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Association of antioxidant monophenolic compounds with β-cyclodextrin-functionalized cellulose and starch substrates

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ABSTRACT

Polysaccharide substrates loaded with antioxidant and antimicrobial compounds, effectively protected by cyclodextrin moieties, can be a long-lasting solution to confer certain properties to fabrics, paper and other materials. β-Cyclodextrin was attached to α-cellulose, bleached pulp and starch by a two-step esterification with a tetracarboxylic acid. The resulting derivatives were characterized by spectroscopy, thermal degradation analysis and capability of phenolphthalein inclusion. The carriers, containing between 89 and 171 μmol of β-cyclodextrin per gram, were loaded with carvacrol, cuminaldehyde, cinnamaldehyde and hydroxytyrosol. From a stoichiometric addition, the percentage of compound retained ranged from 49% (hydroxytyrosol in pulp–cyclodextrin) to 92% (carvacrol in starch–cyclodextrin). Finally, the release rate to aqueous ethanol was measured over eight days and fitted to kinetic models. From the analysis of the mean dissolution time, it can be concluded that inserting β-cyclodextrin units enhanced the long-term holding of phenolic active compounds in carbohydrate matrices.

1. Introduction

Cyclodextrins are cyclic oligosaccharides with amazing properties, their hydrophobic cavity together with their water solubility being the most remarkable one (Szejtli, 1998). Alongside their very low toxicity and the abundance of hydroxyl groups in their structure, allowing a multitude of reactions, these molecules are highly attractive for applications in different fields such as pharmacy (Loftsson & Brewster, 2010), textiles (Valdes, Ramos, Beltran, & Garrigos, 2018), food chemistry (Tian et al., 2020), wastewater treatment (Cova, Murtinho, Pais, & Valente, 2018) and detergency (Valente & Söderman, 2014).

Beta-cyclodextrin (β-CD), the most used natural cyclodextrin, is composed by seven glucopyranose units and its cavity allows to host a wide variety of small molecules (Khatmi, Bezzina, & Humbel, 2020; Nilsson, Valente, Olofsson, Söderman, & Bonini, 2008). Many publications have dealt with the immobilization of β-CD onto fabrics, aiming to add functionalities to textile surfaces (Bhaskara-Amrit, Agrawal, & Warmoeskerken, 2011; Buschmann, Knittel, & Schollmeyer, 2001; Mihailiasa et al., 2016). This way, the guest molecules that confer these functionalities are protected from UV radiation, oxidation and fast release (Yoshii, Furuta, Fujwara, & Linko, 2003). Cyclodextrins can be bound onto wool, polyester and polyamides by intermolecular and/or ionic interactions, but their long-term immobilization onto glucopyranose-based polymers, such as cellulose and starch, likely requires the formation of covalent bonds (Bhaskara-Amrit et al., 2011). Commonly, studies on cellulose–cyclodextrin linkages are aimed towards functionalized cotton fabrics (Cabrales, Abidi, Hammond, & Hamood, 2012; Ibrahim, Abd El-Ghany, Eid, & Mabrouk, 2018). Even starch–cyclodextrin binding, scarcely described in the literature, has been proposed for textile sizing (Hebeish, Higazy, El-Shafei, & Sharaf, 2006).

While the possibilities of polysaccharide–β-CD derivatives for textiles have been widely studied, as described above, the potential of these combinations for the paper industry has been somewhat overlooked (Aguado, Murtinho, & Valente, 2019). Nonetheless, some important contributions must be mentioned. For instance, β-CD has been covalently bonded to Japanese washi paper (Furuta et al., 2006), and 1,4-butanediol diglycidyl ether has been tested as a crosslinking agent between β-CD and filter paper (De Bergamasco, Zanin, & De Moraes, 2007). Recently, Ares et al. (2019) functionalized β-CD with *N*-

Abbreviations: BTCA, 1,2,3,4-butanetetracarboxylic acid; SHPI, sodium hypophosphite; AE, association efficiency; CDEE, cyclodextrin entrapment enhancement; DF, dilution factor; MDT, mean dissolution time.

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(hydroxymethyl)acrylamide and grafted the resulting derivative to cellulose paper.

The most common ways to bind β-CD to a cellulose substrate involve either the previous functionalization of the CD with monochlorotriazinyl moieties (Ibrahim et al., 2018; Ibrahim, E-Zairy, & Eid, 2010) or the use of certain crosslinking agents, namely citric acid, 1,2,3,4-butanetetracarboxylic acid (BTCA) and epichlorohydrin (Dong et al., 2014; Liu, Luo, & Huang, 2016; Medronho et al., 2013).

In the present work, we have synthesized cellulose–β-CD and starch–β-CD substrates by covalent bonding with BTCA. Aiming to avoid excessive polymer–polymer linkages, the substrates were synthesized in a two-step esterification process. The functionalized polymers were characterized by thermogravimetric analysis, FTIR spectroscopy, phenolphthalein inclusion and NMR spectroscopy where possible. Cellulose–β-CD and starch–β-CD were associated to four active compounds: cuminaldehyde, cinnamaldehyde, carvacrol, and hydroxytyrosol. These compounds show antimicrobial, antioxidant and fragrance properties (Wishart et al., 2018). The hypothesis that the insertion of β-CD units would enhance the potential of cellulose and starch as carriers of phenolics was tested in two ways: determining the encapsulation efficiency from electronic absorption spectra, and measuring and evaluating the release kinetics of the different active compounds.

2. Materials and methods

2.1. Reagents

Hydroxytyrosol (98%), carvacrol (98%) and cuminaldehyde (97%) were acquired from Sigma-Aldrich. Cinnamaldehyde was supplied by BDH Chemicals. Some properties of these monophenolic compounds are summarized in Table 1.

β-Cyclodextrin (98%), ethanol (absolute, anhydrous), phenolphthalein and phosphate salts (NaH_2PO_4 and Na_2HPO_4) were purchased from Acros Organics, Carlo Erba Reagents, Honeywell and Merck, respectively. We received α-cellulose powder (average molecular weight 7.3×10^4 g mol^{-1}), BTCA (99%), sodium hypophosphite (SHPI, 99%) and sodium hydroxide (NaOH) from Sigma-Aldrich. Bleached eucalyptus kraft pulp (BEKP, Schopper-Riegler 35°) and industrial corn starch (27.3% amylose, 3.2×10^5 g mol^{-1}) were kindly supplied by The Navigator Company (Portugal). D_2O (99.8% D), NaOD (40% in D_2O) and 3-(trimethylsilyl) propionic-2,2,3,3-d4 acid sodium salt were obtained from Eurisotop.

All carbohydrate sources were dried at 50 °C for at least 72 h before use.

Table 1
Relevant properties of the active compounds used in this study. *MW*: molecular weight; s_w: aqueous solubility at 25 °C; P_{ow}: logarithm of the octanol/water partition coefficient.

Guest compound	MW (g mol^{-1})	s_w (g/L)	P_{ow}	Functions	References
Carvacrol	150.2	1.25	3.49	Antimicrobial, antioxidant, strong activator of TRPV3 channel	Kfoury et al., 2016 Wishart et al., 2018
Cuminaldehyde	148.2	0.24[a]	2.93	Antimicrobial, antioxidant, antidiabetic (α-glucosidase inhibitor)	Cui, Siva, & Lin, 2019 Wishart et al., 2018
Cinnamaldehyde	132.2	1.42	1.9	Antimicrobial, antioxidant, insecticide, corrosion inhibitor	Sharma et al., 2017 Wishart et al., 2018
Hydroxytyrosol	154.2	50	1.1	Antioxidant, anti-inflammatory, antimutagenic	López-García, López, Maya, & Fernández-Bolaños, 2010 Rietjens, Bast, De Vente, & Haenen, 2007

[a] This study. See Supplementary Material.

2.2. Synthesis of the polysaccharide–cyclodextrin substrates

β-CD (4 g) and BTCA (3 g) were dissolved in 50 mL of distilled water by stirring at 60 °C for 10 min. Then, SHPI (1.5 g), used as catalyst, was added. The obtained solution was poured onto a stainless steel surface, left until complete evaporation of the solvent, and placed inside an oven at 140 °C, for 10 min. The product of this solid phase esterification will be referred to as BTCA–β-CD (Scheme 1a). This is actually a mixture of compounds including mono- and polysubstituted β-CD, and even oligomers resulting from homopolymerization. Unreacted BTCA was removed by repeatedly washing with ethanol (96%, v/v). Afterwards, BTCA–β-CD was dissolved in water at 60 °C, adding again 1.5 g SHPI.

For the synthesis of cellulose–BTCA–β-CD (cellulose-CD) or BEKP–BTCA–β-CD (BEKP-CD), cellulose or BEKP (4 g) were suspended in the BTCA–β-CD solution under stirring. After 30 min, the suspension was filtered and the filtration cake was pre-dried at 120 °C for 15 min, followed by grafting, at 180 °C for 20 min (Scheme 1b). The modified fibers were washed with water.

To obtain starch–BTCA–β-CD, corn starch (4 g) was dispersed in the BTCA–β-CD solution. After solvent evaporation, dry grafting was carried out at 160 °C for 5 and 10 min, resulting in starch-CD_5 and starch-CD_{10}, respectively (Scheme 1b). The functionalized polymer was washed with an ethanol-water mixture (1:1, v/v).

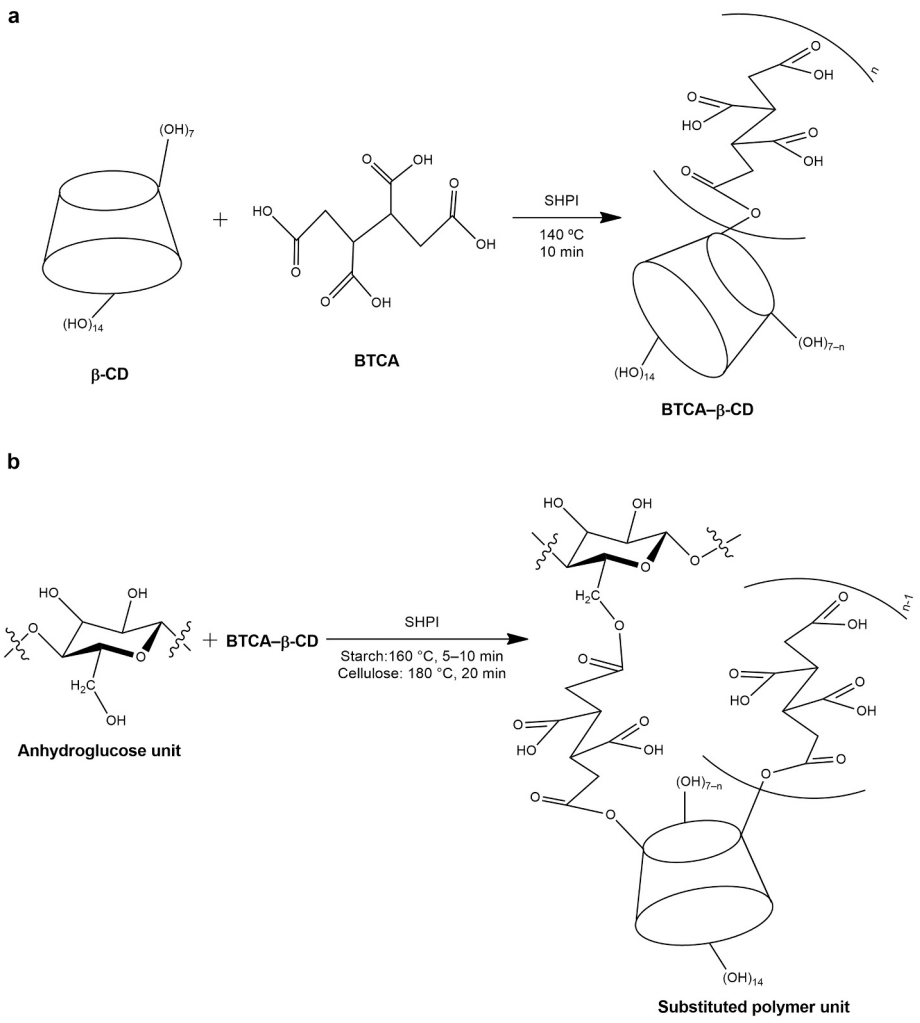
2.3. Characterization of the substrates

The ester bonds of BTCA–β-CD, cellulose–CD and starch–CD_{10} were confirmed by ATR-FTIR spectroscopy, by using an Agilent Cary 630 FTIR spectrometer. A previous washing step with NaOH 0.1 M provided the free carboxyl groups with counterions so that the band for ester linkages could be assigned separately (Medronho et al., 2013). The same alkaline treatment was performed on samples to be analyzed by the phenolphthalein inclusion test (De Bergamasco et al., 2007; Goel & Nene, 1995). Electronic absorption spectra were recorded by means of a Shimadzu spectrophotometer, model UV-2450.

Thermogravimetric analysis was performed on a thermomicrobalance TG 209 F3 Tarsus, from Netzsch Instruments. The heating rate was set at 10 °C min^{-1}, from 40 °C to 600 °C, under flow of nitrogen (20 mL min^{-1}). It should be noted that, for the sake of correct comparison, the tests for unmodified starch, cellulose and pulp implied a previous treatment of suspension in hot water, drying, being placed in an oven and washing, in prevision of possible changes in the supramolecular structure of polysaccharides (decrystallization, retrogradation, fibril collapse). This way, the process for their CD-containing derivatives was imitated in each case, but without the addition of BTCA–β-CD.

^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra from soluble samples were collected from a Bruker Biospin GmbH spectrometer, using D_2O as solvent and 3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid sodium salt as internal reference. The dissolution of starch-CD, BTCA–β-CD and starch was achieved with a small addition of NaOD. The cellulose derivative was insoluble.

Micrographs of starch granules, starch-CD_{10}, cellulose and cellulose-CD were obtained by scanning electron microscopy (SEM). Backscattered-electron imaging was carried out by means of a tabletop microscope TM4000 Plus from Hitachi.



Scheme 1. Key esterification reactions involved in the reaction of starch or cellulose with β-CD, including the synthesis of BTCA–β-CD (a) and the subsequent linkage to a glucopyranose unit of a polysaccharide (b). It is worth clarifying that, although not intended, intramolecular crosslinking (a) and linkages with a second carbohydrate chain (b) cannot be ruled out.

2.4. Binding between active compounds and cyclodextrin-containing polymers

The interaction of carvacrol, cuminaldehyde, hydroxytyrosol and cinnamaldehyde with β-CD or its derivatives has been reported in aqueous solution in a 1:1 stoichiometry (Cui et al., 2019; Kfoury et al., 2016; López-García et al., 2010). Thus, to assess the binding of the four active compounds on the CD-containing polymers, the number of available CD moieties in starch–CD_{10}, BEKP–CD and cellulose–CD (μmol per gram), as determined by the phenolphthalein method, was initially taken into account to calculate the stoichiometric weight.

The required weight of each monophenolic compound was dispersed or dissolved in Milli-Q water, applying ultrasound (35 kHz) until complete homogeneisation. While cuminaldehyde and carvacrol could not be fully dissolved, they formed stable emulsions that did not settle down when undisturbed.

In each experiment, 150 mg of substrate were suspended in 5 mL of the stock solution or emulsion. The mixtures were shaken at 25 °C for 48 h in a ZWY-103B shaking incubator from Labwit. The free liquid was separated by centrifugation at 900*g* for 30 min in an IEC Centra-3C centrifuge. A sample of the guest-loaded material was recovered and freeze-dried by means of a Labconco Freeze Dryer Freezone 4.5.

The quantification of the active compounds in the free liquid was carried out by UV spectroscopy before and after their interaction with the polymer. Then, the association efficiency (*AE*) was computed by using the following equation:

$$AE(\%) = 100 - 100\frac{A_f\ DF_f}{A_0\ DF_0} \quad (1)$$

where A_f and A_0 are the absorbance values of the free solution after complexation and the loading solution, respectively, while DF_f and DF_0 are their dilution factors.

Here, "association" comprises adsorption to the solid matrix and complexation, not only with the cylodextrin moieties but also to the polysaccharide backbone. In order to obtain the proportion of guest that was entrapped in the CD group, the experiment was performed for the native polymers as well, weighting the same amounts of substrate and active compound. Noting, once again, that native polymers had undergone the same thermal treatment as their derivatives, the cyclodextrin-entrapment enhancement (*CDEE*) can be expressed as:

$$CDEE = AE_{P-CD} - AE_P \quad (2)$$

where AE_{P-CD} and AE_P are the association efficiency of the modified and native polymers, respectively.

Clearly, Eq. (1) cannot be applied to soluble systems, as in the case of β-CD bound to these monophenolic compounds. Hence, the entrapment efficiency (*EE*) of β-CD and BTCA–β-CD was estimated by following the method of Tao, Hill, Peng, and Gomes (2014) for inclusion complexes of

β-CD and thymol.

2.5. Release kinetics in water

An important question that must be addressed is how much and how fast the active compound can be released from the CD-containing polymer. Release experiments were performed in such a way that all possible ways of chemical degradation, namely photoinduced oxidation and hydration of aldehydes in acidic or basic aqueous media, were avoided. 250 mg of substrate were loaded with excess active compound (200 μmol) in 10 mL of a phosphate buffer solution (100 mM, pH 7.1).

The loaded substrates were suspended without agitation in 10 mL of aqueous ethanol (10%, v/v), also known as food simulant A (European Commission, 2017). They were precipitated by mild centrifugation (900*g*, 10 min) and the first sample was taken from the supernatant immediately after this step. Then, tubes were covered with aluminum foil and gently shaken (120 rpm) in vertical position, not disturbing the polysaccharides at the bottom. All samples were analyzed by UV spectrophotometry.

The release rate was evaluated by fitting to different rate equations. Pseudo-first-order (Eq. (3)) and pseudo-second-order (Eq. (4)) equations may be applicable from the suspension in aqueous alcohol to the equilibrium, and do not need the establishment of initial and border conditions:

$$c_t/c_\infty = 1 - e^{-k_1 t} \tag{3}$$

$$c_t = \frac{{c_\infty}^2\, k_2\, t}{c_\infty\, k_2\, t + 1} \tag{4}$$

where k_1 and k_2 are the corresponding rate constants and c_t and c_∞ are the cumulative active compound release at time t and at equilibrium, respectively.

Furthermore, for assessing the release mechanism for short range times, the power law equation has been applied (Korsmeyer, Gurny, Doelker, Buri, & Peppas, 1983):

$$c_t/c_\infty = k_d\, t^n \;\forall\; c_t/c_\infty \lesssim 0.6 \tag{5}$$

where k_d and n are fitting constants, being n related with the release mechanism (Costa & Sousa Lobo, 2001). The effect of the retarding efficiency of the polymeric matrix towards the essential oil release has been characterized by computation of the mean dissolution time (MDT), by using the equation (Möckel & Lippold, 1993):

$$MDT = \frac{n}{n+1}\, {k_d}^{-1/n} \tag{6}$$

where k_d and n are those calculated by Eq. (5).

3. Results and discussion

3.1. Qualitative analysis of cyclodextrin-bound polysaccharides

The presence of ester bonds was confirmed by ATR-FTIR spectra, as shown in Fig. 1. The band at 1718 cm^{-1}, with no appreciable absorption found for any native carbohydrate compound but with noticeable peaks for the BTCA-functionalized derivatives, is associated with C═O stretching in ester bonds. The absorption band at 1560 cm^{-1}, likewise, corresponds to C═O stretching in carboxylate (—COO^-) functional groups. Because BTCA–β-CD was not treated with NaOH, carboxyl groups are mostly protonated (—COOH) and their vibration band is overlapped with that of the ester bonds. Furthermore, since the functionalization of cellulose and starch was not carried out with further additions of BTCA but with BTCA–β-CD, absorption bands assigned to ester bonds confirm not only the polymer-BTCA linkages but also, indirectly, the presence of CD moieties.

TG and DTG curves, displayed in Fig. 2 (no DTG curve is shown for

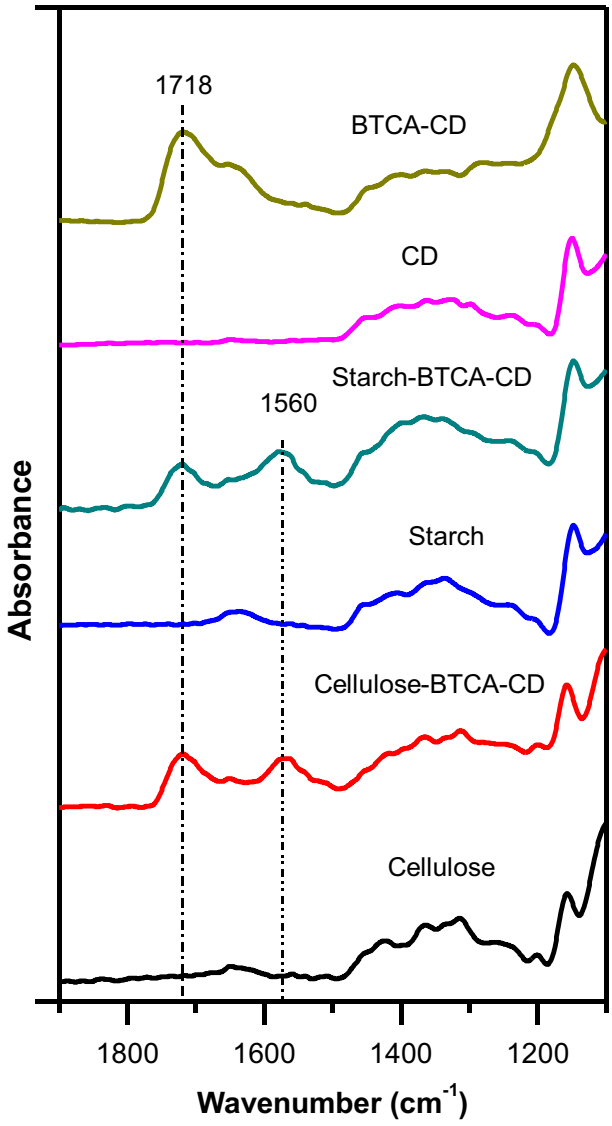


Fig. 1. ATR-FTIR spectra of cellulose, cellulose–BTCA–β-CD, starch, starch–BTCA–β-CD, β-CD, and BTCA–β-CD. Bands assigned to ester bonds (1718 cm^{-1}) and carboxylate groups (1560 cm^{-1}) are highlighted.

BEKP, since it is qualitatively identical to that of cellulose), also supports the crosslinking with CD groups. The pyrolysis of the derivatives under inert atmosphere starts after the water loss and at lower temperatures than the thermal degradation of any of the unmodified carbohydrates. Likewise, the temperature corresponding to the minimum in the DTG curves (T_{max}) is always lower for the ester derivatives: 310 °C < 347 °C (cellulose, Fig. 2e); 280–285 °C < 309 °C (starch, Fig. 3b). Medronho et al. (2013) attributed this phenomenon to an increase in the disorder of the molecular packing. Moreover, an appreciable amount of char remains at 600 °C for cellulose–CD (Fig. 2a), for starch–CD_{10} (Fig. 2c) and, to a lower extent, for BEKP–CD (Fig. 2b). It is suggested that the abundance of new bonds, including double bonds, confers certain thermal stability to this residue.

Electronic micrographs of native and functionalized substrates are presented in Fig. 3. Native starch particles encompass spherical granules of diameter 1–10 μm, lenticular granules and small quasi-polyhedral aggregates. This semicrystalline structure, related to intra- and intermolecular hydrogen bonds between amylose and amylopectin chains, was partially disrupted by the reaction and the thermal treatment (Fig. 3b), even though the material was not dissolved at any point.

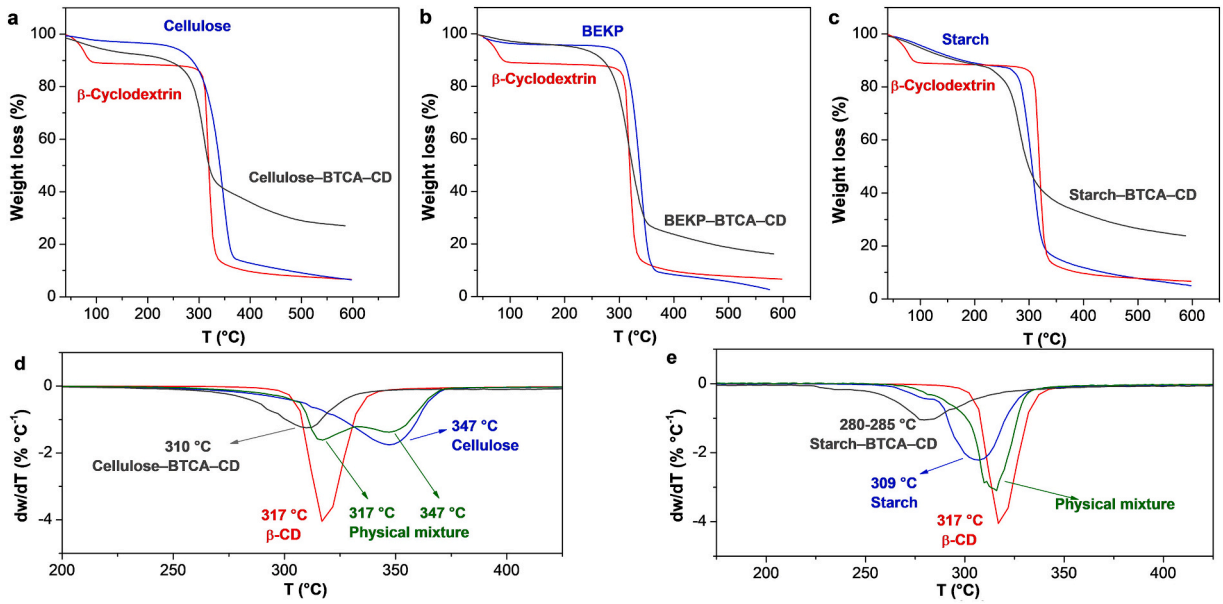


Fig. 2. Thermal degradation curves (a, b, c) and DTG curves (d, e) of cellulose, BEKP, starch, their derivatives with β-CD, unmodified β-CD, and physical mixtures of native polysaccharides with β-CD.

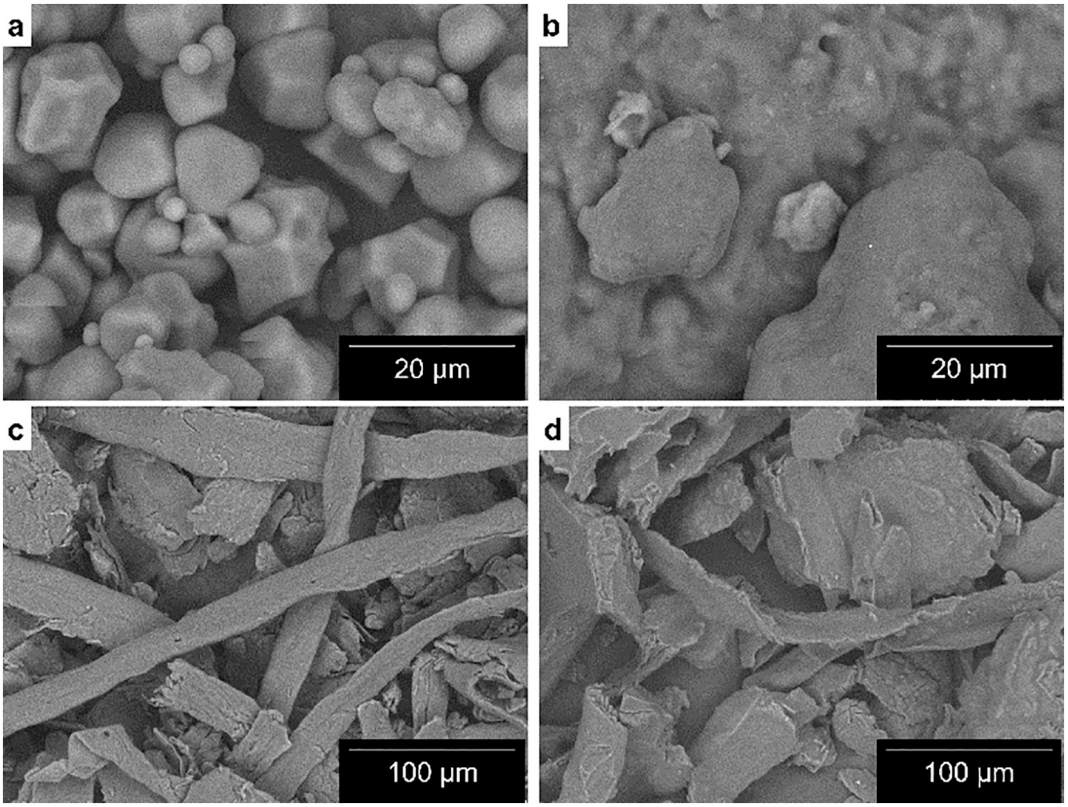


Fig. 3. SEM images of: a) starch; b) starch-CD_{10}; c) cellulose; d) cellulose-CD.

Cellulose, on the other hand, underwent less noticeable changes (Fig. 3c and d). The fiber structure was kept after esterification, but aggregates emerged as a result of inter-fiber collapse. Moreover, external fibrillation and damage on the fiber wall to a certain extent can be appreciated.

3.2. Quantification of cyclodextrin and tetracarboxylic acid units

Fig. 4 displays the ^{1}H NMR spectra of the samples that could be dissolved. The shielding of protons in anhydroglucose units was evidenced by an upfield displacement of all signals. The large shift upfield of the H-1 doublet of β-CD (up to −0.3 ppm) hinders its quantification by this method, at least for starch–CD_{10}, due to the proximity of this signal to that of HDO. For starch–CD_5, the degree of substitution could be

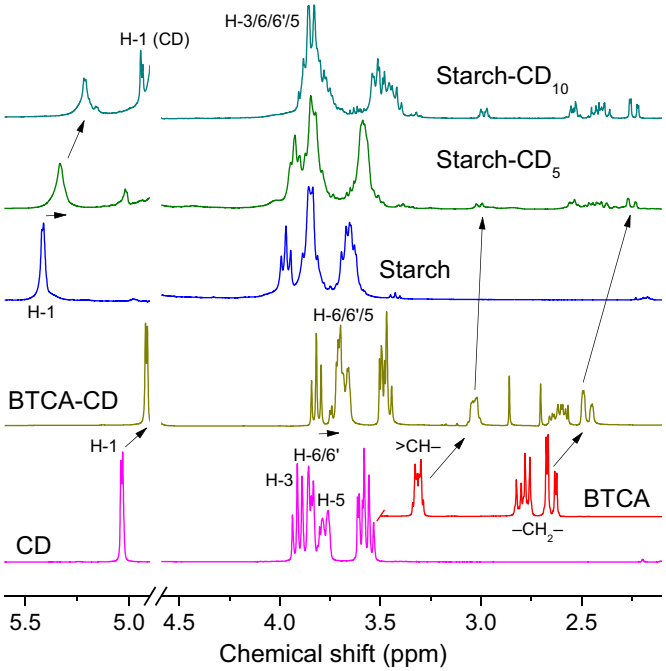


Fig. 4. Stacked ^{1}H NMR spectra of (a) starch-CD$_5$, (b) starch, (c) BTCA–β-CD, (d) BTCA, and (e) β-CD.

estimated as 0.027, corresponding to 131 μmol of CD per gram of polymer.

In any case, with the area assigned to the >CH– or –CH$_2$– hydrogens of BTCA, it was possible to quantify reliably the polycarboxylic moieties in BTCA–CD, yielding a degree of substitution of 2.4. Methylene hydrogens underwent a greater displacement (up to −0.41 ppm) than methanetriyl hydrogens. Moreover, among the non-anomeric protons of β-CD, the doublet for H-6,6′ was particularly shifted upfield, overlapping the H-5 triplet.

^{13}C NMR spectrum of BTCA–CD (100 MHz, D2O): δ(ppm) = 37.3, 41.2 (–CH$_2$–, BTCA); 48.6, 50.6 (>CH–, BTCA); 61.8 (C-6); 73.3, 74.5, 75.3, 83.2 (C-2, C-3, C-4, C-5); 104.2 (C-1); 182.6 (C═O, ester); 183.7 (C═O, acid). ^{13}C NMR spectrum of starch–CD$_{10}$ (100 MHz, D2O): δ(ppm) = 37.5, 41.4 (–CH$_2$–, BTCA); 48.8, 50.8 (>CH–, BTCA); 61.8–62.0 (C-6, starch and CD); 72.9–83.4 (C-2, C-3, C-4, C-5, starch and CD); 103.0 (C-1, starch); 104.4 (C-1, CD); 182.8 (C═O, ester); 183.9 (C═O, acid). It should be noted that the signal for C-6 of β-CD was displaced up to +5.8 ppm as compared to the native saccharide. Most likely, esterification took place preferentially between OH-6 and terminal carboxyl groups.

The weight gain and the suppression of absorbance at 557 nm are shown in Table 2 for all samples. The micromoles of CD moieties per gram of substrate, n_{CD}, are estimated from Eq. (7) for the weight gain method and from Eq. (8) from the phenolphthalein inclusion method:

Table 2
Measurements of the number of CD moieties bound to polysaccharides.

Sample	Weight method		Phenolphthalein method	
	Gain (%)	n_{CD} (μmol CD g^{-1})	$\Delta A_{blank} - \Delta A_{sample}$	n_{CD} (μmol CD g^{-1})
Cellulose-CD	39	171	0.314	137
BEKP-CD	17	89	0.151	66
Starch-CD$_5$	22	110	0.251	110
Starch-CD$_{10}$	35	158	0.318	139

$$n_{CD}\ (\mu\text{mol g}^{-1}) = 10^{6}\frac{Gain(\%)}{1653\ (Gain(\%) + 100\,)} \quad (7)$$

$$\Delta A_{blank} - \Delta A_{sample} = 10^{-6}\ n_{CD}\ (\mu\text{mol g}^{-1})\ \varepsilon'\frac{m(\text{g})}{V(\text{L})} \quad (8)$$

where 1653 corresponds to the molecular weight (in g mol^{-1}) of β-CD with the average number of BTCA substituents (2.4), ΔA_{blank} (negative number) is the change of absorbance of phenolphthalein in a cellulose or starch suspension, ΔA_{sample} (also negative) is the change of absorbance of phenolphthalein in a CD-substituted derivative, and ε' (L mol^{-1}) is determined from a calibration curve (see Supplementary Material, Fig. S1).

Typically, the weight gain informs of the total number of CD units attached, while phenolphthalein tests allow for an estimation of the available sites for inclusion. While the latter, as obviously expected, was generally lower than the former, both resulted in 110 μmol g^{-1} for starch–CD$_5$ (Table 2), whereas the ^{1}H NMR spectrum allowed for a calculation of 131 μmol g^{-1}. Indeed, the loss of a part of this derivative during washing, being more water-soluble or more easily dispersible than the others, implied an underestimation by the weight gain method. It is worth remarking that the absence of heteroatoms, the fact that cyclodextrins share empirical formula with both starch and cellulose, the insolubility of some samples and the displacement of signals in ^{1}H NMR spectra hinder the use of more direct methods (De Bergamasco et al., 2007). All things considered, the phenolphthalein method seemed to provide the most consistent and uniform measurement, and thus it was used to load each substrate in a 1:1 stoichiometry with each of the monophenolic active compounds.

On a side note, the higher reactivity of starch was evident, as starch–CD$_{10}$ could attain roughly the same number of CD moieties that cellulose–CD, with shorter grafting time and lower temperature.

3.3. Efficiencies of active compound loading

The compliance of aqueous solutions of the active compounds with the Beer-Lambert law is shown in Fig. S2 (Supplementary Material). The molar extinction coefficients were estimated, being equal to 2059 (±26), 2928 (±47), 5827 (±26) and 23,252 (±330) mol^{-1} dm^3 cm^{-1}, for carvacrol, hydroxytyrosol, cuminaldehyde and cinnamaldehyde, respectively. Likewise, Fig. S3 displays the electronic absorption spectra in the near UV region of the solutions, before and after loading, of the active compounds onto and/or into the modified substrates. The resulting AE values (Eq. (1)) are shown in Table 3, along with the EE calculated for β-CD and BTCA–β-CD.

Despite their structural similarities, their main association mechanisms with aromatic compounds are completely different. Cellulose is known to be a good adsorbent for substituted aromatics, not only because of its ubiquous interaction with lignin, but also because this adsorption has been deeply studied (Da Silva Ferez, Ruggiero, Morais, Machado, & Mazea, 2004). However, starch not only adsorbs, but also forms inclusion complexes. The helical structure of amylose chains forms a relatively hydrophobic cavity capable to form complexes with lipophilic compounds (Lebail, Buleon, Shiftan, & Marchessault, 2000).

Cellulosic fibers were found out to be excellent adsorbents for cuminaldehyde, whereas their association with cinnamaldehyde was very poor. Likely, the isopropyl group of the former notably contributed to the adsorption energy onto cellulose and, perhaps more importantly, contributed to cuminaldehyde's hydrophobic character. It should be noted that the compounds whose P_{ow} is high (Table 1), *i.e.*, both cuminaldehyde and carvacrol, became more associated with cellulose, pulp and starch in an aqueous medium than the least lipophilic ones (hydroxytyrosol and cinnamaldehyde).

Again, regarding the enhancement of the association efficiency achieved by cyclodextrins, this was greater for cuminaldehyde and

Table 3
Entrapment efficiencies (EE) of phenolic compounds in β-CD and BTCA–β-CD (freeze-drying method), association efficiencies (AE) of phenolic compounds in cellulose and starch, AE in the cellulose–CD and starch–CD derivatives, and the resulting enhancement (CDEE).

Guest active compound	EE(%) in cyclodextrins		AE (%) in native substrates			AE (%) in modified substrates			CDEE (%)		
	β-CD	BTCA-β-CD	Cellulose	Starch	BEKP	Cellulose–CD	Starch–CD	BEKP–CD	Cellulose–CD	Starch–CD	BEKP–CD
Carvacrol	86.0	89.1	50.4	56.7	38.9	83.3	92.2	60.9	32.9	35.5	22
Cuminaldehyde	84.2	88.0	69.6	60.0	66.7	87.0	91.3	82.0	17.9	31.6	15.3
Cinnamaldehyde	67.5	62.4	41.5	34.1	44.0	68.1	69.9	60.7	26.6	35.8	16.7
Hydroxytyrosol	65.6	80.8	33.4	40.1	38.1	56.0	65.7	49.2	22.6	25.6	11.1

carvacrol (Table 3). Furthermore, this enhancement was consistently higher for the starch derivatives, probably due to the ease of diffusion through the starch granules, as compared to fibers, to reach the internal β-CD moieties. The free carboxyl groups seemed to play an important role as well, especially for hydroxytyrosol, whose entrapment efficiency was clearly higher with BTCA–β-CD than with β-CD.

3.4. Modeling of release kinetics

The evolution of the cumulative release of active compounds to the aqueous-alcoholic medium is displayed in Fig. 5. As a general approach, it can be seen that a high percentage of the active substance is freed in the first couple of hours, indicating a fast release. However, the equilibrium (*i.e.*, $C_t/C_\infty \approx 0.8$) is reached at different times depending on the active substance. Thus, while for carvacrol and cuminaldehyde it takes *ca.* 2 h to reach 80% of the cumulative release, for cinnamaldehyde and hydroxytyrosol it lasts longer than 37 h. This shows that the hydrophobic/hydrophilic character of these active substances plays an important role on the release process. In fact, the first two compounds have hydrophobic character, as shown by their high water-octanol partition coefficient (Table 1). On the other hand, cinnamaldehyde and hydroxytyrosol have low hydrophobic properties (Rietjens et al., 2007; Sharma et al., 2017).

To evaluate the release kinetics, pseudo-first order (Eq. (3)) and pseudo-second order (Eq. (4)) models have been applied; the corresponding fitting parameters are shown in Table 4. Generally, the relase follows a pseudo-second order model, indicative of a process dependent not only of the concentration gradient of the active substance but also due to active compound-polymeric matrix interaction. However, it is not possible to conclude whether that interaction occurs with the CD units or with the polymer backbone (cellulose or starch). Comparing the release rate constants, k_2, for the polymer with and without CD, it can be concluded, as a general trend, that k_2 decreases for the functionalized polymer. Such decrease is statistically more evident for the most hydrophobic active substances, suggesting a higher interaction with the CD, *via* supramolecular hydrophobic interactions (Saokham, Muankaew, Jansook, & Loftsson, 2018).

A deeper assessment on the release mechanism is obtained by using the power law equation. The fitting of this equation to the experimental data, for short-range times, indicates a pseudo-Fickian mechanism (Sitta et al., 2014). This evidence might suggest that the initial release is mainly due to the active substance sorbed by the polymeric matrix.

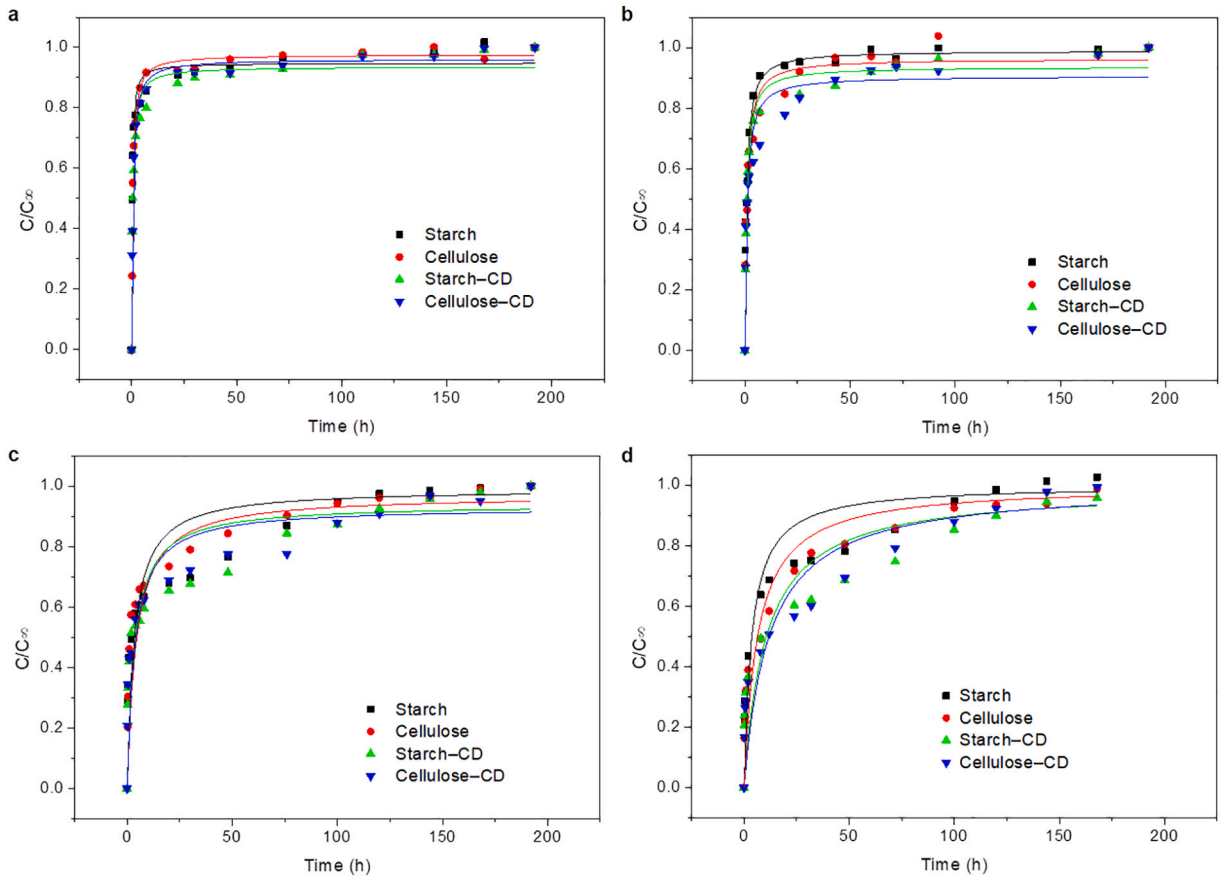


Fig. 5. Release curves of carvacrol (a), cuminaldehyde (b), cinnamaldehyde (c) and hydroxytyrosol (d) in water, from native and modified substrates.

Table 4
Fitting parameters and correlation coefficients for each of the substrates and active compounds.

		Eq. (3)		Eq. (4)		Eq. (5)			MDT/h
Guest	Substrate	k_1/h^{-1}	R^2	$k_2/M^{-1}\ h^{-1}$	R^2	k_d/h^{-n}	n	R^2	
Carvacrol	Cellulose	1.1 ± 0.1	0.944	1.8 ± 0.2	0.984	0.61 ± 0.05	0.4 ± 0.1	0.912	1.7
	Cellulose–CD	0.9 ± 0.1	0.926	1.4 ± 0.1	0.981	0.59	0.38 ± 0.04	0.987	1.1
	Starch	1.9 ± 0.3	0.853	3.0 ± 0.4	0.948	0.74	0.27 ± 0.04	0.995	0.7
	Starch–CD	1.0 ± 0.1	0.826	1.5 ± 0.2	0.939	0.59	0.27	0.998	1.5
Cuminaldehyde	Cellulose	0.58 ± 0.09	0.942	1.0 ± 0.1	0.962	0.50	0.29 ± 0.04	0.963	2.4
	Cellulose–CD	0.45 ± 0.09	0.740	0.7 ± 0.1	0.892	0.46	0.22 ± 0.03	0.968	6.2
	Starch	0.67 ± 0.07	0.942	1.2 ± 0.1	0.982	0.52	0.36	0.974	1.6
	Starch–CD	0.60 ± 0.09	0.878	1.0 ± 0.1	0.960	0.49	0.34 ± 0.03	0.987	2.1
Cinnamaldehyde	Cellulose	0.52 ± 0.12	0.850	0.8 ± 0.2	0.936	0.41 ± 0.03	0.34 ± 0.06	0.949	3.5
	Cellulose–CD	0.40 ± 0.11	0.793	0.7 ± 0.2	0.889	0.38	0.25 ± 0.03	0.972	9.6
	Starch	0.45 ± 0.14	0.744	0.8 ± 0.2	0.854	0.42	0.25	0.997	6.4
	Starch–CD	0.49 ± 0.16	0.715	0.80 ± 0.27	0.833	0.40	0.20 ± 0.02	0.979	16.3
Hydroxytyrosol	Cellulose	0.11 ± 0.03	0.863	0.25 ± 0.07	0.928	0.29 ± 0.02	0.28 ± 0.03	0.976	18.2
	Cellulose–CD	0.05 ± 0.02	0.785	0.11 ± 0.04	0.854	0.28	0.24 ± 0.02	0.984	38.9
	Starch	0.39 ± 0.12	0.841	0.5 ± 0.1	0.941	0.34	0.30	0.995	8.4
	Starch–CD	0.32 ± 0.13	0.773	0.4 ± 0.2	0.854	0.30	0.24 ± 0.02	0.995	29.2

Hence, the release is driven by diffusion, while at long-range times the release occurs from active substance–CD complexes. Finally, MDT values (Eq. (6)) were computed and evaluated (Costa, Albuquerque, Queiroz, & Valente, 2019). It can be concluded that, with the exception of the carvacrol/cellulose-based matrix, the functionalization of CD is a key factor, contributing to a two-fold or three-fold increase of MDT, reaching the maximum value of 39 h (for hydroxytyrosol/cellulose–CD).

4. Conclusions

Cellulose, kraft pulp and starch were successfully functionalized with β-cyclodextrin (β-CD) in a two-step process, preventing an excessive increase of the molecular weight that could affect their usability in paper, cosmetic and textile industries. In the first step, β-CD was esterified with 1,2,3,4-butanetetracarboxylic acid (BTCA), yielding an average degree of substitution of 2.4 as evaluated by ^{1}H NMR spectra. In the second step, the polymers were crosslinked with the β-CD–BTCA derivative in solid state, reaching incorporations of β-CD close to 170 μmol g^{-1} for starch (160 °C, 10 min) and cellulose (180 °C, 20 min). While certain degree of crosslinking between polymer chains was unavoidable, due to the polysubstitution of β-CD in the first step, only slight macrostructural changes were observed.

Association of these substrates with cuminaldehyde and carvacrol, with a higher lipophilic character, was more successful than with hydroxytyrosol and cinnamaldehyde. Besides increasing the association efficiency, the insertion of cyclodextrins slowed down the release rate of guest compounds to ethanol-water (1:9) media, as shown by lower mean dissolution times.

CRediT authorship contribution statement

Roberto Aguado: Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Dina Murtinho:** Conceptualization, Validation, Investigation, Writing – review & editing, Formal analysis, Resources, Project administration. **Artur J.M. Valente:** Conceptualization, Methodology, Writing – review & editing, Formal analysis, Resources, Supervision, Project administration, Funding acquisition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.carbpol.2021.118189.

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