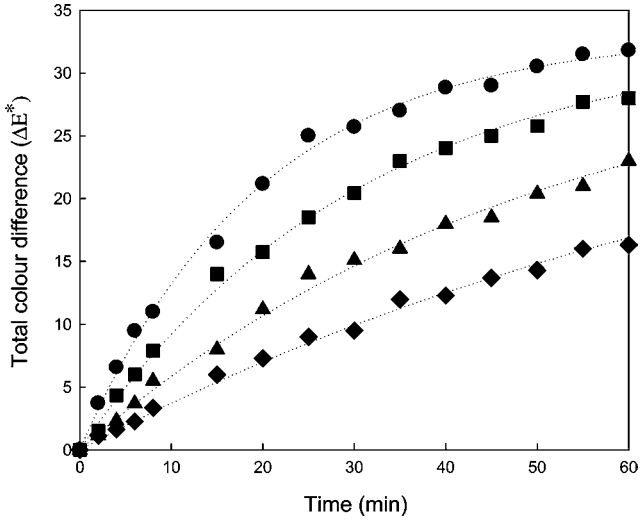

Figure 2. Effect of α-CD concentration on the evolution of total color difference (Δ*E**) in peach juice at 25 °C in the absence of α-CD (●) and in the presence of α-CD: 10 mM (■); 30 mM (▲); 60 mM (◆). Each data point is the mean of three replicates.

Δ*E**. As can be observed, in the presence of α-CD, the Δ*E** evolution of peach juice color during storage is strongly dependent on the CD concentration, and the addition of α-CD resulted in lower variations in Δ*E** during the 60 min measured than when this CD was absent. Moreover, the evolution of this parameter when α-CD was added to the medium was more linear than with no CD.

Effect of Addition of *β*-CD on Color Evolution of Peach Juice. When the effect of *β*-CD on peach juice browning was evaluated, a lower concentration was used because this is the least soluble of the three CDs used in this work. **Figure 3** shows the principal color parameters of peach juice in the absence and in the presence of *β*-CD.

As can be seen, there is no significant difference in the evolution of the lightness parameter when *β*-CD was added to the peach juice at any concentration used (0−10 mM), so the strong decay of *L** observed in the absence of any additive cannot be reduced by the addition of this type of CD. Moreover, the behavior of the other scalar coordinates (*a** and *b**) was very similar to that of the control at all concentrations of *β*-CD used.

The effect of *β*-CD addition on the total color difference (Δ*E**), the single value that takes into account the differences between *L**, *a**, and *b** of the sample and standard, of peach juice was not significant (**Table 3**). As is shown in **Table 3**, regardless of the presence of this type of CD, Δ*E** increased rapidly during the first 20 min, after which the increase slowed significantly until 60 min. Neither did the presence of *β*-CD in the medium produce any variation in the angular coordinates (*H** and *C**) at the concentrations assayed (**Table 3**).

If we compare the results obtained for *β*-CD with those shown for α-CD at the same concentration (10 mM), we can see that the efficiency of α-CD to slow the peach juice enzymatic browning was better than that observed for *β*-CD. Indeed, the reduction observed in the total color difference value in the presence of α-CD (Δ*E** = 28.0) is higher than that determined in the presence of *β*-CD (Δ*E** = 33.1) with respect to the total color difference value observed in the absence of any agent (Δ*E** = 31.8) for the first hour after juicing. Moreover, the lightness value determined in the presence of 10 mM α-CD (*L** = 45.0) increased with respect to the control (*L** = 41.1).

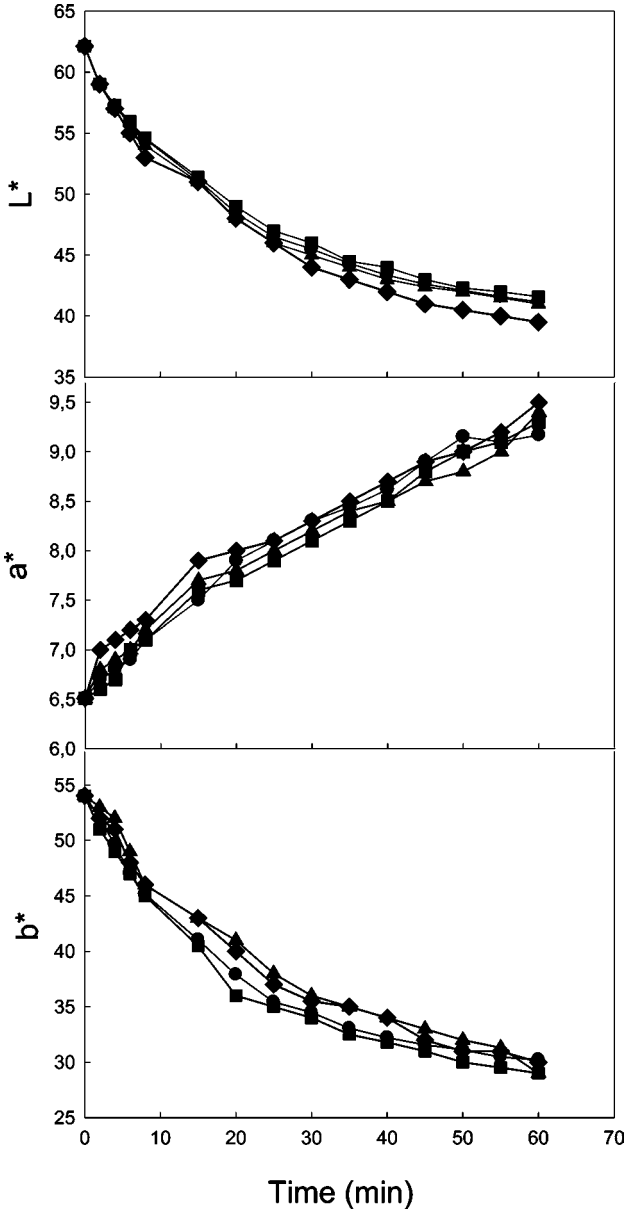

Figure 3. Effect of *β*-CD concentration on the evolution of *L**, *a**, and *b** color parameters in peach juice at 25 °C in the absence of *β*-CD (●) and in the presence of *β*-CD: 3 mM (■); 5 mM (▲); 10 mM (◆). Each data point is the mean of three replicates.

However, when *β*-CD is added to the medium at the same concentration, no increase is observed in lightness value (*L** = 39.5).

For these reasons, it can be concluded that the optimum structure of these natural and generally recognized as safe CDs to inhibit peach juice enzymatic browning is that formed by six molecules of glucose, that is, α-CD, probably due to two reasons: (1) the lower concentration of *β*-CD used because of its poor solubility in aqueous medium and (2) the size of the CD cavity. The inner diameter of the hydrophobic cavity is approximately 0.47−0.53 and 0.60−0.65 nm for α-CD and *β*-CD, respectively (*18*). Therefore, "goodness of fit" between α-CD and the mixture of phenols present in peach juice is more favorable than that presented by *β*-CD. With respect to the mixture of phenols present in peach juice, several works have been published stating that the main phenols present in peach juice are catechin, caffeic acid, chlorogenic acid, and epicatechin (*10*, *33*). These works studied the presence of different types

Table 3. Evolution of Total Color Difference (ΔE^*), Hue Angle (H^*), and Chrome (C^*) of Peach Juice in the Absence and in the Presence of Different β-CD Concentrations

reaction time (min)	Hunter values		
	ΔE^*	H^*	C^*
	Without β-CD		
0	0	83.12 ± 2.25	54.42 ± 2.31
20	21.16 ± 1.22	78.22 ± 3.01	38.71 ± 1.65
40	28.84 ± 1.31	75.02 ± 2.54	33.35 ± 1.76
60	31.81 ± 1.78	73.12 ± 2.25	31.59 ± 1.82
	3 mM β-CD		
0	0	83.12 ± 2.25	54.42 ± 2.31
20	22.32 ± 1.43	77.92 ± 3.73	36.81 ± 1.35
40	28.74 ± 1.73	75.03 ± 3.26	32.91 ± 1.64
60	32.47 ± 1.23	72.21 ± 3.54	30.45 ± 1.78
	5 mM β-CD		
0	0	83.12 ± 2.25	54.42 ± 2.31
20	19.24 ± 1.38	79.22 ± 3.27	41.73 ± 1.56
40	27.75 ± 1.82	75.96 ± 3.83	35.04 ± 1.38
60	32.87 ± 1.91	72.04 ± 3.43	30.48 ± 1.84
	10 mM β-CD		
0	0	83.12 ± 2.25	54.42 ± 2.31
20	19.95 ± 1.01	78.69 ± 3.76	40.79 ± 1.76
40	28.46 ± 1.34	75.64 ± 3.43	35.09 ± 1.65
60	33.12 ± 1.27	72.42 ± 3.32	31.46 ± 1.28

of phenols in some peach cultivars and showed that the concentrations of catechin and epicatechin were higher than those of other phenols tested, including chlorogenic acid. Therefore, the data presented in this paper show the complexation of the mixture of these phenols present in peach juice by CDs.

Effect of Addition of Maltosyl-β-CD on Color Evolution of Peach Juice. In recent years, the use of modified CDs to complex different guest molecules has been improved because many different chemical moieties may be introduced into the CD molecule by reaction with the hydroxyl groups lining the upper and lower ridges of the toroid, for example, methyl, hydroxypropyl, carboxymethyl, acetyl, etc. (*34*). Because β-CD was not able to inhibit the enzymatic browning of peach juice, we have used a derivatized β-CD, in this case maltosyl-β-CD, which is more soluble that underivatized β-CD, to observe whether a modification of the natural CD is more effective at maintaining the initial color of peach juice in the first hour following processing.

When the effect of maltosyl-β-CD on lightness (L^*) was evaluated, increasing the concentration of this more soluble derivative up to 30 mM did not lead to the complete inhibition of browning measured after 60 min at 25 °C (**Figure 4**). Moreover, when concentrations higher than 30 mM were tested (60 or 90 mM), there was no further reduction in the L^* parameter and, so, we did not maintain totally the luminous intensity of peach juice in the first 60 min after its juicing, as was previously shown when 60 mM α-CD was used.

Table 4 shows the principal color parameters of peach juice in the absence and presence of maltosyl-β-CD. The effect of maltosyl-β-CD on both scalar parameters (a^* and b^*) was very similar to the effect described for α-CD (**Table 4**). In the absence of any additive in the reaction medium, a slight increase in a^* and a strong decrease in b^* were observed (i.e., a shift toward blue and red colors). However, the presence of maltosyl-β-CD in peach juice moderated both the decay in b^* and the increase in a^* (i.e., there was a shift toward yellow and green colors). Furthermore, when maltosyl-β-CD was used, L^* and

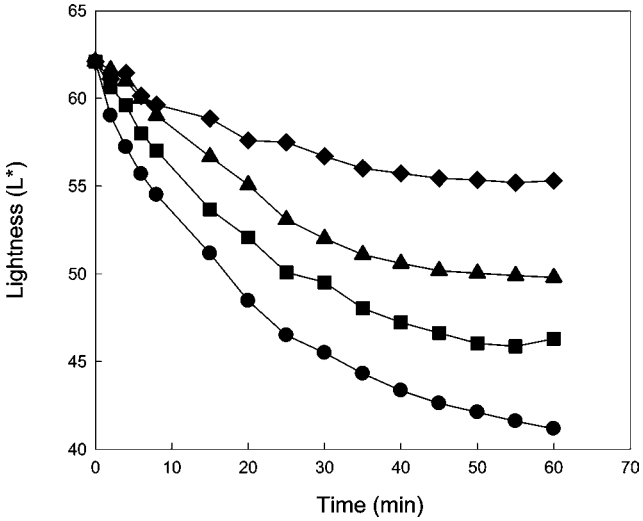

Figure 4. Effect of maltosyl-β-CD concentration on the evolution of lightness (L^*) in peach juice at 25 °C in the absence of CD (●) and in the presence of maltosyl-β-CD: 10 mM (■); 20 mM (▲); 30 mM (◆). Each data point is the mean of three replicates.

Table 4. Evolution of a^*, b^*, C^*, and H^* Coordinates of Peach Juice in the Absence and in the Presence of Different Maltosyl-β-CD Concentrations

reaction time (min)	Hunter values			
	a^*	b^*	H^*	C^*
	Without Maltosyl-β-CD			
0	6.51 ± 0.31	54.03 ± 2.12	83.12 ± 2.25	54.42 ± 2.31
20	7.91 ± 0.42	37.9 ± 1.98	78.22 ± 3.01	38.71 ± 1.65
40	8.62 ± 0.41	32.2 ± 1.05	75.02 ± 2.54	33.35 ± 1.76
60	9.17 ± 0.57	30.23 ± 1.08	73.12 ± 2.25	31.59 ± 1.82
	10 mM Maltosyl-β-CD			
0	6.51 ± 0.31	54.03 ± 2.12	83.12 ± 2.25	54.42 ± 2.31
20	7.50 ± 0.62	40.40 ± 0.36	79.48 ± 0.61	41.09 ± 0.29
40	8.38 ± 0.71	34.88 ± 0.29	76.49 ± 0.65	35.87 ± 0.18
60	9.36 ± 0.79	34.57 ± 0.26	74.85 ± 0.69	35.81 ± 0.17
	20 mM Maltosyl-β-CD			
0	6.51 ± 0.31	54.03 ± 3.12	83.12 ± 2.25	54.42 ± 2.31
20	6.47 ± 0.24	44.00 ± 3.34	84.28 ± 6.75	47.21 ± 3.38
40	6.45 ± 0.37	39.50 ± 2.32	84.33 ± 6.69	43.64 ± 3.32
60	6.52 ± 0.31	37.30 ± 2.29	84.38 ± 6.62	42.64 ± 3.37
	30 mM Maltosyl-β-CD			
0	6.51 ± 0.31	54.03 ± 2.12	83.12 ± 4.25	54.42 ± 2.31
20	4.70 ± 0.22	46.98 ± 3.39	85.32 ± 4.15	48.24 ± 3.92
40	4.30 ± 0.21	43.43 ± 3.34	85.24 ± 4.43	45.41 ± 3.52
60	4.16 ± 0.22	42.43 ± 3.31	85.17 ± 4.5	44.15 ± 3.87

b^* also showed the highest weight in the scalar parameters and the weight of a^* was similar to that observed when α-CD was used.

As shown in **Table 4**, the strong decay of C^*, the parameter that best describes the vividness or dullness of a color, observed in the absence of CDs was slowed by increasing concentrations of maltosyl-β-CD. However, the 60 mM concentration of maltosyl-β-CD did not totally reduce the variation of C^* values observed in its absence. On the contrary, the addition of 20 mM maltosyl-β-CD maintained the initial value of the hue angle (H^*) observed in the absence of any additive.

To study the effect of the concentration of maltosyl-β-CD on total color evolution of peach juice (ΔE^*), increasing concentrations (0−30 mM) were used. As is shown in **Figure 5**, the reduction of ΔE^* during storage was strongly dependent on the CD concentration. The ΔE^* values increased rapidly during the first 10 min in the absence of maltosyl-β-CD, as

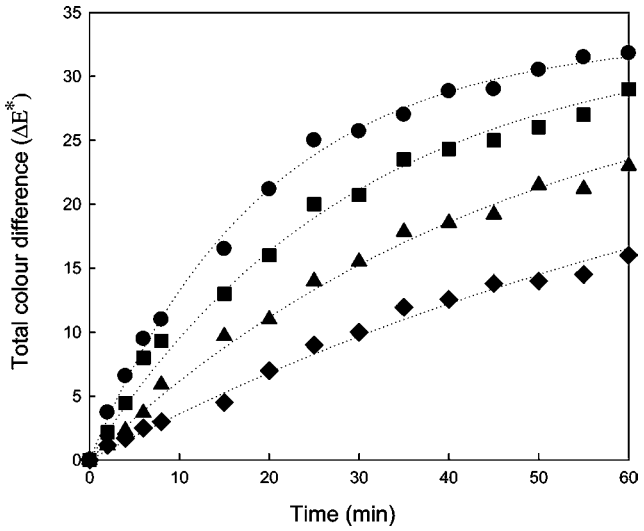

Figure 5. Effect of maltosyl-β-CD concentration on the evolution of total color difference (ΔE^*) in peach juice at 25 °C in the absence of maltosyl-β-CD (●) and in the presence of maltosyl-β-CD: 10 mM (■); 20 mM (▲); 30 mM (◆). Each data point is the mean of three replicates.

shown in **Figure 5**. After this time, the increase in total color difference was significantly lower. The ΔE^* values after 60 min were lower with increasing concentrations of maltosyl-β-CD (**Figure 5**).

Kinetic Model of Peach Juice Color Evolution in the Presence and Absence of CDs. Although the use of CDs has been proposed for the control of enzymatic browning in different products by several authors, the action mechanism of CDs in fruit juices, as mentioned above, has been the subject of controversy (*26*−*30*). For this reason and bearing in mind that there is no information on the effect of CDs on peach enzymatic browning and to evaluate the action's mechanism of CDs on peach juice enzymatic browning, a kinetic model of the browning in the presence of CDs is proposed.

To explain the variations of the total color (ΔE^*) when CD concentration is increased, a velocity equation is proposed as a function of the total CD concentration, which is the only known parameter. Thus, the velocity of ΔE^* evolution (v) can be expressed as

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

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

In the kinetic model proposed in this paper, we have assumed two premises. First, that the free concentration of substrate is negligible with respect to the K_m, that is, $[S]_F \ll K_m$. We can make this assumption because the K_m values of some phenolic substrates of PPO from different fruits are higher than the free concentrations of polyphenolic compounds in the juices elaborated from those fruits (*22*, *35*). Then, the velocity of ΔE^* evolution can be expressed as

$$v = k[S]_F \quad (2)$$

where k is a specific kinetic constant defined as

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

To express the equilibrium of the kinetic model proposed in this work, we have assumed the second premise, that only one molecule of substrate PPO can enter into a CD molecule (stoichiometry 1:1) as is shown in several papers published by our group (*26*, *27*). Moreover, in these research studies we have demonstrated that PPO is able to work only with free substrate and not with the complex between CD and PPO substrates. Therefore, the equilibrium can be expressed as

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

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

$$K_c = \frac{[S]_F[CD]_F}{[CD{-}S]} \quad (4)$$

The mass balance of the substrate and cyclodextrins is represented by (where subscripts T and F denote total and free concentration, respectively)

$$[S]_T = [CD{-}S] + [S]_F \quad (5)$$

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

Taking into account eqs 4−6 and assuming that $[S]_T \ll [CD]_T$ and so $[CD]_T \cong [CD]_F$, $[S]_F$ can be expressed as

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

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

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

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

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

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

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

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 calculate the apparent specific kinetic constant (k_{app}) values and to evaluate whether the two assumptions of our previously described mathematical model were correct, ΔE^* data were fitted (dotted line) to eq 10 by nonlinear regression as shown in **Figure 2**. For this purpose α-CD, a natural CD generally recognized as safe, was utilized. The values obtained for k_{app} at increasing concentrations of α-CD were 0.05107 min^{-1} (in the absence of α-CD), 0.03266 min^{-1} (in the presence of 10 mM α-CD), 0.0195 min^{-1} (in the presence of 30 mM α-CD), and 0.0119 min^{-1} (in the presence of 60 mM α-CD). Moreover, the fitted data confirm the two assumptions of the mathematical

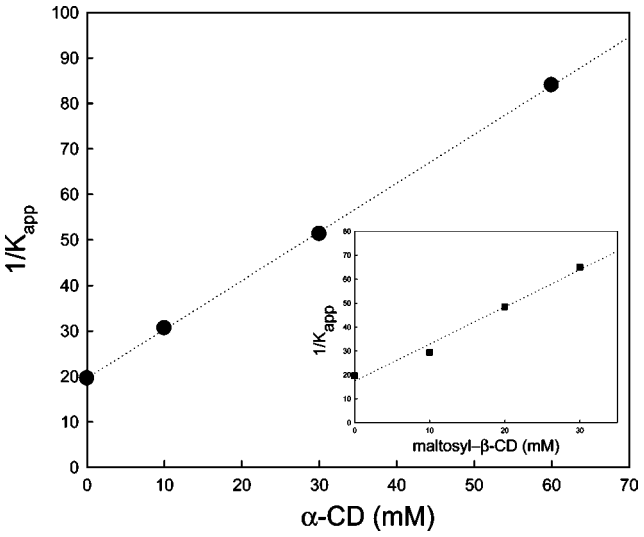

Figure 6. Lineweaver–Burk plot of the effect of the α-CD concentration on k_{app} of the evolution of total color difference (ΔE^*) at 25 °C. (Inset) Lineweaver–Burk plot of the effect of the maltosyl-β-CD concentration on k_{app} of the evolution of total color difference (ΔE^*) at 25 °C. Each data point is the mean of three replicates.

Table 5. Values of the Kinetic Constant (k) and Apparent Complexation Constant (K_c) for the Interaction between Different Types of CDs and Several Fruit Juices

fruit juice	type of CD	k (min^{-1})	K_c (mM^{-1})
peach	α-CD	0.051 ± 0.01	18.31 ± 1.13
	maltosyl-β-CD	0.057 ± 0.01	11.17 ± 1.05
apple[a]	maltosyl-β-CD	0.270 ± 0.01	4.09 ± 0.12

[a] Data obtained from our previous work (ref *31*).

model assumed previously were correct: (1) that the free concentration of substrate is negligible with respect to the K_m and (2) that only one molecule of substrate PPO may enter a CD molecule (stoichiometry 1:1).

To calculate both the kinetic constant, k, and the apparent complexation constant, K_c, the reciprocal of k_{app} was plotted versus the total cyclodextrin concentration ($[CD]_T$) (**Figure 6**). Fitting the data by linear regression using Sigma Plot (SPSS Inc.), the values of k and K_c were determined (**Table 5**).

Effect of CD Structure on Apparent Complexation Constants. In an attempt to characterize at a molecular level the interaction between the phenols and the host CD, the same kinetic model was used to calculate the apparent complexation constants between the mixture of phenolic compounds responsible for enzymatic browning and different types of CDs. As described in a previous section, the absence or presence of β-CD in the reaction medium had no effect on the evolution of color parameters of peach juice due to the low concentration of β-CD used because of it low solubility. For this reason, the apparent complexation constant could not be calculated.

However, as shown previously, when a modified CD (maltosyl-β-CD) is added to peach juice, the inhibition of peach juice enzymatic browning is favored.

Fitting the values of the total color evolution of peach juice in the absence and presence of maltosyl-β-CD to eq 9 (**Figure 5**), the values of the apparent specific kinetic constant (k_{app}) were obtained for increasing maltosyl-β-CD concentrations. These values were 0.05107 min^{-1} (in the absence of maltosyl-β-CD), 0.03398 min^{-1} (in the presence of 10 mM maltosyl-β-CD), 0.02061 min^{-1} (in the presence of 20 mM maltosyl-β-CD), and 0.0154 min^{-1} (in the presence of 30 mM maltosyl-β-CD). Plotting the reciprocal of k_{app} versus the total maltosyl-β-CD concentration (**Figure 6**, inset) provided the values of both the kinetic constant, k, and the apparent complexation constant, K_c (**Table 5**). As can be seen from this table, the apparent complexation constant obtained for peach juice is higher than that shown by López-Nicolás et al. (*36*) for the complexation of the mixtures of diphenols present in apple juice and maltosyl-β-CD.

The fact that maltosyl-β-CD presents higher K_c values than its parent β-CD may be due to the stronger interactions established between this type of modified CD and the guest molecules. Similarly, Veiga et al. (*34*) showed that in a comparison between 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. These differences could be due, in general, to the chemical structure of the substitution group, which allows the formation of a hydrogen bond with the hydration water molecules, because the maltosyl substitution group gives rise to more intense bonding.

For the species α-, β- and maltosyl-β-CD, it can be observed that the highest K_c value was found for α-CD, indicating that the mixture of phenolic compounds responsible for the enzymatic browning of peach juice interact more strongly with this CD (**Table 5**). Moreover, as has been previously shown, α-CD was the only type of CD able to totally eliminate the darkness of peach juice in the first hour after its juicing. This, besides the fact that α-CD enjoys GRAS status (FDA) for use as an additive in food products, makes it the most recommendable CD for the inhibition of peach juice enzymatic browning.

Due to the food industry's growing interest in natural antibrowning agents, we have studied the effect of CD addition on the color evolution of peach juice and proposed a kinetic model to explain the evolution of the color parameters of peach juice in the presence of different types of CDs, during the first hour following juicing. However, further investigation into the stability of phenol−CD complexes in the different food-processing steps is necessary before such complexes can be used as ingredients in fruit juices.

ABBREVIATIONS USED

CD, cyclodextrin; PPO, polyphenol oxidase.

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