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Preparation and characterization of chitosan/HP-β-cyclodextrins composites with high sorption capacity for carvacrol

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ABSTRACT

The aim of this work was to design new polymer-based systems exhibiting an adjustable loading capacity of carvacrol depending on the film formulation. For this purpose, biocomposite films were developed employing chitosan (CS) as the polymer matrix and hydroxypropyl-β-cyclodextrins (HP-βCDs) as an adjuvant to improve the sorption of carvacrol in the polymer matrix. The morphology, optical, mechanical and barrier properties of the resulting films were investigated, and the sorption capacity of carvacrol evaluated. Biocomposites resulted highly transparent with higher mechanical resistance and moisture barrier properties. Sorption of carvacrol was greatly affected by the humidity (RH) and glycerol (G) content of the biocomposites. The highest sorption values were achieved for composites incorporating 35% glycerol and conditioned at 75% these composites retained 216% carvacrol (g/100 g dry matter). These results indicate that inclusion of carvacrol in the films could be occurring by mechanisms other than formation of inclusion complexes.

1. Introduction

Chitosan has been widely studied as a polymer for the design of reservoir delivery systems for the slow release of active compounds over an extended period of time. These regulated delivery systems are effective in minimizing the amount of compound used for a specific application and thus improve efficacy and reduce possible side effects associated with the use of large amounts of bioactives (Pedro, Cabral-Albuquerque, Ferreira, & Sarmento, 2009; Sivakumar, Manjubala, & Rao, 2002). Chitosan employed as a delivery system can find applications in a variety of technological areas, such as agrochemistry, pharmacy, biomedicine, textiles and food packaging. The development of antimicrobial materials and their application in the design of active packaging is creating considerable expectation in the food industry, since food safety is an area of great concern. Although there are many studies in the literature that focus on the use of chitosan films as antimicrobials in contact with food, the use of chitosan films for the release of active compounds has received much less attention.

Because of their antimicrobial properties, many plant extracts and essential oils have found applications as natural preservatives. In this regard, carvacrol (5-isopropyl-2-methylphenol) is a constituent of essential oils of oregano and thyme, with known antifungal, insecticidal, antitoxigenic and antiparasitic activities (Burt, 2004; Veldhuizen, Tjeerdsma-Van Bokhoven, Zweijtzer, Burt, & Haagsman, 2006). Carvacrol is categorized as GRAS (Generally Regarded as Safe) by the FDA (Food and Drug Administration) for food. Volatile active compounds can be added to films to achieve a more effective and rational use of them. This step is especially problematic since a large amount of the compounds is lost or inactivated during processing and the remaining amount in the polymer is not enough to exert its effects on the food. In addition, the active compound must be chemically compatible with the polymer matrix to allow good dispersion in the film but not inhibit its release (Chalier, Ben Arfa, Preziosi-Belloy, & Gontard, 2007; Kurek, Descours, Galic, Voilley, & Debeaufort, 2012). Chitosan films have been loaded with active volatile compounds for several purposes (Abdollahi, Rezaei, & Farzi, 2012; Altiok, Altiok, & Tihminlioglu, 2010). However, loading of volatiles in a chitosan matrix presents several difficulties. Its incorporation as an additive into the water-based chitosan film-forming solution is challenging because of general limited aqueous solubility of volatiles and the inevitable partial loss of the compound by evaporation during the casting and drying of the film. In order to overcome these problems, Presence of cyclodextrins in the chitosan matrix could improve compatibility between the polymer matrix and the agent. Moreover, the volatile could be loaded after film casting to avoid agent losses during the film drying step.

Cyclodextrins (CDs) are donut like oligosaccharides with hydrophobic cavities and hydrophilic outer surface. They are widely used as excipients in pharmacy to solubilize lipophilic drugs

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by means of inclusion complex. However, non-inclusion based aspects of CDs are being studied and their importance to solubilization by formation of self-assemble aggregates or surfactant like effects.

The aim of this work was to develop chitosan films with a selective carvacrol loading capacity. For this purpose, biocomposites based on the addition of HP-βCD, a highly water-soluble CD derivative, into the chitosan film-forming solution were prepared and their physico-chemical properties studied, including morphology and optical, mechanical and barrier properties. The carvacrol loading capacity of the films was also studied as a function of the water and glycerol content.

2. Materials and methods

2.1. Materials

Carvacrol (kosher >98%) and low-molecular-weight chitosan (CS) were supplied by Sigma (Barcelona, Spain). Hydroxypropyl-β-cyclodextrin, HP-βCD (CAVASOL® W7-HP) was supplied by Wacker Ibérica (Barcelona, Spain). Glycerol (G) and acetic acid were purchased from Aldrich (Barcelona, Spain).

2.2. Film preparation

First, a 1.5% chitosan (w/w) solution in an aqueous 0.5% (w/w) acetic acid solution was prepared and filtrated to eliminate impurities. Pure chitosan (CS) films were prepared by casting, that is, pouring a suitable amount of the solution into a flat polystyrene tray and allowing it to dry under controlled environmental conditions (36 h, 40.0 ± 1.5 °C and 20 ± 9% RH). Glycerol-plasticized films were produced by adding glycerol at 20% or 35% (g glycerol/100 g dry matter) to the film-forming solution.

Chitosan/hydroxypropyl-β-cyclodextrin composites (CS–CD) were obtained by adding HP-βCD to the chitosan solution in a 1:1 proportion (w/w) with respect to CS, stirring at 1500 rpm and 37 °C until complete dissolution and submitting the solution to the casting process. Plasticized CS–CD films were prepared by adding glycerol at 20% or 35% (g glycerol/100 g dry matter) to the film-forming solution.

2.3. Film thickness

The film thickness of each sample was individually measured using a digital micrometre (Mitutoyo Manufacturing Co. Ltd., Tokyo, Japan) with a sensitivity of 1 μm. Five readings were taken for each sample, one at the sample centre and four measurements around the perimeter. Average thickness of the films was 55 ± 5 μm.

2.4. Optical properties

The colour of the films was measured with a CR-300 Minolta Chroma meter® (Minolta Camera Co., Ltd., Osaka, Japan). The film samples were placed on a white standard plate; the results were expressed in accordance with the CIELAB system with reference to illuminant D65 and a visual angle of 10°. The measurements were performed through a 6.4-mm-diameter diaphragm containing an optical glass, monitoring L^*, a^*, b^*, chroma ($C^*_{ab} = (a^{*2} + b^{*2})^{1/2}$) and hue ($h_{ab}$ = arctan(b^*/a^*)). The samples were measured in triplicate by eight measurements in different locations for each film sample.

The apparent opacity was evaluated (Agilent 8453 UV-visible spectrophotometer (Agilent, Barcelona, Spain)) as the integrated area under the curve, which was calculated using UV-WIN-Lab software and expressed as the product of absorbance value (A) and wavelength (nm). Samples were measured in triplicate.

2.5. Morphology

Films were fractured under liquid nitrogen and the cross-section surface morphology studied by field emission (FE) scanning electron microscopy (SEM) using a HITACHI S-4100 unit equipped with a secondary electron (SE) detector and an EMIP 3.0 image capture system (HITACHI, Madrid, Spain). Samples were coated under vacuum with gold–palladium in a sputter coating unit and their fracture surface was investigated. Images were captured at 10 kV, at a distance of 14 cm, with 1000× magnification.

2.6. Thermogravimetric analysis (TGA)

TGA of films was carried out using a Mettler Toledo TGA/SDTA/851 (Columbus, OH, USA). Samples of approximately 10 mg were heated from room temperature to 900 °C at 10 °C/min and held at an isotherm for 3 min under a nitrogen atmosphere. The TGA data were plotted as weight per cent versus temperature and the decomposition temperature was measured from the first derivative of weight per cent versus temperature (DTGA).

2.7. Moisture content

Samples (0.4–0.5 g) of each film were cut into pieces and placed on aluminium plates. They were placed in desiccators containing saturated solutions of magnesium nitrate 6-hydrate (Sigma, Barcelona, Spain), sodium chloride (Scharlau, Barcelona, Spain) and barium chloride 2-hydrate (Fluka, Madrid, Spain) in a chamber conditioned at 23 ± 1 °C in order to maintain a relative humidity (RH) of 53.0 ± 0.5, 75.0 ± 0.5 and 90.0 ± 3.0%, respectively (ASTM, 2007). These values were confirmed by direct RH measurements with hygrometers (Hygrodynamics, Newport-Scientific Inc. Jessup, MD, USA). After reaching weight equilibrium, in approximately 2 weeks, they were weighed and placed in desiccators with phosphorus pentoxide (Sigma, Barcelona, Spain) for dehydration for 2 more weeks. The tests were done in triplicate.

2.8. Barrier properties

2.8.1. Water vapour permeability (WVP)

WVP tests were carried out at two RH gradients (0/53% and 0/75%) and 23 ± 1 °C using permeability cups (Elcometer, Manchester, England) in accordance with ASTM E96/E96M-10 for flexible films (ASTM, 2010c). To ensure the necessary relative humidity, the cups were stored in desiccators containing salt solutions: magnesium nitrate 6-hydrate and sodium chloride for 53% and 75% RH, respectively. The cups were weighed daily, and the plot of the weight increase vs. time provided the water vapour transmission rate. These values were then divided by the water pressure gradient and multiplied by the sample thickness to obtain the water vapour permeability value.

2.8.2. Oxygen permeability

The oxygen permeation rates of the materials were determined at 50 and 75% RH and 23 ± 1 °C using an OXTRAN Model 2/21 ML Mocon (Lippke, Neuwied, Germany) based on the ASTM standard (ASTM, 2010b). The film samples were previously conditioned at the RH of the experiment. After conditioning the samples in the OXTRAN cells for 6 h, the transmission values were determined every 45 min until constant.

2.9. Mechanical properties

A Mecmesin MultiTest 1-í universal test machine (Landes Poli Ibérica, S.L., Barcelona, Spain) equipped with a 100-N static load cell was used to evaluate the maximum tensile strength (σ_m), percentage of elongation at break (ε_b) and Young's modulus (E) of the films according to ASTM D882-09 18 (ASTM, 2010a). Films were conditioned at 53 and 75% RH for one week before testing. Sample films were cut into 25.4 mm × 130 mm strips. Grip separation was set at 100 mm and cross-head speed at 25 mm/min. Twenty replicates from each sample were tested.

2.10. Conditioning and immersion in carvacrol

Circular film samples 55 mm in diameter were stored in glass desiccators at 0 (with phosphorus pentoxide to dry films), 53.0 ± 0.5, 75.0 ± 0.5 and 90.0 ± 3.0% RH (ASTM, 2007) in a temperature-controlled room at 23 ± 1 °C. After reaching equilibrium water sorption, the films were immersed in carvacrol the necessary time to achieve equilibrium.

2.11. Sorption of carvacrol

The analysis of the concentration of carvacrol retained in the materials was performed by thermal desorption coupled to gas chromatography using a Dynatherm Thermal Desorber Model 890/891 (Supelco, Teknokroma, Barcelona, Spain) connected in series to the column of an HP5890 gas chromatograph Series II Plus (Agilent Technologies, Barcelona, Spain) via a heated transfer line. A cut piece of the film was cleaned with a paper tissue to remove any excess of volatile compound on the film surface and then inserted into an empty desorption tube (11.5 cm × 0.39 cm I.D.). The tube was placed in the desorber chamber, which was immediately sealed. Conditions for desorption were as follows: desorption temperature, 210 °C; transfer line, 230 °C; desorption time, 7 min; He desorption flow, 8.15 mL/min. The GC was equipped with a TRB5 (30 m, 0.32 mm, 0.25 μm) column (Teknokroma, Barcelona, Spain) and a flame ionization detector. The chromatographic conditions were: 260 °C detector temperature, 7 min at 45 °C, heating ramp to 220 °C at 18 °C/min, and 1 min more at 220 °C. After the analysis, the film sample was recovered from the desorption tube and weighed on an analytical balance (Voyager V11140 model, Ohaus Europe, Greifensee, Switzerland).

2.12. Data analysis

Statistical analysis of the results was performed with SPSS commercial software (SPSS Inc., Chicago, IL, USA). A two-way analysis was applied to compare the effect of different amounts of glycerol in the same matrix CS or CS–CD. Additionally one-way analysis of variance was carried out for the other data. Differences between means were assessed on the basis of confidence intervals using the Tukey-b test at a level of significance of $P \leq 0.05$. The data are represented as average ± standard deviations. The data were analyzed and plotted using the Sigma-plot 10.0 software (Systat Software Inc., Richmond, CA, USA).

3. Results and discussion

3.1. Optical properties

The influence of the presence or absence of HP-βCDs and the different proportions of glycerol added to the film-forming solution on the colour parameter values (L^*, C^*_{ab}, h_{ab}, a^*, b^*) are presented in Table 1. In all the materials, the high values of L^* (>94) are indicative of high lightness, while slightly negative values of a^* and positive b^* are indicative of a yellow-green colour. The addition of glycerol did not significantly affect the colour coordinates ($P > 0.05$) within the glycerol range tested. However, when 50% of the CS content in the film was replaced by HP-βCD, significant changes in colour parameters were observed, with higher values of L^* and lower values of a^*, b^*, C^*_{ab} and h_{ab}. These results suggest that HP-βCDs dilute the CS matrix, reducing the colour and increasing the lightness of the composite films.

Both films had high transmittance, greater than 80% in the visible region, indicative of transparent films. The addition of HP-βCDs to the films resulted in higher transmittance for wavelengths between 190 and 600 nm and a decrease in opacity, as the values of these parameters in Table 1 show. These results correlated well with the L^* values and were indicative of the previously mentioned dilution effect caused by the addition of HP-βCDs to the film matrix.

3.2. Morphology

In a visual inspection, the CS–CD composite films were homogeneous, with no observed phase separation and with smooth surfaces and high transparency. SEM images (not shown) indicate compact surfaces, smooth and homogeneous, without pores or discontinuities, indicating a good miscibility of the three components, biopolymer, oligosaccharide and plasticizer. No differences

Table 1
Colour parameter values of chitosan (CS) films and chitosan/hydroxypropyl-β-cyclodextrin composite (CS–CD) plasticized with different concentrations (%) of glycerol and transparency parameters obtained from transmittance (T%) in the UV–vis region.

Material films	Colour				
	L^*	a^*	b^*	C^*_{ab}	h_{ab}
CS	$94.5 \pm 0.1^{a,x}$	$-1.31 \pm 0.08^{a,x}$	$10.9 \pm 0.4^{a,x}$	$10.9 \pm 0.5^{a,x}$	$96.9 \pm 0.2^{a,x}$
CS-20G	$94.3 \pm 0.8^{a,x}$	$-1.41 \pm 0.15^{a,x}$	$11.3 \pm 0.7^{a,x}$	$11.4 \pm 0.7^{a,x}$	$97.1 \pm 0.4^{a,x}$
CS-35G	$94.7 \pm 0.4^{a,x}$	$-1.32 \pm 0.09^{a,x}$	$10.7 \pm 0.4^{a,x}$	$10.8 \pm 0.4^{a,x}$	$97.0 \pm 0.3^{a,x}$
CS–CD	$95.5 \pm 0.2^{a,y}$	$-0.27 \pm 0.06^{a,y}$	$5.9 \pm 0.3^{a,y}$	$5.9 \pm 0.3^{a,y}$	$92.7 \pm 0.5^{a,y}$
CS–CD-20G	$95.5 \pm 0.2^{a,y}$	$-0.33 \pm 0.05^{a,y}$	$5.9 \pm 0.2^{a,y}$	$5.9 \pm 0.3^{a,y}$	$93.3 \pm 0.3^{a,y}$
CS–CD-35G	$95.3 \pm 0.3^{a,y}$	$-0.48 \pm 0.07^{a,y}$	$6.2 \pm 0.3^{a,y}$	$6.2 \pm 0.3^{a,y}$	$93.4 \pm 0.4^{a,y}$

Transparency				
Material films	Opacity (AU × nm)	T (%) at 280 nm	T (%) at 325 nm	Average T (%) (400–800 nm)
CS	31.8 ± 1.6^{n}	12.7 ± 1.4^{m}	22.9 ± 1.8^{m}	83.4 ± 0.8^{m}
CS–CD	26.6 ± 1.7^{m}	36.5 ± 1.7^{n}	44.9 ± 1.3^{n}	85.8 ± 0.3^{m}

[a]No statistically significant differences between means ($P > 0.05$) were found by Tukey's test when comparing different amounts of glycerol in the same matrix CS or CS–CD.
Different letters (x and y) in the same column indicate a statistically significant difference ($P \leq 0.05$) comparing CS and CS–CD.
Different letters (m and n) in the same column indicate a statistically significant difference ($P \leq 0.05$) comparing CS and CS–CD.

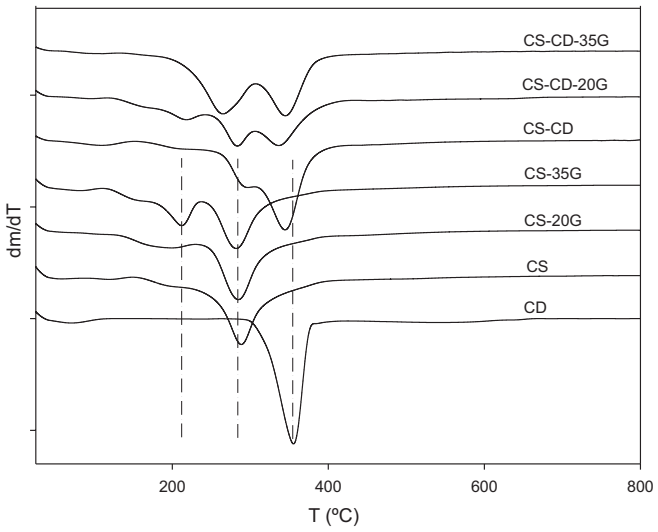

Fig. 1. Temperature derivative of sample mass obtained by TGA for selected composites and components.

were observed between samples, which indicates high dispersion and solubilization of the HP-βCDs in the CS matrix. Films formed without plasticizer showed a similar morphology, without pores or cracks.

3.3. Thermogravimetric analysis

Dry samples were analyzed by TGA to determine the thermal stability of the samples and to detect potential interactions between components. Fig. 1 shows the weight loss with temperature (d*m*/d*T* derivative) for selected samples including pure HP-βCD and CS. As can be seen, CS–CD composites presented two main features at temperatures close to those of their two components. The addition of HP-βCDs to CS appeared to produce a delay of ca. 6 °C in CS thermal degradation and an advance of 10 °C in HP-βCD degradation. These observations are indicative of a certain degree of interaction between the two components. The addition of 35% glycerol to the CS matrix reduced the temperature at which maximum weight loss was measured from 289 to 282 °C (285 °C with 20% glycerol, thermogram not shown). A previous transition at 212 °C is due to the degradation of glycerol. Finally, the composite films plasticized with 20% glycerol showed the three transitions which could be expected from the addition rule. In contrast, the sample with 35% glycerol presented only two transitions, as can be seen in Fig. 1. The transition assigned to the HP-βCDs appeared at the same temperature as in the unplasticized film. However, a new intermediate feature appeared between those of CS and glycerol, indicating that the two compounds degrade and volatilize together.

3.4. Moisture content

The properties and behaviour of the composites were expected to be dependent on environmental humidity, as occurs with most hydrocolloid-based films. The water gained by the composites was measured at three RHs: 53, 75 and 90%. Table 2 resumes the results for various films and components, including bibliographic data for glycerol (Bell & Labuza, 2000). HP-βCDs showed the lowest water gain at all the humidities tested. Plain CS films had higher water affinity than HP-βCDs. The CS–CD composite films had intermediate values which are in agreement with the additivity principle. Glycerol was the component with the highest water uptake and therefore the plasticized films had higher water uptake values than the corresponding unplasticized samples, as shown in the table. This effect was more noticeable at 75 and 90% RH. Compared with the results derived from the application of the additivity principle, water sorption by the plasticized composites was below the theoretical value in all cases. This is interpreted as a result of interaction between the glycerol and the film matrix, especially with CS, which reduces the ability of the compound to retain water and limits the accessibility of water molecules in the matrix. This result is also in agreement with the information gathered from the thermal analysis.

3.5. Barrier properties

Permeability to gases and vapours is one of the most important properties of materials with potential application in food packaging design. In this work, permeability to water vapour and to oxygen were measured at 23 ± 1 °C and at two RH conditions.

3.5.1. Water vapour permeability (WVP)

The effect of the various matrix components and the humidity gradient on the resistance to permeation of water vapour was evaluated for the films and the values are presented in Table 3. The images show various features. First, glycerol in the films gave rise to greater values of permeability to water vapour through both CS- and CS–CD-based materials. As a plasticizer, glycerol reduces the fragility of the polymer material by reducing interchain interactions. Glycerol interacts via hydrogen bonds with the $-NH_2$, C=O and −OH substituents of the macromolecules (Brown et al., 2001; Quijada-Garrido, Iglesias-González, Mazón-Arechederra, & Barrales-Rienda, 2007), thus increasing chain flexibility and mobility, which leads to lower resistance to the diffusion of permeants. The higher the concentration of glycerol, the more noticeable is the increase in water vapour permeability for a given sample and humidity gradient.

The presence of HP-βCDs in the matrix resulted in an improvement in the water vapour barrier provided. The presence of HP-βCDs appeared to produce an antiplasticizing effect on the film, as has been reported after the incorporation of fillers in polymers. Polymer–CD interactions and the structural rigidity of HP-βCDs could create steric hindrance and decrease segmental mobility,

Table 2
Water vapour uptake by films and components at 23 ± 1 °C and various relative humidities (%).

Material films	53%RH	75%RH	90%RH
Glycerol (G)	27.00[a]	82.00[a]	215.00[a]
Hydroxypropyl-β-cyclodextrin (HP-βCD)	9.30 ± 0.07	11.30 ± 0.30	28.70 ± 0.13
CS	16.98 ± 0.60	28.60 ± 0.30	45.95 ± 0.50
CS-20G	18.10 ± 0.20	33.10 ± 0.90	64.20 ± 1.50
CS-35G	18.90 ± 0.60	37.70 ± 1.60	75.50 ± 0.40
CS–CD	12.25 ± 0.25	19.30 ± 0.20	39.04 ± 0.30
CS–CD-20G	13.20 ± 0.30	28.80 ± 0.10	61.00 ± 0.40
CS–CD-35G	13.70 ± 0.30	34.80 ± 0.20	70.00 ± 0.35

[a] Data obtained from Bell and Labuza (2000).

Table 3
Water vapour permeability of CS and CS–CD films obtained with 0–53% and 0–75% humidity gradient at 23 ± 1 °C and oxygen permeability of CS and CS–CD films obtained at 50% and 75% RH at 23 ± 1 °C.

Material films	Water vapour permeability ($\times 10^{-11}$ g m/(m^2 s Pa))		Oxygen permeability (cc m/(m^2 s Pa))	
	0–53%RH	0–75%RH	50%RH	75%RH
CS	$2.49 \pm 0.13^{a,y,m}$	$5.77 \pm 0.37^{a,y,n}$	$(2.80 \pm 0.12)10^{-14a,x,m}$	$(9.27 \pm 0.06)10^{-14a,x,n}$
CS-20G	$2.57 \pm 0.12^{a,y,m}$	$6.93 \pm 0.62^{b,y,n}$	$(4.98 \pm 0.05)10^{-14b,x,m}$	$(5.40 \pm 0.90)10^{-13b,x,n}$
CS-35G	$5.29 \pm 0.22^{b,x,m}$	$11.11 \pm 0.92^{c,x,n}$	$(1.15 \pm 0.01)10^{-13c,x,m}$	$(1.25 \pm 0.16)10^{-12c,x,n}$
CS–CD	$2.16 \pm 0.03^{a,x,m}$	$3.49 \pm 0.34^{a,x,n}$	$(4.51 \pm 0.13)10^{-14a,y,m}$	$(1.04 \pm 0.10)10^{-13a,y,n}$
CS–CD-20G	$2.35 \pm 0.06^{b,x,m}$	$5.96 \pm 0.38^{b,x,n}$	$(7.98 \pm 0.15)10^{-14b,y,m}$	$(7.17 \pm 0.79)10^{-13b,y,n}$
CS–CD-35G	$6.61 \pm 0.10^{c,y,m}$	$10.37 \pm 1.38^{c,x,n}$	$(2.43 \pm 0.09)10^{-8c,y,m}$	$(1.45 \pm 0.25)10^{-7c,y,n}$

Different letters in the same column (a–c) indicate a statistically significant difference ($P \leq 0.05$) comparing different amounts of glycerol in the same matrix CS or CS–CD.
Different letters in the same column (x–y) indicate a statistically significant difference ($P \leq 0.05$) comparing CS and CS–CD.
Different letters in the same rows (m–n) indicate a statistically significant difference ($P \leq 0.05$) comparing different relative humidities of analysis for the same matrix and glycerol content.

restricting diffusivity of the permeant through the CS matrix. Moreover, as can be seen in Table 2, the addition of HP-βCDs also reduces the water uptake of the matrices. Thus, it might be expected that a reduction in diffusion and in the solubility coefficient would result in a decrease in the permeability values of the biocomposites.

Finally, increasing the humidity gradient yielded higher permeability values for the plain CS and the CS–CD films with or without glycerol. This effect can be correlated to the known water plasticization of hydrocolloids (Caner, Vergano, & Wiles, 1998; Wiles, Vergano, Barron, Bunn, & Testin, 2000). As humidity increases, water uptake also increases, and so does the plasticization of biopolymer chains.

The effects of HP-βCDs and glycerol on the CS films were also affected by the humidity gradient. The barrier improvement caused by the addition of HP-βCDs is more noticeable at the greater gradient (75% RH), as could be derived from the lower water content observed in the composite samples in comparison with the content in plain CS films. On the other hand, the plasticizing effect of glycerol was less perceptible in the WVP values measured at 75% RH, since at high humidity the water uptake plasticizes the polymer film, mimicking the glycerol effect.

3.5.2. Oxygen permeability

Table 3 shows the oxygen permeability values measured for CS- and CS–CD-based films at 23 ± 1 °C and 50 and 75% RH. CS films provided a high barrier to permeation of oxygen in dry and intermediate humidity conditions, but this property worsened with environmental humidity, as happens with other polymers with high cohesive energy density but also high affinity for water, such as EVOH or PVOH (Gallstedt & Hedenqvist, 2006; Kjellgren, Gallstedt, Engstrom, & Jarnstrom, 2006; Mensitieri et al., 2011). A similar effect was caused by the addition of glycerol. The presence of humidity and glycerol plasticized the polymer matrix of CS and CS–CD films and consequently caused a large deterioration in the oxygen permeability.

Contrasting with the effect observed in water permeability, the addition of HP-βCDs produced a significant increase in oxygen permeability ($P \leq 0.05$), which was noticeable in all composites and conditions. The cavities of HP-βCDs might be used as channels for diffusion, which would explain the results obtained. The huge deterioration in the oxygen permeability determined for the CS–CD-35G sample is noteworthy; the barrier worsened by a factor of 100,000. The obtaining of a loose polymer network owing to the glycerol content might be responsible for this mass transport behaviour.

3.6. Mechanical properties

Tensile strength, elongation at break and modulus of elasticity were determined (Table 4). The effect on these properties of the presence of HP-βCDs in CS films and the effect of the humidity and the concentration of glycerol in the films were evaluated.

As shown, the addition of HP-βCDs to the CS matrix produced changes in its mechanical properties. In the absence of glycerol, the tensile strength and modulus of elasticity of CS and CS–CD films did not differ significantly at any of the humidities tested. However, the elongation at break was significantly reduced ($P \leq 0.05$) by the incorporation of HP-βCDs at both 53% and 75% RH. This reduction was more acute in films conditioned at 75% RH. Thus, HP-βCDs act as fillers for the CS matrix, decreasing the strain of the films. It has been reported that fillers produce a rapid decrease in the elongation at break of polymers, especially if there is good adhesion between the phases.

Regarding the effect of moisture, both matrices, CS and CS–CD, showed a decrease in tensile strength and modulus of elasticity when films were conditioned at 75% RH, which can be explained by the plasticizing effect of water on the polymer matrix. The elongation at break of CS films rose with humidity, but the effect of humidity on the elongation at break of the CS–CD composites was much less acute.

Glycerol produced a decrease in tensile strength and modulus of elasticity in both, CS and CS–CD matrices, and higher values of elongation, effects which were more marked for films having a greater content of glycerol. All these effects were predictable, since glycerol acts in any of these matrices as a plasticizer, reducing interchain interactions and cohesion. The addition of this plasticizer had a secondary effect, which was the increment in water content of the matrices at any humidity because of the high hydrophilicity of glycerol, as already shown in Table 2. Since both water and glycerol produced the same effect on the matrices, their effect on mechanical properties was cumulative, the materials being less brittle and more deformable for a greater content in either glycerol or humidity. Regarding the effect of glycerol in the elongation at break of CS or CS–CD films, Table 4 shows that contents exceeding 20% glycerol did not affect this property at 53 and 75% RH. Apparently, the integrity of the matrices is damaged when films are elongated more than 50% of their initial length. Glycerol and humidity produced a similar effect on the tensile strength and modulus of elasticity of both CS and CS–CD films. However, the decline of these properties was more pronounced in the CS–CD composites, indicating a greater plasticizing capacity. It is worth highlighting that the amount of glycerol related to the CS polymer in the composite was double the amount in the plain film.

3.7. Sorption of carvacrol

Samples of CS and CS–CD composite films, prepared with and without glycerol and conditioned at various relative humidities, were immersed in carvacrol for three months. After this prolonged exposure to carvacrol, the films did not break or lose their integrity,

Table 4
Tensile strength, modulus of elasticity and elongation at break of CS and CS–CD films with different glycerol contents at 53 and 75% RH and 23 ± 1 °C.

Material films	53% RH			75% RH		
	Tensile strength (MPa)	Elongation at break (%)	Modulus of elasticity (MPa)	Tensile strength (MPa)	Elongation at break (%)	Modulus of elasticity (MPa)
CS	$57.47 \pm 3.25^{b,x,n}$	$16.39 \pm 4.23^{a,y,m}$	$1635.54 \pm 150.63^{c,x,n}$	$46.45 \pm 3.32^{c,x,m}$	$40.58 \pm 7.01^{a,y,n}$	$1297.0 \pm 121.7^{c,x,m}$
CS-20G	$40.00 \pm 4.74^{a,y,n}$	$57.04 \pm 7.59^{b,x,m}$	$609.00 \pm 70.06^{b,y,n}$	$25.46 \pm 3.46^{b,y,m}$	$54.15 \pm 3.53^{b,x,m}$	$149.2 \pm 38.0^{b,y,m}$
CS-35G	$31.00 \pm 7.24^{a,y,n}$	$56.92 \pm 5.27^{b,x,m}$	$175.95 \pm 52.61^{a,y,n}$	$19.40 \pm 3.26^{a,y,m}$	$53.52 \pm 5.21^{b,x,m}$	$28.7 \pm 4.6^{a,y,m}$
CS–CD	$58.00 \pm 3.88^{c,x,n}$	$5.09 \pm 0.79^{a,x,m}$	$1855.28 \pm 160.00^{c,x,n}$	$44.32 \pm 3.27^{c,x,m}$	$6.19 \pm 0.73^{a,x,m}$	$1440.8 \pm 128.5^{c,x,m}$
CS–CD-20G	$19.91 \pm 1.19^{b,x,n}$	$55.21 \pm 4.27^{b,x,m}$	$198.00 \pm 61.00^{b,x,n}$	$13.21 \pm 1.29^{b,x,m}$	$54.41 \pm 5.24^{b,x,m}$	$46.6 \pm 7.4^{b,x,m}$
CS–CD-35G	$7.87 \pm 2.17^{a,x,m}$	$56.61 \pm 7.39^{b,x,m}$	$14.36 \pm 2.10^{a,x,n}$	$5.00 \pm 1.91^{a,x,m}$	$55.33 \pm 5.08^{b,x,m}$	$8.7 \pm 0.7^{a,x,m}$

Different letters in the same column (a–c) indicate a statistically significant difference ($P \leq 0.05$) comparing different amounts of glycerol in the same matrix CS or CS–CD.
Different letters in the same column (x–y) indicate a statistically significant difference ($P \leq 0.05$) comparing CS and CS–CD.
Different letters in the same rows (m–n) indicate a statistically significant difference ($P \leq 0.05$) comparing different relative humidities of conditioning for the same matrix and glycerol content.

Table 5
Sorption equilibrium of carvacrol in CS and CS–CD films at 23 ± 1 °C.

Materials films	% Sorption of carvacrol (g/100 g dry matter)			
	0%RH	53%RH	75%RH	90%RH
CS	$0.08 \pm 0.01^{a,x,m}$	$0.17 \pm 0.01^{a,x,o}$	$0.19 \pm 0.03^{a,x,o}$	$0.11 \pm 0.01^{a,x,n}$
CS-20G	$0.09 \pm 0.02^{a,x,m}$	$0.23 \pm 0.03^{b,x,n}$	$0.36 \pm 0.01^{b,x,o}$	$0.27 \pm 0.03^{b,n}$
CS-35G	$0.47 \pm 0.07^{b,x,m}$	$0.96 \pm 0.04^{c,x,o}$	$0.92 \pm 0.07^{c,x,o}$	$0.68 \pm 0.07^{c,n}$
CS–CD	$0.26 \pm 0.02^{a,y,m}$	$0.40 \pm 0.01^{a,y,n}$	$0.43 \pm 0.03^{a,y,n}$	$9.97 \pm 1.12^{y,o}$
CS–CD-20G	$0.34 \pm 0.02^{b,y,m}$	$6.13 \pm 0.39^{b,y,n}$	$56.84 \pm 3.52^{b,y,o}$	–
CS–CD-35G	$4.50 \pm 0.26^{c,y,m}$	$133.27 \pm 16.93^{c,y,n}$	$216.00 \pm 22.00^{c,y,o}$	–

Different letters (a–c) in the same column indicate a statistically significant difference ($P \leq 0.05$) comparing different amounts of glycerol in the same matrix CS or CS–CD.
Different letters (x and y) in the same column indicate a statistically significant difference ($P \leq 0.05$) comparing CS and CS–CD with the same glycerol content.
Different letters (m–o) in the same rows indicate a statistically significant difference ($P \leq 0.05$) comparing different relative humidities of conditioning for the same matrix and glycerol content.

they were easy to handle and showed an apparently good mechanical resistance. Differences within samples were evident by visual inspection, since initially colourless composites acquired a yellow/green colour depending on the amount of carvacrol sorbed (Fig. 2). Table 5 shows the sorption of carvacrol by CS and CS–CD films plasticized with different amounts of glycerol (0, 20 and 35%) and conditioned at various RHs (dry film, 53, 75 and 90%).

For the CS films the sorption of carvacrol did not exceed 1%, reflecting the low affinity of this hydrophilic polymer for carvacrol. The presence of water and glycerol in the CS matrix significantly affected the carvacrol retention capacity. It was observed that, in the films conditioned at a given RH and thus having a fixed water content, sorption of carvacrol increased when the glycerol content increased from 0 to 35% (g/100 g dry matter). Glycerol-unplasticized films and films with 35% glycerol conditioned at 90% RH retained less carvacrol than those conditioned at 53 and 75% RH. On the one hand, water and glycerol have a positive effect on the sorption of carvacrol; these compounds act as plasticizers, decreasing polymer-polymer interactions and increasing chain mobility and free volume in the polymer matrix, facilitating sorption of carvacrol. However, the presence of a high water content in the polymer matrix makes the films more polar reducing their affinity for the non-polar phenolic compound carvacrol.

It is worth noting that only CS–CD films without glycerol could be conditioned at 90% RH, since when glycerol was added the films conditioned at that humidity were very sticky and could not be handled. In general, water and glycerol have a similar effect in CS matrices incorporating HP-βCDs. However, retention of carvacrol in the composites ranged from 0.26% for non-plasticized dry film to 216% for films containing 35% glycerol and conditioned at 75% RH, the sorption amount being dependent on the water and glycerol content in the films.

It was expected that HP-βCDs would promote the sorption of carvacrol, owing to their ability to form inclusion complexes with non-polar molecules. Theoretically, assuming the formation of 1:1 or 1:2 β-CD:carvacrol complexes (Locci, Lai, Piras, Marongiu, & Lai, 2004; Ravi & Divakar, 2001) the carvacrol content of the composites would range between 5 and 10% (g carvacrol/100 g dry matter). As shown in Table 5, HP-βCDs enhanced the retention of carvacrol in the biocomposites compared to plain films. Biocomposites without glycerol conditioned at 53 and 75% RH or in a dry environment, and dry biocomposites containing 20% glycerol presented significant increases in carvacrol gain with respect to CS films, but carvacrol retention was below 1%. Biocomposites containing 35% glycerol and conditioned at 53 or 75% RH and those having 20% glycerol and conditioned at 53% RH retained high levels of carvacrol, 133, 216 and 57% carvacrol (g/100 g dry matter), respectively, carvacrol sorption in these biocomposites being much greater than the theoretical sorption expected. Assuming that carvacrol is able to form 1:1 and 2:1 inclusion complexes (guest:HP-βCD), the theoretical amount of carvacrol expected to be sorbed by the film would be <10% (g carvacrol/100 g dry matter). Since amounts of carvacrol above 10%

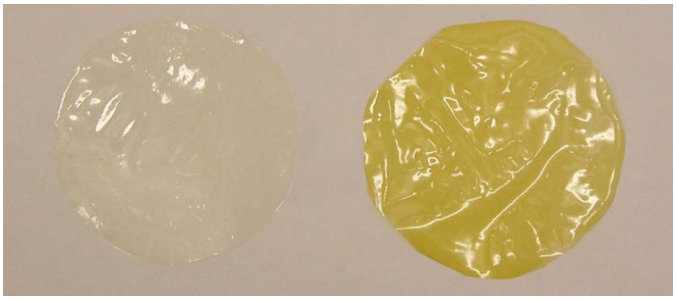

Fig. 2. CS-35G-CD composites conditioned at 75% RH, before (left) and after (right) in contact with carvacrol for three months.

were found in some films, an alternative mechanism to the formation of inclusion complexes must be taking place. These results suggest that sorption of carvacrol in CS–CD films largely plasticized by glycerol and water could be occurring by mechanisms other than formation of inclusion complexes with HP-βCDs. It has been reported that CDs are able to self-assemble to form nanosized complex aggregates, and aggregation happens rapidly with CD concentration (Messner, Kurkov, Jansook, & Loftsson, 2010). Given that the composition of the biocomposites is CS:βCDs 1:1, HP-βCDs occupy a considerable volume in the polymer matrix, which could be forming nanoparticles. Furthermore, a great increase in the free volume of the film is expected as a result of the presence of glycerol and water.

4. Conclusions

Films having good transparency and moderate mechanical properties and permeability to water and oxygen have been developed incorporating 1:1 HP-βCDs into a chitosan matrix. These hydrophilic films are capable of retaining different amounts of the non-polar, volatile compound carvacrol. Sorption depends on the degree of plasticization of the film by glycerol and water, thus it is possible to tailor the amount of the volatile agent in the film. Due to the antimicrobial properties of carvacrol, the film developed could be used as a sustained release device in food packaging, pharmaceutical and agrochemistry applications.

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