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Additional Information

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2 **Immobilization of β -cyclodextrin in ethylene-vinyl alcohol copolymer for active**
3 **food packaging applications**
4

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20

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26
27

ABSTRACT

28

29

30 Current developments in active food packaging are focusing on incorporating agents
31 into the polymeric package walls that will release or retain substances to improve the
32 quality, safety and shelf-life of the food. Because cyclodextrins are able to form
33 inclusion complexes with various compounds, they are of potential interest as agents to
34 retain or scavenge substances in active packaging applications. In this study, β -
35 cyclodextrin (β CD) was successfully immobilized in an ethylene-vinyl alcohol
36 copolymer with a 44% molar percentage of ethylene (EVOH44) by using regular
37 extrusion with glycerol as an adjuvant. Films with 10%, 20% and 30% of β CD were
38 flexible and transparent. The presence of the agent slightly increased the glass transition
39 temperature and the crystallinity percentage of the polymer, that is to say, it induced
40 some fragility and a nucleating effect. The water vapor, oxygen and carbon dioxide
41 barrier properties of the materials containing β CD were determined and compared with
42 those of the pure polymeric material. Permeability to these three permeants increased
43 with the addition of β CD due to the presence of discontinuities in the matrix and to the
44 internal cavity of the oligosaccharide. Also the CO_2/O_2 permselectivity increased with
45 the addition of β CD. Finally, the potential effect of the composites in the food aroma
46 was analyzed. The materials with β CD preferentially sorbed apolar compounds such as
47 terpenes. This characteristic could be useful in active packaging applications for
48 preferentially retaining undesired apolar food components like hexanal or cholesterol.

49

50 Keywords: β -cyclodextrin, EVOH copolymer, extrusion, immobilization, DSC,
51 retention of organic compounds, gas permeation.

INTRODUCTION

52
53

54 Active food packaging is one of the most dynamic emerging technologies that the food
55 industry is investigating as an alternative to traditional food techniques (intense heat
56 treatments, salting, acidification, drying, chemical preservation, etc.) for several uses,
57 especially combined with other technologies such as MAP, radiation, pulsed electric
58 fields, high pressure treatments, etc. Active food packaging is defined as the technology
59 in which the packaging system plays an active role in food preservation and quality
60 during the marketing process. Examples of active packaging technologies include the
61 use of oxygen scavengers, carbon dioxide or ethylene emitters and scavengers, ethanol
62 releasers, amine or aldehyde scavengers, and antimicrobial or antioxidant agents.

63 In many present-day active packaging technologies the active agent is placed in the
64 package with the food, in a small sachet, pad or device manufactured from a permeable
65 material which allows the active compound to achieve its purpose but prevents direct
66 contact with the food product, protecting the food from contamination or degradation.
67 Active packaging developments are now focusing on incorporating the agents into the
68 polymeric matrices which constitute the package walls; the resulting materials act by
69 releasing substances which have a positive effect on the food or by retaining undesired
70 substances from the food or the internal atmosphere of the package.

71 Because cyclodextrins (CDs) are able to form inclusion complexes with various
72 compounds, they present a potential interest as agents to retain or scavenge substances
73 such as odors, bitter compounds, lactose, cholesterol, etc., or to add aromas, colors, or
74 functional ingredients whose release could enhance the quality of the packaged product
75 and extend its shelf-life.

76

77 Cyclodextrins (CDs) are cyclic oligosaccharides composed of several glucose units (6
78 units in α CD, 7 units in β CD or 8 units in γ CD) linked by α -(1-4) bonds. They are
79 enzyme-modified starch derivatives with molecules which present amphipathic ring
80 structures: the hydrophilic groups are on the outside of the molecular cavity, the 2- and
81 3-OH groups on the wider rim and the 6-OH group on the narrower, while the
82 hydrophobic inner surface includes the ether-like anomeric oxygen atoms and the C3-H
83 and C5-H hydrogen atoms. In aqueous solution, this hydrophobic cavity contains about

84 3 (α CD), 7 (β CD) or 9 (γ CD) poorly held and easily displaceable water molecules.¹ The
85 water in the cavity has low density, as the cavities are large enough to accommodate
86 several more molecules. Thus, the cyclodextrin molecules may bind suitably-sized non-
87 polar aliphatic and aromatic compounds such as aroma compounds and lipophilic drugs.
88 They may bind in 1:1, 2:1 and 1:2 ratios, depending on the molecules involved.

89

90 This unique structure enables CDs to form inclusion complexes, entrapping all or part
91 of a 'guest' molecule inside their cavities, principally by means of weak forces such as
92 van der Waals, dipole-dipole interactions, and hydrogen bonding. While the height of
93 the cyclodextrin cavity is the same for all three types, the number of glucose units
94 determines the internal diameter of the cavity and its volume. Based on these
95 dimensions, α CD can typically complex low molecular weight molecules or compounds
96 with aliphatic side chains, β CD will complex aromatics and heterocycles and γ CD can
97 accommodate larger molecules such as macrocycles and steroids. β CD is the seven-
98 glucose cyclic oligosaccharide which has been on the GRAS list since 1992. It has a
99 cavity at the center of the molecule which allows complex formation with a wide
100 variety of organic molecules. β CD is nontoxic, edible, nonhygroscopic, chemically
101 stable and easy to separate, and overall is readily available at a low cost.

102

103 Cyclodextrins have a wide range of applications in the food and pharmaceutical
104 industries. Food ingredients, bioactive compounds, or flavor compounds can be
105 complexed by CDs to protect them against oxidation, light-induced reactions, heat-
106 promoted decomposition, loss by volatility, or sublimation, or to reduce undesired
107 tastes/odours.^{2,3} Empty β CD is also being used in the food industry to entrap or remove
108 undesirable compounds such as bitter components from coffee and tea,⁴ milk casein
109 hydrolysate,⁵ ginseng extract,⁶ or grapefruit or mandarin juices.⁷ Reducing the
110 cholesterol in food is probably the main commercial use of β CD in the food sector.⁸

111

112 Several applications have been described in which cyclodextrins or cyclodextrin
113 derivatives have been immobilized in different polymeric supports. Cyclodextrin
114 glucanotransferase has been immobilized onto a surface-modified polyethylene which
115 has then been used to produce cyclodextrins from corn starch.⁹ Modified cyclodextrins
116 with different substituents (hydroxyl, acetyl, silanes ...) to improve polymer

117 compatibility have been used to increase water permeability and reduce migration in
118 extruded polyethylene and polypropylene films.¹⁰

119

120 In this study, β CD was immobilized in an ethylene-vinyl alcohol copolymer and the
121 resulting materials were studied for their potential use in active packaging. Ethylene-
122 vinyl alcohol copolymers (EVOHs) are a family of semicrystalline random copolymers
123 widely used in the food-packaging sector due to their outstanding properties as gas
124 barriers to oxygen and organic compounds (solvents and food aromas), as well as their
125 considerable chemical resistance and high transparency.¹¹ The major drawback of these
126 materials in this application is their moisture sensitivity, which causes a significant
127 decrease in their gas barrier properties. Nevertheless, this water sensitivity can be useful
128 in the development of active packages for food, as food humidity is a potential
129 mechanism to trigger their activity. EVOH was selected as the vehicle for immobilizing
130 β CD because of its hydrophilicity and polarity. EVOH should present good chemical
131 compatibility with the external surface of the cyclodextrin molecule and the interior can
132 be used to design active packages based on the release of complexed agents or the
133 retention of undesirable food components.

134

135 In this study, EVOH films containing β CD were prepared by conventional extrusion and
136 the resulting materials were studied in order to characterize the effect of cyclodextrin
137 addition on the most relevant properties of EVOH as a packaging material, namely its
138 morphological, thermal and barrier properties.

MATERIALS AND METHODS

139

140

141 **Chemicals and Reagents**

142

143 Ethylene vinyl alcohol copolymer with a 44% ethylene molar content (EVOH44) was
144 gently supplied by The Nippon Synthetic Chemical Company, (Osaka, Japan). Reagent-
145 grade ethanol, α -pinene, d-limonene, linalool, citral, ethyl caproate, 2-nonanone,
146 octanal, methyl-isobuthyl ketone, n-hexanal, n-hexanol, silica gel, magnesium chloride
147 $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, magnesium nitrate $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, sodium nitrite NaNO_2 , and sodium
148 chloride NaCl were provided by Sigma (Madrid, Spain). Nitrogen, carbon dioxide and
149 oxygen were provided by Abelló-Linde (Valencia, Spain)

150

151 β CD was purchased from Wacker Fine Chemicals, S.L.(Barcelona) and glycerol from
152 Sigma (Madrid). Water was obtained from a Milli-Q Plus purification system
153 (Millipore, Molsheim, France).

154

155 **Film Preparation**

156

157 EVOH films containing β CD were obtained by two methods: flat extrusion and
158 compression molding. In both methods, cyclodextrins at different concentrations (10, 20
159 and 30%) were incorporated into a hydrophilic EVOH44 material.

160

161 For the flat extrusion process, a glycerol-CD paste was prepared, mixed with the
162 polymer pellets and melt-blended during extrusion. The mixture of the three
163 components was extruded on a Brabender DSE 20/40 co-rotating twin screw extruder
164 (Plastograph, Dusseldorf, Germany) at 200°C with a screw speed of 100 rpm. The
165 resulting films were ca. 50 micrometers thick, although the thickness of every sample
166 was individually measured with a digital Mitutoyo micrometer (Metrotec, San
167 Sebastian, Spain) before testing.

168

169 For the compression molding process, the EVOH polymer was ground with a knife mill
170 and sieved, then mixed with the cyclodextrin powder and compression-molded in a hot

171 plate Carver press (Carver, Wabash, IN, USA) at a temperature of 190°C. The material
172 was compression-molded at 190°C and 20 bars for 1 minute, then the pressure was
173 increased to 200 bars. After a total of 4 minutes, the material was cooled for 2 minutes.
174 The films obtained were ca. 100 micrometers thick.

175

176 The film samples were vacuum-packed in aluminum/LDPE bags and stored at room
177 temperature until the moment of analysis. The morphology of the composite, the degree
178 of immobilization of β CD, the thermal properties and the gas, water vapor and organic
179 compound barrier properties were studied.

180

181 **Morphology**

182

183 The materials were analyzed by Electronic Scanning Microscopy (SEM), using a
184 Hitachi model S-4100 with a BSE AURATA detector and EMIP 3.0 image trapping.
185 After being completely dried, the samples were broken up to investigate their cross-
186 section. A cylindrical aluminum stub cut like a straight-backed chair, on which the film
187 was fixed using a double-sided copper tape, was used in a specific way to observe the
188 morphology of the cross section. The stubs holding the films were then coated with
189 gold.

190

191 **Migration studies**

192

193 Since cyclodextrins are partially water soluble and the first objective is to use the
194 material for active food packaging, a preliminary study of cyclodextrin release from the
195 films was carried out by determining the global migration from the polymer into water,
196 as an aqueous food simulant, following EU regulations (UNE-EN 1186-3) with slight
197 modifications.^{12,13} In brief, 4-cm² samples of each of the films obtained were immersed
198 in 100 mL of freshly distilled Millipore water for periods of 1 and 10 days at 40°C and
199 the global migration values were determined on days 1 and 10 by calculating the weight
200 loss according to the method prescribed in the applicable EC directives¹⁴⁻¹⁶. All the
201 samples were measured in triplicate.

202

203 **Thermal Analysis**

204

205 Thermal properties were determined with a DSC Model Q2000 from TA Instruments
206 (New Castle, DE, EEUU). Thermograms were obtained from -50°C to 250°C with a
207 10°C/min heating ramp. The glass transition and melting point temperature and enthalpy
208 were calculated.

209

210 **Barrier Properties**

211

212 *Water vapor*

213 WVP tests were carried out at 35, 50, 65, 75 and 100% RH and 23°C using permeability
214 cups (Elcometer, Manchester, UK) according to ISO 2528. Aluminum cups were filled
215 with 7 g of silica gel and sealed with vacuum silicon grease (Sigma, Barcelona, Spain)
216 and the film to be tested. The film was fixed with a flat Viton ring, an aluminum ring
217 and three press-screws. The cups were then stored in desiccators containing saturated
218 salt solutions to ensure the required relative humidity: magnesium chloride
219 $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, magnesium nitrate $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, sodium nitrite NaNO_2 , sodium
220 chloride NaCl and water for humidities of 35, 50, 65, 75 and 100% respectively. The
221 cups were weighed daily, and the plot of the weight increment over time provided the
222 water vapor transmission rate. These values were divided by the water pressure gradient
223 and multiplied by the sample thickness to obtain the water vapor permeability value.

224 The water sorption of the prepared films was also measured. Film samples were cut, put
225 on aluminum dishes and placed in the above mentioned desiccators. The samples were
226 weighed daily until constant. The films were then dried for 48 h at 60°C under vacuum
227 and weighed. The sorption values were calculated as the water gain per 100 g of dry
228 sample.

229

230 *Oxygen*

231 The oxygen permeation rates of the materials were determined at 50 and 90% RH and
232 23°C using a OXTRAN Model 2/21 ML Mocon (Lippke, Neuwied, Germany). The film
233 samples were first conditioned at the RH of the experiment in the desiccators described
234 above. Two identical samples were then placed in the apparatus for analysis. The
235 samples were conditioned in the cells for 6 hours, then the transmission values were
236 determined every 45 min. After the permeation tests were completed, continuous
237 permeation experiments were carried out on each sample to determine the diffusion
238 coefficient (D). From the transmission rate values measured during the transient state,

239 the value of D was assessed from the solution to Fick's second law for the boundary
240 conditions of an isostatic permeation experiment.¹⁷

241

242 *Carbon dioxide*

243 The carbon dioxide permeation rates of the materials were determined at 50 and 90%
244 RH and 23°C using an isostatic permeation test.¹⁸ In brief: a stainless-steel cell with two
245 chambers separated by the film to be tested was used. A constant gas stream was passed
246 through each chamber. The permeant gas, carbon dioxide, flowed through the upper
247 chamber while the carrier gas, nitrogen, flowed through the lower chamber at the
248 required relative humidity and drove the permeated molecules to the detector system.
249 To humidify the gases, a stream of gas was bubbled through a gas washing bottle filled
250 with water and then mixed with a second stream of dry gas. Flowmeters (Dakota
251 Instruments, Orangeburg, USA), needle valves (Swagelok, Solon OH, USA), and digital
252 hygrometers were used to adjust and control the gas streams. At the exit from the lower
253 chamber, the flowrate of carrier gas was measured by a mass flowmeter (Dakota
254 Instruments, Orangeburg, USA). The concentration of CO₂ in this stream was analyzed
255 by gas chromatography. A HP5890 gas chromatograph (Agilent Technologies,
256 Barcelona, SPAIN) equipped with a manual injection valve, a Chromosorb 102, 80/100
257 mesh, 12' x 1/8' column (Teknokroma, Barcelona, Spain), and a thermal conductivity
258 detector was used. The GC was calibrated by injecting known amounts of carbon
259 dioxide. Once the cell had been assembled, the carrier gas was passed through both
260 chambers for at least 6 hours to remove all gases present in the cell during handling and
261 equilibrate the film to the humidity of the test. At time zero, the permeant gas started to
262 flow into the upper chamber. The concentration of carbon dioxide in the lower chamber
263 was monitored until constant, that is to say, until the stationary state was established.
264 The gas permeability (P) was calculated as follows:

$$265 \quad P = \frac{c_{CO_2} \cdot f \cdot \ell}{A \cdot \Delta P}$$

266 Where c_{CO_2} is the volume concentration of CO₂ in the stationary state, f is the carrier
267 gas flow in m³/s, ℓ is the film thickness in m, A is the film area in m², and ΔP is the
268 difference in the partial pressure of CO₂ in the stationary state between the two
269 chambers, measured in Pa.

270

271 *Organic compounds*

272

273 The organic compound retention capacity of the films and the effect of the addition of
274 β CD were also studied. A hydroalcoholic solution (5% ethanol) of several organic
275 compounds commonly found in food aromas was prepared at an initial concentration of
276 200 ppm (mg/L). The organic compounds selected were α -pinene, d-limonene, linalool,
277 citral, ethyl caproate, 2-nonanone, octanal, methyl-isobuthyl ketone, n-hexanal and n-
278 hexanol. The solution was then further diluted (to 5 ppm) with distilled water. The β CD
279 and the EVOH-based film materials were exposed to this solution as follows:

280 a) To determine the preferential sorption of the β CD, 1 g of cyclodextrin was added to
281 30 mL of the same solution. The vial was closed with a PTFE/rubber septum and stored
282 at room temperature for 15 days with constant stirring. The concentration of β CD
283 exceeded the molar concentration of the different compounds by a factor of 500.

284 b) To determine the sorption capacity of the films, 10 pieces measuring 2x2 cm,
285 separated by glass rings, were threaded onto a stainless steel wire, immersed in 30 mL
286 of distilled water and stored for 1 day at 40°C to remove all releasable β CD molecules
287 from the samples. Then, the threaded samples were removed from the vial, thoroughly
288 rinsed with water, dried for 48 hours under vacuum and immersed in 30 mL of a 5ppm
289 solution of hexanal, d-limonene and α -pinene, and stored for 15 days at room
290 temperature. Parallel samples were prepared by dispersing 0.02 g of β CD in 30 mL of
291 the solution, which corresponds to the molar content of organic compounds in the
292 sample and is similar to the β CD content in the EVOH-10 β CD sample. All the samples
293 were prepared in triplicate.

294 The concentration of organic compounds present in the organic solution after the
295 storage period was evaluated by gas chromatography. Three 5 mL fractions of the
296 hydroalcoholic solution from each sample were placed in glass vials closed with
297 PTFE/rubber septa. A Supelco 65- μ m DVB/PDMS solid phase microextraction (SPME)
298 fiber (Teknokroma, Barcelona, Spain) was exposed to the liquid solution for 10 min and
299 immediately desorbed for 5 min in the injector of a HP5890 gas chromatograph (Agilent
300 Technologies, Barcelona, Spain) equipped with a 30 m, 0.32 mm, 0.25 μ m TRB-5
301 capillary column (Teknokroma, Barcelona, Spain). The chromatographic conditions
302 were as follows: He as carrier gas, 1/20 split injection, 210 and 300 °C injector and
303 detector temperatures, 5 min at 40°C, first heating ramp to 60°C at 3°C/min, second
304 heating ramp to 200°C at 10°C/min, and 5 min at 200°C. The gas chromatograph was

305 calibrated by measuring hydroalcoholic solutions with known concentrations of the
306 organic compounds.

307

308

309

310

311

RESULTS AND DISCUSSION

312

313 The objective of this work was to immobilize β CD molecules in the polymer matrix and
314 produce films with functional characteristics suitable for the production of active
315 packages for foods.

316

317 In a first attempt, EVOH powder was mixed with β CD and films were obtained by
318 compression molding at 190°C and 20 bar for four minutes. The resulting films were
319 translucent, brittle and heterogeneous, and the cyclodextrins were visible and
320 concentrated in some areas due to an insufficient mixture of polymer chains and
321 oligosaccharide molecules. Besides, the high migration results ruled out these materials
322 for food packaging applications. Therefore, no further tests were done on the materials
323 obtained by compression.

324

325 In the second procedure, the EVOH films were obtained by extrusion. To improve the
326 CD addition results, the CDs were first mixed with glycerol, then the resulting paste
327 was manually mixed with the polymer pellets before being sent to the extruder hopper.
328 The films obtained by this method were transparent and flexible, although some white
329 spots could occasionally be observed due to the presence of β CD aggregates. The final
330 thickness was in the 50 ± 10 μ m range. This technique was used to obtain materials with
331 10, 20 and 30% β CD and 20% of glycerol, designated as EVOH-10 β CD, EVOH-20 β CD
332 and EVOH-30 β CD, respectively. Blank samples of pure EVOH (EVOH) and EVOH
333 with 20% of glycerol (EVOH-20G) were also extruded for comparison and submitted to
334 the characterization and migration studies.

335

336

337 **Morphological analysis**

338

339 The phase morphology of the prepared materials was studied through scanning electron
340 microscopy. The cryo-fracture surface was examined after drying the samples; Figure 1
341 shows an example of the SEM images obtained. Figure 1A presents the morphology of
342 the EVOH-20G sample used as control. As can be seen, the figure shows a continuous
343 homogeneous matrix without phasing. Figure 1B is the SEM image of an extruded

344 sample of EVOH with 30% of β CD (EVOH-30 β CD). As can be seen, β CD particles of
345 different sizes (0.1-1.5 μ m) are dispersed within the polymer matrix, showing signs of
346 low compatibility at the interface. Also, the image shows the presence of particle
347 aggregates, which would explain the white spots observed in the films with the naked
348 eye. Figure 1C is the image of a similar sample after immersion in water for 10 days.
349 Small holes in the fracture surface and some small particles can be seen in this picture,
350 as in Figure 1B. Nevertheless, in these samples there is no evidence of large particles or
351 particle aggregates, which suggests that they may have been released into the liquid
352 medium before the SEM analysis.

353

354 **Migration**

355

356 Since cyclodextrins are partially water soluble and the first objective is to use them in
357 an active food packaging material, a preliminary study of their release from the films
358 was carried out by determining the global migration from the polymer into water, as an
359 aqueous food simulant, in accordance with EU regulations.¹⁴⁻¹⁶ Film samples were
360 immersed in distilled water and the global migration values were measured on days 1
361 and 10. Figure 2 presents the results of the migration tests. As can be seen, all the CD
362 samples showed high global migration values when exposed to distilled water, the food
363 simulant indicated by EU regulations for non-acid aqueous food products due to the
364 slight water solubility of β CD.

365

366 The samples with glycerol presented global migration values of about 2%. Since pure
367 EVOH samples presented negligible migration (not included in the figure), this
368 percentage can be assigned to the loss of plasticizer. However, it is much lower than had
369 been expected from the results reported for hydrophilic biopolymers such as proteins or
370 polysaccharides,^{19,20} where the glycerol used as the plasticizer was fully extracted from
371 the developed films. The low values observed in the present work can be interpreted as
372 indicating a high chemical affinity between this polyol and the EVOH copolymer,
373 resulting in a partition equilibrium which favors the polymer over the food simulant.
374 Also, the results show that the migration increases with exposure time, indicating a
375 release process controlled by diffusion.

376

377 The EVOH samples with β CD and glycerol presented higher migration values than
378 those containing only glycerol. The amount of substances released by the different films
379 increased with the initial β CD content. The method used in this assay was gravimetric
380 and therefore does not distinguish between glycerol, β CD or other residues and
381 additives. Nevertheless, considering that the release of glycerol should be similar in all
382 the samples, the amount of β CD released from the films was below 50% of the initial
383 content. Also, it was noticeable that the migration values for the samples containing
384 β CD were similar on days 1 and 10. This kind of effect matches up with the case of
385 migration processes limited to the more external zones of the polymeric sample.
386 However, EVOH copolymers are high-barrier materials and although they become
387 plasticized by water, the diffusivity of organic molecules is very slow.²¹ Bearing in
388 mind that β CD molecules are very large, cyclic and rigid, their diffusivity in this
389 material should be practically negligible. Considering the SEM images of the samples
390 before and after exposure to the food simulant (Figure 1), the larger particles and the
391 aggregated particles might be those most exposed to dissolution in the aqueous medium.

392

393 **Thermal Characterization**

394

395 Thermal properties are among the most important attributes of the material to be
396 characterized for any polymer application, but are especially relevant in food packaging
397 design since the materials can be exposed to various thermal processes, either alone or
398 with the food product. DSC thermogram values for EVOH samples are shown in Figure
399 3 and the more relevant thermal properties are given in table 1.

400

401 As Figure 3 shows, the thermograms of the samples containing β CD present several
402 differences with respect to the blank samples. The glass-transition temperature obtained
403 for pure EVOH was 46°C, in agreement with the T_g values reported in the literature on
404 this material.^{22,23} The addition of 20% glycerol brought a reduction in the T_g value of
405 ca. 3 °C. This effect was expected, although the plasticization caused by the addition of
406 this agent was lower than that observed in other hydrophilic biopolymers.^{24,25} The
407 addition of cyclodextrins to this 'plasticized' copolymer caused an increase in rigidity,
408 with 10-15 °C increments in T_g value. The values were calculated by the onset of the
409 transition, since the end was hidden by the endothermic feature ascribed to enthalpy
410 relaxation. Figure 3 also shows that the crystallinity of EVOH was not affected by the

411 addition of glycerol. The melting temperature value and range and the melting enthalpy
412 were nearly identical. However, the incorporation of the cyclodextrins did influence the
413 melting behavior of the composite materials and, therefore, the copolymer morphology.
414 The presence of cyclodextrins in the polymer matrix reduced the melting temperature
415 and process enthalpy values. The endothermic minimum fell by 10-15 °C and the shape
416 of the endothermic depression differed, being wider with the addition of the
417 oligosaccharides. These changes can be interpreted as due to the cyclodextrins having a
418 nucleating effect, leading to a reduction in crystal size and a more imperfect crystalline
419 structure. The same effects of a lower melting temperature and a wider transition
420 temperature range were observable during the second heating. However, the T_g
421 transitions were not perceptible (no relaxation enthalpy), nor were they detected by the
422 equipment software.

423

424 **Barrier Properties**

425

426 Water vapor transport

427

428 The transport of water vapor through the prepared materials was characterized by
429 sorption and permeation experiments. The film samples were exposed to different
430 humidity conditions and the water gain was determined as described in the experimental
431 section.

432

433 Figure 4 shows the results obtained in the sorption experiments. As expected, the water
434 uptake increased with the relative humidity in all the samples. The profile was an S-
435 shape, convex at low humidity values and concave at high RH values, in agreement
436 with previously reported isotherms for pure EVOH copolymers.²⁶ The addition of
437 glycerol caused a slight rise in the water gain of EVOH samples under all the RH
438 conditions tested. Nevertheless, the differences were not statistically different ($p < 0.05$).
439 With respect to the incorporation of cyclodextrins, the amount of sorbed water increased
440 with the β CD content, rising by a factor of 2 for samples with 10% and by a factor of 3
441 for samples with 30% RH. Solubility values were obtained for all the samples and RH
442 conditions. As can be seen, the solubility of the pure EVOH and EVOH-20G samples
443 remained fairly constant at ca. 0.02 Kg/[m³.Pa]. These values are in agreement with
444 published values for EVOH44, although the weak effect of glycerol on water uptake

445 was noticeable, in disagreement with the strong effects observed in other hydrophilic
446 biopolymers. The samples containing β CD presented values of between 0.04 and 0.06
447 Kg/[m³.Pa], well above the control samples. This increment could be a consequence of
448 two effects: a) the presence in the matrix of empty spots, as shown in the SEM images,
449 which could be filled with water molecules, and b) inclusion of water molecules in the
450 cavity of the β CD molecules. According to the literature,¹ the water uptake due to
451 molecule inclusion inside the β CD cavity could account for up to 4% (w/w) for the
452 EVOH-30 β CD samples, close to the amount sorbed by the pure copolymer.

453

454 The water permeability through the films was also measured under different relative
455 humidity conditions; the results are plotted in Figure 5. As can be seen, all the samples
456 presented the same profile, showing the plasticizing effect of water at high humidities.
457 This effect is in agreement with previous reports in which water permeability was rather
458 constant at low and medium humidity and increased rapidly in very humid
459 environments.²⁶ The addition of glycerol to EVOH produced a small increase in
460 permeability in all the conditions tested, which can be related to the presence of glycerol
461 in the sample and a slight plasticization caused by this polyol as reflected by the
462 reduction in Tg. The addition of cyclodextrins increased the water permeability of all
463 the samples, especially those with 20% and 30% β CD. This effect could have two
464 causes: a) higher water-solubility in these matrices, as shown in Figure 4, and b) an
465 increase in water diffusion resulting from the presence of voids in the matrices and in
466 the polymer/ β CD interface. Using the definition of permeability as the product of
467 solubility and the diffusion coefficient (D), the diffusion coefficient can be estimated.
468 At 75% RH, the D values of the control samples were in the $4 \cdot 10^{-14}$ m²/s range, while
469 the D values for the samples with β CD rose to $6-7 \cdot 10^{-14}$ m²/s. At 90% RH, the addition
470 of β CD raised the D values from 8 to $15 \cdot 10^{-14}$ m²/s.

471

472

473 Gas transport

474

475 Permeability to oxygen and carbon dioxide was also evaluated under two relative
476 humidity conditions: 50% and 90%. Figure 6 shows the values for all the samples. As
477 can be seen, the addition of glycerol significantly increased the permeability to oxygen
478 of the EVOH films, especially at low humidity. This effect was greater than for water

479 permeation and is most probably due to the effect of the plasticizer on the diffusivity of
480 this gas. To check this hypothesis, the diffusion coefficient was calculated by applying
481 the analytical solution to Fick's laws for the boundary conditions of an isostatic
482 permeation process.¹⁷ The results collected in Figure 7 show that the addition of
483 glycerol increased the D value by a factor of 10 at 50% RH. This increment was similar
484 to that in the EVOH sample caused by the plasticization of water at 90% RH. The
485 incorporation of cyclodextrins also increased the permeability of the samples to oxygen
486 compared to the pure EVOH sample. However, this increment was not as high as that
487 caused by the plasticizer. The profile obtained at 90% RH was very similar to that
488 obtained at 50%. With respect to oxygen diffusivity, the addition of β CD increased the
489 diffusion coefficient value in proportion to the β CD content.

490

491 The effect of relative humidity on the diffusion coefficient of the EVOH sample shows
492 the plasticization caused by water sorption.²⁷ This effect was less pronounced in the
493 sample containing glycerol, since the material was partially plasticized by this agent.
494 The effect is even smaller in the presence of β CD. Discontinuities in the matrix and
495 transport through β CD cavities may be responsible for the greater diffusivity of the β CD
496 films, where water uptake is a less important factor than in the polymeric matrix.

497

498 Finally, the permeability of these materials to carbon dioxide was also measured, due to
499 the growing importance of this property in current packaging technologies (MAP, high
500 barrier packaging, active packaging, etc.). The results shown in Figure 6 present the
501 same profile as the oxygen permeability values. However, the permselectivity of the
502 EVOH was altered by the presence of β CD: the carbon dioxide permeability was 3-4
503 times higher than the oxygen permeability in the EVOH control samples, but 5-6 times
504 higher in the samples containing β CD. This might be caused by an increase in the
505 solubility of carbon dioxide in the composite matrices. Unfortunately, the experimental
506 method did not allow evaluation of D, which would have helped to confirm this
507 hypothesis.

508

509 *Transport of organic compounds*

510

511 It is well known that polymer-based packages have interactions with organic
512 compounds present in food. This characteristic, known as aroma scalping, may affect

513 the sensory characteristics of the packaged food product, causing a reduction in aroma
514 intensity or an unbalanced aroma due to the preferential retention of certain compounds.
515 Therefore, in order to optimize the packaging design, it is important to study the
516 potential food aroma interaction that a new material may present.

517 In this work, a preliminary experiment was carried out to study the relationship between
518 the polarity of the organic compounds and the complexation capacity of β CD. For this
519 purpose, β CD was added to a hydroalcoholic solution of several organic compounds at a
520 concentration of 5 ppm (w/v). The amount of oligosaccharide added was calculated to
521 achieve a molar ratio of 500/1 β CD/organic compounds. After two days of contact, the
522 samples were filtered and the concentration of non-encapsulated organic compounds
523 (A_{free}) was calculated by gas chromatography. The results were expressed as the value
524 of the equilibrium constant:

$$525 \quad K = \frac{[A_{complexed}]}{[A_{free}]}$$

526 The concentrations of complexed organic compounds ($A_{complexed}$) were estimated by
527 subtraction from the nominal concentrations. Figure 8 presents the complexation
528 constant value as a function of the Hildebrand coefficient (δ), which is related to the
529 polarity of substances.²⁸ As can be seen, the highest complexation values were obtained
530 for the most apolar compounds, those with the lowest Hildebrand coefficient values.
531 Aproximately 90% of the α -pinene and limonene molecules were complexed by β CD.
532 At the other extreme, the most polar compound tested, hexanol, presented the lowest K
533 value. Only 20% of the hexanol molecules formed inclusion complexes with the
534 oligosaccharides. These results are in agreement with previous published results in which
535 terpenes and sesquiterpenes from various plant extracts were highly encapsulated and
536 protected by β CD.^{29,30}

537

538 Another set of experiments was prepared with the materials under development. Control
539 EVOH films and those containing β CD were immersed in water for 24 hours at 40°C
540 and then dried under vacuum for 48 hours to eliminate the cyclodextrine molecules
541 which are released from the films. In this way, the potential organic compound
542 complexation caused by released β CD can be considered negligible. Then, films were
543 immersed in hydroalcoholic solutions of pinene, limonene and n-hexanal. The two
544 terpenes were selected because of their great affinity for the β CD. The hexanal was
545 selected since it is an organic compound which is formed as a consequence of oxidative

546 degradation of fats, and scavenging it could be a potential application of the materials
547 for active food packaging. The amounts of film and of the hydroalcoholic solution were
548 selected to obtain a β CD/organic compounds molar ratio of 1:1, 2:1 and 3:1 for the
549 EVOH-10 β CD, EVOH-20 β CD and EVOH-30 β CD respectively. For the control tests, a
550 similar sample was prepared with EVOH-20G as the control and another by adding
551 β CD in a β CD/organic compounds molar ratio of 1:1. As Figure 9 shows, in all the
552 samples the highest retention was obtained with the most apolar compounds. It is
553 interesting to observe the sorption of components by the polymeric matrix. The quantity
554 retained by the polymer is similar or even higher in comparison to free cyclodextrins.
555 EVOH is known to be a high barrier material for organic compounds when dry, but it
556 has been reported that water plasticization severely damages the barrier properties of
557 this family of copolymers. Thus, EVOH with glycerol is highly plasticized when
558 immersed in the hydroalcoholic solution, and organic compounds can easily diffuse
559 through the matrix.

560 The materials developed in this study presented much higher retention values because
561 of complexation with immobilized cyclodextrins, mainly of terpenic compounds. This
562 high retention also provides confirmation of the availability of the β CD cavity for
563 sorption of compounds. Therefore, it may be concluded that EVOH materials containing
564 immobilized β CD, prepared by regular extrusion, can be used to design active food
565 packaging that uses the β CD cavity for the inclusion or retention of apolar organic
566 compounds. At present, these materials are being tested with real products to observe
567 their capacity to retain undesirable compounds such as hexanal or cholesterol.

568

569

570 **CONCLUSIONS**

571

572 β -Cyclodextrin was successfully immobilized in an ethylene-vinyl alcohol copolymer
573 with a 44% molar percentage of ethylene by regular extrusion with the aid of glycerol to
574 improve the dispersion of the oligosaccharide. Although the composite presented phase
575 separation, the films were continuous and transparent and their thermal properties did
576 not present great changes. The barrier properties to gases and water decreased slightly
577 due to the increase of free volume in the matrix. Also, the CO₂/O₂ permselectivity
578 increased, attribute with interesting uses in MAP. It is noticeable that the presence of

579 β CD increased their apolar organic compound sorption capacity, a characteristic which
580 could be useful in active packaging design.

581

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666 LEGENDS TO FIGURES
667

668 Figure1. Scanning electron micrographs of the materials: EVOH-20G (A), EVOH-
669 30 β CD before migration process (B) and EVOH-30 β CD after migration (C).

670

671 Figure 2. Global migration from EVOH materials obtained by extrusion

672

673 Figure 3. DSC thermograms of the different EVOH-based materials during the first
674 heating.

675

676 Figure 4. Water sorption rates (A) and solubility coefficients (B) of the different
677 EVOH-based materials as a function of relative humidity.

678

679 Figure 5. Water vapor permeability of film samples at 23°C as a function of relative
680 humidity

681

682 Figure 6. Oxygen and carbon dioxide permeability of film samples at 23°C, at 50% and
683 90% RH.

684

685 Figure 7. Oxygen diffusion coefficient values for the different film samples at 23°C, at
686 50% and 90% RH

687

688 Figure 8. Effect of the polarity of aroma compounds on the complexation capacity of
689 β CD.

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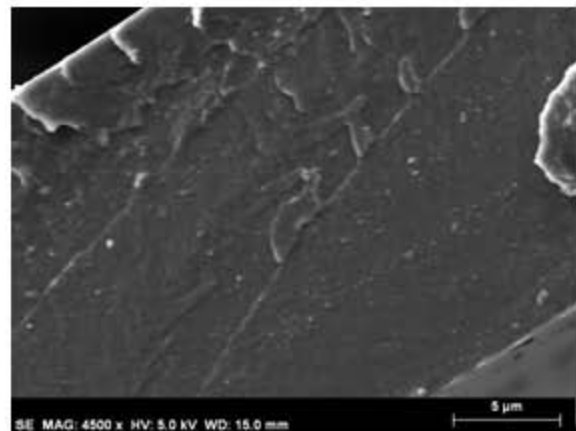
692 Figure 9. Retention of volatile components by the different samples.

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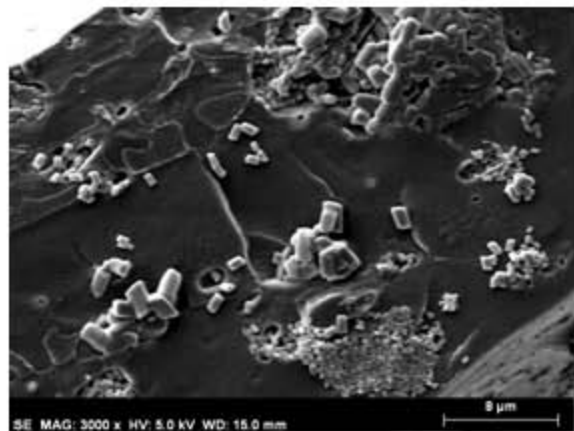
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Table 1. Thermal properties of the materials developed: glass transition temperature (T_g in °C, *: onset value), melting enthalpy (ΔH in J/g), melting temperature (T_m in °C) and melting temperature range (ΔT_m in °C).

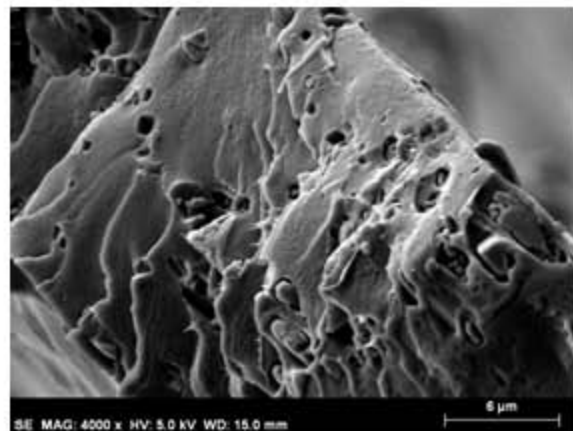
Material	First heating				Second heating			
	T _g	ΔH	T _m	ΔT_m	T _g	ΔH	T _m	ΔT_m
EVOH	46.08	59.09	159.96	43.3	43.28	67.49	161.73	63.6
EVOH-20G	42.89	58.56	160.3	38.9	38.48	68.55	160.77	61.7
EVOH-10 β CD	57.70*	55.42	141.53	64.7		49.25	147.76	64.4
EVOH-20 β CD	56.94*	48.66	143.47	66.6		49.11	149.82	73.8
EVOH-30 β CD	61.6*	47.7	149.15	89.7		45.85	151.87	81



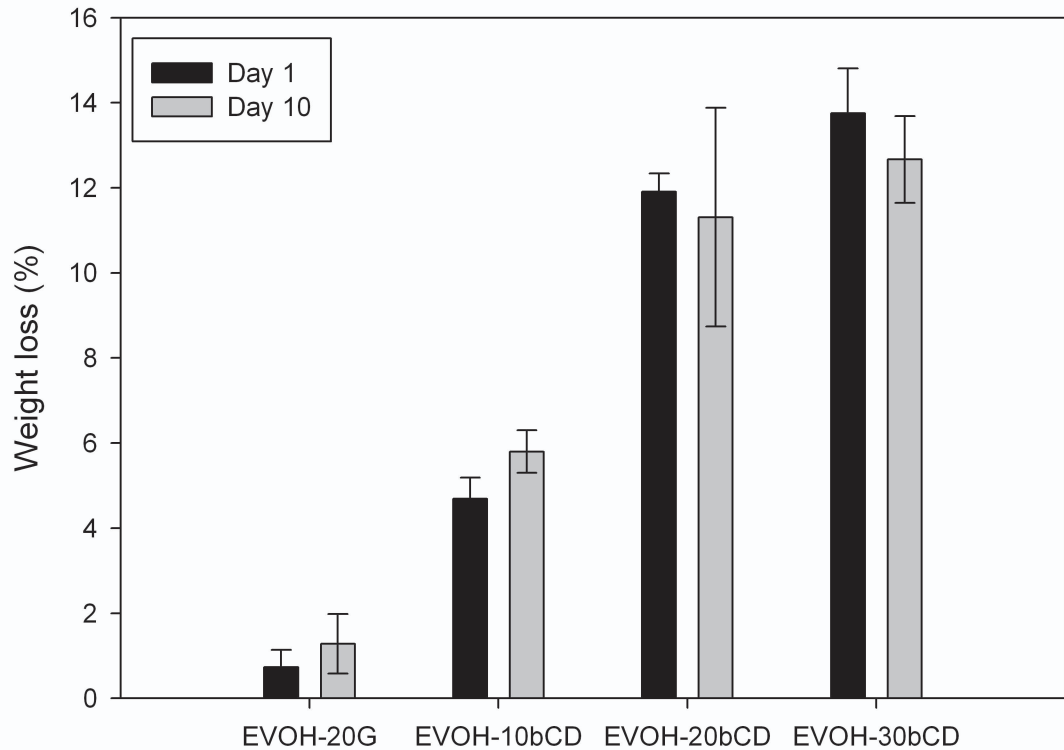
A



B



C



Heat Flow >>Exo>>

