Food and Chemical Toxicology 132 (2019) 110647

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Encapsulation of cinnamon oil in cyclodextrin nanosponges and their potential use for antimicrobial food packaging

Ilaria Simionato[a,b], Fernanda C. Domingues[c], Cristina Nerín[a], Filomena Silva[c,d,e,*]

[a] I3A – Aragon Institute of Engineering Research, University of Zaragoza, Calle Mariano Esquillor s/n, 50018, Zaragoza, Spain
[b] Dipartimento di Ingegneria Chimica (DICAM), Università di Bologna, Viale del Risorgimento, 2, 40136, Bologna, Italy
[c] CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal
[d] ARAID – Agencia Aragonesa para la Investigación y el Desarrollo, Zaragoza, Spain
[e] Faculty of Veterinary Medicine, University of Zaragoza, Zaragoza, Spain

ARTICLE INFO

Keywords:
Cinnamon essential oil
Cyclodextrin nanosponges
Controlled release
Foodborne pathogens
Antimicrobial activity
Active food packaging

ABSTRACT

The main goal of this work is the encapsulation of cinnamon essential oil in cyclodextrin nanosponges and the assessment of their antimicrobial activity against foodborne pathogens. After nanosponge synthesis, a headspace-solid phase microextraction coupled to gas chromatography-mass spectrometry (HS-SPME-GC-MS) method was validated to quantify essential oil major compounds. Results showed that essential oil was successfully encapsulated in cyclodextrin nanosponges with α-NS and β-NS being able to encapsulate higher essential oil amounts. Cinnamon essential oil, alone and encapsulated in nanosponges, proved to have antimicrobial activity against foodborne bacteria. Time-kill assays proved that the essential oil, alone or encapsulated, had a bacteriostatic effect against all bacteria tested, with the exception of *Y. enterocolitica* where a bactericidal action was observed. Furthermore, the controlled release achieved by its encapsulation, allowed cinnamon essential oil to be effective at a much lower concentration in culture medium than when solely dissolved in culture medium. Thus, the results described herein encourage the use of cyclodextrin nanosponges as encapsulating agents for active food packaging applications.

1. Introduction

Some foods, such as poultry meat, ground pork, fish, bread, cheese, fruits and vegetables are very sensitive to spoilage and pathogenic microorganisms and therefore constitute a risk to human health (Berjia et al., 2014; Ölmez, 2016). The pathogenic and spoilage microorganisms either found naturally or post-contaminated are mesophiles, psychrotrophs, coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni*, *Clostridium perfringens*, *Yersinia enterocolitica* and *Bacillus cereus* (Petruzzi et al., 2017; EFSA and ECDC, 2017).

The basic approach to ensure food safety is to minimize the initial microbiological load and/or to inhibit the growth of the remaining microorganisms during post-process applications, like production and storage, by the use of an active packaging (Nerin et al., 2017; Yildirim et al., 2018). In these packaging systems, the desired shelf-life extension of the packaged food can be achieved by regulating the physiological, chemical and physical processes that play a key role in the determination of the food shelf-life (Nerín et al., 2017). The basic underlying principle behind the use of active packaging depends on the incorporation of components inside the polymer packaging material able to fulfil several needs such as the potential to release active agents or to retain compounds or undesirable food components in a controlled way (Nerin et al., 2017). In addition, active packaging came into existence with the aim of satisfying the consumer demands for minimally processed food, new distribution trends and stricter requirements regarding consumer health and safety (Janjarasskul and Suppakul, 2018).

The recent growing trends in 'green' consumerism, namely the development of products derived from plants or plant material to be used in foods, cosmetic and medical products, as an alternative to chemical ingredients or food preservatives, led to a renewed scientific interest on these compounds (Bakkali et al., 2008). In particular, there is a clear need for new methods of food preservation using natural additives, and a very interesting option could be the use of essential oils as antimicrobial additives, because they are categorized as GRAS (Generally Recognized as Safe) by the US Food and Drug Administration and are

* Corresponding author. Facultad de Veterinaria, Calle de Miguel Servet, 177, Universidad de Zaragoza, 50013, Zaragoza, Spain.
E-mail addresses: ilaria.simionato16@gmail.com (I. Simionato), fdomingues@ubi.pt (F.C. Domingues), cnerin@unizar.es (C. Nerín), filomena@unizar.es (F. Silva).

https://doi.org/10.1016/j.fct.2019.110647
Received 8 January 2019; Received in revised form 4 June 2019; Accepted 24 June 2019
Available online 28 June 2019

rich sources of biologically active compounds (Llana-Ruiz-Cabello et al., 2015) with known antimicrobial and antioxidant properties (Manso et al., 2011; Wrona et al., 2015).

Among the most used essential oils, cinnamon essential oil (CEO) is one of the most relevant due to its many applications: as a flavouring agent, as an aroma both in the food and cosmetic industries, and in particular as an antimicrobial agent (Haddi and Oliveira, 2017). In recent years, there has been a growing interest in the antimicrobial properties of this essential oil, as studies have shown that it exhibits a strong antimicrobial activity towards foodborne pathogens (Clemente et al., 2016; Manso et al., 2014), which can be exploited by the food industry to use it as a preservative or to incorporate it in the food packaging as antimicrobial agent.

Since essential oils are highly volatile, meaning that they can easily evaporate and eventually decompose when exposed to light, heat or pressure, one of the possible strategies to circumvent these obstacles is their encapsulation aiming to preserve and protect their functional properties, while providing a controlled release in a given medium (Vergis et al., 2015). Moreover, the controlled release of cinnamon essential oil from an encapsulating material can improve its effectiveness in a given food packaging system (Ghaderi-Ghahfarokhi et al., 2017; Niu et al., 2018). Among all the strategies already proposed to encapsulate cinnamon essential oil such as nanoparticles of shellac/xanthan gum (Muhammad et al., 2018) or chitosan microcapsules (Ghaderi-Ghahfarokhi et al., 2017), cyclodextrins seem to be a viable option (Hill et al., 2013; Munhuweyi et al., 2018) as they are virtually non-toxic and some of them are already approved as food additives or as novel foods.

Cyclodextrins are cyclic oligosaccharides, consisting of glucopyranose units attached by α-(1,4) glucosidal bonds forming a toroidal-shaped molecule. There are two types of cyclodextrins: natural, corresponding to the ones existing in nature, and modified, corresponding to natural cyclodextrins that underwent some chemical modifications in order to improve their characteristics. Cyclodextrins can form inclusion complexes with a wide variety of hydrophobic guest molecules by molecular complexation. In particular, essential oils and volatile compounds can be encapsulated in cyclodextrins in order to improve their water solubility (Yildiz et al., 2018), and to avoid heat-induced degradation (Celebioglu et al., 2018; Ren et al., 2018) and loss of these compounds during processing and storage due to their volatility (Ren et al., 2018), which can result in an improved efficacy of these essential oil-containing complexes (Yildiz et al., 2018). Although being effective encapsulating compounds, over the past years, cyclodextrin molecules have been engineered to improve their properties. One of this engineered solutions is the formation of cyclodextrin polymers, namely cyclodextrin nanosponges (CD-NS). Cyclodextrin nanosponges can be defined as hyper-cross-linked cyclodextrins that can be obtained with α-, β -and γ-cyclodextrins, either alone or as mixtures containing relevant amounts of linear dextrin, cross-linked with a suitable cross-linking agent such as dialdehydes, epoxides, epichlorohydrin, or diacyl chlorides to fulfil the need of improved technological characteristics (Sherje et al., 2017). Cyclodextrin nanosponges present several advantages with respect to native cyclodextrins, such as solubility enhancement, protection of the encapsulated molecules from light and degradation, and an improved controlled delivery (Trotta et al., 2012). Up until now, cyclodextrin nanosponges have been successfully used to encapsulate camptothecin to enhance its solubilisation (Swaminathan et al., 2010), and linalool (Trotta et al., 2012), Babchi oil (Kumar et al., 2018) and oxygen (Cavalli et al., 2010), to increase their stability and prolong their release, among other drugs and natural compounds (Sherje et al., 2017).

Taking all this into consideration, this work is aimed to encapsulate cinnamon essential oil in cyclodextrin nanosponges in order to develop an antimicrobial active packaging capable of providing a controlled release of cinnamon essential oil while maintaining this oil's antimicrobial effectiveness.

2. Materials and methods

2.1. Materials

Cinnamon essential oil was purchased from Matières Premières Essentielles (Grasse, France). For CD-NS synthesis, α-cyclodextrin (α-CD, Mw = 972.84 g/mol), β-cyclodextrin (β-CD; KLEPTOSE®, Mw = 1134.98 g/mol), hydroxypropyl-β-cyclodextrin (HP-β-CD; KLEPTOSE® HPB, Mw = 1399 g/mol) and maltodextrin (LC; KLEPTOSE® Linecaps, Mw = 12000 g/mol) were kindly provided by Roquette Freres S.A. (Lestrem, France) . The following chemicals were used as standards for the analysis of cinnamon essential oil: (R)-(+)-Limonene (> 97%; CAS 5989-27-5; Sigma-Aldrich, Spain), *trans*-Cinnamaldehyde (> 99%; CAS 14371-10-9 Sigma-Aldrich, Spain) and (−)-Verbenone (> 97%; CAS 1196-01-6; Chemika®, Sigma-Aldrich, Spain).

2.2. Synthesis of cyclodextrin nanosponges

CD-NS synthesis was performed according to a previously described method (Castiglione et al., 2013; Trotta and Cavalli, 2009) using carbonyldiimidazole (1:4 molar ratio) as cross-linking agent. After synthesis, four nanosponges (NS) polymers were obtained, namely alpha-nanosponge (α-NS), beta-nanosponge (β-NS), hydroxypropyl-beta:beta-cyclodextrin 1:2 molar ratio nanosponges (HP-β:β-NS) and maltodextrin nanosponges (LC-NS).

2.3. Encapsulation of cinnamon essential oil in cyclodextrin nanosponges

In order to evaluate the most suitable conditions for cinnamon essential oil encapsulation in CD-NS, two variables were tested, namely the solvent used for encapsulation and the encapsulation time. For this purpose, three solvents with different polarities were selected (ethanol, methanol and acetone) as well as four encapsulation times (24, 48, 72 and 96 h). For the encapsulation assays, 50 ± 0.2 mg of each nanosponge were weighted and placed in 2 mL microtubes. Then, 50 μL of cinnamon essential oil together with 950 μL of one of the afore-mentioned solvents were added to each tube. Encapsulation was carried out by magnetic stirring of the samples at 1000 rpm and room temperature. After each encapsulation time, samples were centrifuged at 10000 rpm for 10 min at room temperature in order to recover the solid loaded nanosponges and the solvent containing the remaining (not incorporated) essential oil was discarded. After centrifugation, the supernatant was removed and the loaded nanosponges were stored at -80 °C before freeze-drying. Samples were freeze-dried (HETO Loteen FDB E1, LaboAragón, Spain) at - 35 °C under vacuum in order to sublimate ice and to achieve stability of the substances.

2.4. Analysis of cinnamon essential oil encapsulation by cyclodextrin nanosponges

10 ± 0.2 mg of each loaded nanosponge were weighted and placed in 2 mL microtubes. Then, to each sample, 500 μL of acetone were added and NS lyophilized samples were stirred for 24 h at 1000 rpm and room temperature. After 24 h, each sample was centrifuged for 10 min at 10000 rpm and room temperature in order to recover the acetone supernatant containing the released cinnamon oil.

2.5. Head-space solid microextraction (HS-SPME) coupled with gas-chromatography-mass spectrometry analysis of cinnamon essential oil

SPME analysis was performed in the headspace environment of 20 mL glass vials using a fused silica fibre coated with polydimethylsiloxane (PDMS) of 100 μm diameter and 1 cm length (SUPELCO, Sigma - Aldrich®, Spain) suitable for volatile compound analysis. Sample extraction (20 μL) was performed at 40 °C for 15 min and 500 rpm and desorption was carried out at 250 °C for 2 min.

Verbenone (m/z: 107.1, 91) was chosen as internal standard and 5 μL of a 10 ppm solution were added to each sample prior to extraction. SPME procedure was performed automatically using a CTC Analytics autosampler system from Agilent Technologies (Santa Clara, USA). The gas chromatograph system (Agilent Technologies, Spain) was a HP 6890 series connected to a HP 5973 series mass selective detector. Chromatographic separation was carried out on a Carbowax pp 20 column (30 m × 0.25 mm x 0.25 μm) (SGE Analytical Science, Belgium). All samples were injected in splitless mode. The initial column temperature was set at 60 °C and held for 3 min, then raised to 220 °C at a rate of 10 °C/min and held for 2 min. Helium (99.999%, Air Liquide, Madrid, Spain) was used as carrier gas at a flow rate of 1 mL/min. For quantitative cinnamon essential oil analysis, calibration plots of its major compound (> 90%), cinnamaldehyde, and other bioactive compound, limonene, were obtained from appropriate dilutions in acetone of limonene (m/z: 68.1, 93) and cinnamaldehyde (m/z: 131.1, 103) standard solutions detected in single ion monitoring (SIM mode). The retention times obtained for limonene, verbenone and cinnamaldehyde were 8.988, 13.092 and 14.028 min respectively. This method was validated according to the Food and Drug Administration (FDA, 2001) and to the International Conference of Harmonization guidelines (ICH, 2005) and the parameters studied were linearity, intermediate, intra- and interday precision and accuracy, as well as SPME recovery.

2.6. CEO-loaded cyclodextrin nanosponges antimicrobial activity

2.6.1. Bacterial strains

Antibacterial activity of cinnamon oil and CEO-loaded nanosponges was tested against four reference bacterial strains from the Colección Española de Cultivos Tipo (CECT)e: two Gram negative (*E. coli* O157:H7 CECT 5947 and *Y. enterocolitica* CECT 500) and two Gram-positive bacteria (*B. thermosphacta* CECT 847 and *L. monocytogenes* CECT 911). All bacterial strains were stored in Brain Heart Infusion (BHI) broth with 30% (v/v) glycerol at −80 °C. Prior to susceptibility testing, each strain was inoculated on Mueller – Hinton Agar (MHA; Scharlau, Spain) or Brain Heart Infusion Agar (BHIA: Scharlau, Spain) to ensure optimal growth and purity.

2.6.2. Determination of cinnamon oil MIC and MBC

A microdilution broth susceptibility assay for bacteria was used (Becerril et al., 2012). This assay was performed to evaluate the amount of cinnamon essential oil in liquid medium required to inhibit bacterial growth. All tests were performed in Mueller–Hinton Broth (MHB) supplemented with DMSO (maximum final concentration of 2%, v/v) to enhance the oil solubility, with the exception of *L. monocytogenes* MIC and MBC determination, where BHI broth was used to facilitate growth visualization.

Bacterial colonies were suspended in 0.9% (w/v) NaCl to obtain a cell density of 1–2 × 10^8 CFU/mL. These cell suspensions were diluted to obtain a cell density of 1 × 10^6 CFU/mL and were further diluted to obtain a final density of 5 × 10^5 CFU/mL in each test tube. Geometric dilutions ranging from 2000 to 15.625 ppm of the essential oil were prepared in test tubes. Growth conditions (MHB or BHI with 2%, v/v, DMSO with tested micro-organism) and sterility of the medium (MHB or BHI) were checked. Oil sterility was checked by substituting the inoculum with MHB or BHI medium and performing the oil serial dilutions previously described. The tubes were incubated under normal atmospheric conditions at 37 °C for 24 h, with the exception of *B. thermosphacta*, where plates were incubated at 25 °C. The MIC was defined as the lowest concentration of oil, which prevented visible growth. The MBC was defined as the lowest concentration of oil where 99.9% or more of the initial inoculum was killed and was determined by subculture on MHA or BHIA plates of two 20 μL drops from each tube where visible growth was prevented. Each experiment was repeated at least three times at each test concentration and the modal MIC and MBC values were selected.

2.6.3. Vapour phase activity assay

The aim of this assay is to evaluate microbial growth inhibition caused by the volatile compounds present in cinnamon essential oil. For this assay, MHA or BHIA plates were inoculated by striking a cotton swab previously dipped in a 1–2x10^8 CFU/mL bacterial suspension in 0.9% (w/v) NaCl. For the placement of the paper disks, a glass coverslip was attached at the center of the inside of the lid; then, the paper disk was placed on top of the coverslip and loaded with 10 μL of cinnamon essential oil. Plates were sealed with Parafilm® and incubated at 37 °C for 24 h at atmospheric conditions, with the exception of *B. thermosphacta* plates, that were incubated at 25 °C and growth inhibition halos were recorded with a digital calliper.

2.6.4. Time – kill assays

For the time-kill assays, cinnamon essential oil (MIC value) and 5 mg/mL of each CEO-loaded cyclodextrin nanosponges were used to detect differences in killing. Inocula were prepared from overnight cultures grown in MHA or BHIA plates. Glass tubes (15 mL) containing MHB or BHI medium with cinnamon essential oil or the appropriate loaded nanosponges were inoculated with the test strains to a final cell density of 1 × 10^6 cells/mL. Tubes were incubated at 37 °C or 25 °C, in the case of *B. thermosphacta*, under aerobic conditions, with aliquots being removed at 0, 1, 2, 4, 6, 8 and 24 h for the determination of viable cell counts. Serial dilutions were prepared in sterile 0.9% (w/v) NaCl and drop (20 μL) plated onto Plate Count Agar (PCA; Scharlau, Spain). Plates were incubated at 37 °C or 25 °C, for *B. thermosphacta*, for 24 h, and the number of colonies was determined. The detection level of this plating method was 2 × 10^2 CFU/mL.

Killing curves were constructed by plotting the $\log_{10}$ CFU/mL versus time over 24 h, and the change in bacterial concentration was determined. Bactericidal activity was defined as a reduction of 99.9% (3 $\log_{10}$) or more of the number of CFU/mL in the original inoculum (CLSI, 1999); while bacteriostatic activity was defined as the maintenance of the original inoculum concentration or a reduction inferior to 99.9% of the cell concentration (CFU/mL) in the original inoculum (Silva et al., 2011). Control experiments with cells incubated in culture media with or without nanosponges and cinnamon essential oil treated under the same conditions, were carried out in parallel.

2.6.5. Cinnamon essential oil release kinetics from nanosponges in culture medium

To evaluate the kinetics of cinnamon essential oil release in MHB or BHI culture media, glass tubes (15 mL) containing 5 mg/mL of each loaded nanosponges and 4 mL of culture medium where incubated in the same conditions used for the time-kill assays. Culture medium aliquots were removed 0, 1, 2, 4, 6, 8 and 24 h of incubation and centrifuged at 10000 rpm for 10 min to remove any nanosponges particle; then the supernatant was recovered and stored at −20 °C prior to HS-SPME GC-MS analysis as described previously. As the amounts of limonene released from the nanosponges were negligible when compared to cinnamaldehyde concentrations obtained, cinnamon essential oil in this case is just expressed as means of cinnamaldehyde release.

2.7. Statistical analysis

Data analysis was performed using the SPSS software package, version 13.0 (SPSS Inc., Chicago, IL). When the association between categorical and continuous variables was dichotomous, the Mann-Whitney *U* test or Student's t-test were used. In the case of variables with 3 or more categories, analysis of variance (one-way ANOVA) was used. Statistically significant differences between 2 variables were accepted when the probability of significance (p value) was < 0.05.

3. Results and discussion

To promote the use of essential oils, one has to circumvent their limitations in terms of solubility and stability. For instance, essential oils are lipophilic, immiscible with water and at the same time, they are sensitive towards the chemical modification under the effect of some external factors such as: temperature, light, presence of oxygen, among other factors (Dima et al., 2014). In order to minimize these hurdles, in this work cyclodextrin nanosponges were evaluated as an encapsulation strategy to promote the controlled release of this compound while also maintain its antimicrobial activity.

3.1. Method validation

Gas chromatography in combination with mass spectrometry is the most widely used technique for the determination of the chemical composition of essential oils with volatiles being commonly separated on fused silica capillary columns with different stationary phases, such as the nonpolar methyl polysiloxanes and methyl-phenylpolysiloxanes; and polar polyethyleneglycol (Smelcerovic et al., 2013) such as the one used in this study. Taking into consideration the inherent volatility of CEO and the development of a versatile method, we chose to couple GC-MS chromatographic detection to a HS-SPME that can be used both for solid or liquid samples (Licciardello et al., 2013). So, a HS-SPME coupled to GC-MS methodology was developed and validated in terms of linearity, recovery, intermediate, intra- and interday precision and accuracy, following a 5-day validation protocol. The chromatographic and SIM detection conditions described allowed the successful separation of limonene, verbenone (IS) and cinnamaldehyde at 8.988, 13.092 and 14.028 min, respectively (Fig. 1).

To evaluate the method's linearity, several solutions of limonene (LIM) and cinnamaldehyde (CINN) with concentrations ranging from 0.25 for LIM and 0.5 for CINN to 25 ppm were prepared (eight calibrators evenly distributed and five replicates) and analysed as described above. Together with each calibration curve, three quality control samples (QC) at low (LQC: 0.5 μg/mL) and medium (MQC: 10 μg/mL) and high (HQC: 25 μg/mL) concentrations (n = 3) were also analysed. Calibration curves were obtained by plotting the peak–area of each analyte against IS concentration. The acceptance criteria included a determination coefficient of at least 0.99 and the calibrators' accuracy within a ± 15%, with the exception of the lower limit of quantitation (LLOQ), 0.25 and 0.5 ppm, where ± 20% was accepted. Due to the wide calibration range, weighted least squares regressions were adopted. Six weighting factors were evaluated for each analyte ($1/\sqrt{x}$, $1/x$, $1/x^2$, $1/\sqrt{y}$, $1/y$, $1/y^2$), and the one originating the best results was selected. Using each of those factors, the mean relative errors of each calibrator were calculated and their absolute value was summed. The weighting factor $1/y$ and $1/x$ were chosen for limonene and cinnamaldehyde, respectively, since the sum of errors obtained was smaller while presenting simultaneously a mean R^2 value of at least 0.99 (Table 1).

By means of these weighted least squares regressions, linear relationships were obtained ($R^2 \geq 0.99$) (Table 1), and the relative error [mean relative error (bias) between measured and spiked concentrations] was in accordance with the above-mentioned criteria, ranging from 0.06 to 10.63%. The lowest concentration used for quantification [0.25 ppm (LIM) or 0.5 ppm (CINN)] for each analyte are within the range already described by other authors (Friedman et al., 2000; Miller et al., 2008) but the automatized SPME extraction procedure is advantageous when compared to the more laborious extraction procedures described in these works.

Interday precision and accuracy (Table 2) were evaluated at eight concentrations that were different for each analyte (LIM or CINN). The calculated coefficients of variation (CVs) were lower than 11% for all compounds at all concentration levels, while accuracy (in terms of mean relative error) was within a ± 8% interval. The CVs presented can be considered moderately higher and could be related to the fact that an extraction procedure was performed, which is usually considered a source for variability. Intra-day precision and accuracy (Table 2) were determined using 4 standard concentrations prepared and analysed as mentioned above (six replicates for each concentration). The obtained CVs were lower than 6% for all the compounds at all tested concentrations, presenting a mean relative error within a ± 11% interval. Additionally, intermediate precision and accuracy (Table 2) were assessed at 3 concentrations (2, 4 and 8 ppm) and performed in triplicate over the 5-day validation period (n = 15). The results showed that the obtained CVs were always lower than 9% and the relative error was within a ± 6% interval from the target concentration.

Furthermore, analyte recovery was assessed at three concentration levels (0.5; 2.5 and 10 ppm) (Table 3) and the results obtained showed that the HS-SPME extraction procedure is effective with recovery values ranging from 87 to 103% of recovery with variation not higher than

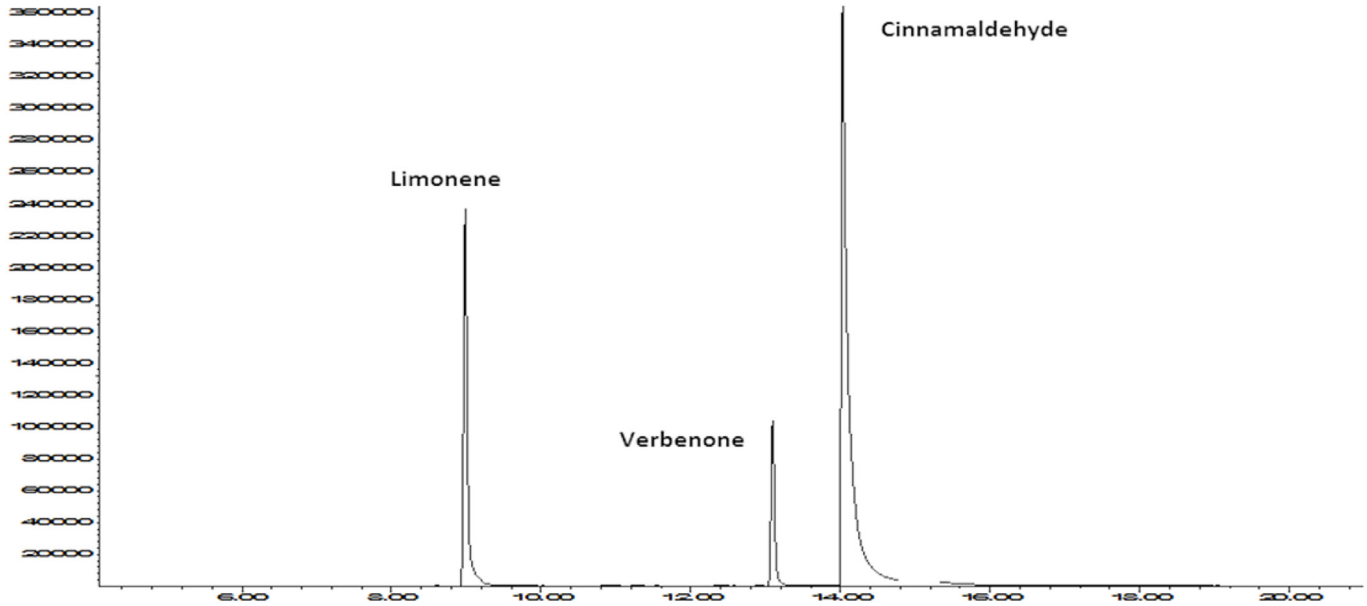

Fig. 1. HS-SPME GC-MS limonene and cinnamaldehyde quantification linearity data: a) typical GC-MS chromatogram (SIM mode).

Table 1
Linearity data (n = 5). All concentrations are in ppm; CV, coefficient of variation; RE, relative error [(measured concentration − spiked concentration/spiked concentration) × 100]. When applicable, values are presented as mean values ± standard deviation.

Compound	Weight	Linearity	Lower limit of quantification (LLQ)	Slope	Intercept	R^2
Limonene	$\frac{1}{y}$	0.25–25	0.25	0.083 ± 0.003	0.11 ± 0.01	0.996 ± 0.002
Cinnamaldehyde	$\frac{1}{x}$	0.5–25	0.5	0.39 ± 0.01	−0.027 ± 0.002	0.9909 ± 0.0008

Table 2
HS-SPME GC-MS limonene and cinnamaldehyde quantification validation data: inter-day precision and accuracy (n = 5), intra-day precision and accuracy (n = 6) and intermediate precision and accuracy (n = 15). All concentrations are in ppm; CV, coefficient of variation; RE, relative error [(measured concentration − spiked concentration/spiked concentration) × 100]. When applicable, values are presented as mean values ± standard deviation.

Inter-day precision and accuracy				
Compound	Spiked	Measured	CV (%)	RE (%)
Limonene	0.25	0.25 ± 0.03	10.81	−5.26
	0.5	0.53 ± 0.02	2.84	3.60
	1	1.03 ± 0.10	10.09	−0.69
	2.5	2.42 ± 0.19	7.95	−4.53
	5	5.32 ± 0.48	8.97	4.39
	7.5	7.29 ± 0.47	6.45	−4.04
	10	10.17 ± 0.72	7.07	0.42
	25	25.25 ± 0.66	2.60	−0.06
Cinnamaldehyde	0.5	0.56 ± 0.02	4.46	8.09
	2.5	2.44 ± 0.2	8.24	−3.26
	5	4.88 ± 0.15	3.06	−3.06
	7.5	7.15 ± 0.17	2.44	−5.54
	10	9.82 ± 0.59	6.06	−2.68
	15	15.75 ± 0.7	4.43	3.13
	20	19.71 ± 0.87	4.44	−2.20
	25	25.83 ± 0.82	3.17	2.54
Intra-day precision and accuracy				
Limonene	0.5	0.52 ± 0.03	6.01	1.55
	2.5	2.82 ± 0.08	2.83	10.63
	10	10.02 ± 0.34	3.43	−0.81
	25	26.27 ± 1.23	4.69	3.72
Cinnamaldehyde	0.5	0.51 ± 0.03	5.04	0.37
	2.5	2.63 ± 0.09	3.44	4.43
	10	9.73 ± 0.35	3.64	−3.37
	25	23.68 ± 0.71	3.01	−6.31
Intermediate precision and accuracy				
Limonene	2	1.92 ± 0.16	8.46	−5.94
	4	3.98 ± 0.29	7.32	−1.80
	8	8.49 ± 0.69	8.13	4.32
Cinnamaldehyde	2	2.11 ± 0.19	9.07	3.94
	4	4.08 ± 0.31	7.56	0.97
	8	8.16 ± 0.61	7.45	0.94

Table 3
HS-SPME limonene and cinnamaldehyde recovery data. Values are expressed as a percentage between the measured concentration versus spiked concentration in blank cyclodextrin nanosponges samples. When applicable, values are presented as mean values ± standard deviation.

Compound	Spiked	Recovery (%)	CV (%)
Limonene	0.5	100.58 ± 8.21	8.16
	2.5	97.08 ± 8.06	8.30
	10	98.71 ± 10.62	10.76
Cinnamaldehyde	0.5	103.42 ± 5.44	5.26
	2.5	87.64 ± 15.48	17.67
	10	98.47 ± 12.54	12.73

17%.

Overall, the HS-SPME GC-MS method described allowed the quantification of LIM and CINN and was successful validated regarding all the parameters evaluated, since CV and relative error values were always lower than 15% for all criteria, with the exception of the recovery values.

3.2. CEO incorporation in CD-NS

Although CEO has already been encapsulated in cyclodextrins (Hill et al., 2013; Petrovic et al., 2010), in this work polymeric cyclodextrin structures, cyclodextrin nanosponges were preferred due to the advantages they offer in comparison to monomeric cyclodextrins, such as increased loading capacity, better controlled release and increased stability of the compounds encapsulated due to the molecular interactions that can be established (Caldera et al., 2017). These features make them the perfect candidates to be applied as encapsulation agents in active food packaging, opening a vast new field of application for this technology that, so far, has only been exploited for pharmaceutical drug delivery (Caldera et al., 2017). Since little is known about the interactions of cyclodextrin nanosponges with essential oils, in this work we synthetized four types of CD-NS containing different monomeric CDs. Usually, CD-NS are synthetized using just one type of monomeric cyclodextrins but, in this work, we also synthetized CD-NS using two different monomeric CDs, namely β-CD and HP-β-CD as an attempt to create a more diverse crosslinking pattern inside the NS, as a consequence of the different size between these two CD molecules. CEO encapsulation is measured only by the CINN amount encapsulated, as the amount of limonene was negligible in all cases, with values not higher than 1 μg/mg NS (data not shown).

It is known that several factors interfere with the complexation and binding of guest compounds to CD and CD-NS. In the case of CD monomers, one of those factors is the polarity of both the guest compound and solvent chosen for the encapsulation, as hydrophobic solvent molecules might compete with the guest molecule to access the hydrophobic core of the CD molecule (Charumanee et al., 2016). On the other hand, CD-NS have different mesh polarities due to hydrophobic cavities of cyclodextrin which are surrounded by hydrophilic nanochannels of the polymeric network allowing substantial interactions with guest molecules of different structures and lipophilicities (Trotta et al., 2012). Given this fact, we evaluated three different solvents with different polarities (ethanol, methanol and acetone) for their ability to potentiate or not CEO encapsulation in the synthetized CD-NS. Encapsulation results (Fig. 2) showed that, independently of the solvent used, maltodextrin NS (LC-NS) were the least effective in encapsulating CEO, with a maximum amount of approximately 10 μg CINN/mg LC-NS; while all the other CD-NS (α-NS, β-NS and HP-β:β-NS) were capable of encapsulating 50–70 μg CINN/mg CD-NS when using ethanol or methanol as solvents.

The choice of a less polar solvent (acetone) clearly had a deleterious effect in CEO encapsulation, by significantly ($p < 0.05$) decreasing the amount of CINN encapsulated in β-NS and HP-β:β-NS. This finding is corroborated by other authors that also stated that the increased hydrophobicity in the encapsulation medium led to a decreased complexation of guest molecule and HP-β-CD (Junquera and Aicart, 1997). However, in the case of α-NS and LC-NS, this change in solvent polarity does not seem to significantly affect encapsulation in almost all cases, probably because the dynamic equilibrium between α-NS/LC-NS and CEO is not so dependent on the hydrophobic interactions formed between the two.

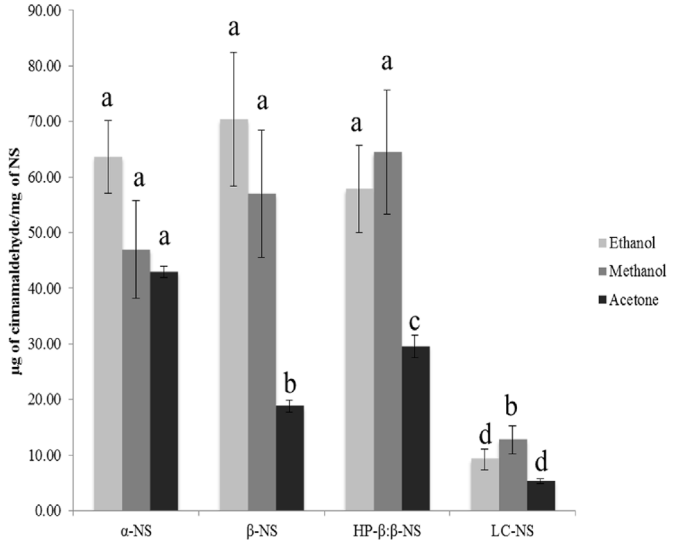

Fig. 2. Effect of different solvents (ethanol, methanol and acetone) in the encapsulation of CEO by α-NS, β-NS, HP-β:β-NS and LC-NS. Results are expressed as the amount of major CEO compound cinnamaldehyde encapsulated by mg of NS. Error bars correspond to SD values of at least three independent replicates. Results with the same letter within a graphic bar are not significantly different ($p < 0.05$) using one-way ANOVA.

CD complexation usually involves an initial equilibrium where the water molecules are displaced from the CD cavity, followed by a second equilibrium step where there is an increase in the interaction between guest molecule and CD cavity (Del Valle, 2004). The first part of the equilibrium is very fast, happening within minutes, but the final equilibrium takes much longer to reach (Del Valle, 2004). Therefore, we investigated the encapsulation kinetics of CEO in CD-NS in one of the most polar solvents tested, ethanol, during a 96 h incubation period. As can be seen by the results obtained (Fig. 3), equilibrium is reached after 24 h of incubation with further 24 h incubation periods having no significant ($p < 0.05$) effect on the amount of CINN entrapped inside the CD-NS network, which is in agreement with the results described by other authors for CD-NS complexation (Swaminathan et al., 2010).

3.3. CEO antimicrobial activity

In this section of the work, the antimicrobial susceptibility of four foodborne pathogenic or spoilage bacteria, namely two Gram-positive (*B. thermosphacta* and *L. monocytogenes*) and two Gram-negative bacteria (verotoxigenic *E. coli* and *Y. enterocolitica*), to cinnamon essential oil was evaluated both in liquid medium and in the vapour phase due to the inherent volatility of essential oils. The MIC values obtained ranged from 125 to 500 ppm (Fig. 4a), with *B. thermosphacta* being the most susceptible bacteria to CEO.

Overall, CEO seems to be equally effective against Gram-positive and Gram-negative bacteria, as the MIC values obtained for *E. coli*, *Y. enterocolitica* and *L. monocytogenes* (500 ppm) are the same. Although it is usually difficult to compare among antimicrobial susceptibility testing results due to differences in culture media, inoculum concentration, etc., the results obtained are in agreement with the ones obtained by other authors. For instance, Raeisi and collaborators (Raeisi et al., 2015) described MIC and MBC CEO values of 2500 ppm for *E. coli*. These higher values might be related with the fact that in this study no solvent was used to increase the dispersion of the essential oil in water. In another study performed against foodborne bacteria, CEO major compound, cinnamaldehyde, proved to be effective against *E. coli* O157:H7, *L. monocytogenes* and *B. thermosphacta* with MIC values ranging from 125 to 250 ppm (Mith et al., 2014), which are very similar to the ones obtained in our work for CEO; although the MBC values obtained in that work are slightly lower (250–1000 ppm) than the ones obtained herein.

According to the data obtained in the vapour phase activity assays (Fig. 4b), cinnamon essential oil yielded growth inhibition in vapour phase for all four bacteria tested. Additionally, for *L. monocytogenes* and *E. coli,* a delay halo (Fig. 4c) was also visible, meaning that cinnamon essential oil was also capable of slowing down bacterial growth without preventing it. Similar delay halos have also been obtained by other authors testing antifungal vapour phase activity of CEO (Manso et al., 2013). Goñi and collaborators (Goni et al., 2009) also evaluated the vapour phase activity of CEO against foodborne pathogens and they also describe the evidence of a growth reduction halo for *E. coli* and *L. monocytogenes*. Nevertheless, the results obtained in our work show higher inhibition halos than the ones obtained in that work, probably by the differences in plate inoculation and inoculum concentration.

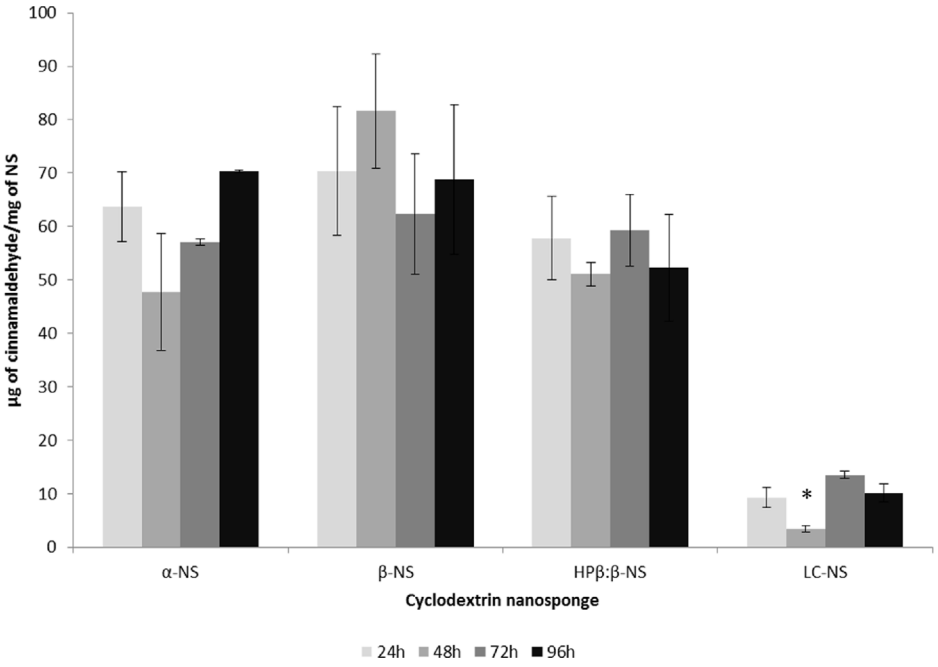

Fig. 3. Effect of encapsulation time (24, 48, 72 and 96 h) in the encapsulation of CEO by α-NS, β-NS, HP-β:β-NS and LC-NS. Results are expressed as the amount of major CEO compound cinnamaldehyde encapsulated by mg of NS. Error bars correspond to SD values of at least three independent replicates. *Statistically significantly different ($p < 0.05$) results using one-way ANOVA.

a)

Bacterial strain	MIC (ppm)	MBC (ppm)
L. monocytogenes CECT 911	500	1000
B. thermosphacta CECT 847	125	1000
E. coli O157:H7 CECT 5947	500	1000
Y. enterocolitica CECT 500	500	2000

b)

Bacterial strain	Inhibition halo (mm))
L. monocytogenes CECT 911	49.37 ± 3.27
B. thermosphacta CECT 847	38.87 ± 0.14
E. coli O157:H7 CECT 5947	49.87 ± 0.99
Y. enterocolitica CECT 500	55.88 ± 3.83

c)

Bacterial strain	Growth delay halo (mm))
L. monocytogenes CECT 911	60.01 ± 3.64
E. coli O157:H7 CECT 5947	51.22 ± 6.45

Fig. 4. Antimicrobial susceptibility of test strains to CEO: a) minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values obtained by broth dilution; inhibition halos (b) and growth delay halos (c) obtained by the vapour activity assay. When applicable, values are presented as mean values ± standard deviation of at least three independent replicates.

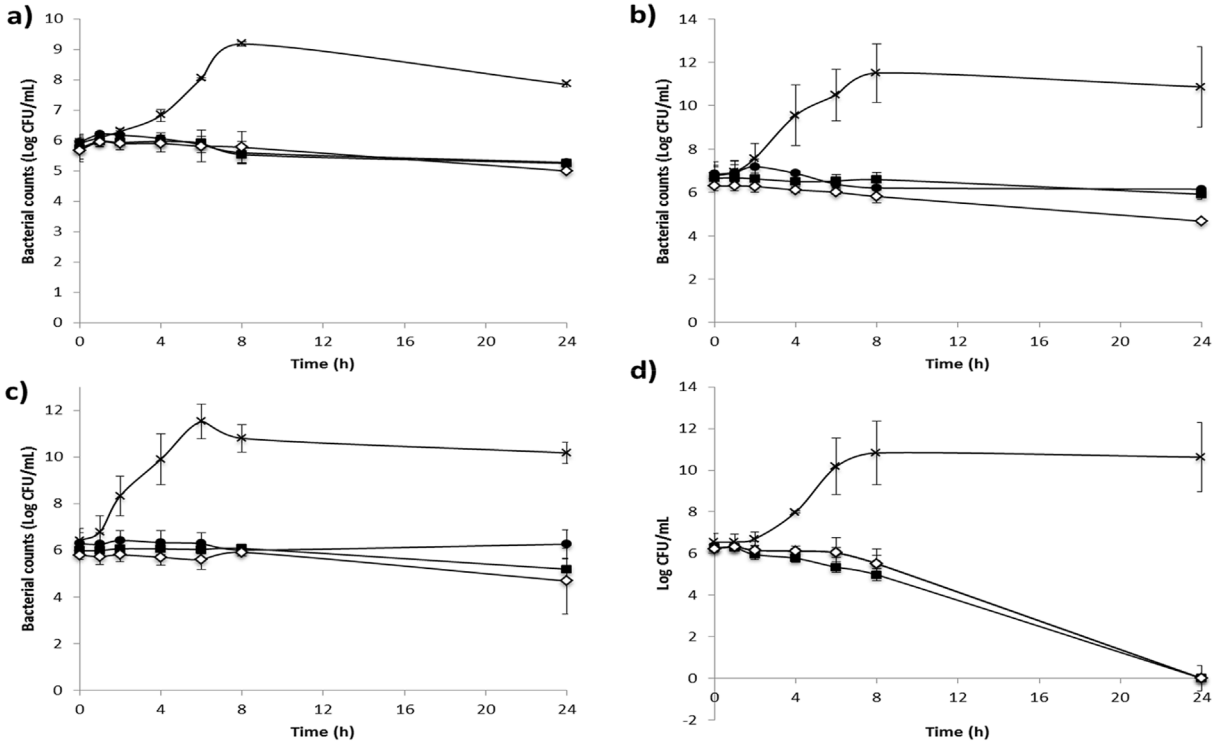

Fig. 5. Time-kill curves of test strains: a) *B. thermosphacta*, b) *L. monocytogenes*, c) *E. coli* O157:H7 and d) *Y. Enterocolitica* incubated with CEO (1xMIC) and CEO-loaded CD-NS during a 24 h period. Values are presented as mean values ± standard deviation of, at least, three independent assays. Growth control (–*–), CEO (–◊–), CEO-loaded α-NS × (–■–) and CEO-loaded β-NS (–●–) during a 24 h period.

3.4. CEO-loaded CD-NS antimicrobial activity

For the assessment of CD-NS containing CEO antimicrobial activity, a kinetic time-kill study was conducted using the two CD-NS able to encapsulate the higher absolute amounts of CEO, namely α-NS and β-NS. Bacterial growth was measured during a 24 h period through

Table 4
CEO release kinetics from α-NS and β-NS in the different culture media and growth conditions used in the time-kill assays. Maximum CEO concentration refers to the higher CEO concentration achieved in the respective culture medium and time refers to the incubation period at which that maximum concentration is attained.

Culture medium	Growth temperature (°C)	CD-NS	Maximum CINN concentration loaded (ppm)	Maximum CINN concentration released (ppm)	CINN release (%)	Incubation time (h)
MHB	**37**	α-NS	318.27 ± 17.65	123.20 ± 23.49	38.71	8
		β-NS	408.32 ± 10.42	79.63 ± 10.64	19.50	8
	25	α-NS	318.27 ± 17.65	125.76 ± 30.58	39.51	6
		β-NS	408.32 ± 10.42	82.32 ± 7.45	20.16	6
BHI	**37**	α-NS	318.27 ± 17.65	84.74 ± 10.34	26.63	4
		β-NS	408.32 ± 10.42	56.78 ± 4.61	13.91	4

bacterial colony counting. CEO release into the culture medium was also evaluated during that 24 h period, to evaluate the release kinetics of CEO into the culture medium and final CEO concentrations. As can be observed by the time-kill curves obtained (Fig. 5), both CEO alone and CEO-loaded CD-NS were able to inhibit the growth of all four bacterial species tested. In terms of mode of action, it can be seen that CEO encapsulation in CD-NS did not affect its mechanism of action against bacterial cells, since the bacteriostatic or bactericidal activities of CEO were maintained after encapsulation.

In particular, a bacteriostatic action was observed for *E. coli*, *L. monocytogenes* and *B. thermosphacta*, for the essential oil either alone or loaded in the two nanosponges. For *Y. enterocolitica*, at the end of the 24 h period, a bactericidal action was observed for both CEO and CEO-loaded NS. No significant differences were observed between both CEO-loaded NS and CEO alone antimicrobial activity for each bacterium, although CEO concentrations release into the culture media were different (Table 4).

α-NS was able to release a higher CEO amount into the culture media than β-NS independently of the growth medium or incubation temperature. This may be due to the fact that the β-cyclodextrin has a bigger cavity (0.6–0.8 nm) with respect to α-cyclodextrin (0.5–0.6 nm) (Brewster and Loftsson, 2007), so the release in this last case may be easier. In fact, β-cyclodextrin is typically the one chosen for essential oil encapsulation (Petrovic et al., 2010; Saini et al., 2017). Furthermore, it can be seen that CEO concentration in the culture medium reaches its maximum after 4–6 h of incubation, when the cells are in the exponential growth phase. This is of great relevance, as several authors have described that cells are more susceptible when they are exponentially-growing (Ferreira et al., 2014), meaning that the fact that CD-NS are able to yield a controlled release of CEO with maximum values being obtained at exponential growth, could potentiate CEO action in the bacterial cells.

Furthermore, it can be seen that the controlled release of CEO by CD-NS in aqueous medium promotes CEO antimicrobial action against *E. coli*, *L. monocytogenes* and *Y. enterocolitica*, as the concentrations released into the culture media (57–125 ppm) are remarkably lower than the concentrations used for CEO alone (125–500 ppm) which correspond to the MIC values obtained for each bacteria. Overall, it can be concluded that not all the cinnamon essential oil previously encapsulated was released by the two loaded nanosponges and that the concentration of cinnamon essential oil released in the liquid medium was lower than the MIC and MBC values first determined for the cinnamon essential oil alone. Nevertheless, the concentration released was equally effective in microbial growth inhibition as demonstrated by the time-kill curves. This can be due to a more targeted oil delivery to the cells, as already observed for cyclodextrin monomer inclusion complexes (Silva et al., 2018) and a more sustained oil release, as it has been shown that the stability of essential oils in liquid culture media is low (Golus et al., 2016).

4. Conclusions

Our results showed that the synthetized CD-NS were able to successfully encapsulate and release CEO as monitored by the HS-SPME GC-MS method previously validated. CEO-loaded CD-NS had an effective antibacterial action against the bacteria tested, with a bacteriostatic action against *B. thermosphacta*, *L. monocytogenes* and *E. coli*, and a bactericidal action against *Y. enterocolitica*. Although the CEO concentration released into the culture medium was much lower than the concentration of CEO alone in DMSO, the concentration released was equally effective in microbial growth inhibition as demonstrated by the almost overlapping time – kill curves obtained. This result is extremely important because it seems that the encapsulation of cinnamon essential oil in cyclodextrin nanosponges not only preserves but also enhances the antimicrobial action of the oil.

To the best of our knowledge, this is the first study demonstrating the potential of cyclodextrin nanosponges to encapsulate and promote a controlled release of essential oils and their major compounds into aqueous media, which in turn promoted essential oils's antibacterial activity. In sum, the physical, chemical and antimicrobial properties of CEO-loaded NS described in this work, makes them ideal candidates to be used in active food packaging application where solid and stable active agent formulations are required for the control of foodborne bacteria in food products, thus confirming the hypothesis previously made.

Acknowledgements

During part of this work, Filomena Silva acknowledged a post-doctoral fellowship [grant number SFRH/BPD/79250/2011] from Fundação para a Ciência e a Tecnologia within the scope of QREN – POPH – Advanced Formation programs co-funded by Fundo Social Europeu and MEC. This work was partially funded by FEDER funds through Programa Operacional Factores de Competitividade – COMPETE and by National Funds through FCT - Fundação para a Ciência e Tecnologia within the scope of project PEst-C/SAU/UI0709/2011. The authors also acknowledge the funding provided by the Ministerio de Economía, Industria y Competitividad (AGL-2015-67362-P) and the Gobierno de Aragón and Fondo Social Europeo to the GUIA group [financiación grupo GUIA T-10].

References

Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M., 2008. Biological effects of essential oils – a review. Food Chem. Toxicol. 46, 446–475. https://doi.org/10.1016/j.fct.2007.09.106.

Becerril, R., Nerín, C., Gómez-Lus, R., 2012. Evaluation of bacterial resistance to essential oils and antibiotics after exposure to oregano and cinnamon essential oils. Foodb. Pathog. Dis. 9, 699–705. https://doi.org/10.1089/fpd.2011.1097.

Berjia, F.L., Poulsen, M., Nauta, M., 2014. Burden of diseases estimates associated to different red meat cooking practices. Food Chem. Toxicol. 66, 237–244. https://doi.org/10.1016/j.fct.2014.01.045.

Brewster, M.E., Loftsson, T., 2007. Cyclodextrins as pharmaceutical solubilizers. Adv. Drug Deliv. Rev. 59, 645–666. https://doi.org/10.1016/j.addr.2007.05.012.

Caldera, F., Tannous, M., Cavalli, R., Zanetti, M., Trotta, F., 2017. Evolution of cyclodextrin nanosponges. Int. J. Pharm. 531, 470–479. https://doi.org/10.1016/j.ijpharm.2017.06.072.

Castiglione, F., Crupi, V., Majolino, D., Mele, A., Panzeri, W., Rossi, B., Trotta, F., Venuti, V., 2013. Vibrational dynamics and hydrogen bond properties of β-CD nanosponges: an FTIR-ATR, Raman and solid-state NMR spectroscopic study. J. Inclusion Phenom.

Macrocycl. Chem. 75, 247–254. https://doi.org/10.1007/s10847-012-0106-z.
Cavalli, R., Akhter, A.K., Bisazza, A., Giustetto, P., Trotta, F., Vavia, P., 2010. Nanosponge formulations as oxygen delivery systems. Int. J. Pharm. 402, 254–257. https://doi.org/10.1016/j.ijpharm.2010.09.025.
Celebioglu, A., Yildiz, Z.I., Uyar, T., 2018. Thymol/cyclodextrin inclusion complex nanofibrous webs: enhanced water solubility, high thermal stability and antioxidant property of thymol. Food Res. Int. 106, 280–290. https://doi.org/10.1016/j.foodres.2017.12.062.
Charumanee, S., Okonogi, S., Sirithunyalug, J., Wolschann, P., Viernstein, H., 2016. Effect of cyclodextrin types and co-solvent on solubility of a poorly water soluble drug. Sci. Pharm. 84, 694–704. https://doi.org/10.3390/scipharm84040694.
Clemente, I., Aznar, M., Silva, F., Nerin, C., 2016. Antimicrobial properties and mode of action of mustard and cinnamon essential oils and their combination against foodborne bacteria. Innov. Food Sci. Emerg. Technol. 36, 26–33. https://doi.org/10.1016/j.ifset.2016.05.013.
Clinical & Laboratory Standards Institute (CLSI), 1999. Methods for Determining Bactericidal Activity of Antimicrobial Agents: Approved Guideline M26-A. Natl. Comm. Clin. Lab. Stand. Pa.
Del Valle, E.M., 2004. Cyclodextrins and their uses: a review. Process Biochem. 39, 1033–1046. https://doi.org/10.1016/S0032-9592(03)00258-9.
Dima, C., Cotarlet, M., Balaes, T., Bahrim, G., Alexe, P., Dima, S., 2014. Encapsulation of coriander essential oil in beta-cyclodextrin: antioxidant and antimicrobial properties evaluation. Rom. Biotechnol. Lett. 19, 9128–9140.
European Food Safety Authority and European Centre for Disease Prevention and Control (EFSA and ECDC), 2017. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. EFSA J. 15, e05077. https://doi.org/10.2903/j.efsa.2017.5077.
Food and Drug Administration (FDA), 2001. Guidance for Industry: Bioanalytical Method Validation. http://www.fda.gov/downloads/Drugs/GuidanceCompilanceRegulatoryInformation/Guidances/ucm070107.pdf.
Ferreira, S., Silva, F., Queiroz, J.A., Oleastro, M., Domingues, F.C., 2014. Resveratrol against *Arcobacter butzleri* and *Arcobacter cryaerophilus*: activity and effect on cellular functions. Int. J. Food Microbiol. 180, 62–68. https://doi.org/10.1016/j.ijfoodmicro.2014.04.004.
Friedman, M., Kozukue, N., Harden, L.A., 2000. Cinnamaldehyde content in foods determined by gas chromatography-mass spectrometry. J. Agric. Food Chem. 48, 5702–5709. https://doi.org/10.1021/jf000585g.
Ghaderi-Ghahfarokhi, M., Barzegar, M., Sahari, M.A., Ahmadi Gavlighi, H., Gardini, F., 2017. Chitosan-cinnamon essential oil nano-formulation: application as a novel additive for controlled release and shelf life extension of beef patties. Int. J. Biol. Macromol. 102, 19–28. https://doi.org/10.1016/j.ijbiomac.2017.04.002.
Golus, J., Sawicki, R., Widelski, J., Ginalska, G., 2016. The agar microdilution method - a new method for antimicrobial susceptibility testing for essential oils and plant extracts. J. Appl. Microbiol. 121, 1291–1299. https://doi.org/10.1111/jam.13253.
Goni, P., Lopez, P., Sanchez, C., Gomez-Lus, R., Becerril, R., Nerin, C., 2009. Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils. Food Chem. 116, 982–989. https://doi.org/10.1016/j.foodchem.2009.03.058.
Haddi, K., Oliveira, E., 2017. Green Pesticides Handbook: Essential Oils for Pest Control. pp. 2017.
Hill, L.E., Gomes, C., Taylor, T.M., 2013. Characterization of beta-cyclodextrin inclusion complexes containing essential oils (trans-cinnamaldehyde, eugenol, cinnamon bark, and clove bud extracts) for antimicrobial delivery applications. LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 51, 86–93. https://doi.org/10.1016/j.lwt.2012.11.011.
International Conference on Harmonization (ICH), 2005. Validation of Analytical Procedures: Methodology ICH Q2B.
Janjarasskul, T., Suppakul, P., 2018. Active and intelligent packaging: the indication of quality and safety. Crit. Rev. Food Sci. Nutr. 58, 808–831. https://doi.org/10.1080/10408398.2016.1225278.
Junquera, E., Aicart, E., 1997. Potentiometric study of the encapsulation of ketoprophen by hydroxypropyl-beta-cyclodextrin. Temperature, solvent, and salt effects. J. Phys. Chem. B 101, 7163–7171. https://doi.org/10.1021/Jp963977s.
Kumar, S., Trotta, F., Rao, R., 2018. Encapsulation of Babchi oil in cyclodextrin-based nanosponges: physicochemical characterization, photodegradation, and *in vitro* cytotoxicity studies. Pharmaceutics 10. https://doi.org/10.3390/pharmaceutics10040169.
Licciardello, F., Muratore, G., Mercea, P., Tosa, V., Nerin, C., 2013. Diffusional behaviour of essential oil components in active packaging polypropylene films by multiple headspace solid phase microextraction-gas chromatography. Packag. Technol. Sci. 26, 173–185. https://doi.org/10.1002/Pts.1969.
Llana-Ruiz-Cabello, M., Pichardo, S., Maisanaba, S., Puerto, M., Prieto, A.I., Gutiérrez-Praena, D., Jos, A., Cameán, A.M., 2015. *In vitro* toxicological evaluation of essential oils and their main compounds used in active food packaging: a review. Food Chem. Toxicol. 81, 9–27. https://doi.org/10.1016/j.fct.2015.03.030.
Manso, S., Nerin, C., Gomez-Lus, R., 2011. Antifungal activity of the essential oil of cinnamon (*Cinnamomum zeylanicum*), oregano (*Origanum vulgare*) and Lauramide Argine Ethyl Ester (LAE) against the mold *Aspergillus flavus* CECT 2949. Ital. J. Food Sci. 23, 151–156.
Manso, S., Cacho-Nerin, F., Becerril, R., Nerin, C., 2013. Combined analytical and microbiological tools to study the effect on *Aspergillus flavus* of cinnamon essential oil contained in food packaging. Food Control 30, 370–378. https://doi.org/10.1016/j.foodcont.2012.07.018.
Manso, S., Pezo, D., Gomez-Lus, R., Nerin, C., Gómez-Lus, R., Nerín, C., 2014. Diminution of aflatoxin B1 production caused by an active packaging containing cinnamon essential oil. Food Control 45, 101–108. https://doi.org/10.1016/j.foodcont.2014.04.031.
Miller, J.A., Hakim, I.A., Thomson, C., Thompson, P., Chow, H.H.S., 2008. Determination of d-limonene in adipose tissue by gas chromatography-mass spectrometry. J. Chromatogr. B-Analytical Technol. Biomed. Life Sci. 870, 68–73. https://doi.org/10.1016/j.jchromb.2008.06.002.
Mith, H., Dure, R., Delcenserie, V., Zhiri, A., Daube, G., Clinquart, A., 2014. Antimicrobial activities of commercial essential oils and their components against food-borne pathogens and food spoilage bacteria. Food Sci. Nutr. 2, 403–416. https://doi.org/10.1002/fsn3.116.
Muhammad, D.R.A., Saputro, A.D., Rottiers, H., Van de Walle, D., Dewettinck, K., 2018. Physicochemical properties and antioxidant activities of chocolates enriched with engineered cinnamon nanoparticles. Eur. Food Res. Technol. 244, 1185–1202. https://doi.org/10.1007/s00217-018-3035-2.
Munhuweyi, K., Caleb, O.J., van Reenen, A.J., Opara, U.L., 2018. Physical and antifungal properties of beta-cyclodextrin microcapsules and nanofibre films containing cinnamon and oregano essential oils. LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 87, 413–422. https://doi.org/10.1016/j.lwt.2017.09.012.
Nerin, C., Astudillo, M., Covián, I., Mujika, R., 2017. Active Packaging that Inhibits Food Pathogens. European patent PCT/ES2007/070039.
Nerín, C., Vera, P., Canellas, E., 2017. Chapter 14 - active and intelligent food packaging. In: Ravishankar, R., Jamuna, A. (Eds.), Food Safety and Protection. CRC Press.
Niu, B., Yan, Z., Shao, P., Kang, J., Chen, H., 2018. Encapsulation of cinnamon essential oil for active food packaging film with synergistic antimicrobial activity. Nanomaterials 8. https://doi.org/10.3390/nano8080598.
Ölmez, H., 2016. Chapter 9 - foodborne pathogenic bacteria in fresh-cut vegetables and fruits. In: Kotzekidou, P. (Ed.), In Food Hygiene and Toxicology in Ready-To-Eat Foods. Academic Press, San Diego, pp. 151–166. https://doi.org/10.1016/B978-0-12-801916-0.00009-1.
Petrovic, G.M., Stojanovic, G.S., Radulovic, N.S., 2010. Encapsulation of cinnamon oil in beta-cyclodextrin. J. Med. Plants Res. 4, 1385–1393.
Petruzzi, L., Corbo, M.R., Sinigaglia, M., Bevilacqua, A., 2017. Chapter 1 - microbial spoilage of foods: fundamentals. In: Bevilacqua, A., Corbo, M.R., Sinigaglia, M. (Eds.), The Microbiological Quality of Food. Woodhead Publishing, pp. 1–21. https://doi.org/10.1016/B978-0-08-100502-6.00002-9.
Raeisi, M., Tajik, H., Yarahmadi, A., Sanginabadi, S., 2015. Antimicrobial effect of cinnamon essential oil against *Escherichia coli* and *Staphylococcus aureus*. Heal. Scope 4.
Ren, X., Yue, S., Xiang, H., Xie, M., 2018. Inclusion complexes of eucalyptus essential oil with β-cyclodextrin: preparation, characterization and controlled release. J. Porous Mater. 25, 1577–1586. https://doi.org/10.1007/s10934-018-0571-x.
Saini, S., Quinot, D., Lavoine, N., Belgacem, M.N., Bras, J., 2017. beta-Cyclodextrin-grafted TEMPO-oxidized cellulose nanofibers for sustained release of essential oil. J. Mater. Sci. 52, 3849–3861. https://doi.org/10.1007/s10853-016-0644-7.
Sherje, A.P., Dravyakar, B.R., Kadam, D., Jadhav, M., 2017. Cyclodextrin-based nanosponges: a critical review. Carbohydr. Polym. 173, 37–49. https://doi.org/10.1016/j.carbpol.2017.05.086.
Silva, F., Ferreira, S., Queiroz, J.A., Domingues, F.C., 2011. Coriander (*Coriandrum sativum L.*) essential oil: its antibacterial activity and mode of action investigated by flow cytometry. J. Med. Microbiol. 60, 1479–1486. https://doi.org/10.1099/jmm.0.034157-0.
Silva, F., Domingues, F.C., Nerín, C., Nerin, C., 2018. Control microbial growth on fresh chicken meat using pinosylvin inclusion complexes based packaging absorbent pads. LWT 89, 148. https://doi.org/10.1016/j.lwt.2017.10.043.
Smelcerovic, A., Djordjevic, A., Lazarevic, J., Stojanovic, G., 2013. Recent advances in analysis of essential oils. Curr. Anal. Chem. 9, 61–70. https://doi.org/10.2174/157341113804486464.
Swaminathan, S., Pastero, L., Serpe, L., Trotta, F., Vavia, P., Aquilano, D., Trotta, M., Zara, G., Cavalli, R., 2010. Cyclodextrin-based nanosponges encapsulating camptothecin: physicochemical characterization, stability and cytotoxicity. Eur. J. Pharm. Biopharm. 74, 193–201. https://doi.org/10.1016/j.ejpb.2009.11.003.
Trotta, F., Cavalli, R., 2009. Characterization and Applications of new hyper-cross-linked cyclodextrins. Compos. Interfac. 16, 39–48. https://doi.org/10.1163/156855408X379388.
Trotta, F., Zanetti, M., Cavalli, R., 2012. Cyclodextrin-based nanosponges as drug carriers. Beilstein J. Org. Chem. 8, 2091–2099. https://doi.org/10.3762/Bjoc.8.235.
Vergis, J., Gokulakrishnan, P., Agarwal, R.K., Kumar, A., 2015. Essential oils as natural food antimicrobial agents: a review. Crit. Rev. Food Sci. Nutr. 55, 1320–1323. https://doi.org/10.1080/10408398.2012.692127.
Wrona, M., Bentayeb, K., Nerín, C., 2015. A novel active packaging for extending the shelf-life of fresh mushrooms (*Agaricus bisporus*). Food Control 54, 200–207. https://doi.org/10.1016/j.foodcont.2015.02.008.
Yildirim, S., Rocker, B., Pettersen, M.K., Nilsen-Nygaard, J., Ayhan, Z., Rutkaite, R., Radusin, T., Suminska, P., Marcos, B., Coma, V., 2018. Active packaging applications for food. Compr. Rev. Food Sci. Food Saf. 17, 165–199. https://doi.org/10.1111/1541-4337.12322.
Yildiz, Z.I., Celebioglu, A., Kilic, M.E., Durgun, E., Uyar, T., 2018. Fast-dissolving carvacrol/cyclodextrin inclusion complex electrospun fibers with enhanced thermal stability, water solubility, and antioxidant activity. J. Mater. Sci. 53, 15837–15849. https://doi.org/10.1007/s10853-018-2750-1.