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3 ***Food applications of active packaging EVOH films containing***
4 ***cyclodextrins for the preferential scavenging of undesirable compounds***
5

6
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12
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14
15 **Abstract**

16
17 Novel ethylene-vinyl alcohol copolymer (EVOH) films containing beta-cyclodextrins
18 (β CD) with potential application in active food packaging have been tested as materials
19 for the preferential retention of undesired food components. The films were immersed
20 on pasteurized milk and UHT milk and stored at 4 and 23°C, respectively. The films
21 containing β CD presented a significant reduction in cholesterol concentration, achieving
22 a 23% reduction in UHT milk exposed to EVOH films containing 30% β CD. Despite
23 the immobilization of the β CD and the large molecular size of cholesterol, 15% of the
24 β CD molecules added to the films were involved in the formation of β CD/cholesterol
25 inclusion complexes. In another set of experiments, the films were used to reduce the
26 presence of aldehydes (substances which develop as a result of oxidative processes) in
27 packaged fried peanuts. The films containing β CD brought a significant reduction in
28 hexanal, reaching a 50% decrease over short periods (1-5 weeks). At longer storage
29 times (10 weeks) the retention capacity of the developed films was exhausted and no
30 differences were observed between the samples.

31
32 **Keywords:** active packaging; cyclodextrins; EVOH; cholesterol scavenging; aldehydes
33 retention; lipid oxidation

35 1. Introduction

36

37 Active packaging is one of the emerging technologies which is being developed as an
38 alternative to traditional food processing (intense heat treatments, salting, acidification,
39 drying and chemical preservation). Active packaging does more than simply provide a
40 barrier to external detrimental factors, as the packaging system plays an active role in
41 food preservation and quality during the marketing process (López-Rubio et al., 2004).
42 Active packaging materials are designed to deliberately incorporate components that
43 release or absorb substances into or from the packaged food or the environment
44 surrounding the food to extend the shelf-life or to maintain or improve the condition of
45 the packaged food (Regulation (CE) No 450/2009 (29/05/2009)). Nowadays, active
46 material developments are focusing on polymeric matrices which release active agents
47 (antimicrobials, antioxidants, etc.) and/or retain substances such as oxygen, ethylene or
48 water (Charles et al., 2006; [Flores et al., 2007](#)) or undesired food components. While the
49 scavengers used in the latter applications are mainly based on inorganic metals or salts,
50 which have mechanisms of action based on irreversible reactions, the present work
51 studies the use of cyclodextrins as potential scavengers.

52

53 Beta-cyclodextrin (β CD) is an inexpensive enzyme-modified starch derivative,
54 composed of seven glucose units linked by $\alpha(1\rightarrow 4)$ glycosidic bonds in a cylindrically
55 shaped cavity with a hydrophobic inner surface and a hydrophilic outer surface. The
56 hydrophobic cavity is able to form inclusion complexes with a wide range of organic
57 guest molecules principally by means of weak forces, such as van der Waals, dipole-
58 dipole interactions, and hydrogen bonding. The use of cyclodextrins has increased
59 annually in the food sector (Astray et al., 2009) mainly to remove cholesterol but also as
60 carriers for molecular encapsulation of flavors and other sensitive ingredients. Several
61 reviews have been published describing their possible applications in food processing
62 and as food additives with different aims (Cravotto et al., 2006), although their use is
63 mainly based on the direct addition of the oligosaccharides to the liquid food, and
64 precipitation and separation of the resulting inclusion complexes. In this work, the
65 retention capacity of β CD immobilized in packaging structures is explored.

66

67 Cholesterol (cholest-5-ene-3- β -ol) is an apolar molecule and its size is compatible with
68 filling the β -cyclodextrin cavity. In the literature it is possible to find cholesterol/ β CD

69 complexation ratios ranging from 1:1 to 1:3 (Yamamoto et al., 2005). The main driving
70 force for complex formation is the release of enthalpy-rich water molecules from the
71 cavity. Water molecules are displaced by more hydrophobic guest molecules present in
72 the solution to attain an apolar-apolar association and a decrease of cyclodextrin ring
73 strain resulting in a more stable lower energy state. Cholesterol has already been
74 removed from milk and dairy products by a β CD -based process, and the resulting low-
75 cholesterol butter and cheese are indistinguishable from untreated products (Schroder &
76 Baer, 1990).

77

78 Complexation with a wide range of organic molecules has already been researched and
79 demonstrated. Cyclodextrins (CDs) have been used to retain and/or release volatile
80 compounds, such as aromes, aldehydes, ketones, etc (Almenar et al., 2007, Szejtli,
81 1982). Previous analysis showed that complexation is related to the polarity of
82 substances, the most apolar compounds having the highest complexation values (López
83 [de Dicastillo et al., 2010](#)). Lipid oxidation of stored peanuts leads indirectly to the
84 formation of aliphatic aldehydes, ketones and alcohols, (Burrioni et al., 1997; Wambura
85 and Yang, 2010; Williams et al., 2006). Lipids are the major components in peanuts,
86 where approximately 80% are unsaturated. Oxidation products and rancid flavors
87 decrease the sensory quality of peanut products at very low concentrations, making
88 them unacceptable to consumers, even before the end of their shelf life.

89

90 In a previous work (López de [Dicastillo et al., 2010](#)), different concentrations β CD
91 were incorporated by extrusion into a hydrophilic ethylene-vinyl alcohol copolymer
92 (EVOH). The films obtained, when exposed to a mixture of organic compounds,
93 presented preferential sorption capacity for apolar compounds. The aim of this work
94 was to use these materials with real food products to reduce the levels of undesired
95 components. Concretely, the films were used to scavenge undesired compounds through
96 the formation of inclusion complexes: a) reducing the cholesterol content of milk by
97 direct contact of the food product with the packaging films, and b) reducing the
98 oxidation byproducts in the package headspace of fried peanuts.

99

100 **2. Materials and methods**

101

102 *2.1. Chemicals and reagents*

103

104 The ethylene vinyl alcohol copolymer with a 44% ethylene molar content (EVOH) was
105 kindly supplied by The Nippon Synthetic Chemical Company, (Osaka, Japan). β CD was
106 obtained from Wacker Ibérica (Barcelona, Spain), glycerol from Sigma (Madrid, Spain)
107 and, cholesterol, 5 α -cholestane and potassium hydroxide from Fluka Biochemika
108 (Barcelona, Spain). Hexane and methanol were from Merck (Barcelona, Spain) and
109 pentanal, hexanal, heptanal, 2-heptenal, octanal, and 2-octenal from Sigma (Madrid,
110 Spain). Molecular weights and sizes of β CD cyclodextrins, cholesterol and selected
111 aldehydes have been included in Table 1.

112

113 Table 1. Physicochemical characteristics of cyclodextrins and undesirable compounds.

Compound	MW (g/mol)	Volume (Å³)	Diameter (Å)
cholesterol	386.65	428.11	8.08
cholesterol tail*¹	113.23	147.29	5.42
pentanal	86.13	98.43	4.88
hexanal	100.16	115.41	5.23
heptanal	114.18	132.41	5.04
2-heptenal	112.17	124.70	5.67
octanal	128.21	149.40	5.15
2-octenal	126.10	141.67	5.58
β-cyclodextrin*²	1135.00	262.00	6.00-6.50

114 *¹ Corresponds to the alquiliic chain of cholesterol molecule (6-methylheptyl).

115 *² Corresponds to the inner cavity of the cyclodextrin.

116

117 Water was treated by a Milli-Q Plus purification system (Millipore, Molsheim, France).
118 Hacendado™ fried peanuts and pasteurized whole milk and UHT whole milk were
119 acquired from a local supermarket (Mercadona, Valencia, Spain).

120

121 2.2. Film preparation

122

123 β -cyclodextrins were incorporated at two different concentrations (20 and 30% w/w)
124 into the hydrophilic EVOH material by flat extrusion. Polymer pellets were previously
125 dried during two days at 60 °C under vacuum. In a previous trial, the direct addition of
126 β CD into the extruder hopper produced very deficient films with holes and β CD
127 agglomerates and a poor distribution of the oligosaccharides. For this reason, a glycerol-
128 β CD 1:1 (w:w) paste was prepared, mixed with the polymer pellets, and melt blended

129 during extrusion in a Brabender DSE 20/40 co-rotating twin screw extruder
130 (Plastograph, Dusseldorf, Germany) with a screw speed of 100 rpm and with the
131 following thermal profile: 160 °C, 180 °C, 200 °C, 190 °C in the barrel and 190 °C at the
132 flat die. The addition of glycerol improved the miscibility of the β CD and the polymer
133 during the extrusion process and a more homogeneous distribution of the
134 oligosaccharide was achieved. The resulting films were ca. 50 μ m thick, although the
135 thickness of every sample was individually measured with a digital Mitutoyo
136 micrometer (Metrotec, San Sebastian, Spain) at 10 positions in the measured area before
137 conducting the experiments.

138 To avoid contaminations, film samples were vacuum-packed in aluminum/LDPE bags
139 and stored at room temperature until the moment of analysis.

140

141 The transparency of the films was determined through the surface reflectance spectra in
142 a spectrophotometer CM-3500d (Minolta Co, Tokyo, Japan) with a 30 mm illuminated
143 sample area. Measurements were taken from three samples in each formulation by using
144 both a white and a black background. The transparency was determined by applying the
145 Kubelka–Munk theory for multiple scattering to the reflection spectra. As each light
146 flux passes through the layer, it is affected by the absorption coefficient (K) and the
147 scattering coefficient (S). Transparency (K/S) was calculated, as indicated by Hutchings
148 (1999), from the reflectance of the sample layer on a known reflectance background and
149 on an ideal black background. Also, the internal transmittance was evaluated. Further
150 details on the procedure can be found in Fabra et al. (2010).

151

152 *2.3. Cholesterol in milk*

153

154 *2.3.1. Milk sample preparation*

155 In the present work, prior to any test, the film samples were immersed in water under
156 agitation for 24 hours, to eliminate any polymer residues (including cyclodextrins)
157 which could migrate out of the film, and then dried with a tissue paper and subsequently
158 in a vacuum oven at 40 °C for 24 hours. This way, no retention or scavenging could be
159 attributed to inclusion complexes formed outside of the active film.

160

161 To simulate the conditions of a conventional package for pasteurized whole milk, a
162 piece of film was placed in contact with real milk at a surface/volume ratio equivalent to
163 that of a 1 L carton.

164

165 After sterilizing the film surfaces by UV irradiation for 15 min, approximately 12.8 cm²
166 of each of the films were immersed in 20 mL of pasteurized whole milk and kept at 4 °C
167 for one week in a closed vial covered by aluminum foil to avoid any potential effect of
168 light.

169

170 In a second experiment, the UV irradiated film samples were immersed in 20 mL of
171 UHT whole milk and kept at 23 °C for one week. In this experiment, the milk package,
172 films, vials and milk were handled under sterile conditions to prevent the risk of
173 bacterial contamination and milk spoilage.

174 The experiments were carried out in quintuplicate.

175

176

177 *2.3.2. Cholesterol determination*

178 The determination of cholesterol in milk was carried out by a simple and rapid method
179 based on direct saponification of the samples with methanolic KOH solution (Fletouris
180 et al., 1998). A 0.3 g sample of milk was accurately weighed into a sample preparation
181 vial and 5 mL of 0.5 M KOH methanolic solution were added, followed by 40 µL of a
182 hexanolic solution of 5 α -cholestane 1 mg/mL as the internal standard. The vial was
183 closed tightly and vortexed for 15 s. The vial was then immersed in a 75 °C bath under
184 agitation for 25 min. Several vials with different samples could be handled conveniently
185 by placing them in a wire basket. Following heating, the vials were cooled to room
186 temperature, 1 mL of water and 5 mL of hexane were added, and the contents were
187 vortexed vigorously for 1 min and then centrifuged for 3 min at 1500 rpm. An aliquot of
188 the upper phase was injected for GC analysis.

189

190 The cholesterol concentration was determined in a fused capillary column (30 m x 0.22
191 mm x 0.22 µm) model TRB-STEROL (Teknokroma S. Coop. C. Ltda., Barcelona,
192 Spain) using an HP 5890 gas chromatograph (Agilent Technologies, Barcelona, Spain)
193 equipped with a flame ionization detector. The chromatographic conditions were as
194 follows: He carrier gas, 4 µL sample injection volume, 1/20 split ratio, isothermal

195 running at 285 °C for 15 minutes, injection port temperature 300 °C, and flame
196 ionization detector temperature 300 °C. The cholesterol was quantified through a 5 point
197 calibration curve with 5 α -cholestane as the internal standard. A linear peak
198 area/concentration response ($r = 0.998$) was observed within the tested range (1-300
199 mg/L). The analysis was carried out in triplicate and the cholesterol in the control/blank
200 samples was determined for each batch of test samples. The results are expressed as the
201 average \pm standard deviation concentration (w/v).

202

203 *2.4. Monitoring oxidation by-products from fried peanuts*

204

205 25 g of fried peanuts and a 10 cm x 8 cm piece of the developed films were placed in a
206 100 mL glass vial, hermetically closed with a twist-off closure equipped with a
207 sampling port, and stored at 37 °C for 10 weeks. Vials with no material, named
208 “peanut”, and with EVOH without cyclodextrins, named “blank”, were prepared in
209 order to check the scalping activity of the pure EVOH copolymer. The tests were
210 carried out in quintuplicate.

211

212 The organic compounds retention of the different films was quantified by gas
213 chromatography with flame ionization detection (GC-FID) at intervals throughout the
214 storage time. A Supelco 65- μ m DVB/PDMS solid phase micro extraction (SPME) fiber
215 (Teknokroma, Barcelona, Spain) was exposed to the vial headspace for 45 min and
216 immediately desorbed for 10 min in the injector of an HP5890 gas chromatography
217 (Agilent Technologies, Barcelona, Spain) equipped with a 30 m, 0.32 mm, 0.25 μ m
218 TRB-MetaX5 capillary column (Teknokroma, Barcelona, Spain). The chromatographic
219 conditions were as follows: He as the carrier gas, splitless injection, 210 °C and 300 °C
220 injector and detector temperatures, 5 min at 40 °C, first heating ramp to 60 °C at 3
221 °C/min, second heating ramp to 200 °C at 10 °C/min, and 5 min at 200 °C.

222

223 The volatile compounds were identified in an HP 5890 series II gas chromatograph
224 equipped with an HP 5972 mass-selective detector. The compounds adsorbed by the
225 fibre were desorbed in the injection port of the GC-MS for 10 min at 210 °C with the
226 purge valve off (splitless mode). The compounds were separated in a 30 m, 0.32 mm,
227 0.25 μ m TRB-5MS capillary column (Teknokroma, Barcelona, Spain) with the same
228 conditions as in the GC-FID analysis. The compounds were identified by comparison

229 with mass spectra from the library database (NIST 98), and by comparison with
230 authentic standards in both GC-MS and GC-FID. No calibration curves were
231 constructed; therefore the results are expressed as peak area units.

232

233 The aldehyde uptake was analyzed with a Dynatherm Thermal Desorber (Supelco,
234 Teknokroma, Spain) connected in series to the column of an HP5890 gas
235 chromatograph (Agilent Technologies, Barcelona, Spain) via a heated transfer line. At
236 the end of the storage time, a cut piece of the film was inserted into an empty desorption
237 tube. The tube was placed in the desorber chamber, which was immediately sealed. The
238 desorption conditions were: desorption temperature 180 °C, transfer line 180 °C,
239 desorption time 300 s. The GC was equipped with a TRB5 (30 m, 0.32 mm, 0.25 µm)
240 column (Teknokroma, Barcelona, Spain) and a flame ionization detector. After the
241 analysis, the film sample was recovered from the desorption tube and weighed on an
242 analytical balance. The Desorber-GC was calibrated by measuring film samples with
243 known amounts of each aroma (measured independently by gravimetry).

244

245 2.5. Statistical analysis

246 One-way analyses of variance were carried out using the SPSS computer program
247 (SPSS Inc., Chicago, IL, USA). Differences in pairs of mean values were evaluated by
248 the Tukey-b test for a confidence interval of 95%. The data are represented as average ±
249 standard deviations.

250

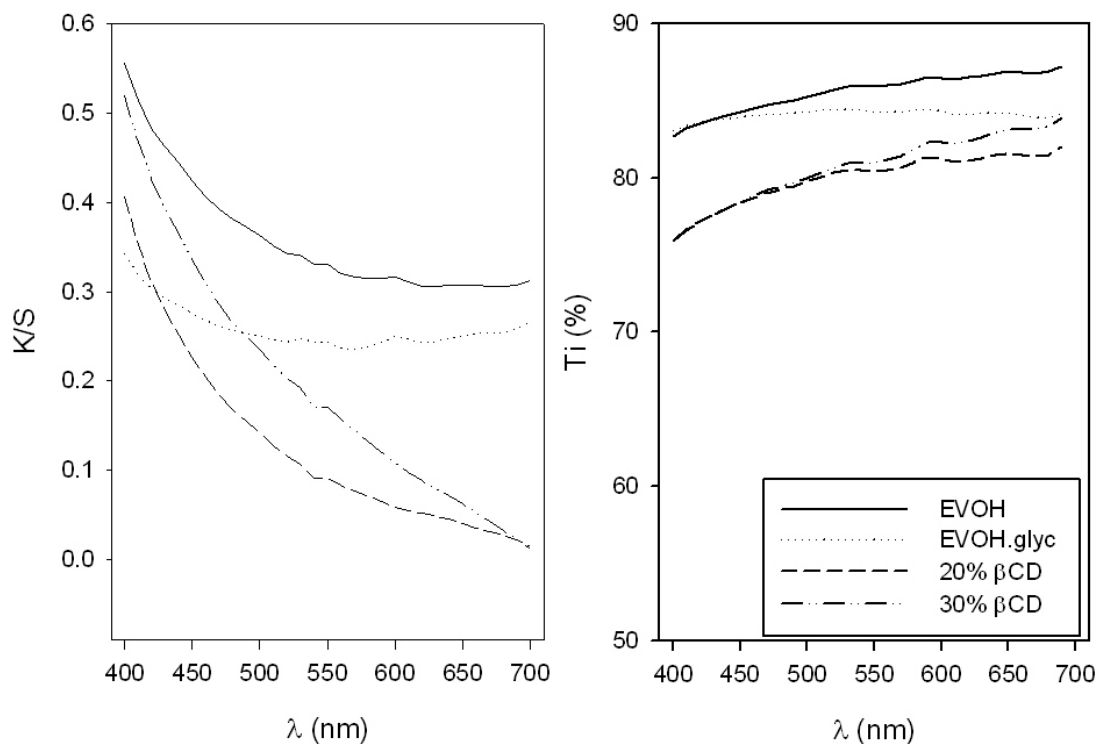
251 **3. Results and discussion**

252

253 In a previous characterization study of EVOH films containing beta-cyclodextrin the
254 level of immobilization was measured by determining the global migration values of the
255 polymer into water (Lopez de Dicastillo et al., 2010). This analysis revealed that films
256 with 20% and 30% of cyclodextrins released approximately 12% and 14% of their
257 weight respectively after one day's immersion in water. Similar values were measured
258 after ten days of storage, indicating a non-diffusion controlled migration process. Since
259 the release of glycerol in the control samples was below 2%, approximately 10% and
260 18% of βCD are actually immobilized in the EVOH film matrix. Film transparency was
261 evaluated through the Kubelka-Munk K/S coefficient, defined as the ratio between light

262 absorption and scattering and the internal transmittance of the film (Ti). An increase in
263 K/S or Ti can be assumed as an increase in transparency.

264 Figure 1 shows the profile of K/S and internal transmittance values as a function of
265 wavelength. As it can be seen, the addition of glycerol reduced the transparency of the
266 film, especially at the lower wavelengths. The incorporation of cyclodextrins results in a
267 slight decrease in the values of K/S that increases with the wavelengths. This result
268 appears to indicate that the presence of the cyclodextrins in the matrix reduces
269 transparency by increasing light scattering. Nevertheless, this decrease is not so relevant
270 when expressed as internal transmittance. Ti values for pure EVOH and EVOH-glycerol
271 films are well above 80% transmittance throughout all the spectra. The incorporation of
272 20-30% of cyclodextrins reduced the transmittance in a 10% at any wavelength.
273 Therefore, the application of the active EVOH-based layer to the packaging structure
274 will imply a reduction in transparency, which will be less noticeable with the reduction
275 of layer thickness.
276



277
278 Figure 1. Values of the Kubelka-Munk transparency parameter (K/S) and of the internal
279 transmittance (Ti) as a function of wavelength.
280

281

282 *3.1. Cholesterol scavenger results*

283 Firstly, the cholesterol concentration in milk was determined by GC following the
284 method described by Fletouris (Fletouris et al., 1998), using 5 α -cholestane as the
285 internal standard. The extraction method was tested by measuring the cholesterol
286 content of a known sample; the cholesterol was dissolved in ethanol and then diluted
287 with distilled water to a final cholesterol concentration of 150 $\mu\text{g}/\text{mL}$. The results
288 showed the method to have good efficacy, with yields of 95.7 \pm 2.3%. The tests carried
289 out on the milk samples as received yielded concentrations of 151.5 \pm 4.3 and 161.6 \pm 3.2
290 $\mu\text{g}/\text{mL}$ for the pasteurized and the UHT milk samples, respectively, which are in
291 agreement with data reported in the literature (Valenzuela et al., 2002, Sterna and
292 Jemeljanovs, 2003).

293

294 For the first set of experiments was carried out at 4 $^{\circ}\text{C}$ with pasteurized milk, the results
295 obtained after 2 and 7 days of exposure are presented in Table 2. At day two, the milk
296 sample exposed to a film without βCD presented a slight reduction that cannot be
297 considered significantly different from the cholesterol level of the control. In contrast,
298 the concentration of cholesterol in the milk exposed to βCD containing EVOH samples
299 decreased significantly ($p < 0.05$) with respect to the control sample, although no effect
300 of βCD concentration could be observed. Similar results were obtained at 7 days, when
301 the lowest cholesterol concentration values were obtained for the samples exposed to
302 the films containing βCD , although the reduction in cholesterol concentration was only
303 significant for the sample with the highest βCD content. However, the most noticeable
304 variation was that observed with storage time. The presence of cholesterol was
305 significantly lower in all the samples, with the largest variations being found in the
306 control samples. This decrease cannot be attributed to the presence of the EVOH films
307 or to the incorporation of the βCD . A decrease in the cholesterol concentration in milk
308 during storage can be due to internal milk reactions caused by lactic bacteria of a
309 proteolytic and lipolytic nature (Varnam and Sutherland, 1995). The reason for the
310 smaller reduction measured in the samples with βCD could be that complexation within
311 the active matrix has a protective effect on the cholesterol molecules. Feigenbaum et al.
312 (1998) described the protective effect of scalping on unstable aroma components in
313 orange juice.

314

315 Table 2. Cholesterol concentration (mg/L) and % of reduction of cholesterol at days 2
 316 and 7 in pasteurized milk exposed to EVOH materials at 4 °C.

	Day 2		Day 7	
	Cholesterol	Reduction (%)	Cholesterol	Reduction (%)
Milk	151.3 ± 7.2 a,x		120.5 ± 5.6 a,y	
Blank	145.6 ± 7.0 a,x	3.8	115.7 ± 5.4 ab,y	4.0
20% βCD	130.9 ± 1.4 b,x	13.5	113.5 ± 4.2 ab,y	6.2
30% βCD	125.5 ± 6.9 b,x	17.1	110.5 ± 2.1 b,y	8.3

317 a, b, c... indicate significant differences in the values of different samples on the same day.
 318 x,y... indicate significant differences in the values of the same sample on different days.

319
 320
 321 A second set of experiments was carried out with UHT milk at 23 °C. In this assay, the
 322 sample with EVOH films without βCD was not included since no scavenging effect by
 323 the pure polymer had been observed. As can be seen in Table 3, a significant reduction
 324 in cholesterol levels was measured for the samples with βCD after two days of storage.
 325 No differences were observed between samples with 20% and 30% of βCD. When
 326 compared with the values shown in Table 2, the active films presented similar efficiency
 327 at the two storage temperatures, with slightly higher values for the pasteurized milk
 328 stored at 4°C.

329
 330 The behavior of the UHT milk samples was similar on day 7. The presence of the active
 331 films resulted in a significant reduction in cholesterol concentration. The cholesterol
 332 scavenging activity increased with the concentration of βCD in the film samples. In this
 333 test with UHT milk, no reduction of cholesterol with time was observed in the control
 334 sample, indicating that the handling of samples in aseptic conditions together with the
 335 use of UHT milk reduced the potential influence of lipolytic microorganisms on the
 336 results. After a week of exposure, the EVOH material containing 30% βCD had
 337 adsorbed nearly 23% of the initial cholesterol content. As mentioned in the introduction,
 338 1:1, 1:2 and 1:3 cholesterol/βCD inclusion complexes have been reported in the
 339 literature. In this work, the low mobility of the cyclodextrin molecules within the matrix
 340 should hinder the formation of inclusion complexes other than 1:1 cholesterol/βCD.
 341 Most probably, the aliphatic chain of the cholesterol molecule is trapped in the βCD
 342 cavity (see dimensions in Table 1). Taking this hypothesis into account and considering

343 that after the washing steps the film samples contained 12% and 18% of β CD, the
 344 percentages of CD molecules involved in cholesterol scavenging after 7 days were
 345 15.5% and 15.7% for the 20% β CD and 30% β CD samples respectively. A reduction in
 346 film thickness could accelerate the diffusion of the large cholesterol molecules and
 347 increase the scavenging efficiency of the films. No significant differences were
 348 observed between the samples containing 20% β CD and 30% β CD.

349

350 Table 3. Cholesterol concentration (mg/L) and % of reduction of cholesterol at days 2
 351 and 7 in UHT milk exposed to EVOH materials at 23 °C.

	Day 2		Day 7	
	Cholesterol	Reduction (%)	Cholesterol	Reduction (%)
Milk	161.4 ± 16.4 a,x		152.5 ± 4.7 a,x	
20% β CD	142.8 ± 2.2 ab,x	11.5	129.2 ± 1.2 b,x	15.3
30% β CD	140.1 ± 3.8b,x	13.2	117.2 ± 5.1 c,y	23.2

352 a, b, c... indicate significant differences in the values of different samples on the same day.

353 x,y... indicate significant differences in the values of the same sample on different days.

354

355

356 3.2. Monitoring peanuts oxidative products

357 Hexanal is one of the principal volatile compounds formed during lipid peroxidation
 358 and its concentration in the package headspace is usually monitored as an index of lipid
 359 deterioration. It has been described as the main oxidation product formed from linoleic
 360 acid, a polyunsaturated fatty acid present in peanuts, and it is chosen as an indicating
 361 chemical for the oxidation of peanuts (Han et al., 2008). In the present study, hexanal
 362 was the most important volatile component identified by GC-MS in the package
 363 headspace over the 10 weeks of storage.

364

365 A previous study of the food aroma retention capacity of β CD (free and incorporated
 366 into EVOH films) showed preferential sorption of compounds with apolar molecules
 367 (Lopez de Dicastillo et al., 2010). In that study, exposing a β CD containing film to a
 368 hydroalcoholic solution resulted in n-hexanal retention of up to 40%. The formation of
 369 β CD inclusion complexes with apolar 'guest' molecules has been described earlier
 370 (Goubet et al., 1998; Szente and Szejtli, 2004).

371

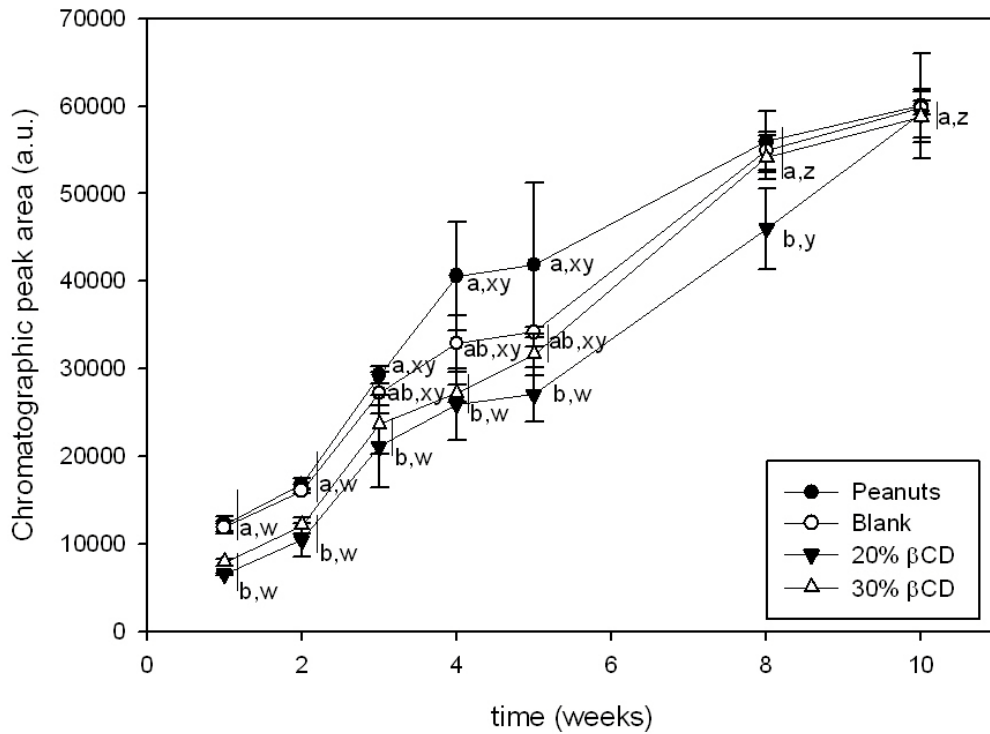
372 Four sets of samples were studied: peanuts (peanuts), peanuts with an EVOH film
373 without β CD to measure the scalping effect of the pure copolymer (blank), peanuts with
374 a film sample containing 20% of β CD (20% β CD) and peanuts with a film sample
375 containing 30% of β CD (30% β CD). Figure 2 shows the evolution of the hexanal
376 concentration in the bag headspace of all the samples over the storage time. As can be
377 seen, the amount of hexanal in the internal atmosphere of the peanuts samples increased
378 rapidly and steadily with time till week eight. From that moment, the concentration of
379 hexanal in the packaged headspace remained constant. This could be caused by a
380 reduction in the release of hexanal and/or because a partition equilibrium between the
381 fried peanuts and the internal atmosphere had been reached. The blank samples
382 presented a similar profile. However, the hexanal concentration in the samples
383 containing β CD increased at a significantly slower pace ($p < 0.05$) than in the peanuts
384 and blank samples. This difference can be attributed to the presence of β CD and the
385 formation of inclusion complexes, since the sample with pure EVOH (blank) did not
386 present significant differences with respect to the peanuts sample. During the first two
387 weeks of storage, the concentration of hexanal in the samples with β CD was half that of
388 the control samples. This reduction continued up to week 5, when the concentration of
389 hexanal in all the samples increased to similar values. At weeks 9 and 10 no significant
390 differences between samples were observed, possibly because the scavenging capacity
391 of the films had been exhausted.

392

393 Additionally, more volatile compounds in the headspace were identified as pentanal,
394 heptanal, 2-heptenal, octanal and 2-octenal. These could be the result of degradation of
395 the vegetable oils used in the frying process. Chung et al. (1993) and Guillen and Ruiz
396 (2005) reported that the main aldehydes generated by oxidation of frying oils were 2-
397 alkenals and n-alkanals. The evolution of these aldehydes is shown in Figure 3. As
398 expected, the concentration of all five compounds increased over the storage time in all
399 the samples. Also, in all the cases the compound formation slope was higher after the
400 fourth week. With respect to the effect of the materials, the results showed that those
401 containing β CD presented the expected scavenging activity, significantly reducing the
402 concentration of all aldehydes after the first week. At the tenth week, the reductions on
403 pentanal, heptanal and 2-octenal concentrations were 28, 33 and 56% in the case of the
404 films with 20% β CD, and 21, 22 and 48% in the case of films with 30% β CD,
405 respectively, being the effect of β CD concentration in the film not statistically

406 significant on the concentration of aldehydes measured at the package headspace. This
 407 result might be a consequence of partition equilibria that occur in the active
 408 film/food/headspace system and that can minimize the effect of scavenging on the
 409 measured concentrations at the headspace.

410



411

412 Figure 2. Hexanal concentration evolution over storage time. a, b, c... indicate
 413 significant differences in the values of different samples on the same day. x,y... indicate
 414 significant differences in the values of the same sample on different days.

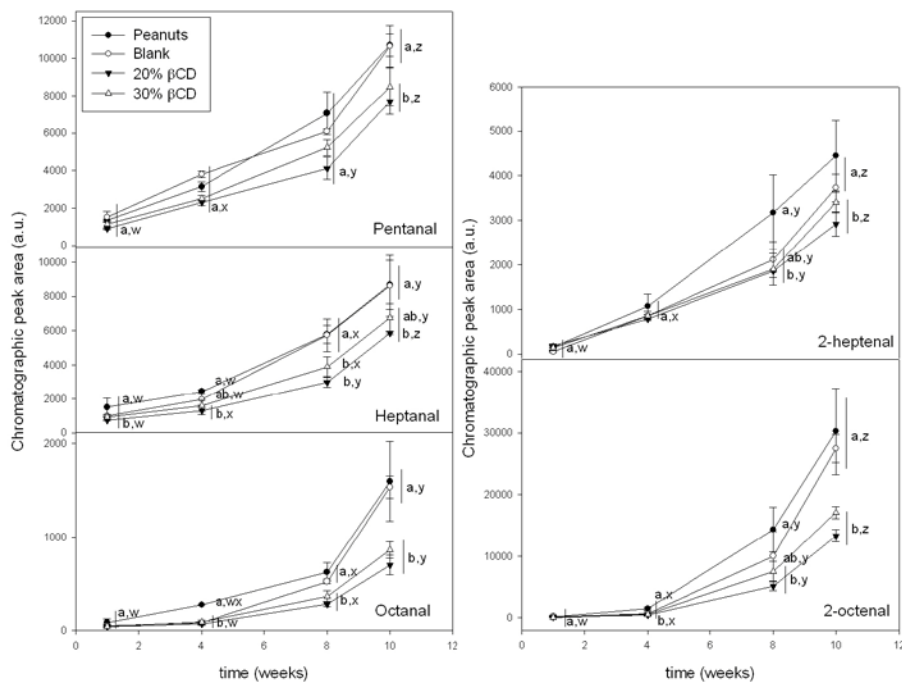
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417

418 These results prove that these EVOH films presented the expected activity in reducing
 419 the concentration of apolar compounds in the package headspace. Since the blank
 420 samples did not show any noticeable scavenging activity, the formation of inclusion
 421 complexes with the β CD molecules appears to be the most plausible action mechanism.
 422 As can be seen in Table 1, all mentioned aldehydes present a maximum diameter below
 423 that of the β CD cavity.

424



425

426 Figure 3. Concentration evolution of pentanal, heptanal, 2-heptenal, octanal and 2-
 427 octenal, over storage time. a, b, c... indicate significant differences in the values of
 428 different samples on the same day. x,y... indicate significant differences in the values of
 429 the same sample on different days.

430

431 To measure the actual amount of aldehydes retained by the film samples at the end of
 432 the storage period, the film was analyzed with a thermal desorber coupled to a GC.
 433 Unfortunately, separation of the different aldehydes could not be achieved and the
 434 chromatogram showed a wide peak. Since the calibration factors of the compounds
 435 were similar, with differences between constant values below 10% and molecular
 436 weights within a 10% range, hexanal (the main compound) was considered the only
 437 aldehyde present in the film. The results showed that the blank film retained 0.37 ± 0.10
 438 mg of hexanal per g of polymer. The 20% β CD and the 30% β CD samples retained
 439 significantly higher amounts: 12.3 ± 0.3 and 15.4 ± 0.3 mg/g. Taking into consideration
 440 the actual β CD concentrations in these films (10% and 18%) and the formation of 1/1
 441 aldehyde/ β CD inclusion complexes, the percentage of cyclooligosaccharides involved
 442 in the scavenging processes were $115 \pm 23\%$ and $97 \pm 15\%$ for the 20% CD and 30%
 443 CD samples respectively. This result confirmed that the cyclodextrins were successfully
 444 incorporated into the EVOH films, with the internal cavity available for the retention of
 445 apolar organic compounds and that the films with 30% CD retained higher amounts of
 446 aldehydes than those containing 20% even though it was not noticeable in the measured
 447 aldehyde concentrations in the headspace. The high polarity of the EVOH polymer

448 segments appeared to interact only with the polar external surface of the
449 oligosaccharides. The smaller molecular size of these aldehydes (see Table 1) compared
450 to the cholesterol molecules increased the availability of β CD for the formation of
451 inclusion complexes.

452

453 **4. Conclusions**

454

455 This study explored the capacity of EVOH films containing β -cyclodextrins to retain
456 undesirable substances present in food or in the surrounding headspace. The films
457 containing 20 and 30% of β CD successfully retained cholesterol from pasteurized and
458 UHT milk at 4 and 23°C, respectively. To check their applicability to scavenging
459 oxidative byproducts, these films were also tested with fried peanuts. As expected, the
460 inclusion of β CD in the EVOH films resulted in a lower aldehyde concentration in the
461 package headspace.

462

463 Similar applications of EVOH- β CD films can be derived to retain undesired apolar
464 compounds. Other potential applications could be to reduce migration by retaining
465 apolar migrants within the film structure, or to incorporate active agents or functional
466 components into the film in the form of inclusion complexes which would protect them
467 during package manufacture and then release them into the food product.

468

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470

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475

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