Effect of hydroxypropyl-β-cyclodextrin and coadjuvants on the sorption capacity of hydrophilic polymer films for monoterpene alcohols

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Abstract

Chitosan films filled with hydroxypropyl-β-cyclodextrin at a 1:1 weight ratio and plasticized with 35 or 50% glycerol or 35% propylene glycol were prepared by casting and conditioned at different relative humidities to achieve a similar water content. Sorption properties of the films for various monoterpene compounds with phenolic, or with linear or cyclohexyl alcohol structures were studied after their immersion in the volatile liquids. In general, the films presented a considerable capacity to retain monophenolic compounds, with sorption values ranging from 455% for meta-cumenol to 193% for guaiacol, for chitosan films with the same formulation. These values were two orders of magnitude higher than those of compounds without the phenol group. The affinity for monophenolic compounds decreased in films plasticized with hydrophilic propylene glycol, whereas no changes were observed in the retention of non-phenolic monoterpenes. Replacement of chitosan with polyvinyl alcohol polymer considerably decreased the retention of monophenolic compounds, with the exception of isoeugenol. Finally, the antimicrobial activity of monoterpenes and films loaded with them was

28 evaluated *in vitro* by the microatmosphere test against *E. coli* and *S. aureus*. The present
29 study shows that hydroxypropyl-β-cyclodextrin hydroxypropyl-β-cyclodextrin and the
30 plasticization level achieved by hydrophilic films can be used to regulate loading
31 capacity and sorption selectivity of naturally occurring antimicrobial compounds.
32
33 **Keywords:** chitosan, polyvinyl alcohol, hydroxypropyl-β-cyclodextrin, films, sorption
34 selectivity, antimicrobial activity.
35

36 **1. Introduction**

37 Nowadays consumers demand natural products that undergo minimal processing and
38 free of synthetic preservatives. However, these products often have a short
39 microbiological shelf life, which makes it necessary to find new alternatives. In this
40 regard, hurdle technology employs combined treatments and their synergies to preserve
41 food more efficiently. Active packaging technologies combined with the use of
42 naturally occurring preservatives could be an approach to hurdle technology for the
43 preservation of minimally processed foods.
44 Among the antimicrobial agents used as food preservatives, there is a growing tendency
45 to employ natural compounds from plant extracts and essential oils (Burt, 2004; Lang &
46 Buchbauer, 2012). Essential oils and their components are considered food additives
47 and classified by the JECFA (Joint FAO/WHO Expert Committee on Food Additives)
48 as flavourings. These compounds have a great potential to be used as active agents in
49 the development of antimicrobial active packaging technologies, which are a
50 complementary method for increasing the microbial safety of packaged foods.
51 Antimicrobial food packaging technologies which are based on the incorporation of
52 active volatiles in polymer matrices do not require the film to be in contact with the

78 control the kinetics of the release (Islam & Yasin, 2012; Jiang et al., 2012; Kumar,
79 2000; Lavorgna, Piscitelli, Mangiacapra & Buonocore, 2010; Muhd Julkapli, Akil &
80 Ahmad, 2011; Rahman, Sin, Rahmat & Samad, 2010; Yu, Song, Shi, Xu & Bin, 2011;
81 Zuber, Zia & Barikani, 2013).

82 In the design of polymer systems capable of retaining and releasing antimicrobial
83 volatile organic compounds it is necessary that a minimal amount of volatile be
84 entrapped in the film to provide it with antimicrobial properties. The major drawback in
85 the development of these systems is that a high percentage of the active agent is
86 evaporated or inactivated during film processing. The alternative method of absorption
87 for loading the volatile into the formed film has low efficacy. This is currently due to
88 the fact that most organic volatile compounds are hydrophobic and thus have low
89 compatibility with hydrophilic films (Balaguer, Gavara & Hernández-Muñoz, 2012).
90 Kurek, Descours, Galic, Voilley and Debeaufort (2012) recently studied how the
91 composition of the film-forming solution and process parameters affect the retention of
92 liquid volatile carvacrol. They found that glycerol and gum arabic were the most
93 effective additives to improve retention of carvacrol, whereas the effect of nanoclays
94 and emulsifiers was weak.

95 β-cyclodextrins are cyclic oligosaccharides composed of seven glucopyranose units
96 with a truncated cone shape characterized by a hydrophilic external surface and a
97 predominantly hydrophobic cavity. This unique structure enables cyclodextrins to form
98 inclusion complexes, entrapping all or part of "guest" molecules inside their cavities,
99 and presenting potential interest as agents to retain or release entrapped substances.
100 However, enhancement of the solubility of hydrophobic compounds by non-inclusion
101 aspects of cyclodextrins is currently being studied, such as solubilization by formation
102 of self-assembled aggregates or surfactant-like effects (Messner, Kurkov, Jansook &

128 Low molecular weight chitosan (CS) was supplied by Sigma (Barcelona, Spain).
129 Polyvinyl alcohol (PVOH, Gohsenol type AH-17, saponification degree 97–98.5% mol
130 and viscosity 25–30 mPa·s) was obtained from The Nippon Synthetic Chemical Co.
131 (Osaka, Japan). Hydroxypropyl-β-cyclodextrin (HPBCD, CAVASOL® W7-HP) were
132 supplied by Wacker Ibérica (Barcelona, Spain). Carvacrol (kosher >98%), L-carveol
133 >95% mixture of *cis* and *trans*, dihydrocarveol kosher >96%, isopulegol >99%,
134 isoeugenol >98% mixture of *cis* and *trans*, nerol kosher >97%, guaiacol and dimethyl
135 sulfoxide 99.9% ACS reagent (DMSO) were supplied by Sigma (Barcelona, Spain).
136 *meta*-Cumenol ≥97% and *ortho*-cumenol ≥98% were purchased from Fluka (Madrid,
137 Spain). *R*-Myrtenol >95%, glycerol (GRO), propylene glycol (PG) and acetic acid were
138 obtained from Aldrich (Barcelona, Spain). Sodium nitrite, sodium chloride, potassium
139 chloride and barium chloride dehydrate were supplied by Sigma-Aldrich (Madrid,
140 Spain). Peptone Water (PW, 0.1%), Tryptone Soy Agar (TSA) and Tryptone Soy Broth
141 (TSB) were supplied by Scharlau (Barcelona, Spain).

142 **2.2. Film preparation**

143 Films based on CS were prepared from 1.5% (w/w) CS solution dissolved in 0.5%
144 (w/w) acetic acid, stirred at 40 °C for 1 h and filtered to eliminate impurities. For films
145 based on PVOH, a 4% (w/w) PVOH solution was prepared in distilled water and stirred
146 at 85–90 °C for 2 h. For all the formulated films HPBCD was added to the film-forming
147 solution in a 1:1 proportion (w/w) with respect to CS or PVOH; the solution was stirred
148 at 37 °C until complete dissolution. Then, GRO or PG plasticizer was added at the
149 corresponding % [(g plasticizer/100 g dry matter (polymer + HPBCD)] to the film-
150 forming solution. Films were formed by casting on polystyrene plates and dried at 37 °C
151 and 40% relative humidity (RH) for 36 h. Film thickness was measured using a digital
152 micrometer (Mitutoyo Manufacturing Co. Ltd., Tokyo, Japan) with a sensitivity of 1

178 **2.4. Sorption method for loading monoterpenes into CS and PVOH films**

179 Films with different matrix compositions as described in section 2.2. were immersed in
180 different pure volatile liquids at 23 °C and the amount of the compound sorbed in the
181 film was measured over time until sorption equilibrium was reached.

182 **2.5. Determination of monoterpene sorbed in a film**The amount of volatile liquid in a
183 film was determined by thermal desorption coupled to gas chromatography using a
184 Dynatherm Thermal Desorber Model 890/891 (Supelco, Teknokroma, Barcelona,
185 Spain) connected in series to the column of an HP5890 gas chromatograph Series II
186 Plus (Agilent Technologies, Barcelona, Spain) via a heated transfer line. A cut piece of
187 the film was cleaned with a paper tissue to remove any excess of volatile compound on
188 the film surface and then inserted into an empty desorption tube (11.5 × 0.39 cm I.D.).
189 The tube was placed in the desorber chamber, which was immediately sealed.
190 Conditions for desorption were as follows: desorption temperature, 210 °C; transfer
191 line, 230 °C; desorption time, 7 min; He desorption flow, 8.15 mL/min. The GC was
192 equipped with a TRB5 (30 m, 0.32 mm, 0.25 μm) column (Teknokroma, Barcelona,
193 Spain) and a flame ionization detector. The chromatographic conditions were: 260 °C
194 detector temperature, 7 min at 45 °C, heating ramp to 220 °C at 18 °C/min, and 1 min
195 more at 220 °C. After the analysis, the film sample was recovered from the desorption
196 tube and weighed on an analytical balance (Voyager V11140 model, Ohaus Europe,
197 Greifensee, Switzerland). The thermal desorption-gas chromatography system was
198 calibrated with a film of polyethylene containing different known amounts of the
199 volatile liquid under study, previously measured by gravimetry. Sorption values are
200 given as grams of compound retained in the film per 100 grams of dry film (Balaguer,
201 Gavara, & Hernández-Muñoz, 2012).

202 **2.6. Antimicrobial assays**

228 volatile agent and incubated upside down at 37 °C for 24 h. At the end of the incubation
229 period, the antimicrobial activity of the volatile liquids was determined by measuring
230 the diameter of the zone on the surface of the agar where there was no microbial
231 growth. The minimum inhibitory dose is defined as the lowest amount of active
232 compound that yields inhibition of microbial growth on the agar surface. Each assay
233 was performed in triplicate.

234 **2.6.3. Antimicrobial activity of the films**

235 The procedure for determining the antimicrobial activity of the films was similar to that
236 described above. In this case, films which were of the same size as the filter papers and
237 loaded with the compound were placed on the centre of the Petri lid. After the
238 incubation period, the diameter of the resulting inhibition zone was measured. Each
239 assay was performed in triplicate.

240 **2.7. Data analysis**

241 The data are represented as average ± standard deviation. The data were graphically
242 plotted with SigmaPlot software (Systat Software Inc., Richmond, CA, USA).

243 **3. Results and discussion**

244 Films based on CS or PVOH as the polymer matrix incorporating HPBCD at a 1:1
245 weight ratio and plasticized with GRO or PG were successfully obtained by casting. To
246 the naked eye the films were homogeneous, with smooth surfaces, very transparent and
247 easy to handle. The thickness of the films was about 55 ± 5 μm.

248 **3.1. Equilibrium moisture content of the films**

249 A study was made of the effect of incorporating HPBCD, and the polarity and amount
250 of the plasticizer added (GRO or PG), on the sorption properties of CS films for various
251 monoterpene compounds with phenolic or non-phenolic linear or cyclohexyl alcohol
252 structures. Moreover, in order to investigate the effect of the hydrophilicity of the

278 (33.5 vs. 30.1 $MPa^{1/2}$), so it would be expected to have a greater affinity for water (δ =
279 47.9 $MPa^{1/2}$).

280 When PVOH was used as the polymer matrix in the PVOH:HPBCD-35GRO
281 formulation, the films had to be conditioned at a higher RH than the corresponding
282 films made with CS (84% vs. 75% RH, respectively) to achieve a similar water content
283 (32.4 ± 1.7%). The solubility parameter is frequently used to study compatibility in
284 polymer–plasticizer, polymer–drug and polymer–aroma systems. With regard to the
285 polymer–water system studied in the present work, the experimental Hildebrand
286 solubility parameter of PVOH ranges from 25.8 to 29.1 $MPa^{1/2}$ compared with 38
287 $MPa^{1/2}$ for CS, whereas δ for water is 47.9 $MPa^{1/2}$, which is in accordance with the
288 lower moisture sorption of PVOH films and the greater RH required to make their water
289 content equal to that of CS films. A further factor to be taken into account is that PVOH
290 is a semicrystalline polymer, which also limits its water sorption capacity.

291 **3.2. Miscibility studies**

292 Plasticizers are low-molecular compounds, chemically compatible with the polymer to
293 be plasticized, which at appropriate concentrations impart flexibility and facilitate film
294 handling. In a plasticization process, the plasticizer molecules are accommodated in the
295 polymer matrix by disrupting intermolecular forces between polymer chains, spacing
296 them apart and increasing the free volume, thus acting as diluents. Plasticization of
297 hydrophilic polymer matrices is commonly carried out by polyols, among which G and
298 PG are commonly used for this purpose. Most plasticizers employed in polysaccharide
299 films, such as polyols, are frequently employed in the flavouring industry as solvents
300 and liquid supports for flavours. The presence of hydroxyl groups in GRO make it a
301 good solvent for many ingredients used in pharmaceutical preparations and flavour
302 compounds. G can behave as a binder of relatively polar volatile compounds such as

327 greater polarity of GRO limits its compatibility with them. This feature could modify
328 the sorption properties of the films, depending on the plasticizer used.
329 **Table 1** shows the solubility parameters of monoterpenes. It is expected that the closer
330 the parameters for a sorbent and a polymer, the greater the sorption affinity will be.
331 Hydrophilic components of the films – CS, PVOH, GRO, PG and water (38, 25.8–
332 29.1,33.5, 30.1 and 47.9, respectively) – had high values of δ compared with those of
333 the monoterpenes. This means that phenolic monoterpenes with δ values closer to those
334 of the film components (ranging from 23.6 to 26.5 $MPa^{1/2}$) are expected to be retained in
335 the films to a greater extent than cyclic and linear non-phenolic monoterpene alcohols
336 with lower solubility parameters, comprised between 19.3 and 21 $MPa^{1/2}$.
337 Absorption of volatile liquids in a film will be affected by chemical affinity between the
338 sorbate and the film components. Sorption properties of the films for the various
339 compounds are shown in **Table 2**. A common feature for all the films formulated was
340 that phenolic monoterpenes were sorbed in greater amounts than non-phenolic cyclic
341 and linear monoterpenes. The presence of the benzene ring increases affinity of the
342 molecule for the film, owing to the affinity between the double bonds of benzene and
343 polar groups of the film (hydroxyl, amino, acetamido and carbonyl groups of the chain
344 end). It could also be hypothesized that these phenolic compounds due to their
345 amphiphilic nature could self-assamby inside the chitosan membrane in the presence of
346 HPBCD. Sorption of phenols increased in the following order: *meta*-cumenol > *ortho*-
347 cumenol > carvacrol > guaiacol > isoeugenol). *meta*-Cumenol was sorbed to a slightly
348 greater extent than *ortho*-cumenol, one possible explanation for which is stearic
349 hindrance of the isopropyl group, hindering hydrogen bonding through hydroxyl groups
350 of *ortho*-cumenol and the hydrophilic matrix of CS or PVOH. In fact, swelling and loss
351 of dimensional stability were observed in CS:HPBCD-35GRO films loaded with *meta*-

377 than carveol (2.92 vs. 2.55). The cyclohexene ring in carveol increased polarity and
378 sorption affinity for the films compared with the cyclohexane ring of dihydrocarveol,
379 whereas the sorption of positional isomer isopulegol decreased considerably, which
380 might be due to the isopropenyl substituent next to the hydroxyl group.
381 It can be concluded that the presence of a benzene group in the molecular structure of
382 monoterpenes allowed high sorption values and plasticization of the films. Plasticization
383 by benzene of hydrophilic pervaporation membranes has been reported in the literature
384 (Villaluenga & Tabe-Mohammadi, 2000).
385 In a comparison of the sorption properties of the films formulated with CS:HPBCD
386 possessing approximately the same water content and differing in the amount of GRO,
387 greater sorption values were observed for *ortho*- and *meta*-cumenol in CS:HPBCD films
388 with 35% GRO. In a previous study regarding the sorption capacity of CS films blended
389 with HPBCD and different amounts of GRO and water, it was demonstrated that
390 sorption is almost suppressed in unplasticized films, requiring the presence of HPBCD
391 and plasticization by GRO and water to retain carvacrol (Higueras, López-Carballo,
392 Cerisuelo, Gavara & Hernández-Muñoz, 2013). In that work, it was concluded that G
393 enhances sorption of carvacrol more than water does. In the present study, it was
394 observed that, at a fixed water content of 33–34% (g/g dry film), increasing the G
395 content from 35 to 50% did not affect the sorption of phenolic monoterpenes with the
396 exception of *ortho*- and *meta*-cumenol, which reduced their sorption. This might be
397 related to an excess of film plasticization, restricting the very high sorption values of
398 cumenol isomers. In an unpublished previous study it was found that, for a lower water
399 content in the films (around 15% when CS:HPBCD films were conditioned at 53%
400 RH), the content of sorbed carvacrol increased with the amount of GRO in the film,
401 giving sorption values of 6.13, 133.27 and 300% when plasticized with 20, 35 and 50%

427 capacity. Sorption of cumenol isomers, carvacrol and guaiacol was lower in the
428 PVOH:HPBCD-35GRO films than in the films with CS as the polymer matrix. Owing
429 to its high hydrophilicity, GRO has a greater capacity to plasticize more polar CS
430 compared with PVOH, which might explain the lower sorption values obtained for
431 phenolic monoterpenes with the exception of isoeugenol. Moreover, PVOH has a
432 certain degree of crystallinity, which restricts sorption. Similar sorption values were
433 found for isoeugenol and non-phenolic monoterpenes in the CS:HPBCD-35GRO and
434 PVOH:HPBCD-35GRO films; the less hydrophilic nature of PVOH tended to increase
435 its sorption ability for more hydrophobic compounds as compared with CS. In fact,
436 **Figure 2** shows that the affinity between the PVOH films without HPBCD and
437 carvacrol was greater than for CS films, reaching carvacrol sorption values of 4.4 and
438 23% for films plasticized with 20 and 35% GRO, respectively, whereas carvacrol
439 sorption in CS films without HPBCD was lower than 1%, irrespective of their water and
440 G contents (Higueras, López-Carballo, Cerisuelo, Gavara & Hernández-Muñóz, 2013).

441 **3.4. Antimicrobial capacity of monoterpenes in vapour phase**

442 The minimum inhibitory dose of monoterpenes against *S. aureus* and *E. coli*, evaluated
443 in vapour phase, is given in **Table 3**. Carvacrol showed the greatest antimicrobial
444 activity against both microorganisms, with 1 mg of carvacrol being needed to produce
445 clear inhibition of growth on agar plates, followed by *ortho*- and *meta*-cumenol.
446 However, it was necessary to use more than 1 mg of these compounds to see a clear
447 effect, and the inhibition halo created by *ortho*-cumenol was larger (29 vs. 19 mm). The
448 greater vapour pressure of *ortho*-cumenol compared with that of *meta*-cumenol (**Table**
449 **1**) might explain the higher activity in vapour phase; the greater antimicrobial activity of
450 *ortho*- and *meta*- isomers compared with *para*-isomers of some drugs is reported in the
451 literature (Biava et al., 1999). Phenolic compounds present in essential oils have been

477 greater antimicrobial activity. The antimicrobial capacity of isoeugenol was lower than
478 that of molecules with an isopropylphenol structure and also than that of
479 dihydrocarveol, carveol and nerol. In a comparison of monoterpenes with similar
480 structures but possessing a cyclohexanol or phenol group, molecules with a
481 methylisopropylphenol structure (cumenol isomers and carvacrol) showed higher
482 activity than compounds with a methylisopropenylcyclohexanol (dihydrocarveol,
483 isopulegol) or methylisopropenylcyclohexenol (carveol) structure. This highlights the
484 above-mentioned importance of the phenol group in the antimicrobial activity of the
485 molecule.

486 Isoeugenol, nerol and carveol showed similar antimicrobial activity, whereas it was
487 slightly higher for dihydrocarveol and lower for myrtenol. With regard to the
488 compounds derived from *para*-methylisopropenylcyclohexanol, isopulegol and
489 dihydrocarveol, the microorganisms presented lower sensitivity against isopulegol than
490 against dihydrocarveol, the only difference between them being the hydroxyl group
491 position. Thus, 8.0 mg of isopulegol was needed compared with 2.0 mg of
492 dihydrocarveol to produce inhibition of bacterial growth.

493 Lipophilicity has been used as the descriptor with the strongest influence on
494 antimicrobial activity owing to the great affinity of lipophobic compounds for cell
495 membrane (Dambolena, Lopez, Meriles, Rubinstein & Zygadlo, 2012). This property is
496 specially considered for compounds with log P between 3 and 4. However, in the
497 present study, monoterpenes with similar log P values exerted different antimicrobial
498 activities. These results indicate that other factors besides hydrophobicity are involved,
499 such as the presence and hydrogen-donating ability of compounds with a phenol
500 chemical structure.

501 **3.5. Antimicrobial capacity of films loaded with monoterpenes**

than the amount required to cause a microbial inhibitory effect in vapour phase. Apparently, the antimicrobial activity of the films with myrtenol, nerol, dihydrocarveol and carveol was similar, owing to the similar sorption values and similar antimicrobial activity of these compounds, although slight differences were found. For example, CS:HPBCD-50GRO films with 3.9 mg of myrtenol produced an inhibition zone of 10 mm, while the film with 3.8 mg of nerol produced an inhibition zone of 30 mm, the minimal dose necessary to produce inhibition being 3.2 mg for myrtenol and 2.4 mg for nerol.

4. Conclusions

The present study shows that HPBCD together with low molecular weight plasticizers GRO and PG, and moisture are capable of regulating the sorption capacity of hydrophilic chitosan films for various monoterpene alcohols. The sorption affinity for monoterpene phenolic compounds was dramatically higher than that for compounds possessing a cyclohexanol structure or linear alcohols. This was associated with the greater polarity of the benzene ring and its affinity for chitosan polar groups. Participation of HPBCD in self-assembly of phenolic terpenes inside a greatly plasticized hydrophilic polymer matrix is also proposed. It was also found that sorption properties of positional isomers differed slightly, which might be related to phenolic or cyclohexanol substituents impeding interactions between the hydroxyl group and polar groups in chitosan. Increasing the affinity of the plasticizer for monoterpene alcohols did not increase sorption properties. On the contrary, sorption values were lower for phenolic compounds cumenol isomers, carvacrol and guaiacol when GRO was replaced by PG. This indicated that the plasticizer plays a more important role in swelling the polymer matrix than in increasing film affinity for sorbates. When PVOH was employed as the polymer matrix the sorption behaviour of the films for monoterpenes

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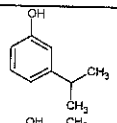
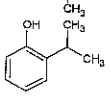
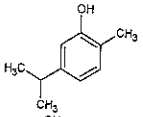
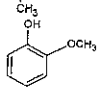
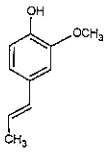
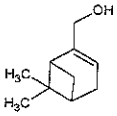
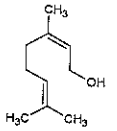
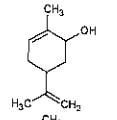
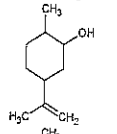
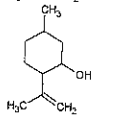
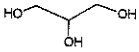
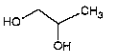
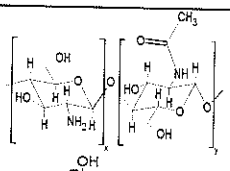
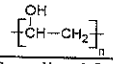
Table 1. Physico-chemical properties and molecular structure of volatile liquids, plasticizers, and polymers.

Name	Molecular structure	Molecular mass	[a]Log P	Vapour pressure (Pa)	δ ($MPa^{1/2}$)
meta-cumenol		136.19	2.82	6.67	24.1[b]
ortho-cumenol		136.19	2.82	14.93	24.1[b]
carvacrol		150.22	3.28	4.00	23.6[b]
guaiacol		124.14	1.19	23.86	26.5[b]
isoeugenol		164.20	2.45	0.67	24.3[b]
myrtenol		152.23	3.22	2.40	21.0[b]
nerol		154.25	3.28	1.73	20.9[b]
carveol		152.23	2.55	1.60	19.7[b]
dihydrocarveol		154.25	2.92	2.40	19.3[b]
isopulegol		154.25	2.92	13.20	19.3[b]
glycerol		92.09	-1.84	<0.01	33.5[b]
Propylene-glycol		76.09	-1.05	27.20	30.1[b]
water	H_2O	18.02	-1.38	3263.01	47.9[c]
CS		50 - 190 KDa		<0.01	38[b]
PVOH					from 25.8 to 29.1[d]

Note: molar volume and log P predicted from ACD/Labs.
[a] log P: hydrophobicity of the molecule expressed as the logarithm of octanol/water partition coefficients and estimated using ACD/Labs 12.0 ChemSketch software.
[b] Hildebrand solubility parameter estimated according to Fedors, in van Krevelen and te Nijenhuis (2009).
[c] Solubility parameter of water from Grulke (1989).

Table(s)

Table 2. Sorption capacity of films based on CS or PVOH incorporating hydroxypropyl-β-cyclodextrin in 1:1 weight ratio and plasticized with glycerol or propylene glycol for monoterpenes at 23 °C (%, g/100 g dry film).

	CS:HPBCD-35GRO-75RH	CS:HPBCD-50GRO-65RH	CS:HPBCD-35PG-90RH	PVOH:HPBCD-35GRO-84RH
meta-cumenol	455.06 ±18.88	372.38 ± 18.85	269.94 ± 13.38	274.27 ± 44.19
ortho-cumenol	419.67 ± 21.99	339.40 ± 17.95	227.28 ± 18.42	226.37 ± 8.68
carvacrol	230.11 ± 18.74	224.80 ± 9.85	179.62 ± 3.09	128.23 ± 2.09
guaiacol	193.22 ± 1.32	184.89 ± 7.95	106.59 ± 3.16	85.70 ± 7.48
isoeugenol	12.02 ± 6.72	12.59 ± 3.28	11.50 ± 2.04	13.56 ± 2.56
myrtenol	3.48 ± 0.93	2.16 ± 0.58	2.26 ± 0.97	2.27 ± 0.11
nerol	3.09 ± 0.79	2.48 ± 0.06	2.43 ± 0.79	2.39 ± 0.51
carveol	2.37 ± 0.74	2.41 ± 0.08	2.50 ± 0.40	2.39 ± 0.06
dihydrocarveol	1.27 ± 0.09	1.85 ± 0.08	1.91 ± 0.20	2.0.5 ± 0.27
isopulegol	0.30 ± 0.74	0.52 ± 0.03	0.28 ± 0.01	0.92 ± 0.19

Table 4. Antimicrobial activity of films (25 mm diameter surface) against *S. aureus* and *E. coli* after loading with monoterpenes, measured by the microatmosphere method.

S. aureus

	CS:HPBCD-35GRO-75RH		CS:HPBCD-50GRO-65RH		CS:HPBCD-35PG-90RH		PVOH:HPBCD-35GRO-84RH	
	Amount (mg)	Inhibition zone (mm)	Amount (mg)	Inhibition zone (mm)	Amount (mg)	Inhibition zone (mm)	Amount (mg)	Inhibition zone (mm)
meta-cumenol	327.1	>85	275.4	>85	226.2	>85	169.3	>85
ortho-cumenol	347.5	>85	243.7	>85	222.8	>85	101.5	>85
carvacrol	146.5	>85	184.9	>85	194.9	>85	147.9	>85
guaiacol	106.0	71	121.3	79	96.4	70	194.2	80
isoeugenol	18.7	54	24.1	63	22.2	59	27.0	67
myrtenol	5.7	44	3.9	10	4.5	29	3.3	10
nerol	5.4	40	3.8	30	3.0	12	3.2	30
carveol	3.5	27	4.4	34	4.3	32	4.6	35
dihydrocarveol	3.0	24	2.8	20	3.4	20	4.0	40
isopulegol	1.9	-	0.8	-	2.9	-	1.7	-

E. coli

	CS:HPBCD-35GRO-75RH		CS:HPBCD-50GRO-65RH		CS:HPBCD-35PG-90RH		PVOH:HPBCD-35GRO-84RH	
	Amount (mg)	Inhibition zone (mm)	Amount (mg)	Inhibition zone (mm)	Amount (mg)	Inhibition zone (mm)	Amount (mg)	Inhibition zone (mm)
meta-cumenol	282.9	>85	297.1	>85	244.0	>85	206.7	>85
ortho-cumenol	272.2	>85	211.3	>85	193.2	>85	182.8	>85
carvacrol	120.2	>85	208.3	>85	219.6	>85	152.0	>85
guaiacol	136.0	64	144.2	81	114.6	72	190.1	77
isoeugenol	20.6	50	27.5	65	25.4	63	17.9	51
myrtenol	6.3	40	3.6	20	3.6	20	3.2	8
nerol	5.4	37	3.8	24	2.9	8	3.0	25
carveol	4.7	22	4.4	29	0.5	-	3.4	28
dihydrocarveol	5.0	24	3.2	17	3.9	20	3.2	36
isopulegol	1.8	-	0.8	-	2.9	-	1.2	-

Figure 2. Effect of incorporating hydroxypropyl-β-cyclodextrin and glycerol on equilibrium moisture content and sorption equilibrium of carvacrol in PVOH films conditioned at 75% RH.