Immobilization of β-cyclodextrin in ethylene-vinyl alcohol copolymer for active food packaging applications

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ABSTRACT

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

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

INTRODUCTION

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

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

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

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

3 (αCD), 7 (βCD) or 9 (γCD) poorly held and easily displaceable water molecules.[1] The water in the cavity has low density, as the cavities are large enough to accommodate several more molecules. Thus, the cyclodextrin molecules may bind suitably-sized non-polar aliphatic and aromatic compounds such as aroma compounds and lipophilic drugs. They may bind in 1:1, 2:1 and 1:2 ratios, depending on the molecules involved.

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

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

Several applications have been described in which cyclodextrins or cyclodextrin derivatives have been immobilized in different polymeric supports. Cyclodextrin glucanotransferase has been immobilized onto a surface-modified polyethylene which has then been used to produce cyclodextrins from corn starch.[9] Modified cyclodextrins with different substituents (hydroxyl, acetyl, silanes …) to improve polymer compatibility have been used to increase water permeability and reduce migration in extruded polyethylene and polypropylene films.[10]

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

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

MATERIALS AND METHODS

Chemicals and Reagents

Ethylene vinyl alcohol copolymer with a 44% ethylene molar content (EVOH44) was gently supplied by The Nippon Synthetic Chemical Company, (Osaka, Japan). Reagent-grade ethanol, α-pinene, d-limonene, linalool, citral, ethyl caproate, 2-nonanone, octanal, methyl-isobuthyl ketone, n-hexanal, n-hexanol, silica gel, magnesium chloride $MgCl_2.6H_2O$, magnesium nitrate $Mg(NO_3)_2.6H_2O$, sodium nitrite $NaNO_2$, and sodium chloride NaCl were provided by Sigma (Madrid, Spain). Nitrogen, carbon dioxide and oxygen were provided by Abelló-Linde (Valencia, Spain)

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

Film Preparation

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

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

For the compression molding process, the EVOH polymer was ground with a knife mill and sieved, then mixed with the cyclodextrin powder and compression-molded in a hot plate Carver press (Carver, Wabash, IN, USA) at a temperature of 190ºC. The material was compression-molded at 190ºC and 20 bars for 1 minute, then the pressure was increased to 200 bars. After a total of 4 minutes, the material was cooled for 2 minutes. The films obtained were ca. 100 micrometers thick**.**

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

Morphology

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

Migration studies

Since cyclodextrins are partially water soluble and the first objective is to use the material for active food packaging, a preliminary study of cyclodextrin release from the films was carried out by determining the global migration from the polymer into water, as an aqueous food simulant, following EU regulations (UNE-EN 1186-3) with slight modifications.[12,13] In brief, 4-cm^2 samples of each of the films obtained were immersed in 100 mL of freshly distilled Millipore water for periods of 1 and 10 days at 40ºC and the global migration values were determined on days 1 and 10 by calculating the weight loss according to the method prescribed in the applicable EC directives [14-16]. All the samples were measured in triplicate.

Thermal Analysis

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

Barrier Properties

Water vapor

WVP tests were carried out at 35, 50, 65, 75 and 100% RH and 23ºC using permeability cups (Elcometer, Manchester, UK) according to ISO 2528. Aluminum cups were filled with 7 g of silica gel and sealed with vacuum silicon grease (Sigma, Barcelona, Spain) and the film to be tested. The film was fixed with a flat Viton ring, an aluminum ring and three press-screws. The cups were then stored in desiccators containing saturated salt solutions to ensure the required relative humidity: magnesium chloride $MgCl_2.6H_2O$, magnesium nitrate $Mg(NO_3)_2.6H_2O$, sodium nitrite $NaNO_2$, sodium chloride NaCl and water for humidities of 35, 50, 65, 75 and 100% respectively. The cups were weighed daily, and the plot of the weight increment over time provided the water vapor transmission rate. These values were divided by the water pressure gradient and multiplied by the sample thickness to obtain the water vapor permeability value.

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

Oxygen

The oxygen permeation rates of the materials were determined at 50 and 90% RH and 23ºC using a OXTRAN Model 2/21 ML Mocon (Lippke, Neuwied, Germany). The film samples were first conditioned at the RH of the experiment in the desiccators described above. Two identical samples were then placed in the apparatus for analysis. The samples were conditioned in the cells for 6 hours, then the transmission values were determined every 45 min. After the permeation tests were completed, continuous permeation experiments were carried out on each sample to determine the diffusion coefficient (D). From the transmission rate values measured during the transient state, the value of D was assessed from the solution to Fick's second law for the boundary conditions of an isostatic permeation experiment.[17]

Carbon dioxide

The carbon dioxide permeation rates of the materials were determined at 50 and 90% RH and 23ºC using an isostatic permeation test.[18] In brief: a stainless-steel cell with two chambers separated by the film to be tested was used. A constant gas stream was passed through each chamber. The permeant gas, carbon dioxide, flowed through the upper chamber while the carrier gas, nitrogen, flowed through the lower chamber at the required relative humidity and drove the permeated molecules to the detector system. To humidify the gases, a stream of gas was bubbled through a gas washing bottle filled with water and then mixed with a second stream of dry gas. Flowmeters (Dakota Instruments, Orangeburg, USA), needle valves (Swagelok, Solon OH, USA), and digital hygrometers were used to adjust and control the gas streams. At the exit from the lower chamber, the flowrate of carrier gas was measured by a mass flowmeter (Dakota Instruments, Orangeburg, USA). The concentration of CO_2 in this stream was analyzed by gas chromatography. A HP5890 gas chromatograph (Agilent Technologies, Barcelona, SPAIN) equipped with a manual injection valve, a Chromosorb 102, 80/100 mesh, 12′x 1/8′ column (Teknokroma, Barcelona, Spain), and a thermal conductivity detector was used. The GC was calibrated by injecting known amounts of carbon dioxide. Once the cell had been assembled, the carrier gas was passed through both chambers for at least 6 hours to remove all gases present in the cell during handling and equilibrate the film to the humidity of the test. At time zero, the permeant gas started to flow into the upper chamber. The concentration of carbon dioxide in the lower chamber was monitored until constant, that is to say, until the stationary state was established. The gas permeability (P) was calculated as follows:

$$P = \frac{c_{CO2} \cdot f \cdot \ell}{A \cdot \Delta P}$$

Where c_{CO2} is the volume concentration of CO_2 in the stationary state, f is the carrier gas flow in m^3/s, ℓ is the film thickness in m, A is the film area in m^2, and ΔP is the difference in the partial pressure of CO_2 in the stationary state between the two chambers, measured in Pa.

Organic compounds

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

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

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

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

RESULTS AND DISCUSSION

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

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

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

Morphological analysis

The phase morphology of the prepared materials was studied through scanning electron microscopy. The cryo-fracture surface was examined after drying the samples; Figure 1 shows an example of the SEM images obtained. Figure 1A presents the morphology of the EVOH-20G sample used as control. As can be seen, the figure shows a continuous homogeneous matrix without phasing. Figure 1B is the SEM image of an extruded sample of EVOH with 30% of ßCD (EVOH-30βCD). As can be seen, ßCD particles of different sizes (0.1-1.5 μm) are dispersed within the polymer matrix, showing signs of low compatibility at the interface. Also, the image shows the presence of particle aggregates, which would explain the white spots observed in the films with the naked eye. Figure 1C is the image of a similar sample after immersion in water for 10 days. Small holes in the fracture surface and some small particles can be seen in this picture, as in Figure 1B. Nevertheless, in these samples there is no evidence of large particles or particle aggregates, which suggests that they may have been released into the liquid medium before the SEM analysis.

Migration

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

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

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

Thermal Characterization

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

As Figure 3 shows, the thermograms of the samples containing ßCD present several differences with respect to the blank samples. The glass-transition temperature obtained for pure EVOH was 46ºC, in agreement with the Tg values reported in the literature on this material.[22,23] The addition of 20% glycerol brought a reduction in the Tg value of ca. 3 ºC. This effect was expected, although the plasticization caused by the addition of this agent was lower than that observed in other hydrophilic biopolymers.[24,25] The addition of cyclodextrins to this 'plasticized' copolymer caused an increase in rigidity, with 10-15 ºC increments in Tg value The values were calculated by the onset of the transition, since the end was hidden by the endothermic feature ascribed to enthalpy relaxation. Figure 3 also shows that the crystallinity of EVOH was not affected by the addition of glycerol. The melting temperature value and range and the melting enthalpy were nearly identical. However, the incorporation of the cyclodextrins did influence the melting behavior of the composite materials and, therefore, the copolymer morphology. The presence of cyclodextrins in the polymer matrix reduced the melting temperature and process enthalpy values. The endothermic minimum fell by 10-15 ºC and the shape of the endothermic depression differed, being wider with the addition of the oligosaccharides. These changes can be interpreted as due to the cyclodextrins having a nucleating effect, leading to a reduction in crystal size and a more imperfect crystalline structure. The same effects of a lower melting temperature and a wider transition temperature range were observable during the second heating. However, the Tg transitions were not perceptible (no relaxation enthalpy), nor were they detected by the equipment software.

Barrier Properties

Water vapor transport

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

Figure 4 shows the results obtained in the sorption experiments. As expected, the water uptake increased with the relative humidity in all the samples. The profile was an S-shape, convex at low humidity values and concave at high RH values, in agreement with previously reported isotherms for pure EVOH copolymers.[26] The addition of glycerol caused a slight rise in the water gain of EVOH samples under all the RH conditions tested. Nevertheless, the differences were not statistically different ($p<0.05$). With respect to the incorporation of cyclodextrins, the amount of sorbed water increased with the ßCD content, rising by a factor of 2 for samples with 10% and by a factor of 3 for samples with 30% RH. Solubility values were obtained for all the samples and RH conditions. As can be seen, the solubility of the pure EVOH and EVOH-20G samples remained fairly constant at ca. 0.02 Kg/[m^3.Pa]. These values are in agreement with published values for EVOH44, although the weak effect of glycerol on water uptake was noticeable, in disagreement with the strong effects observed in other hydrophilic biopolymers. The samples containing ßCD presented values of between 0.04 and 0.06 Kg/[m^3.Pa], well above the control samples. This increment could be a consequence of two effects: a) the presence in the matrix of empty spots, as shown in the SEM images, which could be filled with water molecules, and b) inclusion of water molecules in the cavity of the ßCD molecules. According to the literature,[1] the water uptake due to molecule inclusion inside the ßCD cavity could account for up to 4% (w/w) for the EVOH-30ßCD samples, close to the amount sorbed by the pure copolymer.

The water permeability through the films was also measured under different relative humidity conditions; the results are plotted in Figure 5. As can be seen, all the samples presented the same profile, showing the plasticizing effect of water at high humidities. This effect is in agreement with previous reports in which water permeability was rather constant at low and medium humidity and increased rapidly in very humid environments.[26] The addition of glycerol to EVOH produced a small increase in permeability in all the conditions tested, which can be related to the presence of glycerol in the sample and a slight plasticization caused by this polyol as reflected by the reduction in Tg. The addition of cyclodextrins increased the water permeability of all the samples, especially those with 20% and 30% ßCD. This effect could have two causes: a) higher water-solubility in these matrices, as shown in Figure 4, and b) an increase in water diffusion resulting from the presence of voids in the matrices and in the polymer/ßCD interface. Using the definition of permeability as the product of solubility and the diffusion coefficient (D), the diffusion coefficient can be estimated. At 75% RH, the D values of the control samples were in the $4 \cdot 10^{-14}$ m^2/s range, while the D values for the samples with ßCD rose to $6\text{-}7 \cdot 10^{-14}$ m^2/s. At 90% RH, the addition of ßCD raised the D values from 8 to 15 $\cdot 10^{-14}$ m^2/s.

Gas transport

Permeability to oxygen and carbon dioxide was also evaluated under two relative humidity conditions: 50% and 90%. Figure 6 shows the values for all the samples. As can be seen, the addition of glycerol significantly increased the permeability to oxygen of the EVOH films, especially at low humidity. This effect was greater than for water permeation and is most probably due to the effect of the plasticizer on the diffusivity of this gas. To check this hypothesis, the diffusion coefficient was calculated by applying the analytical solution to Fick's laws for the boundary conditions of an isostatic permeation process.[17] The results collected in Figure 7 show that the addition of glycerol increased the D value by a factor of 10 at 50% RH. This increment was similar to that in the EVOH sample caused by the plasticization of water at 90% RH. The incorporation of cyclodextrins also increased the permeability of the samples to oxygen compared to the pure EVOH sample. However, this increment was not as high as that caused by the plasticizer. The profile obtained at 90% RH was very similar to that obtained at 50%. With respect to oxygen diffusivity, the addition of ßCD increased the diffusion coefficient value in proportion to the ßCD content.

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

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

Transport of organic compounds

It is well known that polymer-based packages have interactions with organic compounds present in food. This characteristic, known as aroma scalping, may affect the sensory characteristics of the packaged food product, causing a reduction in aroma intensity or an unbalanced aroma due to the preferential retention of certain compounds. Therefore, in order to optimize the packaging design, it is important to study the potential food aroma interaction that a new material may present.

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

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

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

Another set of experiments was prepared with the materials under development. Control EVOH films and those containing βCD were immersed in water for 24 hours at 40ºC and then dried under vacuum for 48 hours to eliminate the cyclodextrine molecules which are released from the films. In this way, the potential organic compound complexation caused by released βCD can be considered negligible. Then, films were immersed in hydroalcoholic solutions of pinene, limonene and n-hexanal. The two terpenes were selected because of their great affinity for the βCD. The hexanal was selected since it is an organic compound which is formed as a consequence of oxidative degradation of fats, and scavenging it could be a potential application of the materials for active food packaging. The amounts of film and of the hydroalcoholic solution were selected to obtain a βCD/organic compounds molar ratio of 1:1, 2:1 and 3:1 for the EVOH-10βCD, EVOH-20βCD and EVOH-30βCD respectively. For the control tests, a similar sample was prepared with EVOH-20G as the control and another by adding βCD in a βCD/organic compounds molar ratio of 1:1. As Figure 9 shows, in all the samples the highest retention was obtained with the most apolar compounds. It is interesting to observe the sorption of components by the polymeric matrix. The quantity retained by the polymer is similar or even higher in comparison to free cyclodextrins. EVOH is known to be a high barrier material for organic compounds when dry, but it has been reported that water plasticization severely damages the barrier properties of this family of copolymers. Thus, EVOH with glycerol is highly plasticized when immersed in the hydroalcoholic solution, and organic compounds can easily diffuse though the matrix.

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

CONCLUSIONS

β-Cyclodextrin was successfully immobilized in an ethylene-vinyl alcohol copolymer with a 44% molar percentage of ethylene by regular extrusion with the aid of glycerol to improve the dispersion of the oligosaccharide. Although the composite presented phase separation, the films were continuous and transparent and their thermal properties did not present great changes. The barrier properties to gases and water decreased slightly due to the increase of free volume in the matrix. Also, the CO_2/O_2 permselectivity increased, attribute with interesting uses in MAP. It is noticeable that the presence of βCD increased their apolar organic compound sorption capacity, a characteristic which could be useful in active packaging design.

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LEGENDS TO FIGURES

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

Figure 2. Global migration from EVOH materials obtained by extrusion

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

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

Figure 5. Water vapor permeability of film samples at 23ºC as a function of relative humidity

Figure 6. Oxygen and carbon dioxide permeability of film samples at 23ºC, at 50% and 90% RH.

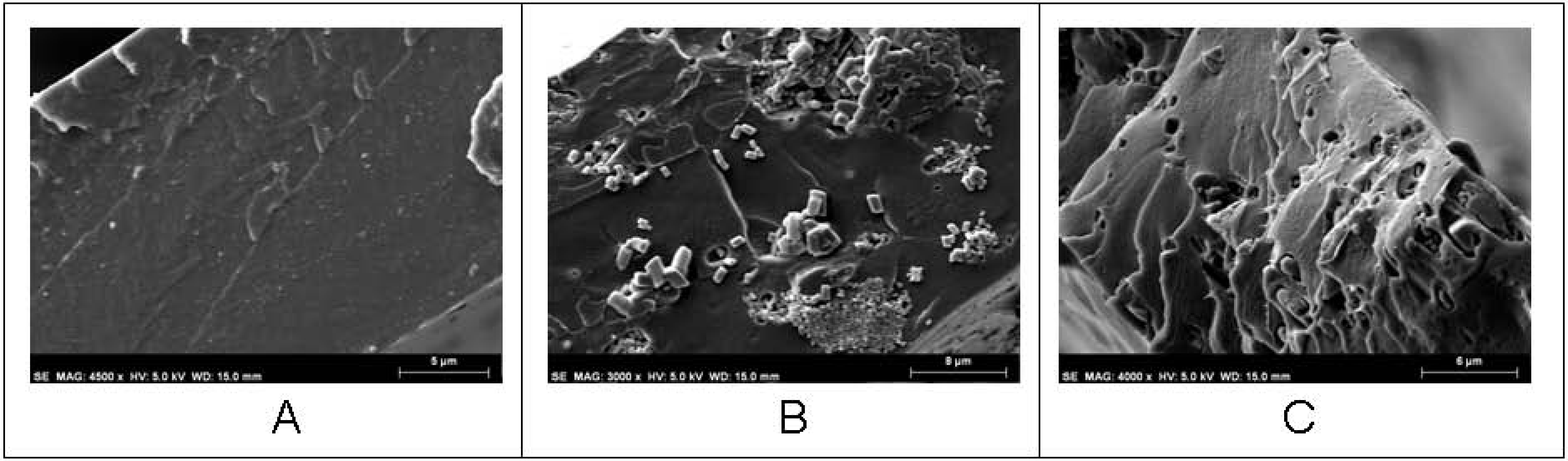
Figure 7. Oxygen diffusion coefficient values for the different film samples at 23ºC, at 50% and 90% RH

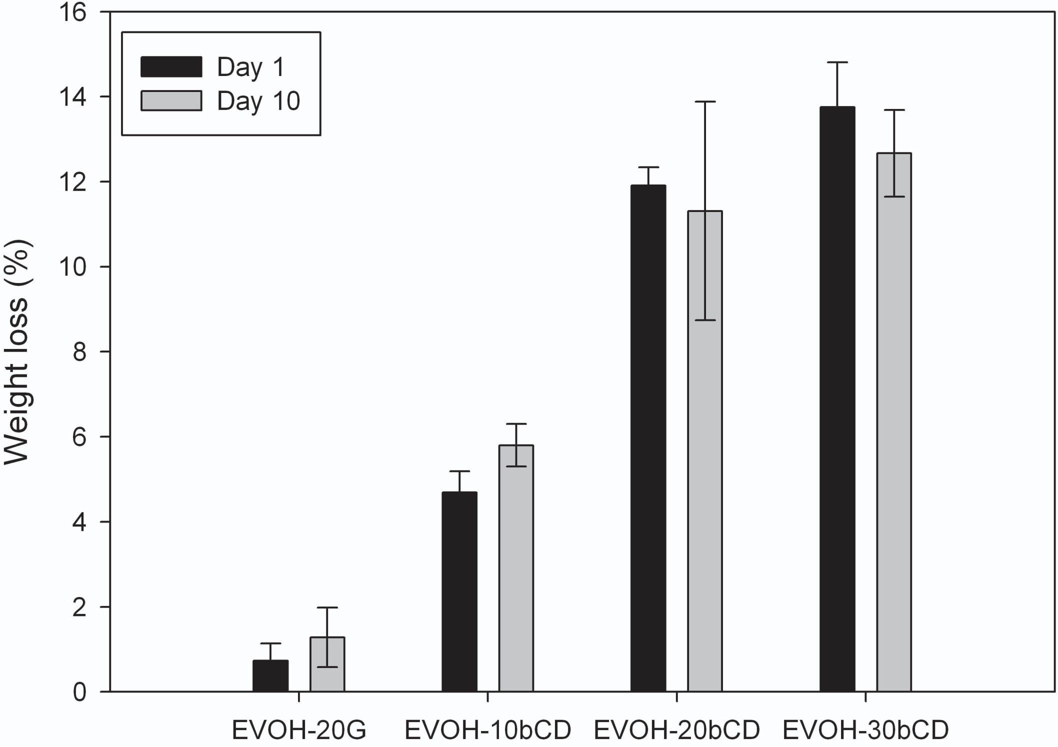
Figure 8. Effect of the polarity of aroma compounds on the complexation capacity of βCD.

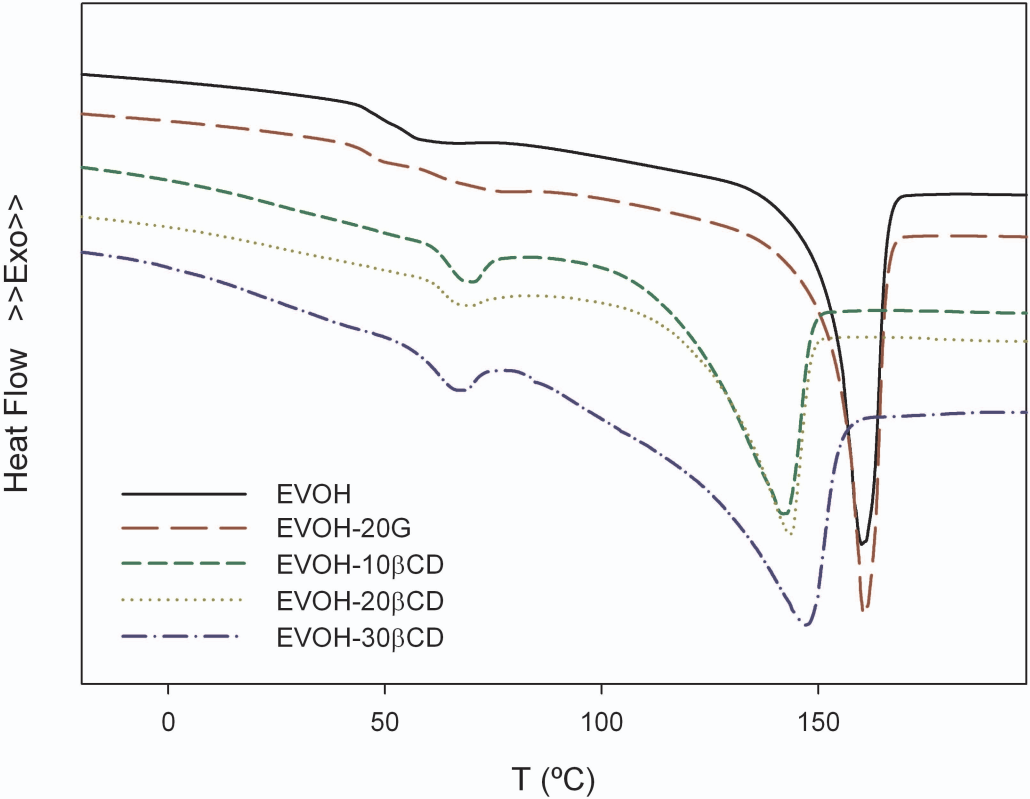
Figure 9. Retention of volatile components by the different samples.

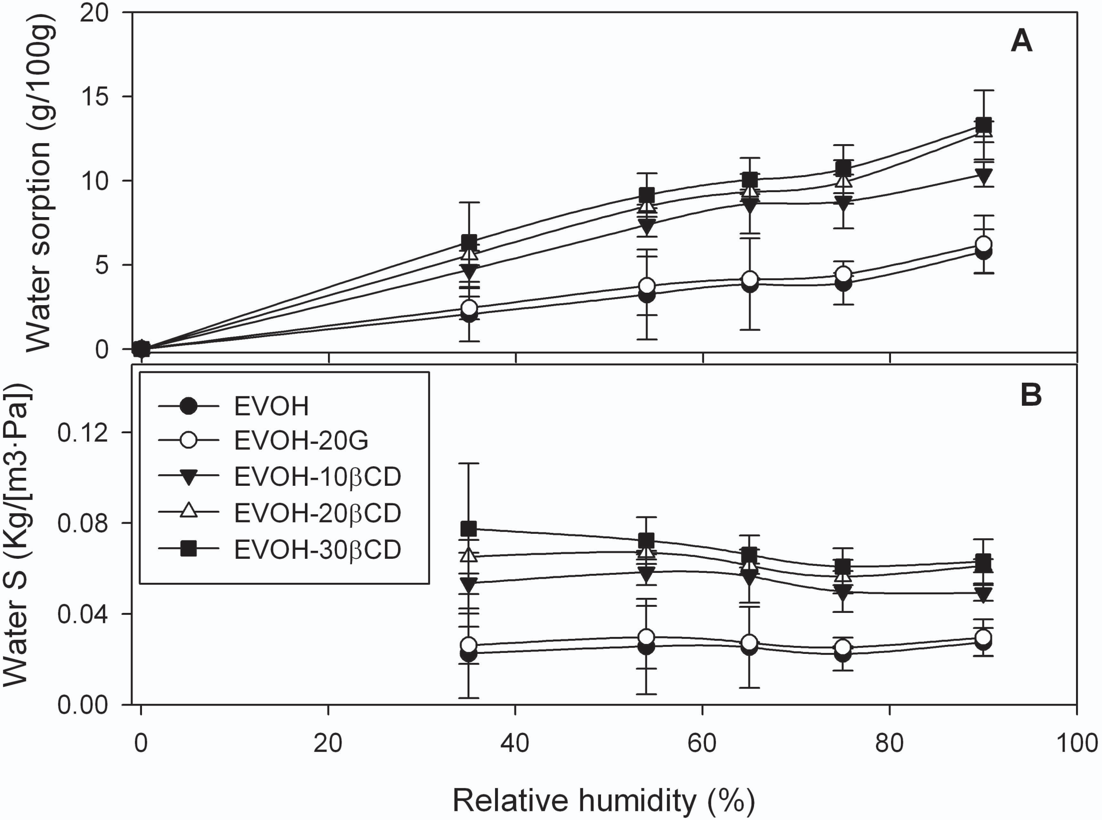
Table 1. Thermal properties of the materials developed: glass transition temperature (Tg in ºC, *: onset value), melting enthalpy (ΔH in J/g), melting temperature (Tm in ºC) and melting temperature range (ΔTm in ºC).

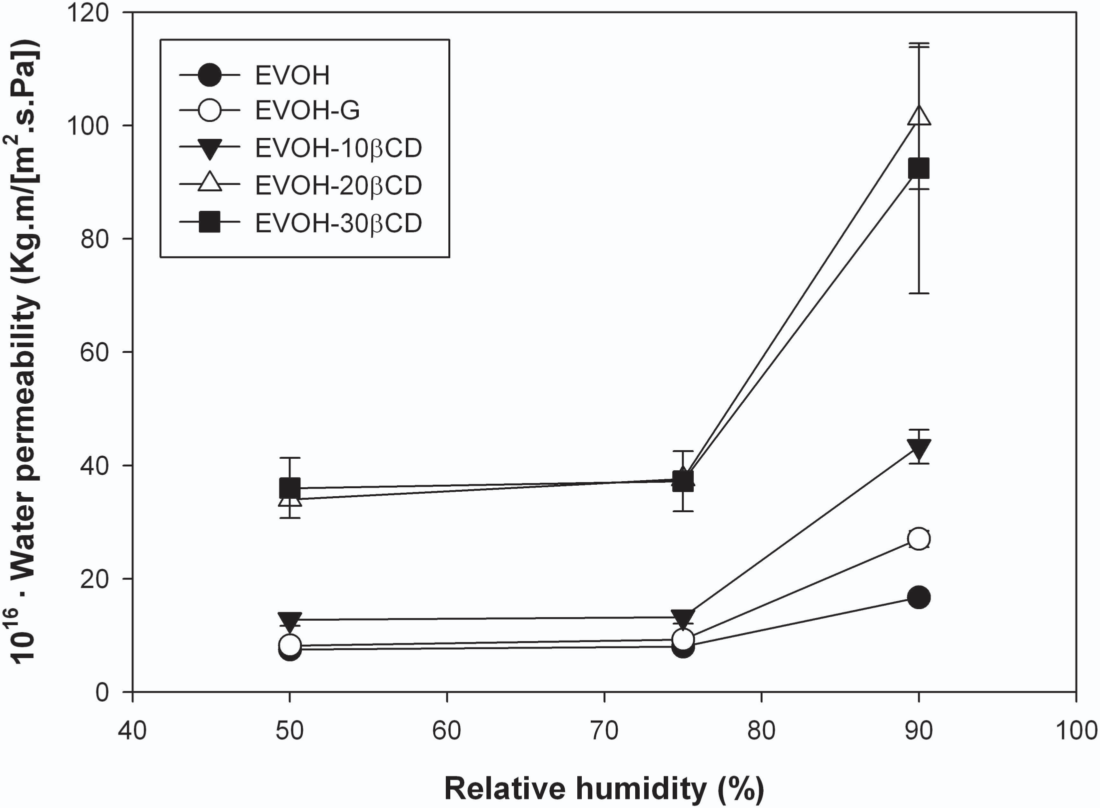
	First heating				Second heating			
Material	Tg	ΔH	Tm	ΔTm	Tg	ΔH	Tm	ΔTm
EVOH	46.08	59.09	159.96	43.3	43.28	67.49	161.73	63.6
EVOH-20G	42.89	58.56	160.3	38.9	38.48	68.55	160.77	61.7
EVOH-10βCD	57.70*	55.42	141.53	64.7		49.25	147.76	64.4
EVOH-20βCD	56.94*	48.66	143.47	66.6		49.11	149.82	73.8
EVOH-30βCD	61.6*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

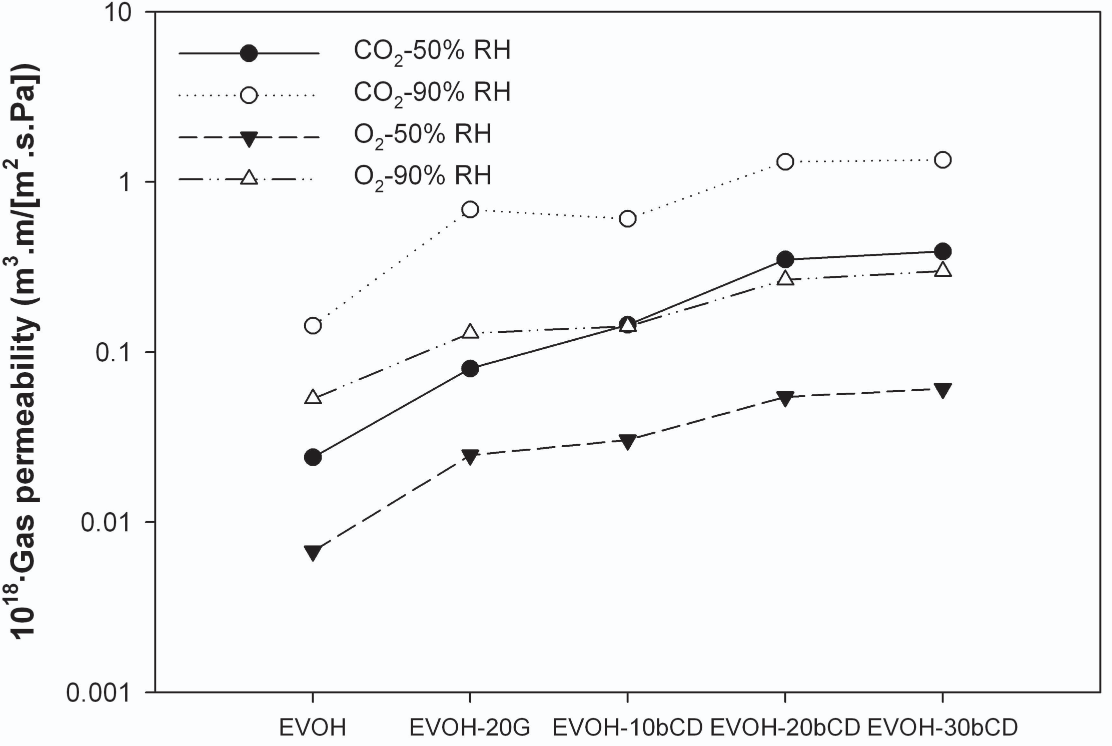

5 µm
SE MAG: 4500 x HV: 5.0 kV WD: 15.0 mm
A
8 µm
SE MAG: 3000 x HV: 5.0 kV WD: 15.0 mm
B
6 µm
SE MAG: 4000 x HV: 5.0 kV WD: 15.0 mm
C

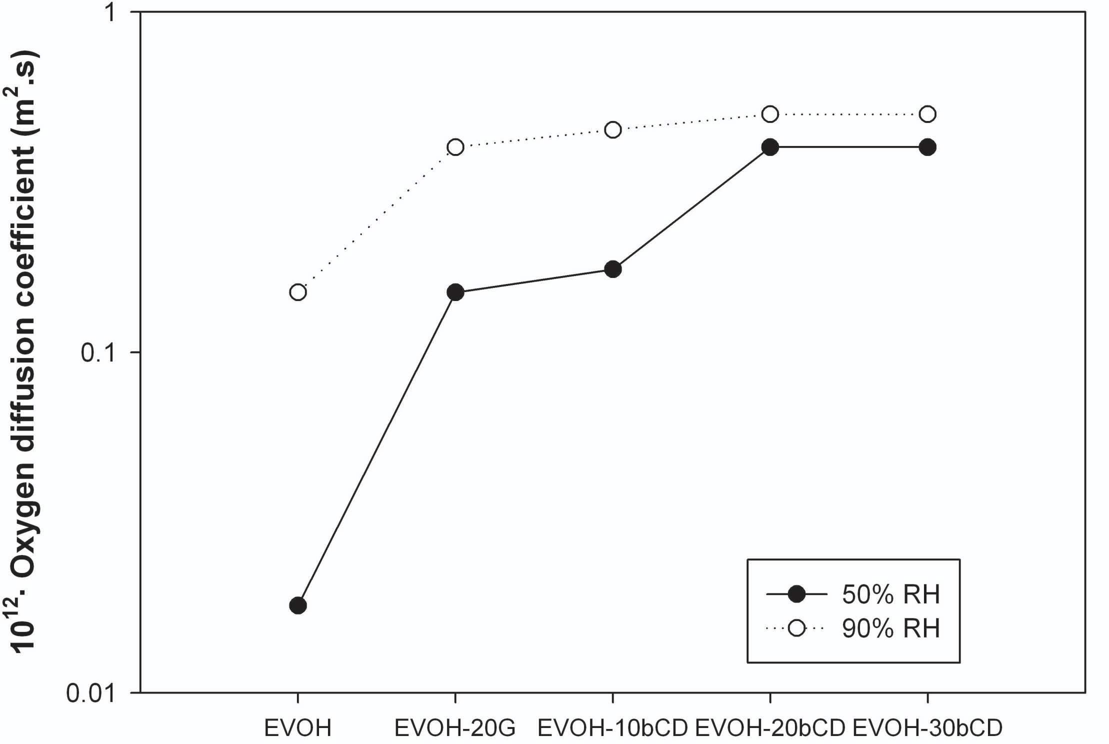

Weight loss (%)
0
2
4
6
8
10
12
14
16
Day 1
Day 10
EVOH-20G
EVOH-10bCD
EVOH-20bCD
EVOH-30bCD

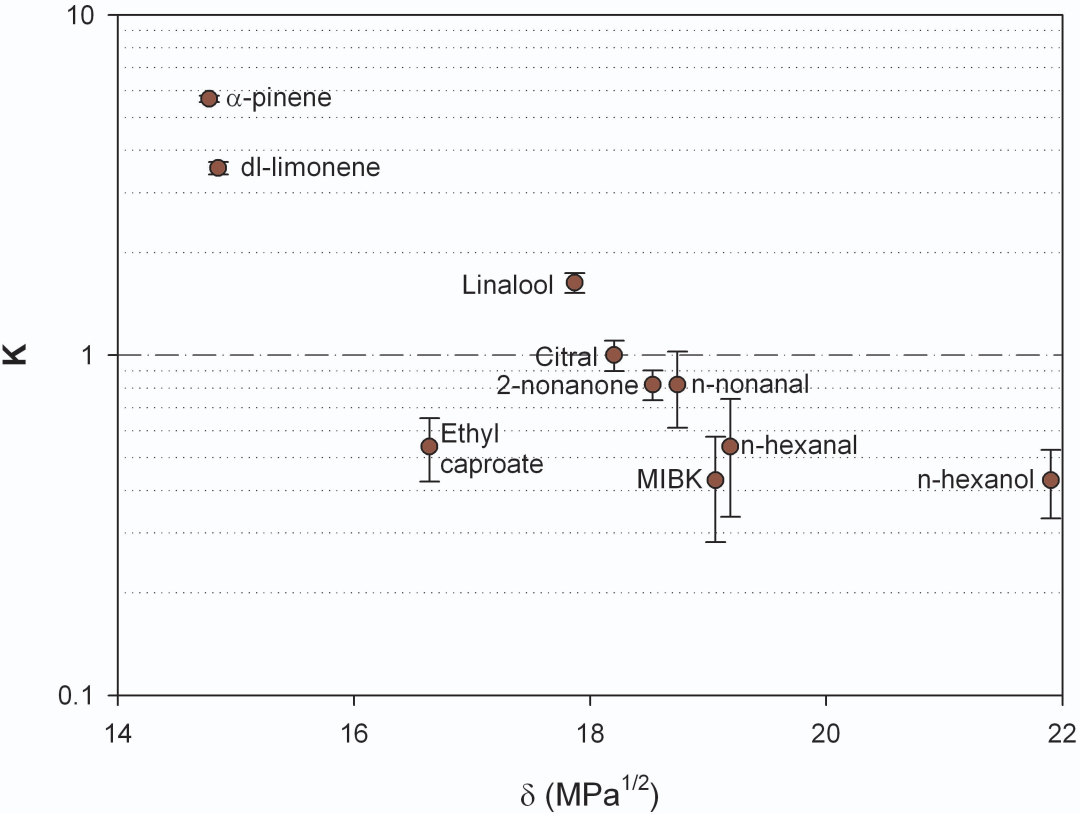

Heat Flow >>Exo>>
EVOH
EVOH-20G
EVOH-10βCD
EVOH-20βCD
EVOH-30βCD
0
50
100
150
T (ºC)

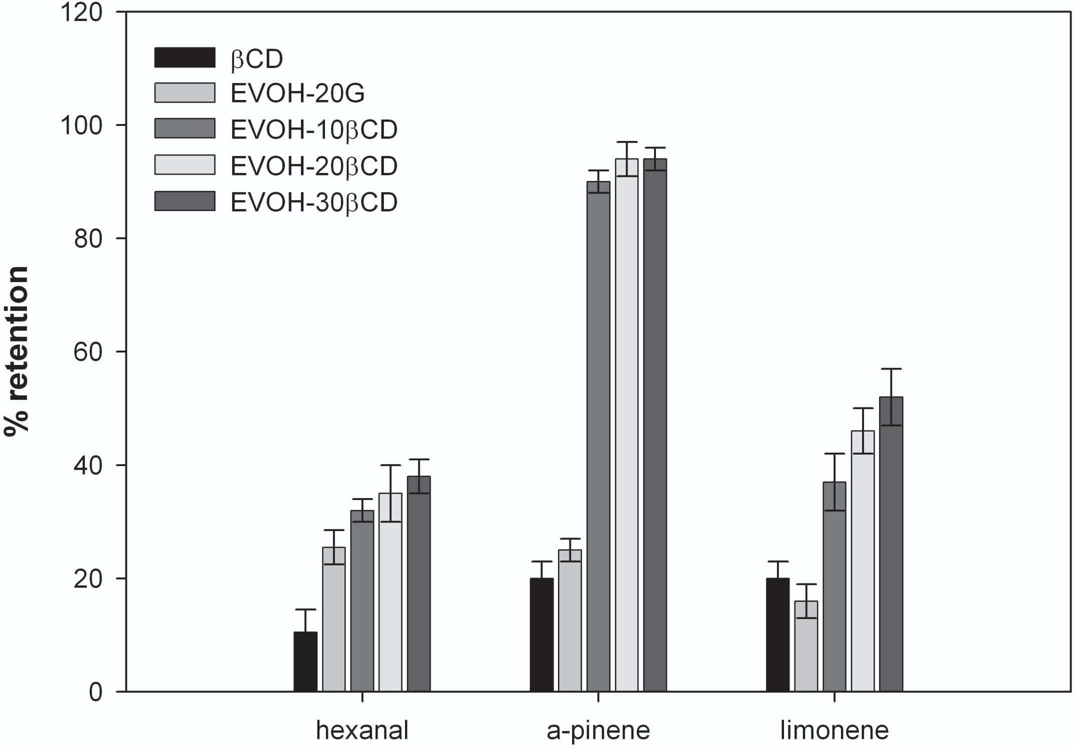

A
B
Water sorption (g/100g)
Water S (Kg/[m3·Pa])
Relative humidity (%)
0
5
10
15
20
0.00
0.04
0.08
0.12
0
20
40
60
80
100
EVOH
EVOH-20G
EVOH-10βCD
EVOH-20βCD
EVOH-30βCD


EVOH
EVOH-G
EVOH-10βCD
EVOH-20βCD
EVOH-30βCD
10^{16} · Water permeability (Kg.m/[m^2.s.Pa])
Relative humidity (%)
0
20
40
60
80
100
120
40
50
60
70
80
90
100


10
1
0.1
0.01
0.001
10^{18}·Gas permeability (m^3.m/[m^2.s.Pa])
CO_2-50% RH
CO_2-90% RH
O_2-50% RH
O_2-90% RH
EVOH
EVOH-20G
EVOH-10bCD
EVOH-20bCD
EVOH-30bCD


1
0.1
0.01
10^{12}. Oxygen diffusion coefficient (m^2.s)
EVOH
EVOH-20G
EVOH-10bCD
EVOH-20bCD
EVOH-30bCD
50% RH
90% RH


10
1
0.1
K
α-pinene
dl-limonene
Linalool
Citral
2-nonanone
n-nonanal
Ethyl caproate
n-hexanal
MIBK
n-hexanol
14
16
18
20
22
δ (MPa^{1/2})


120
100
80
60
40
20
0
% retention
βCD
EVOH-20G
EVOH-10βCD
EVOH-20βCD
EVOH-30βCD
hexanal
a-pinene
limonene