The role of some antioxidants in the HPMC film properties and lipid protection in coated toasted almonds.

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Abstract

Hydroxypropylmethylcellulose based edible films with and without antioxidant additives were characterised as to their microstructure, water vapour and oxygen permeability, mechanical behaviour, optical properties and protective ability against lipid oxidation. The corresponding film forming dispersions were also used to coat toasted almonds in order to test their effectiveness at protecting against rancidity development. The efficiency of three additives (ascorbic acid, citric acid or ginger essential oil) was tested and compared with antioxidant-free coatings. A cross-linking effect in the film matrices containing ascorbic or citric acid was detected through the analysis of the film microstructure, mechanical behaviour and barrier properties to oxygen and water vapour. These films were the most effective protectors against oxidation of almonds, due to both their antioxidant effect and the tighter structure which leads to lower oxygen permeability. In films with ginger oil, the hydrophobic effect markedly reduced water vapour permeability at low temperatures, but protection against lipid oxidation was less effective at long storage times.

Keywords: edible coatings, hydroxypropylmethylcellulose, ascorbic acid, citric acid, ginger oil, almond

1. Introduction

Nuts have been claimed as a healthy food choice, mainly because of their proven cardioprotective effects. Numerous clinical studies have revealed that low-density lipoprotein cholesterol reductions of 10-15% have been observed where walnuts, almonds, macadamias, hazelnuts, pecans or peanuts were incorporated into the diet (Kurlandsky & Stote, 2006).
According to Ahmad (2010), almonds are a highly nutritional source of vitamins (B complex) and minerals (Mg, P, K). Despite containing high levels of fat (51% w/w), they have a favourable fatty acid profile (64-82% oleic acid, 8-28% linoleic acid, 6-8% palmitic acid). The oxidation process of unsaturated fatty acids results in rancid off-flavours, thus shortening the shelf-life of this product under ambient conditions.

The potential interest in the use of biodegradable films has been extensively reviewed by numerous authors (Guilbert, Gontard & Gorris, 1996; Han & Gennadios, 2005; Nussinovitch, 2009). Edible coatings provide a semipermeable barrier against the transfer of moisture, gases and other agents, thus extending the shelf-life and raising the quality of foods, by limiting the deteriorative reactions. Films made from proteins and carbohydrates are excellent barriers to oxygen, because of their tightly packed, ordered hydrogen-bonded network structure (Yang & Paulson, 2000). On the other hand, these materials have poor water vapour resistance due to their hydrophilic nature.

According to Mauer, Smith & Labuza (2000), the interest in biodegradable packaging stems from the drive to find environmentally friendly packaging, to improve the quality of food products and to find new markets for existing materials. Additionally, edible films may be effective carriers of food additives (Lee, Park, Lee & Choi, 2003). Baldwin & Wood (2006) demonstrated the efficacy of cellulose derivative films and several additives (α-tocopherol, citric acid, BHA, BHT) in prolonging the shelf-life of pecan kernels. Ayranci & Tunc (2003) succeeded in reducing the oxygen permeability of methyl cellulose films with the inclusion of ascorbic acid or citric acid, thus slowing down the browning reactions when the coatings were applied.

The aim of this work was to study the impact of some additives with proven antioxidant properties (ascorbic acid, citric acid and ginger oil) on the effectiveness of hydroxypropylmethylcellulose (HPMC) films and coatings as protectors against lipid oxidation. Additionally, the obtained films were characterised as to their relevant functional characteristics like mechanical behaviour, water vapour permeability and optical properties, to know the effect of the additives on the polymer matrix properties.

2. Materials and methods
2.1. Materials

The film-forming dispersions were prepared with hydroxypropylmethylcellulose (HPMC, Sigma-Aldrich, Co. St. Louis, USA), ascorbic acid (Panreac Química, S.A. Barcelona, Spain), citric acid (Fisher Scientific Afora, Valencia, Spain), ginger essential oil (Esential arôms, Torre-Serona, Lleida, Spain) and Tween 80 (Panreac Química, S.A. Barcelona, Spain).

2.2. Preparation of the film-forming dispersions and rheological characterization

HPMC (5% w/w) (average molecular weight 748 g/mol) was dispersed in deionised water at 80°C, and left stirring over night. Ascorbic acid (A), citric acid (C) or ginger oil (G) were added under magnetic stirring at 0.5% w/w (ratio HPMC: additive 1:0.1). Tween 80 was incorporated to reduce the surface tension (HPMC: Tween 80 1:0.05) and contact angle with the almond surface. Homogenization was carried out by using a vacuum high-shear probe mixer (Ultraturax T25, Janke & Kunkel, Germany) at 13,500 rpm for 3 min, and the dispersions were degasified at room temperature. From here onwards, the four formulations will be referred to as H (HPMC), HA (HPMC-ascorbic acid), HC (HPMC-citric acid) and HG (HPMC-ginger oil).

Their viscosity ($\eta$) was determined in triplicate at 25 ºC, with a rotational rheometer (HAAKE Rheostress 1, Thermo Electric Corporation, Karlsruhe, Germany), fitted with a type Z34DIN Ti sensor system of coaxial cylinders. The shear stress ($\sigma$) was measured as a function of shear rate ($\dot{\gamma}$) from 0 to 512 s\(^{-1}\).

2.3. Film casting

The amount of emulsions that would provide 1g of total solids was spread evenly over each casting plate (150mm diameter), thus resulting in a solids distribution of 57 g solids/m\(^2\).

Films were formed by drying on a level surface for approximately 24 h at 45% RH and 20 ºC, and were peeled off from the casting surface. The films were stored in cabinets at 25ºC at two levels of relative humidity (RH), namely 33% and 53%, using oversaturated solutions of MgCl\(_2\) and Mg(NO\(_3\))\(_2\), respectively (Panreac quimica, S.A., Castellar del Vallés, Barcelona, Spain).

2.4. Characterization of the films
2.4.1. Thickness

A Palmer digital micrometer (Comecta, Barcelona, Spain) was used to measure film thickness to the nearest 0.001 mm. Six random positions were considered for WVP tests, and four measurements were taken along the previously cut strips for the tensile tests.

2.4.2. Microstructure

The microstructure of the films was observed by Scanning Electron Microscopy (SEM) using a JEOL JSM-5410 (Japan) electron microscope. Surface and cross images of films were captured and analysed. To obtain the cross-section images, film samples were immersed in liquid nitrogen and cryofractured. After coating with gold, the samples were observed using an accelerating voltage of 10kV.

2.4.3. Water vapour permeability

Water vapour permeability (WVP) was determined at three different temperatures (5, 20, 35°C) and two RH gradients using a modification of the ASTM E96-95 gravimetric method (1995) for flexible films (McHugh et al., 1993). Payne permeability cups of 3.5 cm in diameter (Elcometer SPRL, Hermelle /s Argenteau, Belgium) were filled with oversaturated solutions of Mg(NO$_3$)$_2$ (RH: 51-57% in the tested temperature range) or NaCl (RH: 75-76% in the tested temperature range). Films were selected due to the lack of defects, three round samples per formulation were cut and thickness was measured. Once the films were secured, each cup was placed in a pre-equilibrated cabinet fitted with a fan to provide a strong driving force across the film for water vapour diffusion. The RH of the cabinet was held constant using over-saturated solutions of MgCl$_2$ (RH: 32-33%) or Mg(NO$_3$)$_2$ (RH: 51-57%). The shiny side of the films was exposed to the ambient at the highest RH, and the cabinets were stored at the aforementioned temperatures. The cups were weighed periodically (± 0.00001 g) after steady state had been reached. The slope of the weight loss vs. time plot was divided by the exposed film area to calculate the water vapour transmission rate (WVTR). WVP was quantified using the equations described by Fabra et al. (2009a).

2.4.4. Mechanical behaviour
Rectangular samples (25 x 100 mm) were cut and stored at either 33 or 53% RH and
25°C, in cabinets containing MgCl₂ or Mg(NO₃)₂ saturated solutions. Once equilibrated, film
specimens were mounted in the film-extension grips and stretched at 50 mm min⁻¹ using a
and temperature (25°C) of the testing environment were registered with a thermohygrometer
(testo 60-H2, Testo GmbH & Co., Lenzkirch, Germany). Nine replicates of each formulation
were tested. The elastic modulus (EM), tensile strength at break (TS) and percentage
elongation (%E) were determined according to ASTM standard method D882 (ASTM 2001).

2.4.5. Optical properties

Optical properties were determined on the shiny side of films pre-equilibrated at 33%
RH. The reflectance spectra were obtained with a spectrorcolorimeter MINOLTA, model CM-
3600d (Minolta CO. Tokyo, Japan). Both black and white backgrounds were used and the
diameter of the illuminated sample area was 8mm. The internal transmittance (Tᵢ) and
reflectance for an infinite thickness (Rᵢ) was quantified as described by Fabra et al. (2009a).

Colour CIE-L* a* b* parameters (CIE, 1986) were obtained using illuminant D₆₅ and
observer 10° from Rᵢ values. Colour coordinates chroma (Cₐ₉*), and hue (hₐ₉*), as well as the
whiteness index (WI) of the samples (equation 1), were calculated.

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WI = 100 - \sqrt{(100 - L*)^2 + a^*^2 + b^*^2}
\]  

The gloss of the films was measured at 20, 60 and 85° angles from the normal to the
cocating surface, according to the ASTM standard D-523 (ASTM, 1999) using a flat surface gloss
meter (Multi.Gloss 268, Minolta, Germany). Ten replicates were taken per formulation and
angle. Results were expressed as gloss units, relative to a highly polished surface of black
glass standard with a value near to 100.

2.4.6. Oxygen permeability

The oxygen barrier properties of the films were evaluated by measuring oxygen
permeability (OP) with an Ox-Tran 1/50 system (Mocon, Minneapolis, USA) at 20°C (ASTM
Standard Method D3985-95, 2002). Measurements were carried out at 56% RH, in order to avoid cracking at low RH. Film specimens were placed in precut aluminium masks that allowed a 5 cm² exposure area. Masked films were exposed to pure nitrogen flow on one side and pure oxygen flow on the other side. OP was calculated by dividing the oxygen transmission rate by the difference in oxygen partial pressure between the two sides of the film, and multiplying by the average film thickness. Three replicates per formulation were made.

2.4.7. Protective ability against lipid oxidation

The protective capacity of the films against lipid oxidation was quantified with an accelerated test of oxidative rancidity (Osés et al., 2008). Specially designed methacrylate cells were filled with 18 g of almond oil (Biocesta S.L., Valencia, Spain), covered with the films and hermetically secured. The headspace in each cell was 18.65 cm³, and the oxygen in it was displaced by bubbling nitrogen into the oil. Two films were tested per formulation. Two additional cells were covered with aluminium foil (17 µm thickness) and two more were left uncovered. Each cell was placed in a cabinet at 50% RH and stored in darkness for 85 days at 40°C. Samples of 1 ml almond oil were periodically taken using a syringe through a septum placed in the cell wall. Simultaneously, 1 ml of nitrogen was introduced in the cell through a second septum to maintain the internal pressure constant. The progression of oil oxidation was monitored by analysing the peroxide value (PV) according to the method described in the norm UNE 55-023. PV was expressed as mEq of oxygen per kg of sample.

2.5. Application of the coatings on roasted peeled almonds

2.5.1. Coating procedure

Raw peeled almonds were purchased at a local market, roasted at 160°C for 15 minutes and cooled down using forced air at -20°C. Roasted almonds were coated by dipping in the film-forming formulations for 1 minute, drained for 1 minute and gently air-dried. Surface solid density (SSD), calculated as the ratio between the mass of dry coating and that of the non-coated almond, was determined in quintuplicate for each formulation. All the almonds selected
were similar in size and shape. After coating and drying, almonds were stored at 25°C and two levels of RH, namely 33 and 53%, using MgCl₂ or Mg(NO₃)₂ oversaturated solutions.

2.5.2. Lipid oxidation throughout storage

The lipid fraction of the almonds was extracted from 5 g ground nut samples by Soxhlet extraction equipment (Soxtec T 2055 FOSS Analytical AB, Höganäs, Sweden) using petroleum ether as a solvent, for 1.5 h. Oil oxidation was monitored by analysing the peroxide value (norm UNE 55-023), periodically up to 17 weeks storage time. Peroxide value (PV) was expressed as mEq of oxygen per kg of extracted oil. All analysis was performed in triplicate.

2.6. Statistical analysis

The statistical analysis of the data was performed through analysis of variance (ANOVA) using Statgraphics Plus for Windows 5.1 (Manugistics Corp., Rockville, Md.) Fisher’s least significant difference (LSD) procedure was used.

3. Results and discussion

3.1. Characterization of the films

3.1.1. Microstructure

Figures 1 and 2 show, respectively, the surface and cross-section micrographs of all the obtained films. Small irregular particles on the surface of the films, specially for pure HPMC films (H), can be observed, which can be attributed to some amount of non-dissolved polymer which remains non integrated in the matrix (figure 1). With the addition of acids (HA and HC) the films surface became smoothened, which indicates that a good integration of these compounds occurred into the HPMC matrix, resulting in a very homogenous, tighter structure. Cross-section micrographs (figure 2) are coherent with this; homogenous and smooth cross-section surface are observed. Nevertheless, the structure of HG films show discontinuities in both surface and cross-section images (figures 1 and 2, corresponding to the ginger oil droplets, whose size reached about 20 µm. This reveals that films with ginger oil had a heterogeneous structure, with oil droplets dispersed in the polymer matrix. Moreover, HG films appeared thicker than the other
film, as can be seen in figure 2, which can be attributed to the fact that the oil presence made
the polymer chain aggregation more difficult, given rise to a more open film structure. In fact the
measurement of the film thickness revealed that those containing ginger oil were significantly
(p<0.05) thicker (HG 62 ± 14 µm) that pure H films (55 ± 9 µm). The ginger oil droplets can also
be observed in the irregular surface of HG films (figure 1). The statistical analysis of the film
thickness values also indicated that HA and HC films were very similar (HA: 53 ± 8 µm; HC: 53
± 9 µm) and significantly (p<0.05) thinner than pure H films. This could be related to some
cross-linking effect caused by these acids, which favours the polymer chain aggregation and
tightens the structure of the matrix.

3.1.2. Water vapour permeability

The effect of the RH on WVP was relevant, and the values at RH gradients of about 33-
53% were significantly (p<0.05) lower than those obtained at about 53-75% RH gradients, as
shown in table 1. This is coherent with the results of Villalobos et al. (2006) who reported the
water sorption isotherm of HPMC films at 10ºC, and observed that the equilibrium moisture
increased slowly below 0.62 a_w, whereas a substantial water gain occurs from 0.62 a_w. Hence,
the hydrophilic nature of the HPMC, whose structure became plasticized at the highest moisture
content, allows for more intense water transport at high RH. To assess the effect of temperature
and formulation on WVP, the results at each RH gradient were treated separately for the
statistical analysis. It was found that the effect of both temperature and formulation on WVP was
similar, regardless of RH.

Formulation had a significant impact on WVP (p<0.05) under any RH-T conditions. In
the case of films without additives, the obtained results were consistent with previous studies
(Ayranci et al., 1997; Sánchez-González et al., 2009), showing poor water barrier properties,
coherent with the hydrophilic nature of carbohydrates. The incorporation of A or C resulted in an
improvement of the water barrier properties (p<0.05) and films with both acids behaved similarly
under all conditions (p>0.05). This could be due to the cross-linking between the polymer chains
and the carboxylic acids, a phenomenon previously described by Reddy & Yang (2010).
According to these authors, carboxylic acids cross-link the hydroxyl groups in cellulose. This
would result in the formation of a tighter structure (coherently to the microstructure observed by SEM), thus restricting the movement of the molecules and leading to a WVP decrease.

HG films had a variable response. At low temperatures, G was the most effective WVP depressor due to its hydrophobic contribution to the matrix, whereas at 35ºC it had no significant effect on the poor water barrier properties of the HPMC films, whose structure seemed to undergo some loosening. This could be due to the structural characteristics of HG films which show a heterogeneous structure with a liquid oil dispersed phase and interfaces (see figure 2). Similar results were observed by Atarés et al. (2010) in sodium caseinate films. The liquid state of ginger oil could favour molecular mobility at high temperature, thus promoting the transport of water molecules through the emulsified films.

3.1.3. Mechanical behaviour

Table 2 shows the mechanical parameters of all the films tested. RH had a reduced impact on the mechanical behaviour of the samples. As RH increased from 33% to 53%, the average values of mechanical parameters was either not significantly affected (p>0.05) or showed little variations. Chick & Hernández (2002) found that casein-wax films were 5-fold weaker at 75% RH than at 50% RH. Fabra et al. (2009a) studied the mechanical behaviour of sodium caseinate films incorporated with fatty acids or beeswax, and reported that both EM and TS were significantly lower at 53%RH than those at 33%. However, such studies refer to protein edible films and no studies have been found dealing with the effect of moisture content on the mechanical properties of cellulose derivative films. A possible explanation for this mild effect of moisture content on the mechanical properties of HPMC films could be the limited water content of the films, even at 53% RH, which was 5.2 ± 1.6 (db) without any significant effect of formulation (p>0.05). Villalobos et al. (2006) reported the water sorption isotherm of HPMC films at 10ºC, and observed that the equilibrium moisture increased slowly between 0 and 0.62 a_w, remaining below 0.05 (db) even at 0.53 a_w. The substantial water gain occurs from 0.62 a_w, according to the reported sorption isotherm. So, no important changes in the moisture content of the films occurred between equilibrium at 33% and 53% to provoke a notable plasticization effect that can substantially modify mechanical behaviour.
The addition of A or C resulted in a significant EM increase at low RH, probably due to the afore-mentioned cross-linking with the polymer. This effect results in a more compact, rigid and brittle structure (coherently with that revealed in the microstructural analysis), whose fracture occurs at lower TS and E. On the other hand, films with G tended to be weaker and showed the lowest TS, which must be related to the presence of discontinuities in the film structure, as described in section 3.1.1. The highest elongation percentage was observed in films without additives (H), and it was reduced when A or C were added. The addition of G entailed greater extensibility, which may be due the weakening of the polymer chain aggregation associated to the oil presence and to the liquid state of G at room temperature and the elongation of oil droplets as the structure is stretched. Similar results were obtained with the addition of oleic acid to soy protein films (Monedero et al., 2009).

3.1.4. Optical properties

Optical properties are greatly relevant to the film functionality due to their great impact on the appearance of the coated products (Villalobos et al., 2005). The spectral distribution of the internal transmittance (%) between 400 and 700 nm is shown in figure 3. The film formulation had a significant effect (p<0.05) on this parameter at 400, 500, 600 and 700 nm (table 3). The shape of the Ti curves corresponding to H, HC and HG films was very similar and mostly parallel. HA films behaved differently from the rest, especially at low wavelengths. This relates to the increased absorption in the blue region, caused by ascorbic acid which imparts yellowness to the films. Apparently, the cross-linking taking place in HA films affected the chromophore groups of this acid. The addition of ginger oil reduced the internal transmittance of the HPMC films more than any other additive, as can be observed in figure 3. In fact, HG films were the most opaque of the films, which is related to the presence of lipid droplets in the polymer matrix, which implies anisotropy in the material refractive index.

Table 3 reports the colour coordinates L*, C_{ab}*, h_{ab}* and WI of the films. All of them were significantly affected by the film formulation (p<0.05). There were few differences in clarity, whereas ginger oil provoked a slight film darkening, ascorbic and citric acids enhanced clarity, probably due to the structural effects (more compact matrix) which promote the light surface reflection. Films with ascorbic acid had the highest colour saturation (p<0.05) and the lowest
hue (p<0.05), coherent with the light absorption behaviour described above for Ti. HC films showed the highest WI because of their low colour saturation (the lowest chrome values).

Norm ASTM D523 (1999) defines gloss as the ratio of the luminous flux reflected from a specimen to the luminous flux reflected from a standard surface under the same geometric conditions. As recommended by the aforementioned norm, the geometry 60º was selected from the different incidence angles (table 3), given that most of the values were comprised between 10 and 70 units. H and HG films were the least glossy, whereas A and especially C significantly improved (p<0.05) the gloss of the films. As described in section 3.1.1., the presence of these acids entailed structural changes linked with some smoothing effect on the surface topography of the HPMC films. In the case of HG films, the presence of oil dispersed droplets causes irregularities on the film surface that reduce the specular light reflection (Villalobos et al. 2005; Fabra et al. 2009b). Figure 1 supports these results.

3.1.5. Oxygen permeability

The values of oxygen permeability (OP) are shown in table 3. OP of HPMC films was in the same range as that reported by Miller & Krochta (1997). The addition of ascorbic or citric acids had a relevant impact on OP, which was significantly reduced (p<0.05). This is in agreement with the results of Ayranci & Tunc (2003), who included varying amounts of these acids in cellulosic films and observed the improvement of oxygen barrier properties as the content of both acids increased. The cross-linking taking place between these acids and the polymer chains affected the film structure (as already commented in section 3.1.1.), and led to the reduction of OP.

An opposite effect was observed for films containing ginger oil which showed the highest OP values(p<0.05). The occurrence of ginger oil droplets interrupting the HPMC matrix and the apparent weakening of the polymer chain aggregation in the matrix, give rise to an important OP increase. The liquid state of ginger oil, as well as its more hydrophobic nature, also facilitated the oxygen transport in the matrix and worsened the oxygen barrier properties of the films.

3.1.6. Protective ability against lipid oxidation
Figure 4 shows the PV development as a function of the storage time in almond oil protected with HPMC films (with and without additives), aluminium foil and without any film. The protection provided by aluminium foil was so effective that the corresponding almond oil did not undergo any PV increase throughout time, remaining as low as 4.3 meq O₂/kg oil, even after 85 days of storage. In the rest of the cases, PV rose as storage time progressed after an induction period. After 42 days, all PV values were below 20 and the differences between films and control were not significant (p>0.05). At the end of the storage period, unprotected oil had undergone the most intense oxidation (p<0.05), thus reaching a mean value of 99 meq O₂/kg oil. At the same time, the presence of additives in the films resulted in some PV reduction as compared with HPMC films.

Such a trend towards a better protection of lipids in films with additives could be due to different effects. The antioxidant properties of ascorbic and citric acids are widely known, but their chemical nature may not be the only factor affecting the antioxidant effect of HA and HC films. As commented on above, the cross-linking between acids and polymer chains reduced the oxygen permeability in these films, which would play a key role in protecting the oil against oxidation. In the case of ginger oil films, their oxygen barrier properties were not improved (section 3.1.6.), so their antioxidant effect may be necessarily related to the specific action of the oil antioxidant compounds. According to Golebiowski et al. (2008), the main components of ginger oil are zingiberene, β-bisabolene, β-sesquiphellandrene and curcumene. Shan et al. (2005) reported that phenolic volatile oils, such as gingerol and shogaol are present in ginger essential oil, as well as caffeic acid and other phenolic acids. These authors obtained a highly positive linear relationship (R²>0.96) between the antioxidant capacity and the total phenolic content of 26 spices.

Figure 4 shows how the oil protected with HG films remained very well preserved against lipid oxidation until 64 storage days. At such time, only aluminium foil was more effective than HG films as a protector against lipid oxidation. However, the protective ability of films with ginger oil diminished markedly after this period, and lipid oxidation exhibited some acceleration between 64 and 85 storage days. This is in accordance with the aforementioned antioxidant mechanism in films with ginger oil, since its antioxidant compounds will become increasingly oxidized as time progresses, thus losing their protective ability.
3.2. Application of the coatings on roasted peeled almonds

3.2.1. Coating efficiency

HPMC proved to be a suitable material for the coating of roasted peeled almonds. The coatings were continuous and did not develop visible cracks under any storage conditions, which permitted their capability for protecting the nut against lipid oxidation to be tested.

The application of all the formulations markedly improved the appearance of the almonds by increasing their gloss, at the same time as all the formulations effectively coated the totality of the almond surface, which was easily appreciated with the naked eye. Nevertheless, the surface solid density (SSD), ranging between 6 and 12 mg dry coating / g uncoated almond, was significantly affected by the formulation (p<0.05), as shown in table 4. Several factors affect the final SSD, such as the size and shape of the almonds, the extensibility of the film forming dispersion on the almond surface and their viscosity. The latter influences the gravitational losses of the applied dispersion during the drainage and drying period after dipping. In all cases, dispersions showed a Newtonian behaviour (R^2>0.9996), the η values ranging between 0.121 and 0.143 Pa s. These results are similar to those obtained by Jiménez et al. (2010) and Villalobos-Carvajal et al. (2009), who also observed a Newtonian behaviour of HPMC dispersions up to 4.5% (w/w). HA dispersion, being the least viscous, gave rise to the lowest SSD and so, thinner coatings. On the other hand, the highest viscosity value was obtained for HG emulsions (p<0.05), which relates to the presence of oil droplets, and led to a high SSD value (on average, twice that of HA). Differences in the coating thickness may also affect the film protection capacity.

3.2.2. Lipid oxidation throughout storage

Table 5 shows the development of PV throughout storage time, as a measurement of the lipid oxidation in almonds stored at 33 and 53% RH. Given that nuts are low-moisture products, and as a simulation of the actual storage conditions, the lowest RH (33%) was tested for longer. A shorter storage period at 53% RH (2 weeks) was tested in order to evaluate the role of RH in the protective ability of the films and the oxidation kinetics, aiming to simulate the contact of the product with normal ambient conditions.
RH had a relevant effect on the protective ability of the coatings without additives (H), even after only 14 days of storage. At intermediate RH (53%), the oxidation was promoted as compared to low RH (33%). Such a trend may relate to the hydrophilic nature of HPMC and the increase of the oxygen permeability of this kind of films when their moisture content increases (Hong & Krochta, 2006). In the absence of antioxidant additives, the mechanism of protection of H coatings relies on their barrier properties against oxygen transport through the film, which are extremely dependent on water availability. Hence, the oxidation is more delayed at low RH, because of the reduction of oxygen permeability and the oxygen availability within the nut. Maté et al. (1996) applied WPI/glycerol coatings to dry roasted peanuts, and demonstrated that they were more effective at lower RH. In addition, the kinetics of lipid oxidation was dependent on the water availability; intermediate RH promotes this process to a greater extent than low RH (Labuza, 1980).

Almonds coated with formulations including additives (HA, HC and HG) did not show the same trend. The presence of these agents seems to compensate for the negative effect of water availability on the protection ability of the HPMC films. At intermediate RH, almonds coated with HA, HC or HG remained better preserved (p<0.05) than those without additives. This indicates that, under these conditions and in the proportions tested, these agents act against lipid oxidation, thus preserving the quality of coated almonds and extending their shelf-life. As already pointed out, this could be related with two effects: the structural changes of the matrix due to acid addition which limits the oxygen permeability (as commented in sections 3.1.1. and 3.1.6.) and the specific action of antioxidant components, which appears crucial in the case of HG films.

At low RH, even after 118 days of storage, PV of all coated almonds remained, on average, lower than 10, and rancid smell was not detectable. At this time, differences between the treatments were statistically non-significant (p>0.05). However, the pattern of PV as storage time progressed differed between treatments. In almonds coated with HA or HC, the PV values at all tested times (0, 14, 56 and 118 days) were statistically similar (p>0.05), whereas the PV of almonds coated with H or HG showed a progressive increase (p<0.05). At the end of the storage, the almonds coated with HA or HC showed a tendency to remain better preserved than those coated with H or HG (table 5).
The differences between treatments may be related to the different protection mechanisms taking place in the coatings against lipid oxidation. As commented on above, ascorbic and citric acids may interact with the polymer matrix and cause cross-linking, which results in a reduced permeability and a lower transport of oxygen across the coating (table 3). Additionally, these compounds are known as effective antioxidant agents, and their presence in the coating may reduce the rate of lipid oxidation in the almonds. This would not be the case in almonds coated with HPMC without additives, which are preserved by the mere presence of the polymer network acting as a barrier to oxygen at low moisture content. The presence of ginger oil in HPMC coatings did not improve the film protection capacity against lipid oxidation at low RH conditions. This could be due to two opposing phenomena: at short times, the presence of antioxidant compounds in the oil composition contributes to slow down kinetics of lipid oxidation; in a second period when antioxidants are oxidized, the heterogeneous structure of the HG coating does not imply additional protection against the oxygen transport. So, the antioxidant effect of the ginger oil becomes less and less effective as the antioxidant compounds become increasingly oxidized.

4. Conclusions

HPMC coating of almonds improved their appearance and the addition of antioxidant agents was effective against lipid oxidation. The presence of the antioxidant additives was able to compensate for the negative effect of intermediate relative humidity on lipid oxidation. The protective effect of films and coatings is linked to both the presence of active antioxidant compounds and the reduction of the oxygen permeability, closely related to the structure of the film or coating. Coatings with ascorbic or citric acids showed the best barrier efficiency against oxygen transport, whereas the antioxidant activity of coatings containing ginger oil was exclusively related to the chemical action of the antioxidant compounds. The structural effect allows for a maintained protective effect against the action of oxygen, whereas the action of the mere presence of antioxidant agents is limited in time. More work is necessary in order to improve the efficiency of the coatings to delay rancidity development.

5. Acknowledgements
The authors acknowledge the financial support from the Spanish Ministerio de Educación y Ciencia through Project AGL2007-65503/ALI and from the Universidad Politécnica through Project PAID-06-08-3242. Authors also thank Manuel Planelles, Jose Luis Mova and Mercedes Tabernero from UPV Electronic Microscopy Service for their kind assistance in the use of SEM.

6. References


