Development of chitosan films containing β-cyclodextrin inclusion complex for controlled release of bioactives.

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Abstract

Chitosan films with antibacterial and antifungal properties have been developed with 2-phenyl ethanol. Due to the high volatility of 2-phenyl ethanol, inclusion complexes with cyclodextrins (CDs) have been prepared in order to have a controlled release of 2-phenyl ethanol. β-CD was selected to develop the inclusion complex since it showed higher retention yield (45 %, molar basis) than α- or γ-CDs. Chitosan films incorporated with β-CD:2-phenyl ethanol were homogeneous, transparent and colorless, and showed high mechanical resistance. Furthermore, the release results of the films without the inclusion complex indicated that 2-phenyl ethanol was evaporated during the film preparation, and only an 8 % of the total bioactive was retained in the film, while the 100 % of 2-phenyl ethanol was retained in the films with the inclusion complex.

Keywords: chitosan film; inclusion complex; β-cyclodextrin; 2-phenylethanol.

1. Introduction

The use of active compounds is a strategy to confer functional properties, such as antimicrobial, antioxidant or UV-vis barrier properties, to films. However, many of these compounds, such as vitamins, antioxidants or flavors, are not stable under the preparation, storage and/or use conditions. Therefore, the use of encapsulation agents for active compounds become necessary to protect the compound from volatilization or possible reactions with external agents, and also to control a sustained release (Reineccius, 2009). Among encapsulation processes, spray-drying, spray-cooling, extrusion or molecular inclusion are employed in industrial processes, but only molecular inclusion occurs at molecular level and, thus, one molecule of the active compound is trapped in the cavity of the host molecule (Reineccius, Reineccius, & Peppard, 2002). The union between the active compound and the host molecule is called inclusion complex. One of these host molecules is cyclodextrin, since the Food and Agriculture Organization (FAO) of the United Nations recognizes it as additive (FAO, 2019).

Cyclodextrins (CD) are cyclic oligosaccharides constituted of glucose molecules joined together by α-1,4 bonds. CDs are hollow truncated cone structures with an external hydrophilic character and an internal hydrophobic character (Simionato, Domingues, Nerín, & Silva, 2019; Huang, Xu, Ge, & Cheng, 2019). Therefore, it is a suitable molecule to host a variety of bioactives, including those that are non-soluble in water since interactions between cyclodextrins and guest molecules include hydrophobic forces as well as hydrogen bonding (Zhou, Lu, Zhou, & Liu, 2019).

Depending on the glucopyranose units conforming the molecule, the most common CDs are α-, β- or γ-CDs, composed of 6, 7 or 8 glucopyranose units, respectively (Ayala-Zavala, del-Toro-Sánchez, Alvarez-Parrilla, & González-Aguilar, 2008). Due to the variety of guest molecules that can host and their acceptability as food additives, CDs are widely used in food and pharmaceutical industries. Regarding food industry, CDs can be employed for different applications, such as food supplement for spaghetti,

adding pumpkin oil (Durante et al., 2019), as flavor masking, reducing the high intensity of sourness, bitterness and astringency flavor in lingonberry juice (Kalanne et al., 2019), or as thermal stabilizers (Yoshikiyo et al., 2019). In the pharmaceutical industry, CDs are used to increase drug permeability, solubility and stability and to enhance organoleptic characteristics (Chantasart, & Rakkaew, 2019; Santana, Nadvorny, Passos, Soares, & Soares-Sobrinho, 2019; Yildiz, & Uyar, 2019). Regarding preparation, there are different methods for the inclusion complex preparation, such as freeze-drying, spray drying or coprecipitation (Suzuki et al., 2019).

CDs can be used in a variety of matrixes. Morin-β-CD complexes have been incorporated into gelatin to prepare antioxidant films (Yuan et al., 2019). Also antimicrobials, such as citral, have been used to prepare β-CD inclusion complexes to preserve bioactivity during melt extrusion of EVOH films (Chen, Li, Ma, Mcdonald, & Wang, 2019). In order to provide films with both antioxidant and antibacterial properties, essential oils have been encapsulated into β-CD to promote cumulative release from chitosan films for food packaging applications (Adel, Ibrahim, El-Shafei, & Al-Shemy, 2019). In this regard, several works have been reported in relation to the use of chitosan with CD inclusion complexes for controlled release of bioactives such as resveratrol (Zhang, Cao, Ma, Chen, & Li, 2017), carvacrol (Andrade-Del Olmo, Pérez-Álvarez, Hernáez, Ruiz-Rubio, & Vilas-Vilela, 2019), or gallic acid (Munhuweyi, Caleb, Reenen, & Opara, 2018).

The goal of this work was to assess the effectiveness of cyclodextrin inclusion complexes as stabilizers and release controllers of 2-phenyl ethanol in chitosan films. 2-phenyl ethanol is a fragrance with a pleasant odor of rose petals (Surburg, & Panten, 1985). Although it has bacteriostatic and antifungicidal character, it is very volatile and highly susceptible to oxidation, thus, storing and keeping it stable is a troublesome (Qiu, Tian, Yin, Zhou, & Zhu, 2019; Yadav, & Lawate, 2011). Therefore, 2-phenyl ethanol has been trapped into α-, β-, and γ-CDs in order to characterize and compare the retention yield. Additionally, chitosan films with β-CD:2-phenyl ethanol were developed and the optical,

68 physicochemical and mechanical properties of the films were characterized. In order to
69 assess if the addition of the inclusion complex alters the properties of the film, neat
70 chitosan and chitosan with 2-phenyl ethanol films were prepared. Furthermore, the
71 release of 2-phenyl ethanol from chitosan films into a fatty food simulant was determined.

72 **2. Materials and methods**

73 2.1. Materials

74 Chitosan (CHI), with a molecular weight of 375 kDa and a deacetylation degree above
75 75 %, was supplied by Sigma-Aldrich, Spain. Acetic acid and glycerol (GLY), used as
76 solvent and plasticizer, respectively, were supplied by Panreac, Spain. 2-phenyl ethanol
77 and α-, β- and γ- cyclodextrins (CAVAMAX® w6, w7 and w8, respectively), used for the
78 development of inclusion complexes, were food grade and supplied by Wacker
79 Chemical, Spain.

80 2.2. Inclusion complex preparation

81 The inclusion complexes of 2-phenyl ethanol were prepared according to Barba,
82 Eguinoa, & Maté (2015) with some modifications. Firstly, 10 % (w/w) cyclodextrins were
83 hydratated for 10 min; then, 2-phenyl ethanol was added in equimolar ratio and the
84 mixture was mechanically stirred for 24 h. In order to obtain the dry powder, a B-191,
85 Büchi spray-dryer was used at the following conditions: 100 % aspirator capacity, inlet
86 temperature of 180 ºC, and pump at 4 mL/min. The powder was collected and stored in
87 vials at -20 ºC.

88 2.3. FTIR analysis of α-, β-, and γ-cyclodextrin complexes

89 Fourier-transform infrared (FTIR) spectroscopy was carried out with a Nicolet Avatar 260.
90 Inclusion complexes (α-CD:2-phenyl ethanol, β-CD:2-phenyl ethanol, and γ-CD:2-phenyl
91 ethanol), cyclodextrins (α-CD; β-CD and γ-CD) and 2-phenyl ethanol were milled with

anhydrous KBr and the pellet was formed by compression. The spectra were recorded between 4000-800 cm^{-1} with 32 scans and a resolution of 4 cm^{-1}.

2.4. Retention of 2-phenyl ethanol cyclodextrin

2-phenyl ethanol was extracted from cyclodextrins by liquid-liquid extraction. 0.15 g of the inclusion complex was weighed and added into a centrifuge glass tube and 5 mL hexane and 10 mL distilled water were added. The mixture was shaken energetically for 2 min and then, vortexed for 2 min. The samples were put into a bath at 85 ºC and shacked for 30 min. The organic phase was gathered into a 50 mL flask. For each sample, three extractions were carried out, adding the organic phase into the 50 mL flask. A fourth extraction was done to confirm that the extraction was completed. From a 1:10 solution, 1 µL was injected into a Hewlett-Packard 5890 series II gas chromatography spectrometer (Agilent Technology, Barcelona, Spain), equipped with a flame ionization detector (FID) and a Supra Wax 280 column (1.0 µm x 0.53 mm, 30 m). The gas carrier employed was helium, the injection temperature was 250 ºC, the oven temperature program was started at 40 ºC with a temperature ramp of 5 ºC/min up to 220 ºC (1 min), and the detector temperature was set at 300 ºC. In order to avoid the fluctuation of the signal, an internal standard (1-heptanol) was added to the calibration standards and samples. The retention of 2-phenyl ethanol was calculated as the ratio of experimental concentration over the theoretical content.

2.5. Films preparation

Chitosan films were prepared by solution casting. 1 wt % chitosan was dissolved in 1 wt % acetic acid solution under stirring for 45 min. Then, 10 wt % β-CD:2-phenyl ethanol (based on chitosan) was added and stirring was continued for other 30 min. Finally, 15 wt % glycerol (based on chitosan) was added as plasticizer and other 30 min of stirring were needed until total homogenization of the mixture. The solution was casted into Petri dishes and dried at room temperature for 48 h. In total, six compositions were analyzed:

control films, named as CHI0GLY and CHI15GLY as a function of glycerol content; chitosan films with 2-phenyl ethanol, named as CHI0GLY2PE and CHI15GLY2PE; and chitosan films with β-CD:2-phenyl ethanol inclusion complex, named as CHI0GLYCD:2PE and CHI15GLYCD:2PE.

2.6. Optical properties of films

Color and gloss parameters of the films were analyzed. Color measurements were recorded with a CR-400 Minolta Chroma Meter colorimeter (Konica Minolta, Tokyo, Japan). Ten replicates were carried out for each sample. For the determination of color parameters CIELAB scale was used: L* from 0 to 100 (from black to white), a* from – to + (from greenness to redness), and b* from – to + (from blueness to yellowness).

Multi Gloss 268 Plus (Konica Minolta, Tokyo, Japan) was used for the determination of gloss with an incidence angle of 60º according to ASTM D523-99 (ASTM, 2018). For each composition, ten samples were assessed at room temperature.

Light-barrier capacity of films was measured by using a UV-VIS-NIR Shimadzu 3600 spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan) in the range of 200-800 nm.

2.7. Physicochemical properties of films

A Nicolet Nexus FTIR spectrometer (Thermo Fisher Scientific, Messachusetts, USA) with a Golden Gate ATR sampling accessory was employed for collecting FTIR spectra. The spectra were acquired between 4000 and 800 cm^{-1} with 32 scans for each sample and a resolution of 4 cm^{-1}.

A PANalytic Xpert Pro (PANalytical, Almelo, The Netherlands) X-ray diffraction (XRD) equipment was employed with a diffraction unit at 40 kV and 40 mA. A Cu-Kα source was employed as radiation source and the data were collected between 2.5º and 50º.

2.8. Mechanical and barrier properties of films

In order to determine the tensile strength (TS), elongation at break (EAB) and Young's modulus (E), an Instron 5967 electromechanical testing system (Instron, Massachusetts, USA) was used. Samples were cut into dog bone-shaped of 4.75 mm × 22.25 mm and tests were carried out according to ASTM D1708-93 (ASTM 1993). Five replicates were tested for each sample.

Water vapor permeability (WVP) was tested with a PERME™ W3/0120 chamber (Labthink Instruments Co. Ltd., Shandong, China) in a controlled humidity environment, according to ASTM E96-00 (ASTM, 2000). Film discs were cut with a diameter of 7.40 cm and a test area of 33 cm^2. The temperature and relative humidity were set up at 30 ºC and 90 %, respectively. Water vapor transmission rate (WVTR) was calculated as:

$$WVTR\left(\frac{g}{s\ cm^2}\right) = \frac{G}{t \cdot A}$$

where G is the weight change (g), t is the time (s), and A is the film area that was tested (cm^2).

WVP was calculated by the following equation:

$$WVP\left(\frac{g}{cm\ s\ Pa}\right) = \frac{WVTR \cdot L}{\Delta P}$$

where L is the film thickness (cm) and ΔP is the partial pressure difference of the water vapor across the film (Pa).

Three replicates for each sample were reported for the WVP analysis.

2.9. Bioactive release

Film pieces (1 cm x 2 cm) were submerged into a 95 % EtOH solution, used as a fatty food simulant (Liang et al., 2017), for 4 days under continuous stirring (200 rpm) at room temperature. Aliquots were collected at different times (30 min, 1 h, 2 h, 4 h, 8 h, 1 d, 2 d, 3 d, and 4 d). The bioactive release was analyzed by using a UV-VIS-NIR Shimadzu 3600 spectrophotometer (Shimadzu Scientific Instrument, Kyoto, Japan).

2.10. Statistical analysis

In order to determine significant differences among the samples, analysis of variance (ANOVA) was carried out with SPSS software (SPSS Statistics 25.0). For multiple comparisons, Tukey's multiple range test was used with a statistically significance at the $P < 0.05$ level.

3. Results and discussion

3.1. Characterization of α-, β-, and γ-cyclodextrin inclusion complexes

FTIR spectroscopy was used in order to characterize α-, β-, and γ-cyclodextrin inclusion complexes with 2-phenyl ethanol. As can be seen in Figure 1, the O-H stretching vibration band is observed around 3390 cm^{-1}, indicative of the hydroxyl groups of cyclodextrin. The C-O-C stretching vibrations are observed at 1158 cm^{-1}, associated to the oligosaccharide structure, and the bands at 1080 and 1030 cm^{-1} correspond to the C-C stretching vibrations of the cyclodextrin ring carbons. Additionally, the characteristic band of the α-pyranyl vibration in cyclodextrin appeared at 942.8 cm^{-1} and the characteristic band of α-(1,4) glucopyranose in cyclodextrin appeared at 890 cm^{-1} (Yuan, Ye, Gao, Yuan, Lan, Lou, Wang, 2013). As can be seen, there was no chemical interaction between the cyclodextrin and 2-phenyl ethanol. However, some characteristic bands of cyclodextrin were shifted in the inclusion complex spectra. The O-H stretching vibration displaced from 3383 cm^{-1} for β-cyclodextrin to 3393 cm^{-1} for the inclusion complex. Furthermore, C-O-C stretching vibration band moved slightly to lower wavenumbers, from 1158 cm^{-1} for β-cyclodextrin to 1157 cm^{-1} for the inclusion complex. The shift of these bands suggested physical interactions between cyclodextrins and 2-phenyl ethanol (Xiao, Hou, Kang, Niu, & Kou, 2019).

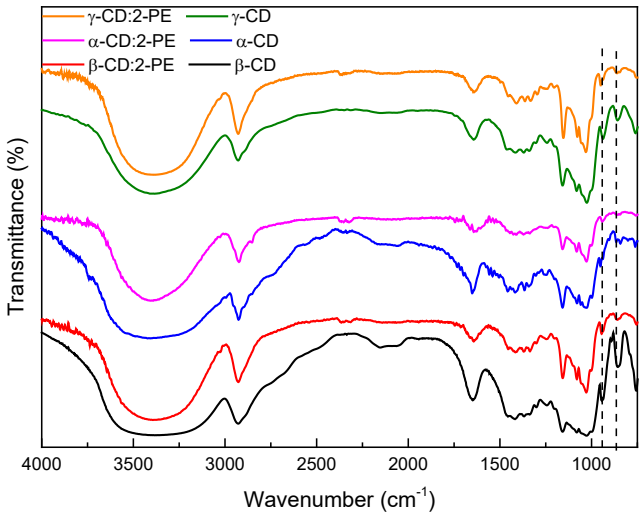


Figure 1. FTIR spectra of α-, β-, and γ-cyclodextrin (CD) inclusion complexes.

3.2. Retention yield of 2-phenyl ethanol

The retention yield of 2-phenyl ethanol for α-, β-, and γ-cyclodextrin inclusion complexes was determined. The highest value was observed for β-clyclodextrin inclusion complex (45 %), followed by γ-cyclodextrin (40 %) and finally, α-cyclodextrin (32 %). These relative low retention values can be related to the fact that 2-phenyl ethanol has only one hydroxyl group to get attached to the cyclodextrin and, thus, the interactions could be weaker. Regarding the differences in retention values, those differences can be associated to the cavity size of CDs. While α-cyclodextrin cavity is the smallest (5.7 Å) to host 2-phenyl ethanol, γ-cyclodextrin cavity is too big (9.5 Å) and, therefore, the interactions between the host and the guest were weaker. However, β-cyclodextrin cavity (7.8 Å) was big enough to host 2-phenyl ethanol and small enough to facilitate physical interactions between them. Taking the above into consideration, chitosan films were prepared with the inclusion complex with the highest retention yield; therefore, β-cyclodextrin:2-phenyl ethanol inclusion complexes were incorporated into chitosan film forming solutions.

3.3. Optical properties of films

All films were transparent and colorless. Regarding CIELab color parameters (Table 1), all films presented L* values close to 100, indicating high lightness of the films; a* parameter values were close to zero and b* parameters close to 3, indicating that the films were colorless but with a greenness tendency. Considering the ΔE* value referred to CHI0GLY, the films did not show differences for the naked eye, since ΔE* values were lower than 1 (Uranga et al., 2019). Furthermore, statistical analysis concluded that there were no significant difference among samples for L*, a* and ΔE* parameters, only a slight difference for b* value, which was not relevant. Therefore, the addition of glycerol, 2-phenyl ethanol, or the inclusion complex did not affect the film color. Regarding gloss values (Table 1), there was a slight increase ($P < 0.05$) with the addition of 2-phenyl ethanol or the inclusion complex, but there was no significant ($P > 0.05$) difference among the films with 2-phenyl ethanol, regardless the presence of CDs. Since gloss values are related to the surface roughness and low values indicate rough surfaces (Luchese, Uranga, Spada, Tessaro, & de la Caba, 2018), the values measured in this work indicated that chitosan film surface was rough.

Table 1. Color (L*, a*, b* and ΔE* parameters) and gloss values for chitosan films.

Film	L*	a*	b*	ΔE*	$Gloss_{60}$ (G.U.)
CHI0GLY	96.9 ± 0.5^{a}	-0.03 ± 0.06^{a}	2.5 ± 0.2^{a}	---	14 ± 2^{a}
CHI15GLY	96.6 ± 0.4^{a}	-0.11 ± 0.08^{a}	3.1 ± 0.3^{b}	0.6^{a}	16 ± 1^{a}
CHI0GLY2PE	96.7 ± 0.3^{a}	-0.08 ± 0.09^{a}	2.8 ± 0.2^{a}	0.3^{a}	$18 \pm 2^{a,b}$
CHI15GLY2PE	96.8 ± 0.3^{a}	-0.11 ± 0.07^{a}	2.9 ± 0.2^{b}	0.4^{a}	20 ± 2^{b}
CHI0GLYCD:2PE	96.4 ± 0.3^{a}	-0.10 ± 0.04^{a}	2.9 ± 0.2^{b}	0.6^{a}	20 ± 1^{b}
CHI15GLYCD:2PE	96.4 ± 0.7^{a}	-0.11 ± 0.08^{a}	2.9 ± 0.2^{b}	0.6^{a}	20 ± 1^{b}

Two means followed by the same letter in the same parameter are not significantly ($P > 0.05$) different through the Tukey's multiple range test.

Additionally, UV-vis spectroscopy was carried out and results are shown in Figure 2. As can be seen, films were transparent since there was no absorption at 600 nm. In particular, there was no absorption in the visible range from 400 to 800 nm, although chitosan films absorbed UV light, especially bellow 250 nm, probably due to some chromophores present in chitosan (Leceta, Guerrero, Ibarburu, Dueñas, & de la Caba, 2013).

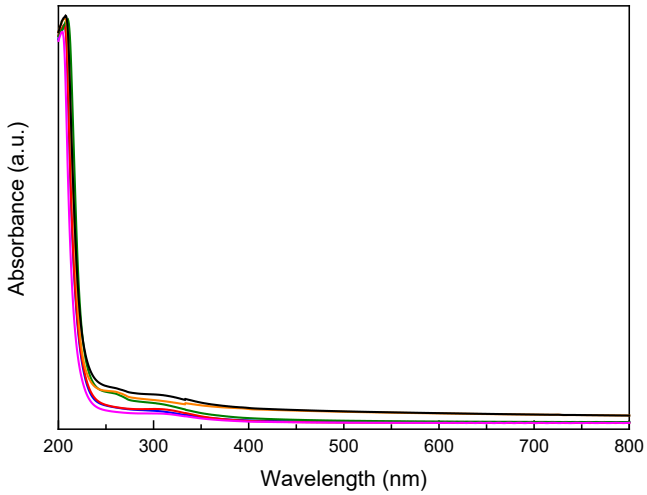
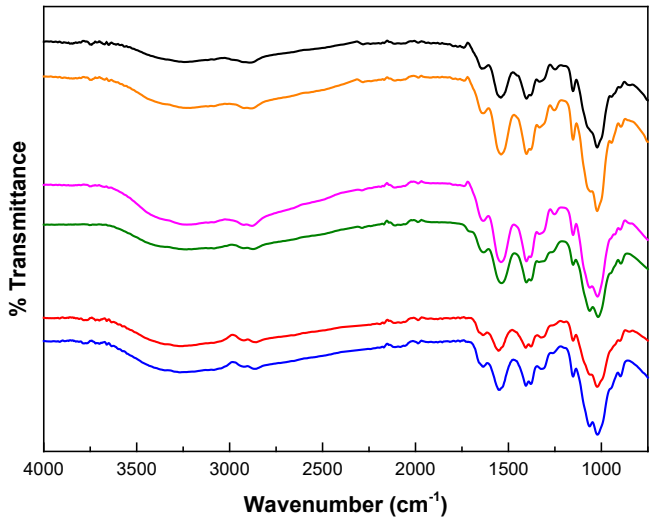


Figure 2. UV-vis light absorption of chitosan films: CHI0GLY, blue line; CHI15GLY, red line; CHI0GLY2PE, green line; CHI15GLY2PE, pink line; CHI0GLYCD:2PE, orange line; and CHI15GLYCD:2PE, black line.

3.4. Physicochemical properties of films

In order to assess the interactions among the components of the films, FTIR analysis was carried out and the FTIR spectra of chitosan films are shown in Figure 3. The characteristic bands of chitosan appeared at 1630 cm^{-1} (amide I band), associated to C=O stretching; at 1530 cm^{-1} (amide II band), related to N-H bending; and at 1310 cm^{-1} (amide III band) assigned to C-N stretching. Moreover, O-H stretching band and C-O-C absorption band were observed at 3250 cm^{-1} and around 1080 cm^{-1}, respectively. No new band was observed between the control spectra and the spectra corresponding to

244 the films with 2-phenyl ethanol, with or without CDs, indicating that no chemical reaction
245 occurred; only slight band displacements were observed, suggesting physical
246 interactions, such as hydrogen bonding or electrostatic interactions, among the
247 components of the films.



248

249 Figure 3. FTIR spectra of chitosan films: CHI0GLY, blue line; CHI15GLY, red line;
250 CHI0GLY2PE, green line; CHI15GLY2PE, pink line; CHI0GLYCD:2PE, orange line; and
251 CHI15GLYCD:2PE, black line.

252 In order to determine the structure of the films, XRD analysis was carried out. As can be
253 observed in Figure 4, films without the inclusion complex present two broad peaks, one
254 at 11.7º and another one around 21.3º, characteristic of chitosan (Pang & Zhitomirsky,
255 2005). These broad peaks indicate the amorphous character of chitosan films. Regarding
256 the films with the inclusion complex, two sharp peaks appeared at 6.2º and 12.3º,
257 characteristic of β-cyclodextrin (Campos et al., 2019; Menezes et al., 2016), indicating
258 the high crystallinity degree of the inclusion complex. However, the intensity of these
259 peaks was different for the films with and withour glycerol, which could be attributed to
260 the change in molecular organization of the cyclodextrin (Campos et al., 2018). As shown
261 by FTIR results, the intermolecular interactions with cyclodextrin molecules occurred by

hydrogen bonding, which changed the chitosan film structure due to the heterogeneous nucleation effect between the inclusion complex and chitosan (Li, & Zhen, 2017) and, as a consequence, the film mechanical behavior also changed.

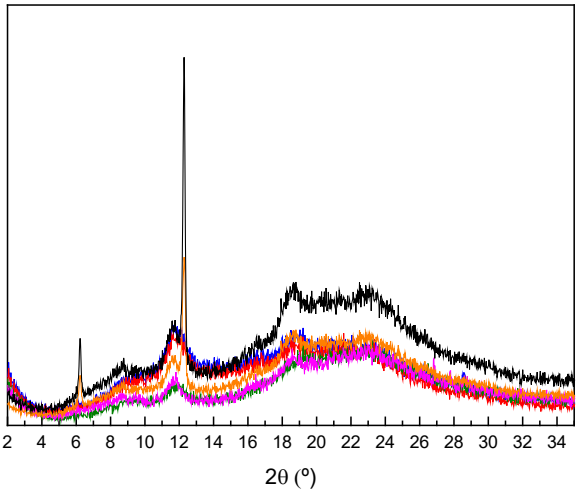


Figure 4. XRD diffractograms of chitosan films: CHI0GLY, blue line; CHI15GLY, red line; CHI0GLY2PE, green line; CHI15GLY2PE, pink line; CHI0GLYCD:2PE, orange line; and CHI15GLYCD:2PE, black line.

3.5. Mechanical and barrier properties

In order to assess the effect of structure changes in the mechanical behavior, tensile tests were performed and TS and EAB values were measured and shown in Table 2. As expected, films plasticized with glycerol showed higher EAB values and lower TS and EM values, indicating more flexible and less rigid films due to the increase of free volume (Rivero, Damonte, Garcia, & Pinotti, 2016). Regarding the addition of 2-phenyl ethanol to the film forming solution, it was observed that this compound did not significantly ($P > 0.05$) change TS or EAB values with respect to the corresponding control film, probably due to the 2-phenyl ethanol evaporation during film preparation when CDs were not used. The most significant change was observed for TS values when the inclusion complex was incorporated into formulations; in particular, TS value significantly ($P <$

0.05) increased from 34.5 to 48.8 MPa for the chitosan films with the inclusion complex but without glycerol. In accordance, EM values also increased and EAB values decreased. This behavior can be related to the interactions among chitosan and CD, as shown by FTIR analysis, which would lead to a more compact network, increasing resistance and rigidity, but decreasing flexibility (Siripatrawan & Vitchayakitti, 2016; Sun et al., 2014).

Table 2. Tensile strength (TS, MPa), elongation at break (EAB, %), Young's Modulus (EM, MPa) and water vapor permeability (WVP, $g \cdot cm^{-1} \cdot s^{-1} \cdot Pa^{-1}$) of chitosan films.

Film	TS (MPa)	EAB (%)	EM (MPa)	WVP ($10^{-12} g \cdot cm^{-1} \cdot s^{-1} \cdot Pa^{-1}$)
CHI0GLY	34.5 ± 2.2^{a}	9.2 ± 2.2^{b}	$1511 \pm 163^{a,b}$	1.08 ± 0.04^{a}
CHI15GLY	32.4 ± 3.1^{a}	16.4 ± 3.1^{a}	1374 ± 125^{a}	1.04 ± 0.02^{a}
CHI0GLY2PE	39.5 ± 3.6^{a}	7.8 ± 1.6^{b}	2205 ± 114^{c}	0.89 ± 0.04^{b}
CHI15GLY2PE	33.7 ± 3.4^{a}	12.3 ± 3.4^{a}	1687 ± 72^{b}	0.82 ± 0.01^{b}
CHI0GLYCD:2PE	48.8 ± 3.2^{b}	7.4 ± 2.2^{b}	2639 ± 173^{d}	0.83 ± 0.03^{b}
CHI15GLYCD:2PE	37.5 ± 4.1^{a}	8.1 ± 2.3^{b}	1960 ± 84^{c}	0.85 ± 0.02^{b}

Two means followed by the same letter in the same parameter are not significantly ($P > 0.05$) different through the Tukey's multiple range test.

Regarding water vapor permeability (WVP), there was no significant difference ($P > 0.05$) between the chitosan films without 2-phenyl ethanol. However, the addition of the active compound significantly ($P < 0.05$) decreased WVP values, probably due to the interactions with chitosan, as shown by FTIR, and also due to the increase of crystallinity, as observed by XRD.

3.6. Release of 2-phenyl ethanol

The release of 2-phenyl ethanol from chitosan films with 2-phenyl ethanol and from chitosan films with β-cyclodextrin:2-phenyl ethanol were carried out in order to compare the delivery trend during the immersion into 95 % EtOH for 4 days (Figure 5). Regarding

CHI0GLY2PE films, it is worth noting that some bioactive content was lost before the analysis, probably during film preparation due to the high volatility of the compound and, as a result, a maximum release of 8 % was achieved. However, chitosan films with the inclusion complex retained the bioactive inside β-cyclodextrin after film preparation and 2-phenyl ethanol was released after 4 h of immersion into 95 % EtOH. The fast release of 2-phenyl ethanol could be explained due to the weak interactions with β-cyclodextrin, since 2-pheyl ethanol has only one hydroxyl group.

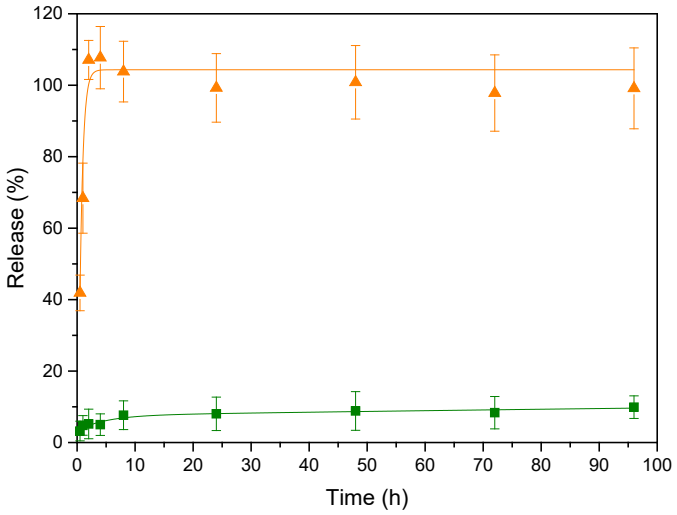


Figure 6. Bioactive release from chitosan films: CHI0GLY2PE, green line; CHI0GLYCD:2PE, orange line.

4. Conclusions

This work showed the good capacity of cyclodextrins to form inclusion complex with 2-phenyl ethanol. Specifically, β-cyclodextrin showed the highest retention yield. The films obtained were homogeneous, transparent and colorless. Moreover, the addition of the inclusion complex into chitosan film forming solutions led to chitosan films with improved properties. In particular, tensile strength reached values up to 48 MPa. The tensile strength increase was related to the physical interactions between chitosan and the inclusion complex, which were corroborated by FTIR and XRD analyses, which indicated

the crystalline structure of chitosan films with the inclusion complex. Finally, the release of 2-phenyl ethanol into 95 % EtOH was carried out in films with and without β-cyclodextrin. Results suggested that the bioactive was evaporated during film preparation for the films without the inclusion complex. In contrast, the films with the inclusion complex preserved the bioactive inside the inclusion complex. These results indicated that CDs were effective to avoid the loss of bioactive compounds during film preparation.

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References

Adel, A. M., Ibrahim, A. A., El-Shafei, A. M., & Al-Shemy, M. T. (2019). Inclusion complex of clove oil with chitosan/β-cyclodextrin citrate/oxidized nanocellulose biocomposite for active food packaging. *Food Packaging and Shelf Life, 20*, 100307.

Andrade-Del Olmo, J., Pérez-Álvarez, L., Hernáez, E., Ruiz-Rubio, L., Vilas-Vilela, J.L. (2019). Antibacterial multilayer of chitosan and (2-carboxyethyl)- β-cyclodextrin onto polylactic acid (PLLA). *Food Hydrocolloids*, 88, 228-236

ASTM D523. (2018). Standard test method for specular gloss. In *Annual book of ATSM standards.* Philadelphia, PA: American Society for Testing and Materials.

ASTM E96-00 (2000). Standard test methods for water vapour transmission of material, E96–00. Annual book of ASTM standards. Philadelphia, PA. American Society for Testing and Materials

Ayala-Zavala, J. F., del-Toro-Sánchez, L., Alvarez-Parrilla, E., & González-Aguilar, G.A. (2008). High Relative Humidity In-Package of Fresh-Cut Fruits and Vegetables: Advantage or Disadvantage Considering Microbiological Problems and Antimicrobial Delivering Systems? *Journal of Food Science, 73 (4),* 41-7.

Barba, C., Eguinoa, A., & Maté, J. I. (2015). Preparation and characterization of β-cyclodextrin inclusion complexes as a tool of a controlled antimicrobial release in whey protein edible films. *LWT-Food Science and Technology, 64*, 1362-1369.

Campos, C. A., Lima, B. S., Trindade, G. G. G., Souza, E. P. B. S. S., Mota, D. S. A., Heimfarth, L., Quintans, J. S. S., Quintans-Júnior, L. J., Sussuchi, E. M., Sarmento, V. H. V., Carvalho, F. M. S., Marreto, R. N., Costa, R. M. R., Nunes, R. S., Araújo, A. A. S., Shanmugam, S., & Thangaraj, P. (2019). Anti-hyperalgesic and anti-inflammatory effects of citral with β-cyclodextrin and hydroxypropyl-β-cyclodextrin inclusion complexes in animal models. *Life Sciences, 229*, 139-148.

Campos, E.V.R., Proença, P.L.F., Oliveira, J.L., Melville, C.C., Della Vechia, J.F., de Andrade, D.J., & Fraceto, L.F. (2018). Chitosan nanoparticles functionalized with β-cyclodextrin: a promising carrier for botanical pesticides. *Scientific Reports*, 8, 2067

Chantasart, D., & Rakkaew, P. (2019). Preparation and characterization of dry β-cyclodextrin-based ternary complexes of haloperidol and lactic acid for drug delivery. *Journal of Drug Delivery Science and Technology, 52*, 73-82.

Chen, H., Li, L., Ma, Y., Mcdonald, T. P., & Wang, Y. (2019). Development of active packaging film containing bioactive components encapsulated in β-cyclodextrin and its application. *Food Hydrocolloids, 90*, 360-366.

Durante, M., Lenucci, M. S., Gazza, L., Taddei, L., Nocente, F., De Benedetto, G. E., De Caroli, M., Piro, G., & Mita, G. (2019). Bioactive composition and sensory evaluation of innovative spaghetti supplemented with free or α-cyclodextrin chlatrated pumpkin oil extracted. *Food Chemistry, 294*, 112-122.

Huang, Z., Xu, R., Ge, G., & Cheng, J. (2019). Complexation of capsaicin with hydroxypropyl-β-cyclodextrin and its analytical application. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 223*, 117278.

Kelanne, N., Laaksonen, O., Seppälä, T., Yang, W., Tuukkanen, K., LoponenJ., & Yang, B. (2019). Impact of cyclodextrin treatment on composition and sensory properties of

lingonberry (Vaccinium vitis-idaea) juice. *LWT - Food Science and Technology, 113*, 108295.

Leceta, I., Guerrero, P., Ibarburu, I., Dueñas, M.T., & de la Caba, K. (2013). Characterization and antimicrobial analysis of chitosan-based films. *Journal of Food Engineering, 116,* 889-899.

Li, Y., & Zhen, W. (2017). Preparation and characterization of benzoylhydrazide-derivatized poly(lactic acid) and γ-cyclodextrin inclusion complex and its effect on the performance of poly(lactic acid). Polymers Advanced Technologies, 28, 1617-1628.

Liang, J., Yan, H., Zhang, J., Dai, W., Gao, X., Zhou, Y., Wan, X., & Puligundla, P. (2017) Preparation and characterization of antioxidant edible chitosan films incorporated with epigallovatechin gallate nanocapsules. *Carbohydrate Polymers, 171,* 300-306.

Luchese, C. L., Uranga, J., Spada, J. C., Tessaro, I. C., & de la Caba, K. (2018). Valorisation of blueberry waste and use of compression to manufacture sustainable starch films with enhanced properties. *International Journal of Biological Macromolecules, 115*, 955-960.

Menezes, P. P., Dória, G. A. A., Araújo, A. A. S., Sousa, B. M. H., Quintans-Júnior, L. J., Lima, R. N., Alves, P. B., Carvalho, F. M. S., Bezerra, D. P., Mendonça-Júnior, F. J. B., Scotti, L., Scotti, M. T., da Silva, G. F., de Aquino, T. M., Sabino, A. R., do Egito, E. S. T., & Serafini, M. R. (2016). Docking and physico-chemical properties of a- and b-cyclodextrin complex containing isopulegol: a comparative study. *Journal of Inclusion Phenomena and Macrocyclic Chemistry,85*, 341-354.

Munhuweyi, K., Caleb, O. J., van Reenen, A. J., & Opara, U. L. (2018). Physical and antifungal properties of β-cyclodextrin microcapsules and nanofibre films containing cinnamon and oregano essential oils. *LWT - Food Science and Technology, 87*, 413-422.

Pang, X., & Zhitomirsky, I. (2005). Electrodeposition of composite hydroxyapatite–chitosan films. *Materials Chemistry and Physics, 94*, 245-251.

Qiu, B., Tian, H., Yin, X., Zhou, Y., & Zhu, L. (2019). Microencapsulation of 2‑phenyl ethanol with methylcellulose/alginate/methylcellulose as the wall material and stability of the microcapsules. *Polymer Bulletin*, 1-13.

406 Reineccius, G. A. (2009). Edible films and coatings for flavor encapsulation. In M. E.
407 Embuscado, & K. C. Huber (Eds.), *Edible films and coatings for food applications* (pp.
408 269-294). New York: Springer.

409 Reineccius, T. A., Reineccius, G. A., & Peppard, T. L. (2002). Encapsulation of flavors
410 using cyclodextrins: comparison of flavor retention in alpha, beta, and gamma types.
411 *Journal of Food Science*, 67, 3271-3279.

412 Rivero, S., Damonte, L., García, M. A., & Pinotti, A. (2016). An Insight into the Role of
413 Glycerol in Chitosan Films. *Food Biophysics, 11*, 117-127.

414 Santana, A. C. S. G. V., Nadvorny, D., Passos, T. D. R., Soares, M. F. L. R., & Soares-
415 Sobrinho, J. L. (2019). Influence of cyclodextrin on posaconazole stability, release and
416 activity: Improve the utility of the drug. *Journal of Drug Delivery Science and Technology,*
417 *53*, 101153.

418 Simionato, I., Domingues, F. C., Nerín, C., & Silva, F. (2019). Encapsulation of cinnamon
419 oil in cyclodextrin nanosponges and their potential use for antimicrobial food packaging.
420 *Food and Chemical Toxicology, 132*, 110647.

421 Siripatrawan, U., & Vitchayakitti, W. (2016). Improving functional properties of chitosan
422 films as active food packaging by incorporating with propolis. *Food Hydrocolloids, 61*,
423 695-702.

424 Sun, X., Siyao Sui, S., Ference, C., Zhang, Y., Sun, S., Zhou, N., Zhu, W., & Zhou, K.
425 (2014). Antimicrobial and Mechanical Properties of β‐Cyclodextrin Inclusion with
426 Essential Oils in Chitosan Films. *Journal of Agricultural and Food Chemistry, 62*, 8914-
427 8918.

428 Surburg, H., & Panten, J. (1985). *Common Fragrance and Flavor Materials. Preparation,*
429 *Properties and Uses.* (5th ed.). Weinheim: WILEY-VCH Verlag GmbH & Co, (Chapter
430 2).

431 Suzuki, R., Inoue, Y., Murata, I., Nomura, H., Isshiki, Y., Hashimoto, M., Kudo, Y.,
432 Kitagishi, H., Kondo, S., & Kanamoto, I. (2019). Preparation, characterization, and study
433 of the antimicrobial activity of a Hinokitiol-copper(II)/γ-cyclodextrin ternary complex.
434 *Journal of Molecular Structure, 1194*, 19-27.

435 Uranga, J., Puertas, A. I., Etxabide, A., Dueñas, M. T., Guerrero, P., & de la Caba, K.
436 (2019). Citric acid-incorporated fish gelatin/chitosan composite films. *Food*
437 *Hydrocolloids, 86*, 95-103.

438 Xiao, Z., Hou, W., Kang, Y., Niu, Y., & Kou, X. (2019). Encapsulation and sustained
439 release properties of watermelon flavor and its characteristic aroma compounds from γ-
440 cyclodextrin inclusion complexes. *Food Hydrocolloids, 97*, 105202.

441 Yadav, G. D., & Lawate, Y. S. (2011). Selective hydrogenation of styrene oxide to 2-
442 phenyl ethanol over polyuria supported Pd–Cu catalyst in supercritical carbon dioxide.
443 *Journal of Supercritical Fluids, 59*, 78-86.

444 Yildiz, Z. I., & Uyar, T. (2019). Fast-dissolving electrospun nanofibrous films of
445 paracetamol/cyclodextrin inclusion complexes. *Applied Surface Science, 492*, 626-633.

446 Yoshikiyo, K., Yoshioka, Y., Narumiya, Y., Oe, S., Kawahara, H., Kurata, K., Shimizu,
447 H., & Yamamoto, T. (2019). Thermal stability and bioavailability of inclusion complexes
448 of perilla oil with γ-cyclodextrin. *Food Chemistry, 294*, 56-59.

449 Yuan, L., Li, S., Zhou, W., Chen, Y., Zhang, B., & Guo, Y. (2019). Effect of morin-HP- β-
450 CD inclusion complex on anti-ultraviolet and antioxidant properties of gelatin film.
451 *Reactive and Functional Polymers, 137*, 140-146.

452 Yuan, Z., Ye, Y., Gao, F., Yuan, H., Lan, M., Lou, K., & Wang, W. (2013). Chitosan-graft-
453 β-cyclodextrin nanoparticles as a carrier for controlled drug release. *International Journal*
454 *of Pharmaceutics,* 446, 191-198

455 Zhang, D., Cao, Y., Ma, C., Chen, S., & Li, H. (2017) Development of Water-Triggered
456 Chitosan Film Containing Glucamylase for Sustained Release of Resveratrol. *Journal of*
457 *Agricultural and Food Chemistry, 65*, 2503-2512.