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ORIGINAL ARTICLE

Complexation of tyrosol with cyclodextrins

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Abstract Tyrosol (TY), 4-(2-hydroxyethyl)phenol, is an olive oil biophenol with antioxidant activity and positive effects on human health. This study has investigated the interactions of TY with cyclodextrins (CD) and a CD polymer. Complexation of TY with β-CD, hydroxypropyl-β-CD (HP-β-CD), and methyl-β-CD (Me-β-CD) has been evaluated both in aqueous solution and in the solid state. The techniques employed in solution to determine the apparent stability constants of the respective complexes were fluorescence and UV–visible spectroscopies. Complexation with β-CD and its derivatives involved an increase of both the UV absorbance and the intrinsic fluorescence of TY; a bathochromic shift of the UV spectrum was detected as well. The apparent stability constants obtained with native β-CD, Me-β-CD and HP-β-CD presented similar values. Complexes in the solid state were obtained by coevaporation and kneading. They were characterised by X-ray diffraction analysis and differential thermal analysis. The interaction of TY with β-CD led to a crystalline complex; the same diffraction pattern was obtained by coevaporation and kneading. The complexes obtained with methyl- and HP-β-CD were amorphous irrespective of the preparation method. In addition, the retention of TY in an insoluble polymer of CD crosslinked with epichlorohydrin has been quantified. In approximately 20 min, 1 mg of TY per gram of polymer was retained.

Keywords Tyrosol · Cyclodextrin · Inclusion complex

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Introduction

A great number of polyphenolic compounds widely distributed in the plant kingdom have attracted attention in the food and pharmaceutical industries; on the one hand, because of their potential health benefits [1–3] and on the other, due to the growing interest in the substitution of synthetic antioxidants by natural ones, not only in the food industry but also in any other which need to prevent deterioration of oxidisable goods such as cosmetics, pharmaceuticals and plastics.

Tyrosol (TY) and hydroxytyrosol (HTY) are biophenols that contribute to the well known beneficial properties of virgin olive oil. It is worthy of note that olive mill waste water can be an excellent source of these natural antioxidants [4] and the recovery of these products would turn a polluting residue into a source of bioactive compounds. Some of the biological effects of HTY, a potent antioxidant, include anti-inflammatory properties [5] and reduction of the risk of coronary disease and atherosclerosis [6]. The activity of TY, the major olive oil biophenol, comprises protection against LDL-induced injury in Caco2 cells and improvement of the intracellular antioxidant defence systems [7, 8].

Cyclodextrins (CD) are cyclic oligosaccharides that present a hydrophilic external part and a relatively hydrophobic cavity in which guest molecules can be encapsulated by inclusion complex formation [9]. Complexation of biophenols with CD can improve different properties such as antioxidant activity, water solubility and stability [10] taking into account that the effectiveness of these compounds depends on preserving their stability, bioactivity and bioavailability. In addition, CD polymers can be obtained by binding CD monomers with a variety of cross-linking agents. The main applications of these CD polymers include

controlled drug release [11, 12] and removal of contaminants from industrial waste waters [13].

The aim of this paper is to characterise the formation of TY inclusion complexes with different β-CD, bearing in mind a future study of stability and antioxidant activity upon encapsulation. In addition, the retention of TY in a CD polymer has been studied in order to evaluate these materials as controlled release devices for antioxidants and also as an adsorbent for the recovery of phenolics from olive oil mill waste water.

Materials and methods

Materials

TY was supplied by Sigma (Barcelona, Spain) β-CD, methyl-β-CD (Me-β-CD) and hydroxypropyl-β-CD (HP-β-CD) were purchased from Cyclolab (Budapest, Hungary); the derivative CD had average substitution degrees of 12 and 4 respectively. An insoluble polymer of β-CD crosslinked with epichlorohydrin was used; it was purchased from Cyclolab (Hungary) in the shape of spherical beads with size ranging between 100 and 300 μm; its CD content was 55 wt% and its swelling capacity 5 mL/g in water. All other reagents and solvents were from Panreac (Barcelona, Spain).

Methods

UV–visible spectrophotometry

The binding constants of the complexes of TY (p$Ka = 10$) [14] with the ligands β-CD, Me-β-CD and HP-β-CD were calculated in aqueous solution at room temperature. In each titration, the absorbance was measured at 278 nm using a Hewlett Packard 8452A diode array spectrophotometer. TY concentration was held constant at 6×10^{-4} M while CD concentrations increased from 2×10^{-3} to 7×10^{-3} M. The absorbance data was fitted to the following equation:

$$\frac{b}{A - A_o} = \frac{1}{S_t \Delta\varepsilon} + \frac{1}{K_{11} S_t \Delta\varepsilon [\mathrm{CD}]}$$

where b is the optical path, A_o is the absorbance of TY alone, A is the absorbance in the presence of each CD concentration, S_t is the total concentration of substrate (TY), $\Delta\varepsilon$ is the difference between the molar absorptivities of complexed and free TY, K_{11} is the binding constant of the complex and [CD] is the concentration of free CD at equilibrium. It is appropriate to employ the total CD concentrations instead equilibrium concentrations when the concentration of CD is larger than S_t. In this work total concentrations have been employed.

Spectrofluorimetric study

Steady-state fluorescence experiments were performed using a FLS929 spectrofluorimeter from Edimburg Instruments. In order to analyse the interactions with β-CD, Me-β-CD and HP-β-CD, the fluorophore concentration was held constant at 5×10^{-5} M while CD concentrations increased from 5×10^{-4} to 5×10^{-3} M. The experimental conditions were $\lambda_{exc} = 276$ nm, $\lambda_{em} = 301$ nm and slit widths of 2 nm.

The experimental data obtained were fitted to the following equation, which assumes a 1:1 stoichiometry, [15]

$$\frac{F_o}{F} = \frac{1 + K_{11}[\mathrm{CD}]}{1 + aK_{11}[\mathrm{CD}]} \quad (1)$$

where F_o represents the fluorescence intensity of free TY, F is the intensity in the presence of CDs, K_{11} is the binding constant and the a parameter is defined by: $a = \phi_c \varepsilon_c / \phi_g \varepsilon_g$, being ε and ϕ the molar absorptivities and fluorescence quantum yields of the complex and the free guest respectively. A linear form of Eq. 1 has been used to calculate K_{11}:

$$\frac{1}{1 - (F_o/F)} = \frac{1}{(1-a)K_{11}[\mathrm{CD}]} + \frac{a}{a-1}$$

Solid state complexation

Solid systems 1:1 TY:CD were obtained by coevaporation and kneading. In addition, physical mixtures were prepared for comparison purposes.

The coevaporated systems were obtained by dissolving 1:1 stoichiometric amounts of TY and CD in a 1/9 V/V ethanol/water solution. The solvent was subsequently eliminated by vacuum evaporation.

The kneaded systems were obtained by adding a small volume of 1/9 V/V ethanol/water solution to an amount of CD (0.3 mol) in order to get a paste; then TY was incorporated to the paste and the mixture was kneaded during 30 min, approximately.

The resulting solid systems were characterised by powder X-ray diffraction analysis using a Bruker D8 Advance diffractometer with CuK_α radiation, 40 kV and 30 mA and also by thermal analysis on a DTA/TGA 851 Mettler Toledo equipment.

Sorption in a β-CD polymer

The kinetic adsorption tests have been performed in unbuffered water at 25 °C. A 6×10^{-4} M aqueous solution of TY was stirred at 150 rpm in the presence of 7.5 g of swollen polymer; this amount corresponds to a 30:1 CD-solute molar ratio. Samples were taken from the supernatant and the residual concentration of the solute was

determined spectrophotometrically at 276 nm using a HP 8452A diode array spectrophotometer.

Results and discussion

Complexation in aqueous solution

The UV absorption spectrum of TY in the presence of the CD studied experiences a slight increase of absorbance upon complexation together with a slight batochromic shift of the absorption band at 276 nm, as can be observed in Fig. 1 for the complex with β-CD. These spectral changes could be attributed to a change of polarity upon inclusion, taking into account that similar spectral changes were observed on the absorption spectrum of aqueous phenol upon the addition of increasing amounts of ethylenglycol [16]. The spectral features of phenolic compounds are strongly dependent on the formation of H-bonds. Thus, from the slight changes detected, it can be inferred that the phenolic group of TY is not included in the cavity but possibly located close to a hydrophilic CD rim, where water or CD hydroxyl groups are available to form H-bonds [17].

The increase of absorbance upon complexation permits the determination of the apparent stability constants of the complexes formed by fitting the experimental data to the equation previously described in the "Methods" section. Figure 2 depicts the plots of $1/(A - Ao)$ as a function of 1/[CD] for the respective complexes of TY. Good linear correlations were obtained ($r > 0.99$), confirming the formation of 1:1 inclusion complexes. Similar values were obtained for the binding constants of the complexes with β-CD, Me-β-CD and HP-β-CD, being 211 ± 13; 271 ± 51 and 264 ± 40 M^{-1}, respectively.

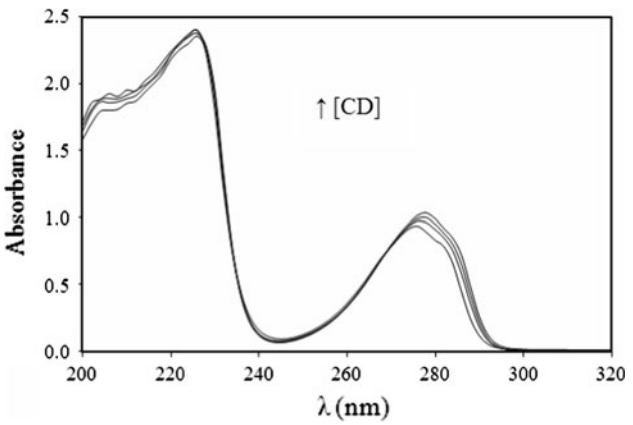

Fig. 1 UV–visible spectra of TY in the presence of increasing concentrations of β-CD

In addition, a spectrofluorimetric study of complexation in aqueous solution has been carried out. The interaction of TY involves an increase of fluorescence intensity, as can be seen in Fig. 3 for the complex with β-CD. This increase of fluorescence can be associated to a protection against collisional quenching, changes of polarity upon inclusion and also to a decrease of vibrational quenching due to an increased rigidity of the included molecule. The experimental data were fitted to the equation mentioned in the "Methods" section and the plots obtained are shown in Fig. 4. The binding constants were calculated with the slope and the intercept determined from the regression analysis of the experimental data ($r > 0.997$ in all the cases). The binding constants obtained for the complexes of TY with β-CD, Me-β-CD and HP-β-CD were 294 ± 63, 294 ± 36 and 265 ± 63 M^{-1}, respectively.

One of the main problems associated with the determination of complexation binding constants is the divergence of the results frequently obtained when the interactions are studied by different techniques [18]. For example, a recent nuclear magnetic resonance study of the complexation of olive oil biophenols with β-CD [19] reported binding constants of 497 and 355 M^{-1} for the complexes of TY and HTY, respectively, but other NMR study determined a binding constant of 93 M^{-1} for the complex of HTY with β-CD [20].

The binding constants obtained in the spectrophotometric and spectrofluorimetric studies of the complexes of TY with the different CD are in the same order of magnitude. Values of 220 M^{-1} [17] and 250 M^{-1} [21] reported in the literature for the complexes of β-CD with *p*-cresol, a compound structurally related to TY, are similar to those determined in the present study.

Although both techniques evidence complexation of TY with the CD assayed, it is important to mention that in the fluorescence study it was possible to employ higher CD/TY ratios, which contribute to make it more reliable than UV spectrophotometry. The reason was that in the UV spectrophotometric assay the absorbance did not increase

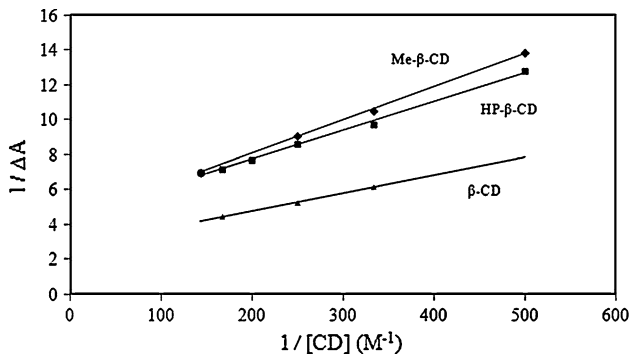

Fig. 2 Plot of the UV–visible spectrophotometric study of TY with β-CD, Me-β-CD and HP-β-CD

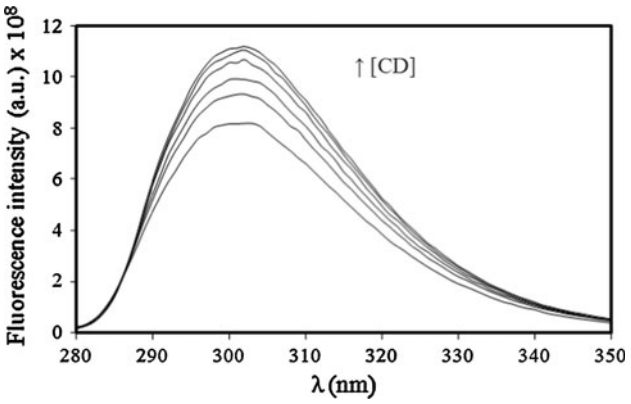

Fig. 3 Emission fluorescence spectra of TY in the presence of increasing concentrations of β-CD

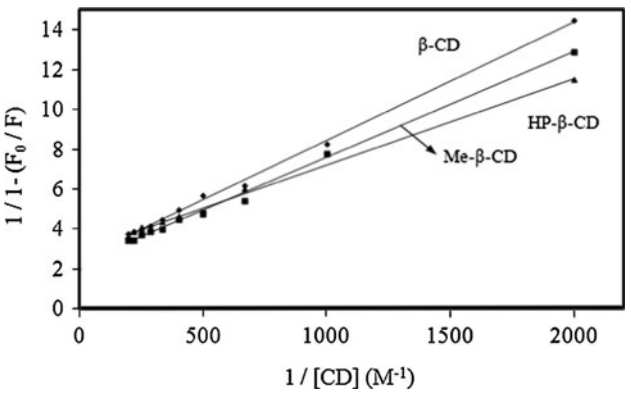

Fig. 4 Plot of the spectrofluorimetric study of TY with β-CD, Me-β-CD and HP-β-CD

further with higher CD concentrations. In addition, from the experimental point of view, it was more difficult to obtain good correlations by UV spectrophotometry because the sensitivity of the technique was lower due to the small changes of absorbance detected upon complexation. For the reasons stated above, fluorescence spectroscopy resulted more reliable for the determination of the apparent stability constants of these complexes.

The binding constants calculated for the three CD are in the same order of magnitude, probably because the hydrophilic nature of TY does not determine differences in its affinity for the three CD and its *para*-substitution does not involve steric problems for inclusion. The next step in our study will be the application of inclusion complex formation to increase the antioxidant properties of TY, as has been reported for the complex of HTY with β-CD [20].

Solid state complexation

Figure 5 shows the X-ray diffraction patterns and the DTA curves of the 1:1 TY–β-CD systems prepared. The systems prepared by coevaporation and kneading exhibit almost identical profiles which are different from that of the physical mixture. The latter presents the main peaks of TY at 10.9 and 21.9° 2θ, which are not present in the coevaporated and kneaded systems. In addition, the reflections of the cage form of β-CD (9.7, 12.7, 13.3 and 18.1° 2θ) are present in the profiles of pure β-CD and also in that of the physical mixture. However, the reflections of the columnar form of β-CD [22] (11.5 and 17.5° 2θ) are present in the coevaporated and kneaded systems.

The X-ray diffraction data together with the fact that the DTA curves of the TY–β-CD coevaporated and kneaded systems do not exhibit the melting endothermic peak of TY, which is present in the physical mixture, are indicative of the formation of a complex between TY and β-CD in the solid state.

The X-ray diffraction patterns and the DTA curves of the 1:1 systems prepared with HP-β-CD and Me-β-CD are shown in Figs. 6 and 7, respectively. Both CD present amorphous profiles characterised by the absence of reflections. The physical mixtures present the peaks corresponding to the crystalline structure of TY. However, these peaks disappeared from the X-ray diffraction profiles of the coevaporated and kneaded systems. The analysis by X-ray diffraction suggests the formation of amorphous complexes of TY with both Me-β-CD and HP-β-CD. The absence of the melting endothermic peaks of TY in the DTA curves of the kneaded and coevaporated systems with HP-β-CD supports the formation of the complex in the solid state. However, this technique was not adequate to evidence complexation with Me-β-CD, because it was not possible to detect the melting peak of TY in the physical mixture. An explanation could be that, occasionally, the formation of complexes in the physical mixture can take place upon heating in the DTA capsule.

Sorption in a β-CD polymer

The sorption of TY in an insoluble β-CD polymer is a rapid process. There is a fast increase of sorption during the first 10 min and the maximum loading is reached in approximately 20 min. The polymer presents a high swelling capacity which enables the expansion of its network and the diffusion of the solute.

The amount of TY retained in the polymeric structure was ca. 1 mg per gram of polymer.

Crosslinked CD polymers show specific sorption characteristics compared to those of epichlorohydrin linear dextrans and this effect can be explained by complex formation [23, 24]. The incorporation of CD to polymeric networks permits an efficient retention of aromatic contaminants in waste waters due to the inclusion phenomena [25].

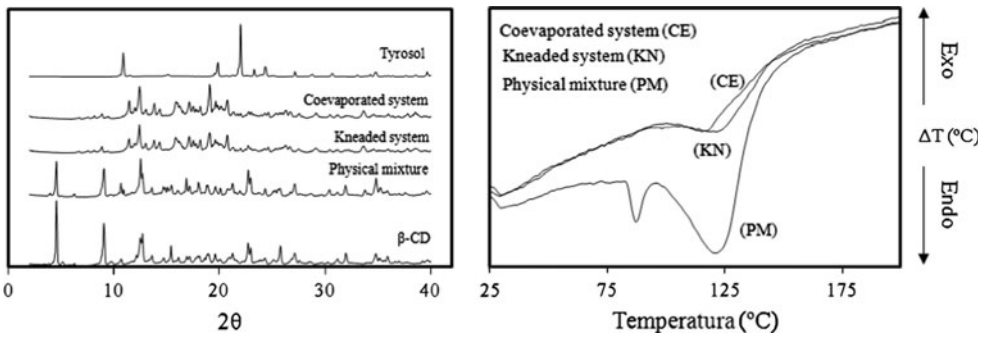

Fig. 5 X ray diffraction patterns and DTA curves of the 1:1 systems TY–β-CD

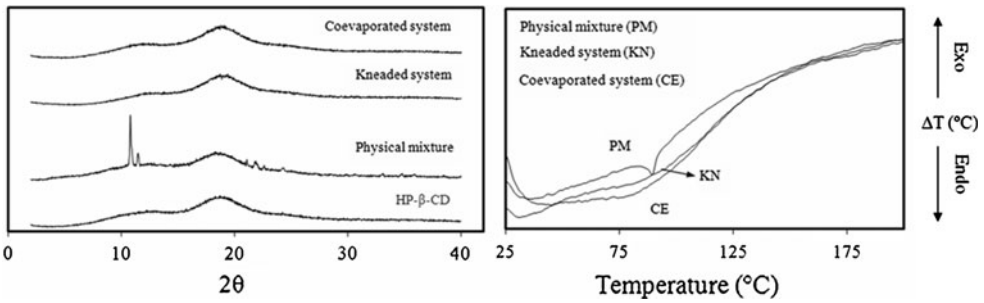

Fig. 6 X ray diffraction patterns and DTA curves of the 1:1 systems TY–HP-β-CD

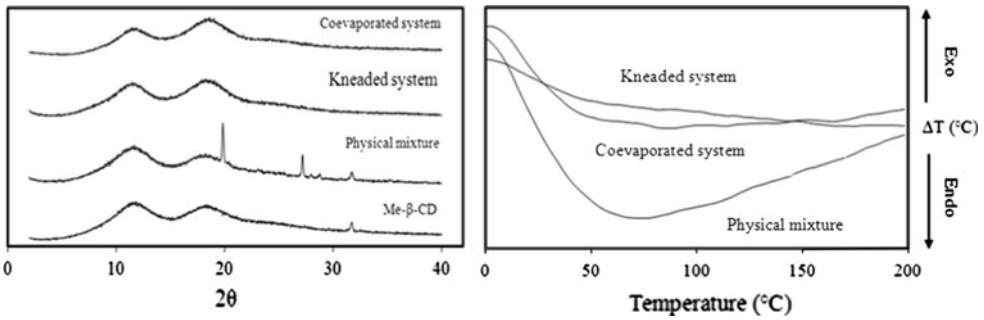

Fig. 7 X ray diffraction patterns and DTA curves of the 1:1 systems TY–Me-β-CD

The next step in our study will be the optimization of the retention of TY in CD polymers for the recovery of antioxidants from olive oil mill waste waters. In addition, these polymers can be applied to develop systems for controlled release of antioxidants.

Conclusion

The combination of UV spectrophotometry and spectrofluorimetry allows a reliable determination of the binding constants of the inclusion complexes of TY with β-CD and its methylated and hydroxypropylated derivatives. The interaction of TY with β-CD in the solid state leads to a crystalline complex, however, the complexes obtained with Me-β-CD and HP-β-CD were amorphous.

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