JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

# Synthesis of a Thiol-β-cyclodextrin, a Potential Agent for Controlling Enzymatic Browning in Fruits and Vegetables

Carmen Manta,*[,†] Gabriela Peralta-Altier,[†] Larissa Gioia,[†] María F. Méndez,[†] Gustavo Seoane,[‡] and Karen Ovsejevi[†]

[†]Cátedra de Bioquímica, Departamento de Biociencias, Facultad de Química, Universidad de la República, Gral Flores 2124, Montevideo, Uruguay,

[‡]Cátedra de Química Orgánica, Departamento de Química Orgánica, Facultad de Química, Universidad de la República, Gral Flores 2124, Montevideo, Uruguay

Supporting Information

**ABSTRACT:** A thiol-β-cyclodextrin was synthesized by a simple and environmentally friendly three-step method comprising epoxy activation of β-cyclodextrin, thiosulfate-mediated oxirane opening, and further reduction of the *S*-alkyl thiosulfate to a thiol group. The final step was optimized by using thiopropyl-agarose, a solid phase reducing agent with many advantages over soluble ones. β-Cyclodextrin thiolation was confirmed by titration with a thiol-reactive reagent, NMR studies, and MALDI-TOF/TOF. Thiolated cyclodextrin had an average value of one thiol group per molecule. Thiol-β-cyclodextrin proved to be an excellent agent for controlling polyphenol oxidase activity. This copper-containing enzyme is responsible for browning in fruits and vegetables. Under the same conditions, thiol-β-cyclodextrin generated a reductive microenvironment that increased the antibrowning effect on Red Delicious apples compared to unmodified β-cyclodextrin.

**KEYWORDS:** *thiol-β-cyclodextrin (thiol-CD), antibrowning agent, polyphenol oxidase (PPO)*

## INTRODUCTION

Cyclodextrins (CDs) are macrocyclic oligosugars shaped like a truncated cone with a hydrophobic internal cavity and a hydrophilic outer surface.[1] The particular significance of CDs lies in their ability to form inclusion complexes with other molecules.[2−9] Moreover, on the basis of the reactivity of their external OH groups, chemically modified CDs can be synthesized to vary their solubility, to modify their complexation properties, and/or to introduce certain specific functional groups.[10] These characteristics facilitate the control of enzymatic activity, not only by encapsulation of substrates or products but also by generating new microenvironments around the enzyme when modified CDs are used.

In particular, β-cyclodextrin (β-CD) has found specific application in the food industry because it has been classified in the list of food additives as "generally recognized as safe" (GRAS) by the U.S. Food and Drug Administration (FDA).[11] In recent years, the potential value of β-CD as an inhibitor of enzymatic browning in fruits and vegetables has been extensively reported. Browning is one of the most important causes of quality loss; organoleptic qualities and nutritional properties may be strongly altered, resulting in product rejection by consumers.[11−13]

The control of enzymatic browning is a challenge to the food industry, and various physical and chemical methods have been used to reduce this oxidative process.[14,15] Polyphenol oxidase is the major enzyme responsible for this process and catalyzes two different reactions in the presence of molecular oxygen: the hydroxylation of monophenols to *o*-diphenols (monophenolase activity) and the oxidation of *o*-diphenols to *o*-quinones (diphenolase activity).[15,16] Several mechanisms are proposed for the CD antibrowning effect; the most well-known is the complexation of PPO substrates by CDs, preventing their oxidation to *o*-quinones and subsequent polymerization to brown pigments. Because PPO activity depends on the oxidative effect of the copper ions present in its active site, the reduction of these metallic ions is an excellent strategy for controlling enzyme activity. It is reported that after the oxidation of *o*-diphenols to *o*-quinones by molecular oxygen, catalyzed by PPO, these *o*-quinones can react with thiol compounds by nucleophilic thiolate addition, giving thiol−diphenol adducts. The formation of these adducts under reducing conditions is another explanation why thiols can be used for controlling enzymatic browning in fruits and vegetables.[17,18]

Several studies have been carried out to search for new natural inhibitors of enzymatic browning, such as sulfur-containing amino acids and their derivatives. These amino acids were evaluated as an alternative to sulfite, which was used as a very effective antibrowning agent until 1986, when its use was limited by the FDA.[19] This search led to thiol-containing compounds, which are promising agents for the control of enzymatic browning in fruits and vegetables; more specifically, cysteine, *N*-acetyl-L-cysteine and reduced glutathione have been reported as effective PPO inhibitors.[20−22]

The present study was focused on the development of a β-CD modified with thiol groups, which could create a reductive

Received: July 15, 2013
Revised: November 8, 2013
Accepted: November 12, 2013
Published: November 12, 2013





dx.doi.org/10.1021/jf403063s | J. Agric. Food Chem. 2013, 61, 11603−11609

environment and simultaneously capture enzyme substrates in its hydrophobic cavity. Furthermore, thiol-cyclodextrin (thiol-CD) could be used as a carrier for natural antioxidant additives, stabilizing them and increasing their antioxidant capacity.

Different strategies for the synthesis of thiolated-β-cyclodextrin are known, with those involving tosylation of CDs being most frequently used.[23−26] Cyclodextrin monotosylate is the most important derivative of this host molecule for access to modifications on the primary hydroxyl side of the macrocycle.[27] However, it is necessary to control the reaction to avoid overtosylation.[28] The synthesis of monotosylated CD is mainly performed according to the method reported by Melton and Slessor[29] using pyridine as solvent; this requires dry conditions, which, if rigorous, cause formation of a cyclodextrin−pyridine gel,[30] with a risk of chlorination during workup.[31] Moreover, the direct sulfonylation at primary positions of cyclomaltooligosaccharides does not usually proceed with high yields and selectivity. Thus, many other alternative routes for synthesizing thiol-CDs start with direct iodination of the cyclic oligosaccharide with triphenylphosphine in dimethylformamide and iodine.[32−35] A different strategy involves cross-linking β-cyclodextrin with epichlorohydrin, giving a polymer with residual hydroxyl groups that are later activated with tosyl chloride.[36]

Here we report a new method for the synthesis of a thiol-CD, involving an initial step in which 1,4-butanediol diglycidyl ether (DGE) was used for the introduction of reactive oxirane groups on β-CD, followed by a thiolation process. This approach was reported by Sundberg et al.[37] for the synthesis of epoxy-activated agarose for the preparation of chromatography adsorbents. On the basis of our previous studies on the introduction of thiol-reactive groups to epoxy-agarose,[38−40] the complete synthesis of thiol-CD was carried out in an aqueous milieu, at room temperature, involving an alkyl thiosulfate intermediate[41] followed by reductive treatment with a solid phase reducing agent (thiopropyl-agarose).[39,42,43] Thiopropyl agarose has many advantages over soluble reducing agents: no contaminant products are liberated, separation from the reduced molecule is easy, as is regeneration, and it can be reused many times.

The antibrowning effect of thiol-CD was evaluated both in a PPO extract and on slices of Red Delicious apples. After the treatment, browning decrease was determined by quantifying PPO residual activity in the extract and by measuring pulp color.

## MATERIALS AND METHODS

β-Cyclodextrin (β-CD), dithiothreitol (DTT), epichlorohydrin (1-chloro-2,3-epoxypropane), 1,4-butanediol diglycidyl ether (DGE), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), phenolphthalein, Triton X-100, and polyvinylpolypyrrolidone (PVPP) were purchased from Sigma (St. Louis, MO, USA). Biotech dialysis membranes, Spectra/Por 500−1000 MWCO, were obtained from Spectrum Laboratories (Rancho Dominguez, CA, USA). Sepharose 4B was from Pharmacia BTG (Uppsala, Sweden).

**Thiopropyl-agarose Preparation.** Thiopropyl-agarose was prepared in our laboratory as reported by Batista et al.[40] Epichlorohydrin (15 mL per 100 g of suction-dried Sepharose 4B) and 100 mL of 1 M NaOH were used for the epoxy activation step. The gel-bound epoxy groups formed were then reacted with sodium thiosulfate, and the resulting alkyl thiosulfate esters were finally reduced to thiol groups by treatment with DTT. Thiopropyl agarose with 1000 μmol SH/g dried gel was obtained.

**Thiol-CD Synthesis.** The method comprises three steps: epoxy activation of β-CD, conversion of the introduced oxirane groups into thiosulfate groups, and their reduction to thiol groups.

*Epoxy Activation of β-Cyclodextrin*.[37] β-CD (1 g) was dissolved in 5.0 mL of 0.6 N sodium hydroxide solution under magnetic stirring at room temperature. Aliquots of DGE were added at a rate of about 100 μL every 5 min over a total period of 50 min, under agitation. After another 40 min, the reaction was ended and excess reagent was removed by extraction with petroleum ether.

*Alkyl Thiosulfate Preparation (Bunte Salt-CD)*.[41] The solution containing the epoxy-cyclodextrin (epoxy-CD) was made pH neutral with hydrochloric acid. Then 2 M sodium thiosulfate in 0.5 M sodium phosphate buffer, pH 6.3, was added, and the resulting solution was stirred for 6 h at room temperature.

Afterward, dialysis was performed to remove salts with membrane Spectra/Por 500−1000 MWCO, checking the absence of thiosulfate by Ellman's reaction.[44]

*Bunte Salt-CD Reduction with Solid Phase Reducing Agent*. Ten milliliters of Bunte salt-CD (dialyzed against 10 mM sodium phosphate buffer, pH 8.0) was incubated with 1.6 g of thiopropyl-agarose (with 53 μmol SH/g suction dried gel) under agitation at room temperature. After 2 h, the gel was removed by filtration on a sintered glass filter, and the resulting filtrate was extensively dialyzed against 10 mM acetate buffer, pH 4.5, using a membrane with 500−1000 MWCO. Then it was frozen for later lyophilization.

**Determination of Oxirane Groups.**[37] Quantitative determination of oxirane groups involved a reaction between the oxirane ring and sodium thiosulfate. Release of $OH^-$ groups was followed by titration with 0.1 M hydrochloric acid. The amount of oxirane groups in the solution was calculated from the amount of hydrochloric acid needed to maintain neutrality. It was necessary to start with a neutral solution of oxiranes.

**β-CD Titration with Phenolphthalein.**[45] Aliquots of sample were incubated with 1.0 mL of phenolphthalein (60 μM in buffer 0.2 M sodium bicarbonate, pH 10.5) for 15 min. Absorbance at 550 nm was determined against a blank of buffer pH 10.5. The presence of β-CD causes a decrease in color intensity leading to negative values of absorbance.

**SH Titration (Ellman's Method).**[46] Sample aliquots were incubated for 15 min with 1.0 mL of 0.1 M buffer sodium phosphate, pH 8.0, and 100 μL of DTNB (4 mg/mL) in the same buffer. Absorbance at 412 nm was determined, and SH groups were calculated as the amount of 2-nitro-5-thiobenzoic acid (TNB) released (molar extinction coefficient at 412 nm of TNB is 13600 $M^{-1}$ $cm^{-1}$).

**MALDI TOF/TOF.** Analysis was carried out in a 4800 MALDI TOF/TOF analyzer (ABI Sciex, Framingham, MA, USA) operating in a reflection mode in the range $m/z$ 900−20000. Dissolved sample was mixed with two different matrix solutions: (1) α-cyano-4-hydroxycinnamic acid in 50% acetonitrile/water and (2) α-cyano-4-hydroxycinnamic acid in 60% acetonitrile and 0.1% trifluoroacetic acid.

**NMR Spectroscopy.** NMR spectra were recorded on a Bruker Avance DPX-400 spectrometer at 303 K, using $D_2O$ as solvent. The chemical shifts are expressed in parts per million, and the signal of HDO at 4.79 ppm was used as reference for $^1H$ NMR spectroscopy. $^{13}C$ NMR spectroscopy was performed using the following acquisition parameters: pulse program, zgpz30; number of raw data points (TD), 64K; number of scans (NS) ranging 18584 to 70947 along different runs; number of dummy scans (DS), 4; spectral window (SWH), 23980.814 Hz (240 ppm); relaxation delay (D1), 2 s; pulse length (P1), 17 μs.

Fully decoupled: composite pulse decoupling sequence (set by CPDPRG2) waltz, 16; nucleus 2 (NUC2), 1H; transmitter power level (PL1), −6.00 db; decoupler power level (PL12), 22.50 db; gated decoupler power level (PL13), 20.26db; decoupler offset (O2), 1600 Hz.

Process parameters: number of real data points (SI), 32K; frequency, 100.6127690 MHz; window function (WDW), EM; line broadening, 1.00 Hz.

**PPO Extraction from Red Delicious Apple.** Organic apples (Red Delicious) harvested at commercial maturity in April 2010 at a

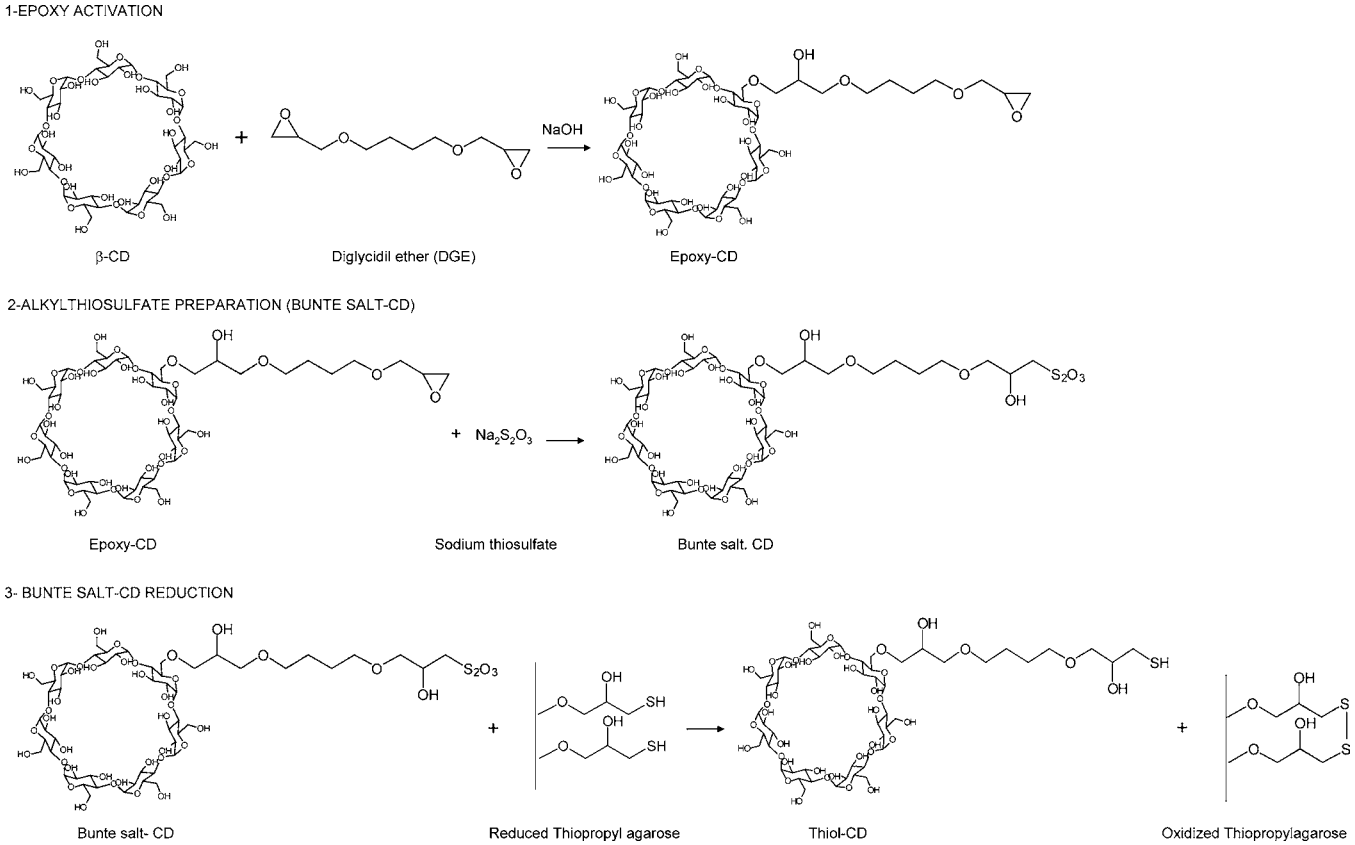


**Figure 1.** Thiol-CD synthesis.

local farm were selected to avoid interferences with pesticides. Representative samples were stored at 0 ± 2 °C and manual peeling, cutting, and washing were performed.

PPO extraction was performed as reported by Yucheng et al.[47] Apple sample (100 g) was blended with a homogenizer for 3 min, with 160 mL of extraction buffer (0.2 M sodium phosphate−citrate, pH 7.4), 2 g of PVPP, and 0.25 mL of Triton X-100. The homogenate was incubated for 90 min at 4 °C and centrifuged for 15 min at 12000*g* at 4 °C.

**Enzymatic Activity Assay.** PPO activity was assayed by mixing 0.9 mL of substrate in activity buffer (0.1 M sodium acetate, pH 4.6) with 0.1 mL of enzyme sample. The change in absorbance at 420 nm/min was determined using chlorogenic acid as substrate.[48] A blank with substrate solution and buffer was run.

One enzyme unit is the amount of enzyme necessary to produce a change in absorbance at 420 nm of 0.001/min at 22 °C and pH 4.6.

**Effect of *β*-CD, Thiol-CD, and DTT on Soluble PPO.** Aliquots of PPO extract were incubated for 1 h at room temperature with each agent (Thiol-CD and DTT 20 $\mu$M, *β*-CD 10 mM) under end-over-end agitation. A control was run with enzyme in activity buffer. Aliquots were taken at intervals of 10 min, and the residual PPO activity was determined.

**Sliced Apple Treatment with PPO Inhibitors.** Organic apples (Red Delicious) were peeled, cored, sliced, and divide into 2 g aliquots in Petri boxes.

One mililiter of test solution (*β*-CD or thiol-CD, both 700 $\mu$M in 0.1 M sodium acetate buffer, pH 4.6) was added to each box. Controls with the same buffer and controlled atmosphere were run at the same time. Apple slices were kept closed in Petri boxes with adherent film at room temperature for 24 h. Afterward, a Konica Minolta CM-700d/600d spectrophotometer was used to assess apple pulp color. The measuring aperture diameter was 8 mm, and CIE/10° was the illuminant/viewing geometry. Three different areas were monitored (slice extremes and middle) by five measurements each. Readings were expressed as $L^*$, $a^*$, and $b^*$ parameters.[49−52] The parameter $\Delta E^*$ was calculated as follows: $\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$.

**Statistical Analysis.** All determinations were replicated at least three times, and analysis of variance (ANOVA) of the data was carried out. The Tukey test was employed to determine the statistical significance of the differences between the means ($p \leq 0.05$).

## RESULTS AND DISCUSSION

**Synthesis of a Thiol-CD.** The present research was based on our previous experience on derivatization of hydroxylated solid phases (e.g., agarose) with thiol groups. Due to the structure of *β*-CD we here report its activation using the same chemistry with modifications.[38−40]The synthesis of thiol-CD involved an epoxy activation, followed by a Bunte salt preparation and a final reduction step (Figure 1).

*Epoxy Activation.* Initially, epoxy activation was carried out with epichlorohydrin in alkaline medium as reported by Renard et al. for *β*-CD polymer preparation.[53] However, higher epoxy activation degree of *β*-CD was achieved using DGE, a reagent reported for agarose activation.[37] Epoxy titration and $^{13}C$ NMR data confirmed the presence of the oxirane residue, for which signals at $\delta$ 44.8 and 51.6 appeared after DGE treatment (see the Supporting Information).

*Bunte Salt Preparation.* This was accomplished by the procedure reported for polysaccharide matrixes using sodium thiosulfate.[37] The elimination of excess reagent by gel filtration or dialysis was important because otherwise this salt will consume the reducing agent used in the next step. NMR data for Bunte salt-CD showed almost the same peaks as the epoxy-CD, except for the absence of the signals corresponding to the oxirane ($\delta$ 44.8 and 51.6) and the presence of a new signal at $\delta$

37.5, which agreed with a $CH_2$ bound to $S_2O_3^{-2}$ (see the Supporting Information).

*Bunte Salt Reduction.* Bunte salt reduction was carried out with thiopropyl-agarose. This insoluble reagent has been extensively used for protein reduction[42] but, to our knowledge, this was the first attempt to reduce oligosaccharides using this solid phase reducing agent. The solid phase reducing agent was selected because its excess can be easily removed by filtration, allowing an easier control of the reaction and avoiding methods that dilute the concentration of the reduced product and lengthen the overall process. Moreover, solid phase reducing agents do not liberate any contaminating byproducts, and it is possible to reuse them many times. Optimization of Bunte salt-CD reduction was carried out by studying kinetic aspects of the reaction for 24 h. As shown in Figure 2, the highest SH titer

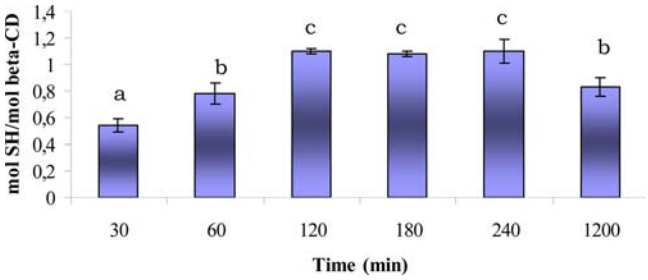


**Figure 2.** Kinetics of Bunte salt-CD reduction on solid phase. Results represent averages of at least three experiments. The same letter indicates no significant difference at the $p \leq 0.05$ level according to Tukey's test.

was achieved between 120 and 240 min. Additionally, the amount of reducing agent was adjusted, because it was found that no significant increase (by Tukey's test) in the SH titer was achieved using more than 6 mol SH/mol $\beta$-CD in the reducing gel (Figure 3). The resulting product had an average of 1 SH

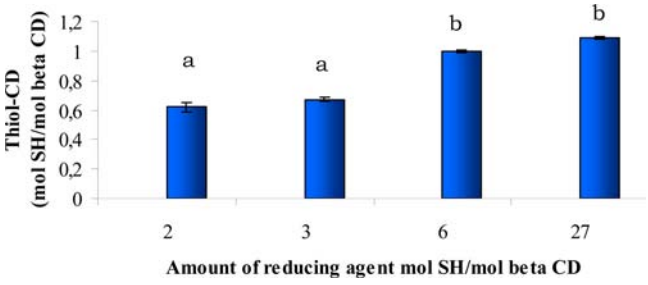


**Figure 3.** Reduction of Bunte salt-CD with different amounts of solid phase reducing agent. Results represent averages of at least three experiments. The same letter indicates no significant difference at the $p \leq 0.05$ level according to Tukey's test.

per unit of $\beta$-CD. This degree of reduction can be explained considering that the alkyl thiosulfate groups on the bulky CD and the thiopropyl groups on the solid phase reducing agent must be in close proximity to react. Consequently, for alkyl thiosulfate groups farther away from the thiopropyl groups the reduction process would be more difficult. This was experimentally confirmed by comparing the mass spectra (MALDI TOF-TOF) of Bunte salt-CD and thiol-CD, because a peak at $m/z$ 1495.43, corresponding to the Bunte salt structure, could be seen in both.

The use of a soluble reducing agent, such as DTT, could increase the number of SH groups per unit of CD, but it would give less control of the reduction process and would hinder scaling up of thiol-CD synthesis because the reducing agent could not be reused.

Different analyses were carried out to confirm the chemical structure of the reduced product, including SH titration (Ellman's reaction), NMR measurements, and MALDI TOF-TOF MS analysis.

Mass spectra in a reflection mode in the range $m/z$ 900−20000 suggested a low degree of polymerization because no peaks of high molecular weight appeared in the $m/z$ range 5000−20000. Thus, most of the chemically modified $\beta$-CD molecules remained as monomers. In addition, only mono-, di-, and trisubstituted compounds (i.e., CD units with one to three side chains) appeared in the MS spectra (peaks at $m/z$ 1393.49, 1627.58 and 1865.70), and the presence of thiol groups was confirmed by titration in solution (Ellman's reaction). An average of 1 mol SH/mol $\beta$-CD was obtained, in agreement with the presence of a heterogeneous population of species including $\beta$-CD without thiolation.

The fragmentation spectra of the monosubstituted peak (M + Na, $m/z$ 1393.49) showed the successive loss of glucose units (sequential loss of 162 mass units) until a last fragment ($m/z$ 421) corresponding to glucose functionalized with the thiolated side chain. Other fragmentation sequences showed that the functionalized glucose could be removed first, followed by the repeated loss of glucose units (Figure 4). Moreover, the mass of the side chain matched exactly that of the proposed structure shown in Figure 1.

From the reactivity point of view it has been reported that in $\beta$-CD the primary C6-OH groups are more reactive than the secondary C2-OH and C3-OH. Therefore, derivatives with substituted primary carbons are expected.[35]

The $^{13}C$ NMR spectra of thiol-CD showed no significant shifts for C2 and C3 compared to $\beta$-CD control, in agreement with the absence of substitution at these positions.[34] However, the signal corresponding to C6 could not be identified because it overlapped with that of the side chain ($\delta$ 60−70) (see the Supporting Information). In brief, all signals indicated the presence of a thiolated cyclodextrin, the mass spectra confirmed that it was mainly a monomer with one to three substituents per $\beta$-CD unit, and the thiol groups could be titrated by specific reaction with Ellman's reagent.

**Thiol-$\beta$-CD as an Antibrowning Agent for Apple PPO.** PPO catalyzes the oxidation of *o*-phenols to *o*-quinones, which then convert to brown melanin pigments, though any event that disrupts these transformations should reduce enzymatic browning. There are different mechanisms to explain the antibrowning effect: direct action on the enzyme (preventing the formation of *o*-quinones) and other actions that affect reaction intermediates (avoiding the transformation of *o*-quinones to brown pigments). Because $Cu^{2+}$ ions in the enzyme are essential for PPO activity and thiol groups have a strong affinity for this metal, thiol-containing compounds are potentially good antibrowning agents. Moreover, nucleophilic addition of the thiolate anion to *o*-quinone intermediates can generate colorless thiol-*o*-quinone adducts instead of brown pigments. Another antibrowning mechanism extensively reported in the literature[11−13,54] is based on the encapsulation of the enzyme's substrates (polyphenols) by cyclodextrins. We aimed to synthesize a derivatized cyclodextrin with thiol groups that could act combining all of these mechanisms and allow the

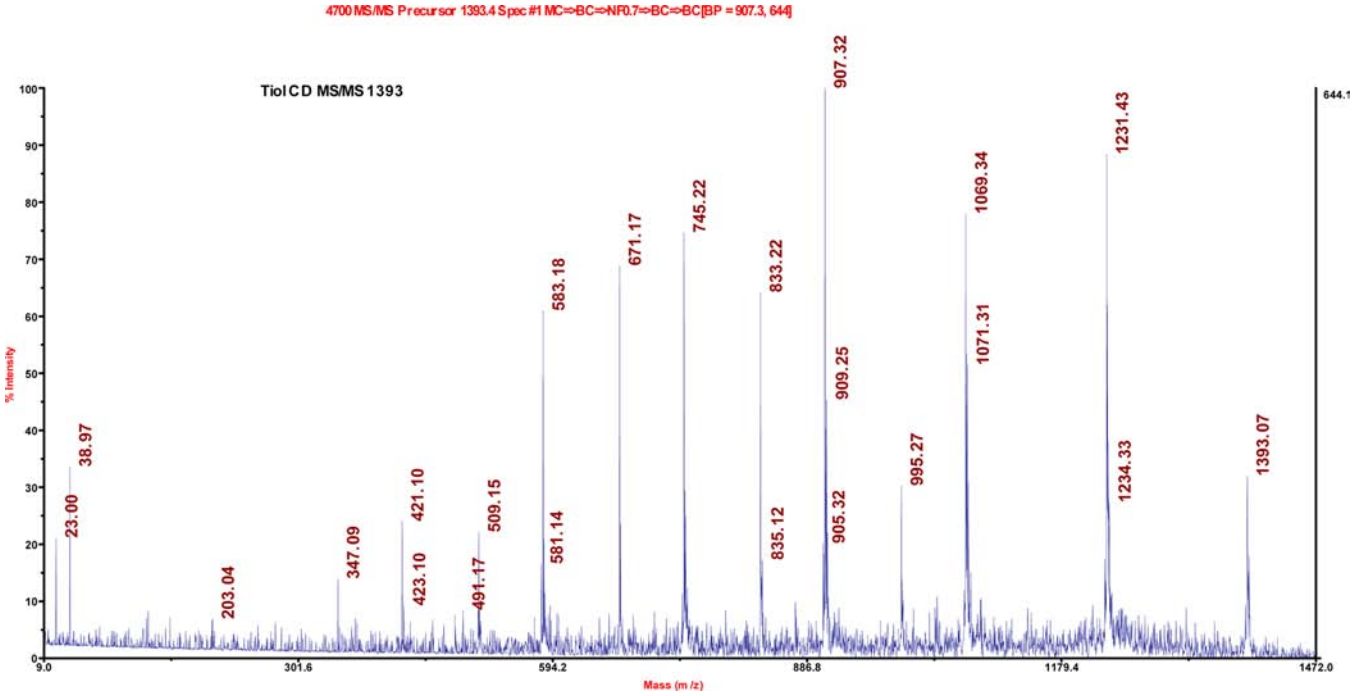


**Figure 4.** Fragmentation spectra (thiol-CD MS/MS 1393.4).

generation of a reducing environment with the concomitant capture of substrates. To elucidate the mechanisms involved, thiol-CD, $\beta$-CD, and DTT were assessed as antibrowning agents on PPO from Red Delicious apples. As can be seen in Figure 5, after PPO incubation with thiol-CD, DTT, and $\beta$-CD,

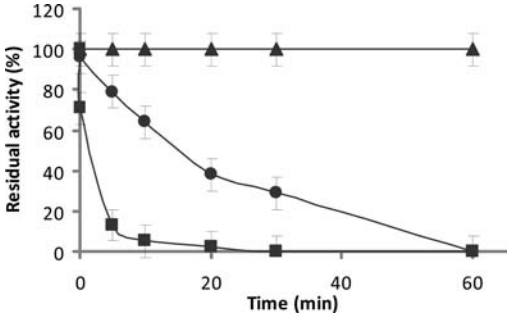


**Figure 5.** Antibrowning effect on soluble PPO: (▲) $\beta$-CD 10 mM; (●) thiol-CD 20 $\mu$M; (■) DTT 20 $\mu$M, in 0.1 M sodium acetate buffer, pH 4.7. Results represent averages of at least three experiments.

the residual activity markedly decreased for both reducing agents, remaining unchanged for $\beta$-CD. These results suggest that the inhibitory effect of thiol-CD and DTT on PPO is due to the interaction of thiol groups with copper ions in the active site of the enzyme. However, DTT showed different kinetics, because residual activity was depleted more rapidly than for thiol-CD. The different chemical structure of those compounds might explain this result, because DTT is a small hydrophilic molecule, whereas thiol-CD is bigger and more hydrophobic. It is important to observe that $\beta$-CD had no effect on protein copper ions even though it was assayed at a concentration (10 mM) almost 500 times higher than that used for both reducing agents.

When effects on the enzyme–substrate reaction were evaluated, it was found that the antibrowning effect achieved with thiol-CD was considerably higher than that found for the same concentration of $\beta$-CD, indicating that substrate encapsulation was not the only mechanism involved when thiol-CD was used.

Moreover, as thiol groups can react with *o*-quinones,[21] another mechanism for explaining the higher antibrowning effect could be the formation of adducts between thiol-CD and *o*-quinone intermediates. In summary, all of these results suggest that the inhibitory effect of thiol-CD on PPO extract was mainly due to the reducing microenvironment generated by its thiol groups, although polyphenol capture contributed to the global result.

For fresh-cut fruit comparative results between modified and unmodified oligosaccharide (both at the same concentration, 700 $\mu$M) were observed. Changes in $L^*$, $a^*$, and $b^*$ were measured to quantify the browning in sliced apple after 24 h with different treatments: 0.1 M sodium acetate buffer, pH 4.7, 700 $\mu$M $\beta$-CD, and 700 $\mu$M thiol-CD (both in the same buffer).

As can be seen in Table 1, the color measurements showed that different effects were achieved by treatment with $\beta$-CD and

**Table 1. Colorimetric Detection of Treated Apple Pulp Color[a]**

| | $L^*$ | $a^*$ | $b^*$ |
|---|---|---|---|
| control[b] | 67.26 ± 0.80 a | 1.32 ± 0.48 a | 26.92 ± 0.45 a |
| $\beta$-CD (700 $\mu$M)[c] | 66.69 ± 1.91 a | 1.55 ± 0.42 a | 26.11 ± 1.20 a |
| thiol-CD (700 $\mu$M)[d] | 70.20 ± 0.84 b | −0.16 ± 0.16 b | 19.92 ± 2.31 b |

[a]Means in the same column followed by the same letter are not significantly different at the $p \leq 0.05$ level according to Tukey's test. The experiment was replicated with three different apples, and five measurements were taken at three different areas (slice extremes and middle). [b]Control (0.1 M sodium acetate buffer, pH 4.7). [c]700 $\mu$M $\beta$-CD in 0.1 M sodium acetate buffer, pH 4.7. [d]700 $\mu$M thiol-CD in 0.1 M sodium acetate buffer, pH 4.7.

thiol-CD. Whereas $a^*$ did not change significantly for $\beta$-CD, a decrease in $a^*$ was found for thiol-CD, corresponding to a reduction in red color and therefore a decrease in enzymatic browning. Moreover, the other color parameters confirmed this fact, because an increase in brightness ($L^*$) and a reduction in yellow color ($b^*$) were also observed.[49−52,55] The parameter $\Delta E^*$ for thiol-CD and $\beta$-CD (7.68 and 1.02, respectively) matched the expected results.

In brief, it was possible to synthesize a thiol-CD by an environmentally friendly method, which has very promising applications for controlling enzymatic browning not only in fresh-cut fruits and vegetables but also in juices and other processed foods. The use of thiol-CD in the food industry will require future studies including the evaluation of its toxicity.

## ■ ASSOCIATED CONTENT

### Supporting Information

$^{13}$C NMR analysis of $\beta$-cyclodextrin, epoxy-CD, Bunte salt-CD, and thiol-CD. This material is available free of charge via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION

### Corresponding Author

*(C.M.) E-mail: carmenluisamanta@gmail.com. Fax: 598 29241906. Phone: 598 29241806.

### Funding

We are grateful for financial support from Comisión Sectorial de Investigación Científica (CSIC Project I+D 415), Universidad de la República, and by the Programa para el Desarrollo de las Ciencias Básicas (PEDECIBA), Montevideo, Uruguay.

### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

We thank Dr. Rosario Durán from Institute Pasteur de Montevideo for MALDI TOF-TOF analysis and Dr. Valerie Dee for linguistic revision.

## ■ ABBREVIATIONS USED

PPO, polyphenol oxidase; CDs, cyclodextrins; $\beta$-CD, $\beta$-cyclodextrin; DMF, dimethylformamide; thiol-CD, thiol-$\beta$-cyclodextrin; DGE, 1,4-butanediol diglycidyl ether; DTT, dithiothreitol; DTNB, 5,5′-dithiobis(2-nitrobenzoic) acid; PVPP, polyvinylpolypyrrolidone; TNB, 2-nitro-5-thiobenzoic acid; epoxy-CD, epoxy-activated cyclodextrin; Bunte salt-CD, alkyl thiosulfate intermediate

## ■ REFERENCES

(1) French, D.; Rundle, R. E. The molecular weights of the Schardinger $\alpha$-and $\beta$-dextrins. *J. Am. Chem. Soc.* **1942**, *64*, 1651−1655.

(2) Koontz, J. L.; Marcy, J. E.; O'Keefe, S. F.; Duncan, S. E. Cyclodextrin inclusion complex formation and solid-state characterization of the natural antioxidants $\gamma$-tocopherol and quercetin. *J. Agric. Food Chem.* **2009**, *57*, 1162−1171.

(3) Varca, G. H. C.; Andreo-Filho, N.; Lopes, P. S.; Ferraz, H. G. Cyclodextrins: an overview of the complexation of pharmaceutical proteins. *Curr. Protein Pept. Sci.* **2010**, *11*, 255−263.

(4) Martin Del Valle, E. M. Cyclodextrins and their uses: a review. *Process Biochem.* **2004**, *39*, 1033−1046.

(5) Mele, A.; Mendichi, R.; Selva, A.; Molnar, P.; Toth, G. Non covalent associations of cyclomaltooligosaccharides (cyclodextrins) with carotenoids in water. A study on the $\alpha$- and $\beta$-cyclodextrin/$\psi$,$\psi$-carotene (lycopene) systems by light scattering, ionspray ionization and tandem mass spectrometry. *Carbohydr. Res.* **2002**, *337*, 1129−1136.

(6) Álvarez-Parrilla, E.; De la Rosa, L. A.; Torres-Rivas, F.; Rodrigo-García, J.; Gonzáles-Aguilar, G. A. Complexation of apple antioxidants: chlorogenic acid, quercetin and rutin by cyclodextrin. *J. Inclusion Phenom.* **2005**, *53*, 121−129.

(7) Hedges, A. R. Industrial applications of cyclodextrins. *Chem. Rev.* **1998**, *98*, 2035−2044.

(8) Kalogeropoulos, N.; Yannakopoulou, K.; Gioxari, A.; Chiou, A.; Makris, D. P. Polyphenol characterization and encapsulation in $\beta$-cyclodextrin of a flavonoid-rich *Hypericum perforatum* (St John's wort) extract. *LWT − Food Sci. Technol.* **2010**, *43*, 882−889.

(9) Chaudhuri, S.; Chakraborty, S.; Sengupta, P. K. Encapsulation of serotonin in $\beta$-cyclodextrin nano-cavities: fluorescence spectroscopic and molecular modeling studies. *J. Mol. Struct.* **2010**, *975*, 160−165.

(10) Mocanu, G.; Vizitiu, D.; Carpov, A. Cyclodextrin polymers. *J. Bioact. Compat. Polym.* **2001**, *16*, 315−342.

(11) Astray, G.; Mejuto, J. C.; Rial-Otero, R.; González-Barreiro, C.; Simal-Gándara, J. A review on the use of cyclodextrins in foods. *Food Hydrocolloids* **2009**, *23*, 1631−1640.

(12) López-Nicolás, J.; Pérez-López, A.; Carbonell-Barrachina, A.; García-Carmona, F. Use of natural and modified cyclodextrins as inhibiting agents of peach juice enzymatic browning. *J. Agric. Food Chem.* **2007**, *55*, 5312−5319.

(13) Hicks, K. B.; Haines, R.; 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.

(14) Li-Qin, Z.; Jie, Z.; Shu-Hua, Z.; Lai-Hui, G. Inhibition of browning on the surface of peach slices by short-term exposure to nitric oxide and ascorbic acid. *Food Chem.* **2009**, *114*, 174−179.

(15) Gorny, J. R.; Hess-Pierce, B.; Cifuentes, R. A.; Kader, A. A. Quality changes in fresh-cut pear slices as affected by controlled atmospheres and chemical preservatives. *Postharvest Biol. Technol.* **2002**, *24*, 271−278.

(16) Valente Mezquita, V. L.; Queiroz, Ch. *Enzymatic Browning. In Biochemistry of Foods*, 3rd ed.; Eskin, N. A. M., Shahidi, F., Eds.; Academic Press: San Diego, CA, 2013; pp 387−418.

(17) Peñalver, M. J.; Rodríguez-López, J. N.; García-Molina, F.; García-Cánovas, F.; Tudela, J. Method for the determination of molar absorptivities of thiol adducts formed from diphenolic substrates of polyphenol oxidase. *Anal. Biochem.* **2002**, *309*, 180−185.

(18) Nikolantonaki, M.; Chichuc, I.; Teissedre, P. L.; Darriet, P. Reactivity of volatile thiols with polyphenols in a wine-model medium: impact of oxygen, iron, and sulfur dioxide. *Anal. Chim. Acta* **2010**, *660*, 102−109.

(19) López-Nicolás, J.; 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-$\beta$-cyclodextrin as secondary antioxidant. *Food Chem.* **2007**, *101*, 1164−1171.

(20) Eissa, H.; Fadel, H.; Ibrahim, G. E.; Hassan, I. M.; Abd Elrashid, A. Thiol containing compounds as controlling agents of enzymatic browning in some apple products. *Food Res. Int.* **2006**, *39*, 855−863.

(21) Friedman, M.; Bautista, F. Inhibition of polyphenol oxidase by thiols in the absence and presence of potato tissue suspensions. *J. Agric. Food Chem.* **1995**, *43*, 69−76.

(22) Guerrero-Beltrán, J.; Swanson, B.; Barbosa-Cánovas, G. Inhibition of polyphenoloxidases in mango puree with 4-hexylresorcinol, cysteine and ascorbic acid. *LWT − Food Sci. Technol.* **2005**, *38*, 625−630.

(23) Yang, J.; Leung, D. K. Facile synthesis of $\beta$-cyclodextrin dithiols. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 169−170.

(24) Fujita, K.; Ueda, T.; Imoto, T.; Tabushi, I.; Toh, N.; Koga, T. Guest-induced conformational change of $\beta$-cyclodextrin capped with an environmentally sensitive chromophore. *Bioorg. Chem.* **1982**, *11*, 72−84.

(25) Nelles, G.; Weisser, M.; Back, R.; Wohlfart, P.; Wenz, G.; Mittler-Neher, S. Controlled orientation of cyclodextrin derivatives immobilized on gold surfaces. *J. Am. Chem. Soc.* **1996**, *118*, 5039−5046.
(26) Bollo, S.; Yáñez, C.; Sturm, J.; Núñez-Vergara, L.; Squella, J. A. Cyclic voltammetric and scanning electrochemical microscopic study of thiolated β-cyclodextrin adsorbed on a gold electrode. *Langmuir* **2003**, *19*, 3365−3370.
(27) Croft, A. R.; Bartsch, R. A. Synthesis of chemically modified cyclodextrins. *Tetrahedron* **1983**, *39*, 1417−1471.
(28) Brady, B.; Lynam, N.; O'Sullivan, T.; Ahern, C.; Darcy, R. 6a-*O*-*p*-Toluenesulfonyl-β-cyclodextrin. *Org. Synth. Coll.* **2004**, *10*, 686.
(29) Melton, L. D.; Slessor, K. N. Synthesis of monosubstituted cyclohexaamyloses. *Carbohydr. Res.* **1971**, *18*, 29−37.
(30) De Rango, C.; Charpin, P.; Navaza, J.; Keller, N.; Nicolis, I.; Villain, F.; Coleman, A. W. βcyclodextrin/pyridine gel systems. The crystal stryctures of a first β-cyclodextrin-pyridine-water compound. *J. Am. Chem. Soc.* **1992**, *114*, 5475.
(31) Defaye, J.; Gadelle, A.; Guiller, A.; Darcy, R.; O'Sullivan, T. Branched thiocyclomalto-oligosaccharides: synthesis and properties of 6-*S*-α- and 6-*S*-β-D-glucopyranosyl-6-thiocyclomaltoheptaose. *Carbohydr. Res.* **1989**, *192*, 251.
(32) Gadelle, A.; Defaye, J. Selective halogenation at primary positions of cyclomalotooligosaccharides and a synthesis of per-3,6-anhydro cyclomalotooligosaccharides. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 78−79.
(33) Rojas, M.; Koniger, R.; Frases Stoddart, J.; Kaifer, A. E. Supported monolayers containing preformed binding sites. Synthesis and interfacial binding properties of a thiolated β-cyclodextrin derivative. *J. Am. Chem. Soc.* **1995**, *117*, 336−343.
(34) Camacho, C.; Matías, J. C.; Chico, B.; Cao, R.; Gómez, L.; Simpson, B. K.; Villalonga, R. Amperometric biosensor for hydrogen peroxide, using supramolecularly immobilized horseradish peroxidase on the β-cyclodextrin-coated gold electrode. *Electroanalysis* **2007**, *19*, 2538−2542.
(35) Peng, K.; Tomatsu, I.; Korobko, A. V.; Kros, A. Cyclodextrin-dextran, based in situ hydrogel formation: a carrier for hydrophobic drugs. *Soft Matter* **2010**, *6*, 85−87.
(36) Camacho, C.; Chico, B.; Cao, R.; Matías, J. C.; Hernández, J.; Palchetti, I.; Simpson, B.; Mascini, M.; Villalonga, R. Novel enzyme biosensor for hydrogen peroxide via supramolecular associations. *Biosens. Bioelectron.* **2009**, *24*, 2028−2033.
(37) Sundberg, L.; Porath, J. Preparation of adsorbents for biospecific affinity chromatography. I. Attachment of group-containing ligands to insoluble polymers by means of bifunctional oxiranes. *J. Chromatogr.* **1974**, *90*, 87−98.
(38) Batista-Viera, F.; Barbieri, M.; Ovsejevi, K.; Manta, C.; Carlsson, J. A new method for reversible immobilization of thiol biomolecules based on solid phase bound thiolsulfonate groups. *J. Appl. Biochem. Biotechnol.* **1991**, *31*, 175−195.
(39) Batista-Viera, F.; Manta, C.; Carlsson, J. Solid phase thiolsulfinates for the reversible immobilization of thiols. *Appl. Biochem. Biotechnol.* **1994**, *44*, 1−14.
(40) Batista-Viera, F.; Manta, C.; Carlsson., J. Covalent binding of thiols to thiolsulfinate-containing supports. *Biotechnol. Appl. Biochem.* **1996**, *24*, 231−239.
(41) Axén, R.; Drevin, H.; Carlsson, J. Preparation of modified agarose gels containing thiol groups. *Acta Chem. Scand. Ser. B* **1975**, *29*, 471−474.
(42) Grazú, V.; Ovsejevi, K.; Cuadra, K.; Betancor, L.; Manta, C.; Batista-Viera, F. Solid-phase reducing agents as alternative for reducing disulfide bonds in proteins. *Appl. Biochem. Biotechnol.* **2003**, *110*, 23−32.
(43) Ovsejevi, K.; Grazú, V.; Cuadra, K.; Batista-Viera, F. Enzyme reduction on solid phase as a tool for the reversible immobilization of yeast β-galactosidase onto a thiol-reactive support. *Enzyme Microb. Technol.* **2004**, *37*, 456−462.
(44) Man, M.; Bryant, R. G. Reactions of thiosulfate and sulfite ions with DTNB: interferente in sulfhydryl group analysis. *Anal. Biochem.* **1974**, *57*, 429−431.
(45) Vikmon, M. Rapid and simple spectrophotometric method for determination of microamounts of cyclodextrins. In *First Symposium on Cyclodextrins*; Szejtli, J., Ed.; Reidel Publishing: Budapest, Hungary, 1982; pp 69−74.
(46) Ellman, G. L. Colorimetric method for determining low concentrations of mercaptans. *Arch. Biochem. Biophys.* **1958**, *74*, 443−450.
(47) Fu, Y.; Zhang, K.; Wang, N.; Du, J. Effects of aqueous clorine dioxide treatment on polyphenol oxidases from Golden Delicious apple. *LWT − Food Sci. Technol.* **2007**, 1362−1368.
(48) Polyphenol oxidase. In *Worthington Enzyme Manual*; Worthington: Freehold, NJ, 1993.
(49) Denoya, G. I.; Pirola, P. M.; Sanow, C. L.; Benítez, C. E. Análisis de la evolución de características cromáticas como parámetro de calidad en vegetales cortados. Chapter A-3. In *World Congress and Exhibition Engineering 2010*, Buenos Aires, Argentina, 2010.
(50) Soliva-Fortuny, R. C.; Oms-Oliu, G.; Martín-Belloso, O. Effects of ripeness stages on the storage atmosphere, color, and textural properties of minimally processed apple slices. *J. Food Sci.* **2002**, *67*, 1958−1963.
(51) Vasantha Rupansinghe, H. P.; Murr, D.; Deell, J.; Odumeru, J. Influence of 1-methycyclopropene and nature seal on the quality of fresh-cut 'Empire' and 'Crispin' apples. *J. Food Qual.* **2005**, *V28*, 289−307.
(52) Perez-Gago, M. B.; Serra, M.; del Río, M. A. Color change of fresh cut apples coated with whey protein concentrate-based edible coatings. *Postharvest Biol. Technol.* **2006**, *39*, 84−92.
(53) Renard, E.; Deratani, A.; Volet, G.; Sebille, B. Preparation and characterization of water soluble high molecular weight β-cyclodextrin-epichlorohydrin polymers. *Eur. Polym. J.* **1997**, *33*, 49−57.
(54) Billaud, C.; Regaydie, E.; Fayad, N.; Richard-Forget, F.; Nicolas, J. Effect of cyclodextrins on polyphenol oxidation catalyzed by apple polyphenol oxidase. In *Enzymatic Browning and Its Prevention*; Lee, C. Y., Whitaker, J. R., Eds.; ACS Symposium Series 600; American Chemical Society: Washington, DC, 1995; pp 295−312.
(55) Chisari, M.; Barbagallo, R. N.; Spagna, G. Charaterization and role of polyphenol oxidase and peroxidase in browning in fresh-cut melon. *J. Agric. Food Chem.* **2008**, *56*, 132−138.