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RESEARCH ARTICLE

# Evaluation of antibacterial activity of caffeic acid encapsulated by β-cyclodextrins

Eva Pinho[1,2], Graça Soares[2], and Mariana Henriques[1]

[1]CEB, Centre for Biological Engineering, LIBRO – Laboratório De Investigacão Em Biofilmes Rosário Oliveira, University of Minho, Campus Gualtar, Braga, Portugal and [2]Centre for Textile Science and Technology (2C2T), University of Minho, Azurém Campus, Guimarães, Portugal

**Abstract**

*Context*: Caffeic acid is described as antibacterial, but this bioactive molecule has some issues regarding solubility and stability to environmental stress. Thus, encapsulation devices are required. *Objective*: The aim of this work was to study the effect of the caffeic acid encapsulation by cyclodextrins on its antibacterial activity. *Materials and methods:* The interactions between the caffeic acid and three cyclodextrins (β-cyclodextrin (βCD), 2-hydroxypropyl-β-cyclodextrin (HPβCD) and methyl-β-cyclodextrin were study. *Results and discussion*: The formation of an aqueous soluble inclusion complex was confirmed for βCD and HPβCD with a 1:1 stoichiometry. The βCD/caffeic acid complex showed higher stability than HPβCD/caffeic acid. Caffeic acid antibacterial activity was similar at pH 3 and pH 5 against the three bacteria (*K. pneumoniae, S. epidermidis* and S. *aureus*). *Conclusions*: The antibacterial activity of the inclusion complexes was described here for the first time and it was shown that the caffeic acid activity was remarkably enhanced by the cyclodextrins encapsulation.

**Keywords**

*Staphylococcus aureus*, inclusion complex, phenolic acid, MIC, wound infection

**History**

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## Introduction

Wounds allow the microorganism deposition and growth, causing skin and soft tissues infections and, consequently, a delay on the healing process. Over the years, antibiotics have been indiscriminately used for the treatment of those infections and aggravated by extended therapies, leading to the development of microbial resistance (Giedraitienė et al., 2011). Thus, antibiotics had been losing their capacity against pathogens and new therapies to control multiresistant bacteria are hard to find. Therefore, natural antibacterial agents have become an alternative to overcome this issue (Saleem et al., 2010).

Caffeic acid (3,4-dihydroxycinnamic acid) is a simple phenolic acid derived from the hydroxycinnamic acid, with some interesting biological properties, such as antibacterial, fungicide and antioxidant, being the latter the most studied (Chen and Ho, 1997; Amorati et al., 2006; Gülçin, 2006; Zhang et al., 2009; Božič et al., 2012).The biological activities of this phenolic acid are related to its electrical charge. In solution, caffeic acid, as well as the other phenolic compounds, lies in equilibrium between the protonated and deprotonated form, according to the environmental pH. At lower pH (2–3.5), the molecule is neutral, and at higher pH, the caffeic acid assumes a charged form (Figure 1) (Zhang et al., 2009). Some authors (Herald and Davidson 1983; Wen et al., 2003) reported that the antibacterial activity of this phenolic acid increase as the pH decrease (pH range: 4.5–7). This property can be compromised by caffeic acid's sensibility to oxidation and acidic environments, as well by its poor solubility in water (Virués et al., 2012).

The improvement of physical, chemical and biological properties of natural bioactive molecules can be accomplished by their encapsulation within cyclodextrin (CD). Moreover, CDs have been described as safe to humans and approved by Food and Drug Administration (FDA) (Pinho et al., 2014). These cyclic oligosaccharides present a hydrophilic external surface and hydrophobic cavity, which make them capable of complex with both hydrophilic and hydrophobic molecules. CDs interact with the bioactive molecules forming an inclusion complex (IC). The complex's stability results from the perfect physical fit between the CDs cavity and the molecule and, also, from interactions established between the two molecules (Buschmann and Schollmeyer, 2002). All these factors contribute to a favourable thermodynamic system that can be characterised by the enthalpy ($\Delta H$), entropy ($\Delta S$) and Gibbs free energy ($\Delta G$) (Hirose, 2001). Moreover, the pH and the solvents are, also, crucial for the efficiency of the complexation process between organic compounds and CDs (Stalin and Rajendiran, 2006; Sankaranarayanan et al., 2010; Pinho et al., 2014). In nature, it is possible to find three native CDs (α, β and γ), but a wide range of modifications have been carried out on native CDs to improve their properties. βCD has been the most applied in industry, as well as its derivatives (Pinho et al., 2014). In the present work, two derivatives were chosen: HPβCD and MβCD. Theses CDs have higher solubility than the βCD (500 and 750 $g.L^{-1}$ at room

Address for correspondence: Eva Pinho, CEB, Centre for Biological Engineering, LIBRO – Laboratório de Investigacão em Biofilmes Rosário Oliveira, University of Minho, Campus Gualtar, Braga, Portugal. Tel: +351 253 604 401. Fax: +351 253 604 429. E-mail: evapinho@deb.uminho.pt

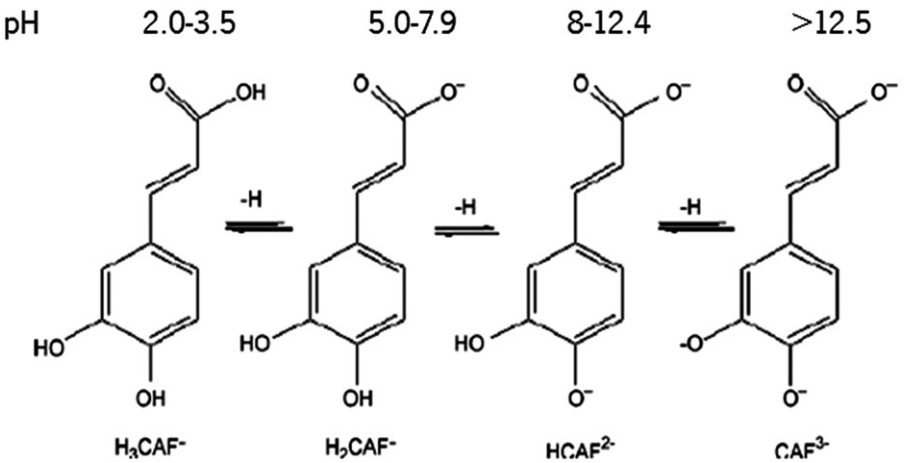


Figure 1. Caffeic acid equilibrium in aqueous solution.

temperature compared to 18 g.L$^{-1}$ for βCD), which enhance the complexation with poor water-soluble molecules (Celik et al., 2011).

Therefore, this work aims to study the effect of the pH on the formation of the IC between caffeic acid and three CDs (βCD, HPβCD and MβCD), characterised by ultraviolet-visible (UV-Vis) spectrometry. Moreover, the effect of the caffeic acid encapsulation, by CDs, on its antibacterial activity was studied.

## Material and methods

### Material

Caffeic acid (3,4-dihydroxycinnamic acid) was purchased from Sigma, β-cyclodextrin (βCD, 1135 g.mol$^{-1}$) and 2-hydroxypropyl-β-cyclodextrin (HPβCD, 1309 g.mol$^{-1}$, MS ≈ 0.6) were acquired from AppliChem, and the methyl-β-cyclodextrin (MβCD, 1310 g.mol$^{-1}$, MS ≈ 1.6) was obtained from Wacker. Caffeic acid stock solutions ($1 \times 10^{-3}$ M or $2.3 \times 10^{-1}$ M) were prepared in ethanol and maintained 30 min at 50°C and 200 rpm. Stock solutions of each CD ($4 \times 10^{-2}$M) were prepared in distilled water. βCD solution was maintained at 50°C and 200 rpm during 30 min to improve it solubility in water. Buffers were prepared by mixing proper amounts of both $H_3PO_4$ ($1 \times 10^{-2}$ M, pH 2.05) and NaOH (1 M, pH 14), until the desired pH (3 or 5).

### Inclusion complex preparation

In order to determine the stoichiometry and stability constant ($K$) of the three ICs, solutions with different concentrations of each CD (between 0 and $6 \times 10^{-3}$ M) were added to the same concentration of caffeic acid ($1 \times 10^{-5}$ M), at each pH (buffer $H_3PO_4$/NaOH pH 3 or 5). The solutions were placed in ultrasounds bath during 30 min, and they were maintained 24 h at 25°C and 50 rpm on dark. Samples of each solution were taken (0 and 24 h) for absorbance measurements.

The absorbance of caffeic acid or IC was measured at the caffeic acid $\lambda_{max}$ (217 nm). The caffeic acid concentration was calculated based on the calibration curve, as previously determined. Moreover, the CDs had no influence on the caffeic acid spectra, at the conditions used. The absorption spectra of each solution were recorded on a Jasco V560 spectrometer (Cremella, Italy), using a 1-cm quartz cuvette.

### Antibacterial activity assessment

The antibacterial activity of caffeic acid on buffer $H_3PO_4$/NaOH at each pH (3 and 5), as well as, the ICs (prepared according with the conditions described previously and with equimolar concentrations ($2.3 \times 10^{-3}$ M) of caffeic acid and CDs, at pH 5) was tested against three bacteria: *Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 11296). The bacteria were grown in tryptic soy agar (TSA, Merck, Germany) for 24 h at 37°C. The cells were, then, inoculated in tryptic soy broth (TSB, Merck, Germany) and incubated for 18 h at 37°C under agitation (120 rpm). Subsequently, bacterial concentration of each strain was adjusted to $1 \times 10^6$ cells.mL$^{-1}$, via absorbance readings, using the correspondent calibration curve.

The MBC (minimal bactericide concentration) was obtained according to the method described by Wiegand et al. (2008), an adaptation of the standard methods published by Clinical and Laboratory Standards Institute and the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2000), using the broth microdilution procedure. Thus, working solutions of 0.28 M of caffeic acid were prepared at pH 3 and pH 5 (buffer $H_3PO_4$/NaOH). Serial dilutions (1:2) of these solutions were made with Mueller–Hinton broth (MHB, Merck, Germany) to a final volume of 50 µL. After, 50 µL of each bacterium suspension was added to a final concentration of $5 \times 10^5$ cell.mL$^{-1}$.Thus, in the first well, the final concentration of caffeic acid was 0.14 M, on the second 0.7 M and so on. Caffeic acid- and bacteria-free controls were, also, included. The plates were incubated for 24 h at 37°C. The number of viable cells was assessed by determination of the number of colony forming unit (CFUs), by plating 10 µL of cell suspension from each well onto TSA and incubated for 24 h at 37°C. The procedure was made in triplicate for each pH and bacteria combination in, at least, three independent assays.

The antibacterial activity of the ICs against the three bacteria was firstly assessed by the disk diffusion method described by the National Committee for Clinical Laboratory Standards, M2-A8 document (M2-A8, 2005), with some modifications. The TSA was the nutritive media used, and 200 µL of each inoculum ($1 \times 10^6$ cells.mL$^{-1}$) was spread on these media plates. Sterile filter paper disks (''Blank Discs'', Liofilchem, Roseto, Italy, 6 mm in diameter) were placed over the petri dish and impregnated with 20 µL of each IC. The plates were incubated at 37°C for 24 h. Thereafter, the size of the inhibition halo was measured.

The ICs capacity to destroy the bacteria was, also, measured quantitatively. A volume of 50 µL of each complex (IC βCD/caffeic acid and IC HPβCD/caffeic acid) was added to 50 µL of $1 \times 10^{-6}$ cells.mL$^{-1}$ of each bacterium on 96-well plates. Bacteria and medium controls were also included. The plates were incubated for 24 h at 37°C. The antimicrobial activity of each solution was assessed by determination of the number of

CFUs, as described previously. Solutions of buffer at pH 5, caffeic acid ($2.3 \times 10^{-2}$ M), βCD ($2.3 \times 10^{-2}$ M) and HPβCD ($2.3 \times 10^{-2}$ M) were also included, to ensure that none of these factors, alone, could have influence on the antibacterial activity of the ICs.

The procedure was made in triplicate for each bacterium in, at least, three independent assays.

## Results and discussion

### Caffeic acid

The UV-Vis spectroscopy analyzes the alterations of the electronic energy levels within the molecules, as a result of electrons transference to different orbitals (Kumar, 2006). In the case of polyphenols, their spectra result from electronic transitions between $\pi$-type molecular orbitals (Anouar et al., 2012). The caffeic acid spectra, usually, shows three peaks: one at 217 nm which is associated with the $\pi$–$\pi$ transition of the phenyl group; the peak near 290 nm attributed to the $\pi$–$\pi$ transition of the phenolic group; and the last one (320 nm) is, normally, associated with the double-bond $\pi$–$\pi$ transition (Górnaś et al., 2006).

Since the pH can change the UV-Vis spectra of polyphenols (Friedman and Jürgens, 2000), the caffeic acid spectra were recorded between 200 and 360 nm for solutions at pH 3 and 5 (Figure 2). Regarding the peaks associated to the $\pi$–$\pi$ transition of the double bound (near 320 nm) and the phenolic group (near 288 nm), the pH increasing induced a reduction of these peaks intensity and had a hypsochromic effect (shift of the peak towards shorter wavelength). A bathochromic effect (peak shift towards longer wavelength) and increase in intensity was observed for the peak attributed to the $\pi$–$\pi$ transition of the phenyl group, when the pH was augmented (Figure 2).

The alterations caused by the pH on the caffeic acid UV-Vis spectra have been attributed to the two adjacent OH groups linked to the benzene ring (Figure 1). Those changes may result from the chemical alteration of the molecule; for instance, the blue shift of the 220 nm peak with pH 5 may be a consequence of the formation of unstable quinone intermediates or other resonance forms (Friedman and Jürgens, 2000; Kumar, 2006).

The biological properties of the phenolic compounds strongly depend on the environmental pH (Friedman and Jürgens, 2000). Therefore, the effect of pH on the antibacterial activity of caffeic acid against three of the most common bacteria isolated from wound infections, *S. epidermidis*, *S. aureus* and *K. pneumoniae*, was assessed. The range of pH tested had no visible influence on the caffeic acid activity. The MBC was the same for pH 3 and 5 for the three bacteria. Being the *S. epidermidis,* the most susceptible bacteria to caffeic acid (0.018 M), and the other two bacteria had the same behaviour when exposed to this phenol, 0.07 M was need to destroys all bacteria cells.

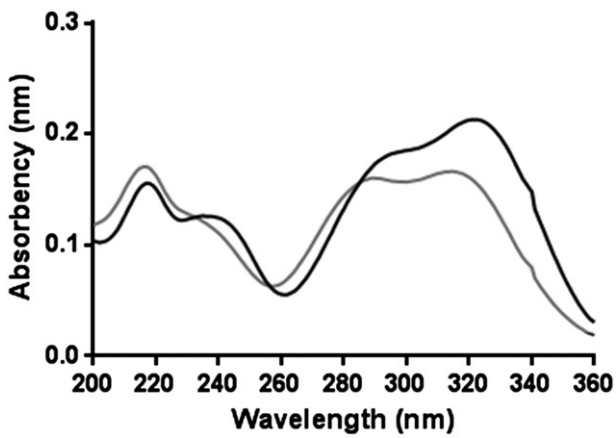


Figure 2. Absorbance spectrum of caffeic acid ($1 \times 10^{-5}$ M) dissolve in $H_3PO_4$/NaOH buffers at pH 3 (black) and pH 5 (grey).

Some studies reported that gram-positive bacteria were more susceptible to antimicrobial activity of various polyphenols than gram-negative bacteria. This higher resistance can be explained on the basis of the different composition of the cell-wall membrane (Canillac and Mourey, 2004; Taguri et al., 2004). However, our results, as well as Almajano et al., (2007), show that the resistance of the tested microorganisms was to caffeic acid was not dependent on the cell wall type.

The antibacterial ability of caffeic acid has been related to its capacity to induce hyperacidification of the environment. The acidification of the plasma membrane, via proton donation, result on disruption of the bacteria cell, and the same effect on the intracellular cytosolic leads to inhibition of the enzyme $H^+$-ATPase necessary for the ATP production (Lin et al., 2005; Kwon et al., 2007; Vaquero et al., 2007). Also, caffeic acid can change the electric potential of the bacterial membrane by quench free electrons from the electron transport chain or by interfering with the proton efflux by dehydrogenases inactivation. Because of this, the bacteria growth may be reduced or even inhibited, since the oxidative phosphorylation reaction cannot occurs (Shetty and Wahlqvist, 2004; Kwon et al., 2007).

According to our results, the antibacterial activity of caffeic acid may be related to the phenolic part of the molecule, since the changes observed, at the pH range tested, on the molecule are on the carboxylic group (Figure 1). Furthermore, this part of caffeic acid (carboxylic group) has the ability to donate protons associated with the acidification of the environment.

### Caffeic acid inclusion complexes

Given the results obtained previously, the study of the IC formation between caffeic acid and the three CDs was performed at both pH values. The encapsulation process involving CDs induces alterations on physical–chemical properties of the guest molecule (caffeic acid). In the case of UV-Vis spectrometry, the absorbance intensity of the guest molecule is changed by its presence within the CD cavity (Górnas et al., 2009).

The encapsulation of caffeic acid by MβCD was not detectable at the conditions tested, since the caffeic acid absorbance was not altered by the increase in MβCD concentration (data not shown). Thus, MβCD was not included on the further analysis. Figure 3 displays the UV-Vis absorption spectra of caffeic acid in the presence of different concentrations of βCD and HPβCD. Since the changes on the spectra were more consistent at the peak near 217 nm, this peak was used for the absorbance analysis.

Regardless the pH or the CD, as CDs concentration increased the peak intensity also increased, and a slightly red-shifted (peak shift to a longer wavelength) was observed. The last alteration was more notorious at pH 5. Those changes on caffeic acid spectrum may result from the partial protection of the molecule by the CD cavity, as well as, the increase in the phenolic compound solubility, factors that suggest the IC formation between this phenolic acid and both CDs (Tang et al., 2002).

The complexes were assessed based on the phase-solubility method described by Higuchi and Connors (1965). This technique analyses the alterations of the caffeic acid (guest) properties (by UV-Vis absorbance spectrometry), in mixtures with excess of the guest and different concentrations of the host (CDs). The ICs were characterised based on the stoichiometry, $K$ and $\Delta G$ (Table 1). The first two parameters were calculated using the modified Benesi–Hildebrand equation (1949) (Equations (1) and (2), $\mathbf{[CD]_0}$ CD initial concentration, **A** absorbance intensities

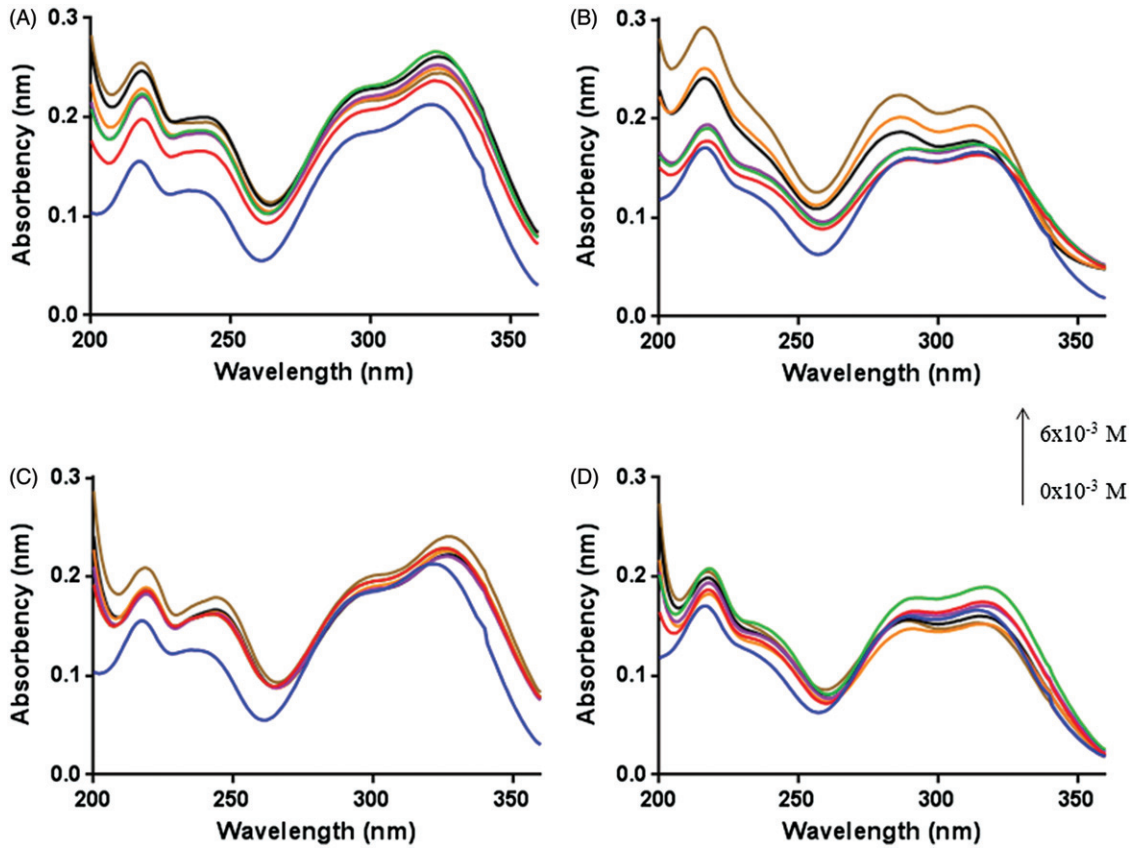


Figure 3. Absorbance spectra of caffeic acid ($1 \times 10^{-5}$ M) in different cyclodextrin concentrations (0–$6 \times 10^{-3}$ M) at pH 3 and 5. (A) βCD/pH 3, (B) HPβCD/pH 3, (C) βCD/pH 5 and (D) HPβCD/pH 5. The procedure was made in triplicate each bacterium in, at least, three independent assays ($n = 3$).

Table 1. Parameters for the inclusion complexes characterisation. $r^2$: coefficient of determination, $K$: stability constant and $\Delta G$: gibbs free energy.

| | βCD | | HPβCD | |
|---|---|---|---|---|
| | pH 3 | pH 5 | pH 3 | pH 5 |
| $r^2$ | 0.923 | 0.979 | 0.548 | 0.924 |
| Stoichiometry | 1:1 | 1:1 | 1:1 | 1:1 |
| $K$ ($M^{-1}$) | 133 | 178 | 10 | 37 |
| $\Delta G$ (kJ) | −12 | −13 | −6 | −9 |

in the presence of CD, $\mathbf{A_0}$ absorbance intensities without CD and $\mathbf{A}'$ limiting intensity of absorption):

$$\frac{1}{A - A_0} = \frac{1}{A' - A_0} + \frac{1}{K(A' - A_0)CD_0} \quad (1)$$

$$\frac{1}{A - A_0} = \frac{1}{A' - A_0} + \frac{1}{K(A' - A_0)CD_0^2} \quad (2)$$

Base on the Equation (1), a double reciprocal Benesi–Hildebrand plot, that is, $\left(\frac{1}{A-A_0}\ vs\ \frac{1}{[CD]}\right)$ were draw (Figure 4). Since the better fit was obtained for this plot, the graphics of $\left(\frac{1}{A-A_0}\ vs\ \frac{1}{[CD]^2}\right)$ were not showed.

Figure 4 shows a straight line with good correlation, indicative of 1:1 molecular complex between caffeic acid and both CDs. The $K$ (Table 1) was obtained based on the slope of the line and can be used as a measurement of the stability of the complexes (Hirose, 2007). Thus, based on our results, βCD appears to be the most suitable for the complexation of caffeic acid. Moreover, the pH had lower influence on the IC formation with the CDs used, but the $K$ value was slight superior at pH 5. $K$ values obtained for the IC with βCD are similar to those reported by (Górnas et al., 2009), as well as the 1:1 stoichiometry. However, in the case of HPβCD, the stoichiometry reported was the same, but the $K$ values were lower than the ones already described. For instances, Zhang et al., (2009) obtained higher pH values for the IC formation with caffeic acid, at pH between 3 and 10. This may be justified by the concentration of cyclodextrin and caffeic acid use in the present work and the sensibility of the method applied to assess the parameters of the IC that were different from the previous work.

The Gibbs free energy was obtained from the Equation (3) where $\boldsymbol{R}$ is the gas constant and $\boldsymbol{T}$ is the temperature in Kelvin.

$$\Delta G = -RTln(K) \quad (3)$$

In concordance with $K$ values, the pH had lower influence on the thermodynamic parameter assessed (Table 1). The pH effect was more obvious for the HPβCD, where the IC was enhanced by pH 5.

The mechanism responsible for the IC formation between CDs and the guest molecule (in our case the caffeic acid) involves the substitution of enthalpy-rich water molecules from the central cavity by caffeic acid. An exothermic reaction occurs as a result of energy release by the system. Thus, the ICs were stabilised mainly by Van der Waals interactions and hydrogen bonds (Górnas et al., 2009; Pinho et al., 2014).

The native CD (βCD), as well as the HPβCD (at the pH range used) are not charged, so hydrophobic interactions between the CD cavity and the caffeic acid, and the formation of hydrogen bonds between caffeic acid with the OH groups of the CDs were the mainly forces responsible by the complex stabilisation. According to our results and other published works (Divakar and Maheswaran, 1997; Górnas et al., 2009; Zhang et al., 2009),

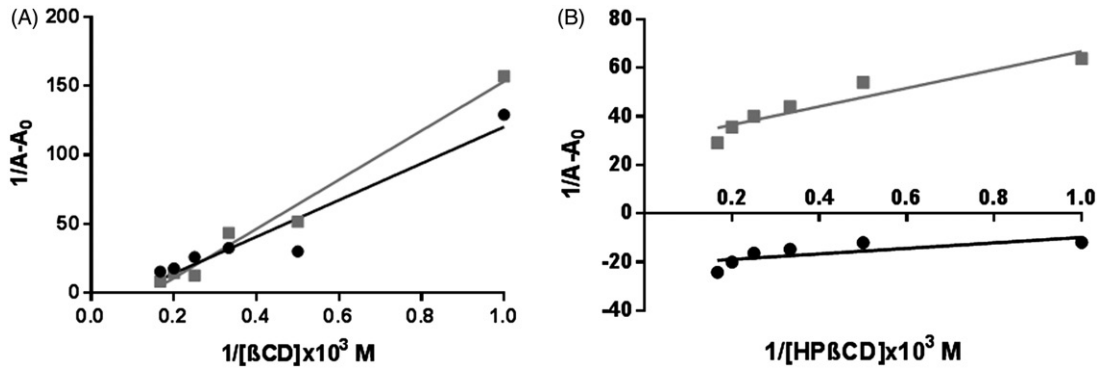


Figure 4. Benesi–Hildebrand plot for the caffeic acid complexation with βCD (A) and HPβCD (B), at pH 3 (black) and pH 5 (grey). The procedure was made in triplicate each bacterium in, at least, 3 independent assays ($n = 3$).

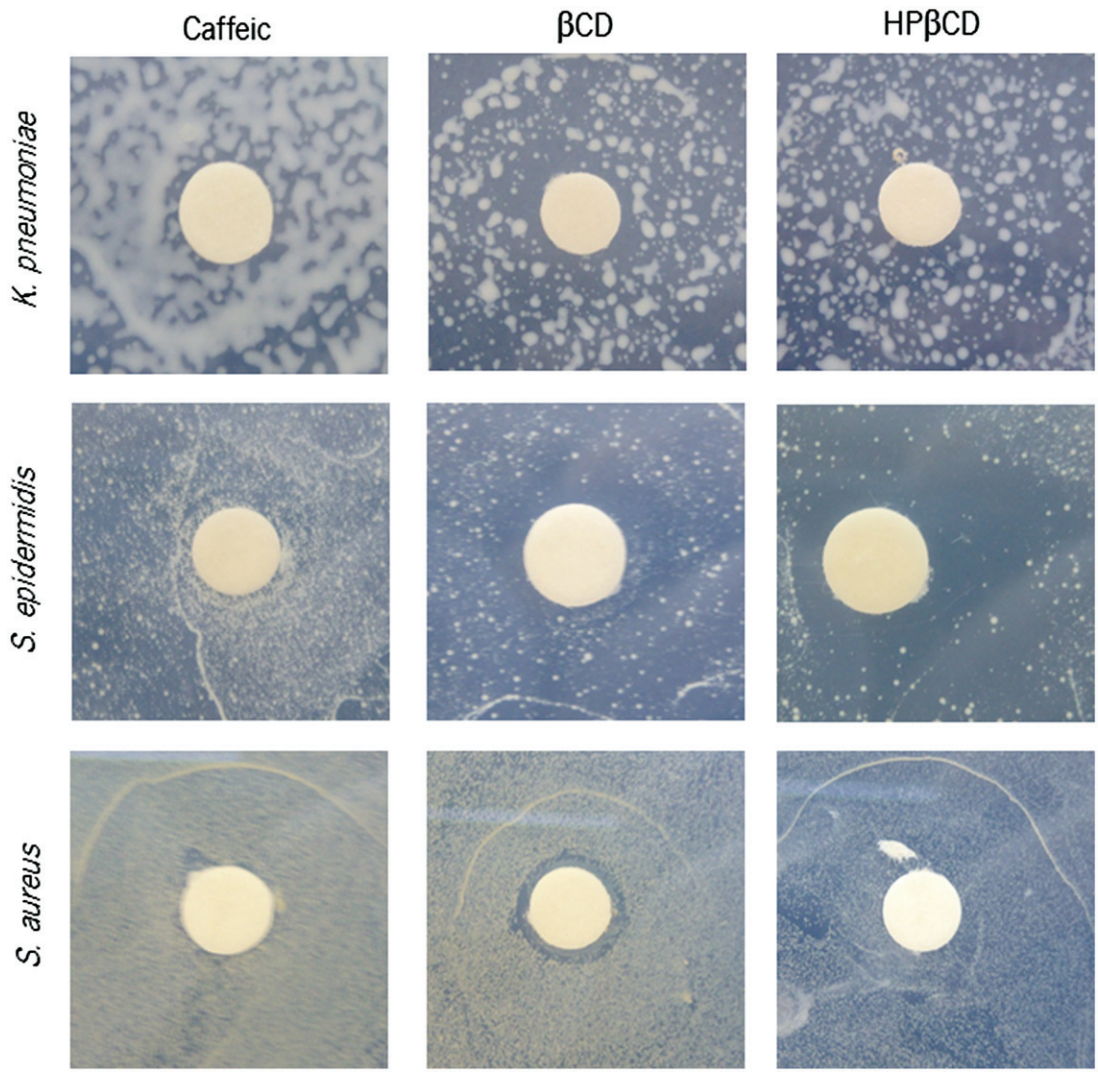


Figure 5. Disk diffusion assay of the βCD/caffeic acid and HPβCD/caffeic acid inclusion complexes against *K. pneumonia*, *S. epidermidis* and *S. aureus* ($1 \times 10^6$ cel.mL$^{-1}$). The inclusion complexes were prepared at pH 5 (buffer $H_3PO_4$/NaOH) with caffeic acid and CDs at the same molecular proportion ($2.3 \times 10^{-2}$ M). The procedure was made in triplicate each bacterium in, at least, three independent assays ($n = 9$).

the caffeic acid aromatic part, including the double bond, was inside of both CDs cavities and the more polar group (carboxylic group) was projected to the water phase.

At pH 3, the neutral form of the caffeic acid (Figure 1) is predominant, and at pH 5, this phenolic acid assumes a monoanionic form, and thus, the hydrophobicity of the phenolic molecule reduces with the increase in pH (Zhang et al., 2009). This justifies the fact that pH 5 was more suitable for the formation of a IC more stable, as also reported by Górnas et al., (2009).

Based on the above results, the βCD appears to be the most appropriate CD for the encapsulation of caffeic acid. Since, the changes induced on the UV-Vis spectra of caffeic acid by the concentration of this CD were more notorious than the ones

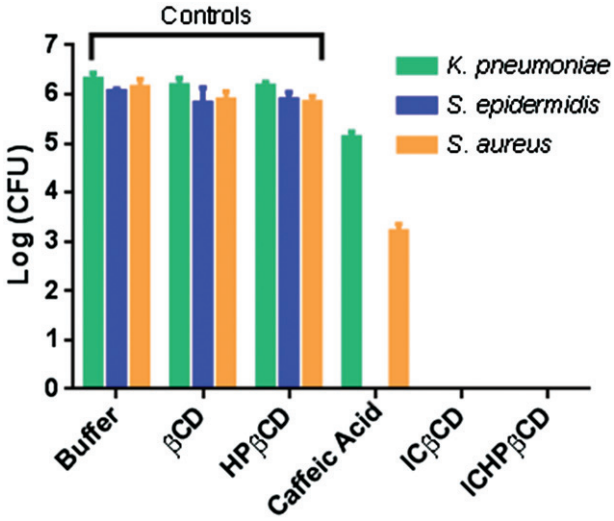


Figure 6. Quantitative analysis of the antibacterial activity of the complexes βCD/caffeic acid and HPβCD/caffeic acid against *K. pneumonia*, *S. epidermidis* and *S. aureus* ($5 \times 10^5$cel.mL$^{-1}$). The inclusion complexes were prepared at pH 5 (buffer $H_3PO_4$/NaOH) with caffeic acid and CDs at the same molecular proportion ($2.3 \times 10^{-2}$ M). The procedure was made in triplicate for each bacterium in, at least, three independent assays ($n = 9$).

observed for HPβCD. Additionally, the $K$ and the $\Delta G$ were also higher for the βCD/caffeic acid complexes. The higher efficiency of complexation by the βCD may be a result of the better fit of caffeic acid molecule into βCD cavity, without the interference of the HPβCD' hydroxypropyl groups.

From author's knowledge, the effect of CDs on the antibacterial activity of caffeic acid was described here for the first time. Since the pH had no significant influence on the caffeic acid activity and the pH 5 allowed a formation of a more stable IC for both CDs (Figure 4 and Table 1), this pH was used for the assessment of the antibacterial activity of the IC (βCD/caffeic acid and HPβCD/caffeic acid).

As reported earlier, the IC formed between caffeic acid and βCD or HPβCD were 1:1, and thus, solutions with the $2.3 \times 10^{-2}$ M of each intervener on the reaction was used. The concentration of caffeic acid used was lower than the MBC detected for *K. pneumoniae* and *S. aureus.,* since βCD has a solubility limit in water of 0.02 M at room temperature (Celik et al., 2011).

The qualitative assessment of the antibacterial activity of the complexes (Figure 5) exhibited the presence of growth inhibition halo for all the IC, higher than the halo obtained for the caffeic acid alone. The only exception was the IC HPβCD/caffeic acid when in contact with *S. aureus*, no halo was observed. This type of method has some drawbacks regarding the diffusion of the antibacterial agents (Cushnie and Lamb, 2005), in this case the complexes. Therefore, a complementary quantitative analysis was made.

Figure 6 displays the antibacterial activity of the two ICs (βCD/caffeic acid and HPβCD/caffeic acid) as well as the controls (buffer, caffeic acid, βCD and HPβCD), at pH5. As expected, the buffer and the CDs alone had no antibacterial activity. The caffeic acid was only capable of killing all the *S. epidermidis* and induced a slight reduction on the growth of the other two bacteria, since the concentration used was six times less ($1.15 \times 10^{-2}$ M) than MBC ($7 \times 10^{-2}$ M) for *K. pneumoniae* and *S. aureus*, as explained earlier.

Interestingly, the IC of the caffeic acid with both CDs were capable of destroy all the cells from the three bacteria (Figure 6), even if we used less than half of the MBC concentration for the *S. aureus* and *K. pneumoniae* on the IC preparation. Thus, the encapsulation of caffeic acid by the CDs enhanced it antibacterial activity against *S. aureus* and *K. pneumoniae*.

The antibacterial activity of caffeic acid appears to be deeply linked with the molecule ability to interact with electrons presents on the bacteria surface or inside the cytoplasm (Luís et al., 2014). The enhanced antibacterial activity of the βCD/caffeic acid and HPβCD/caffeic acid may be a result of the improved solubility of the caffeic acid and a decrease in aggregates formation and, also, by the higher availability of the caffeic acid to interact with bacteria, all induced by the CDs encapsulation.

Therefore, the encapsulation of caffeic acid by the βCD and HPβCD appears to be viable option for the improvement of this phenolic acid' antibacterial activity and further application as antibacterial agent.

## Conclusions

In this work, the IC between caffeic acid and the native CD (βCD) or two derivatives (HPβCD and MβCD) was characterised based on the changes on UV-Vis absorption spectra of the phenolic compound. The results obtained showed that the MβCD was not capable of complex with caffeic acid, at the conditions tested. Moreover, the pH (3 and 5) showed lower influence on the complexation process with βCD and HPβCD, being the higher pH the most suitable. The complexes obtained had 1:1 stoichiometry regardless the CDs, but βCD complexes exhibited higher stability parameters.

The caffeic acid was more effective against *S. epidermidis* than *K. pneumoniae* or *S. aureus* and the pH had no influence on its antibacterial capacity. Therefore, the carboxylic group may not be involved in the interaction caffeic acid – bacteria. Moreover, the antibacterial activity of the caffeic acid was enhanced by the complexation with βCD and HPβCD.

Although the encapsulation of caffeic acid by both βCD and HPβCD enhanced its antimicrobial activity, the IC βCD/caffeic acid appears to be the best option to increase the applicability of this phenolic as antibacterial agent against skin infections, due to its greater stability.

## Declaration of interest

The authors have declared no conflict of interests. The authors are grateful for the FCT Strategic Project PEst-OE/EQB/LA0023/2013 and the Project ''BioHealth – Biotechnology and Bioengineering approaches to improve health quality,'' Ref. NORTE-07–0124-FEDER-000027, co-funded by the ''Programa Operacional Regional do Norte'' (ON.2 – O Novo Norte), QREN, FEDER. The authors also acknowledge the project ''Consolidating Research Expertise and Resources on Cellular and Molecular Biotechnology at CEB/IBB'', Ref. FCOMP-01–0124-FEDER-027462. This work is, also, funded by FEDER funds through the Operational Programme for Competitiveness Factors - COMPETE and National Funds through FCT - Foundation for Science and Technology under the project PEst-C/CTM/UI0264/2011. Additionally, the authors would like to thank the FCT for the grant for E. Pinho (SFRH/BD/62665/2009).

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