Food applications of active packaging EVOH films containing cyclodextrins for the preferential scavenging of undesirable compounds

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Abstract

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

Keywords: active packaging; cyclodextrins; EVOH; cholesterol scavenging; aldehydes retention; lipid oxidation

1. Introduction

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

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

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

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

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

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

2. Materials and methods

2.1. Chemicals and reagents

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

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

*[1] Corresponds to the alquilic chain of cholesterol molecule (6-methylheptyl).
*[2] Corresponds to the inner cavity of the cyclodextrin.

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

2.2. Film preparation

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

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

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

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

2.3. Cholesterol in milk

2.3.1. Milk sample preparation

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

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

After sterilizing the film surfaces by UV irradiation for 15 min, approximately 12.8 cm^2 of each of the films were immersed in 20 mL of pasteurized whole milk and kept at 4 ºC for one week in a closed vial covered by aluminum foil to avoid any potential effect of light.

In a second experiment, the UV irradiated film samples were immersed in 20 mL of UHT whole milk and kept at 23 ºC for one week. In this experiment, the milk package, films, vials and milk were handled under sterile conditions to prevent the risk of bacterial contamination and milk spoilage.
The experiments were carried out in quintuplicate.

2.3.2. Cholesterol determination

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

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

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

2.4. Monitoring oxidation by-products from fried peanuts

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

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

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

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

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

2.5. Statistical analysis

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

3. Results and discussion

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

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

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

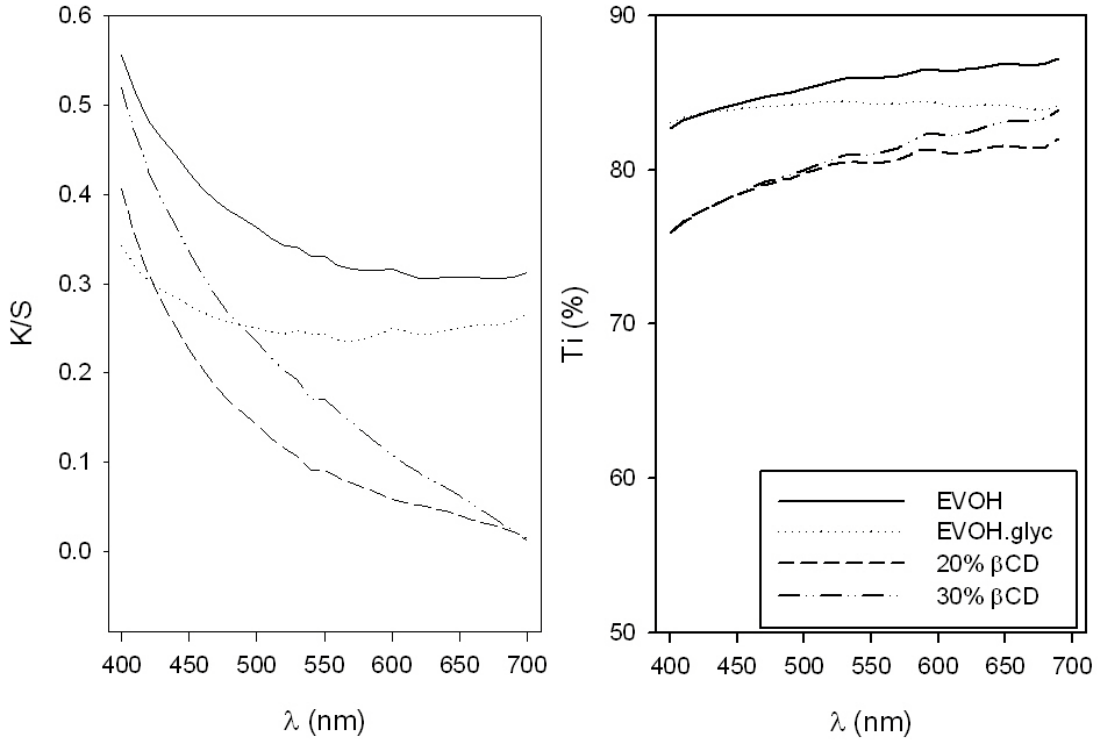

Figure 1. Values of the Kubelka-Munk transparency parameter (K/S) and of the internal transmittance (Ti) as a function of wavelength.

3.1. Cholesterol scavenger results

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

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

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

	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

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

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

The behavior of the UHT milk samples was similar on day 7. The presence of the active films resulted in a significant reduction in cholesterol concentration. The cholesterol scavenging activity increased with the concentration of βCD in the film samples. In this test with UHT milk, no reduction of cholesterol with time was observed in the control sample, indicating that the handling of samples in aseptic conditions together with the use of UHT milk reduced the potential influence of lipolitic microorganisms on the results. After a week of exposure, the EVOH material containing 30% βCD had adsorbed nearly 23% of the initial cholesterol content. As mentioned in the introduction, 1:1, 1:2 and 1:3 cholesterol/βCD inclusion complexes have been reported in the literature. In this work, the low mobility of the cyclodextrin molecules within the matrix should hinder the formation of inclusion complexes other than 1:1 cholesterol/βCD. Most probably, the alquilic chain of the cholesterol molecule is trapped in the βCD 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 βCD, the
344 percentages of CD molecules involved in cholesterol scavenging after 7 days were
345 15.5% and 15.7% for the 20% βCD and 30% βCD 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% βCD and 30% βCD.
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 ºC.

	Day 2		Day 7	
	Cholesterol	Reduction (%)	Cholesterol	Reduction (%)
Milk	161.4 ± 16.4 a,x		152.5± 4.7 a,x	
20% βCD	142.8 ± 2.2 ab,x	11.5	129.2 ± 1.2 b,x	15.3
30% βCD	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 βCD (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 βCD containing film to a
368 hydroalcoholic solution resulted in n-hexanal retention of up to 40%. The formation of
369 βCD inclusion complexes with apolar ‘guest’ molecules has been described earlier
370 (Goubet et al., 1998; Szente and Szejtli, 2004).
371

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

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

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

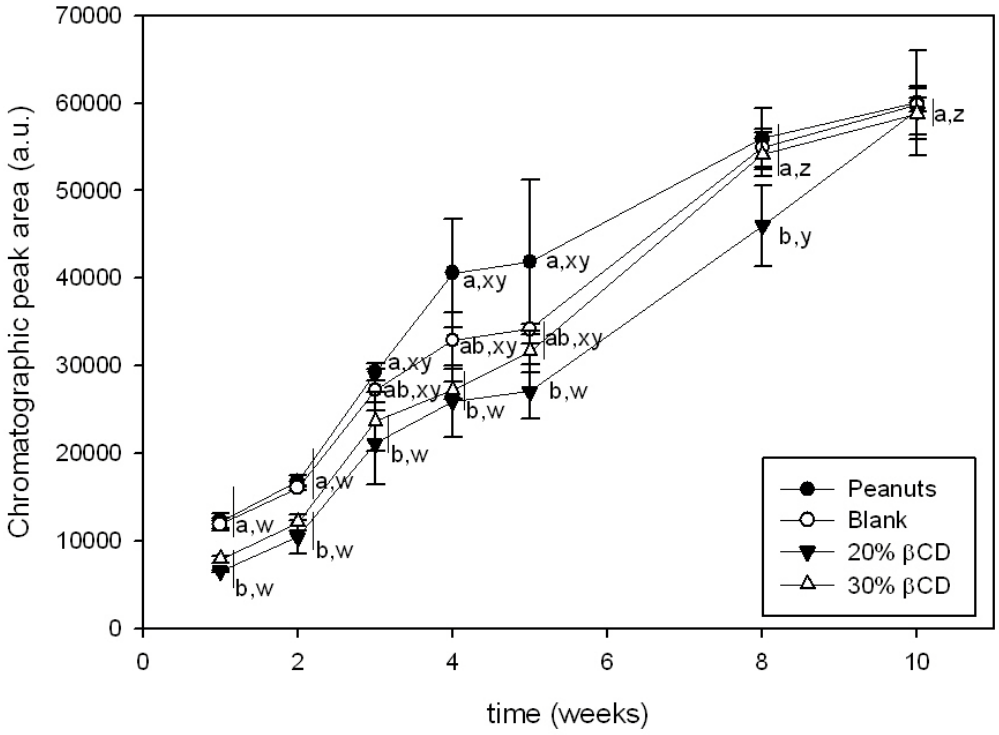

Figure 2. Hexanal concentration evolution over storage time. a, b, c… indicate significant differences in the values of different samples on the same day. x,y... indicate significant differences in the values of the same sample on different days.

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

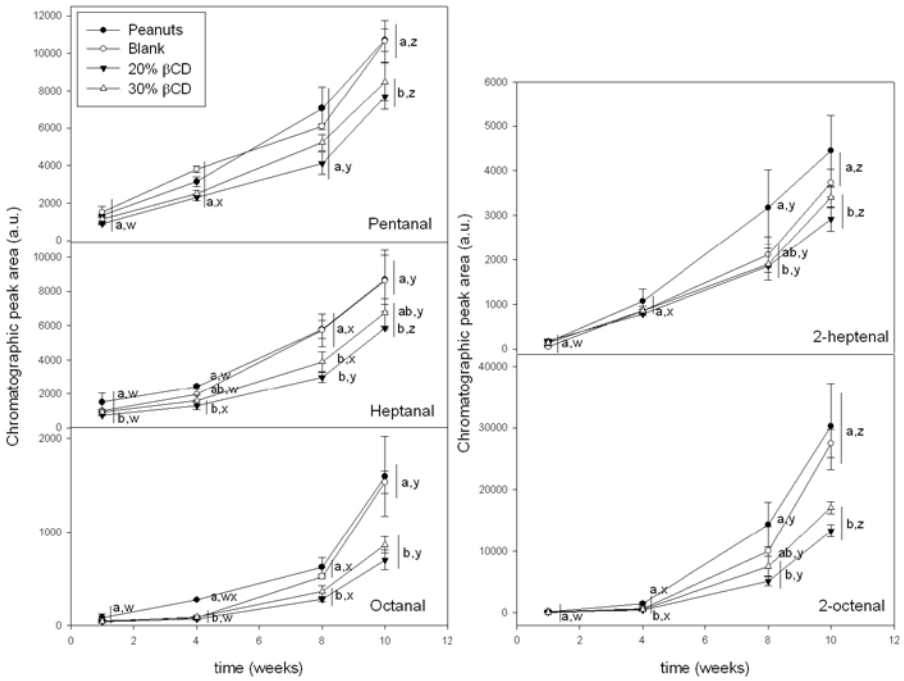

Figure 3. Concentration evolution of pentanal, heptanal, 2-heptenal, octanal and 2-octenal, over storage time. a, b, c… indicate significant differences in the values of different samples on the same day. x,y... indicate significant differences in the values of the same sample on different days.

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

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

4. Conclusions

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

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

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