Natural antimicrobial – containing EVOH coatings on PP and PET films: functional and active properties characterization

Josep Pasqual CERISUELO, Rafael GAVARA*, Pilar HERNÁNDEZ-MUÑOZ

Packaging Lab, Instituto de Agroquímica y Tecnología de Alimentos, IATA-CSIC, Av. Agustín Escardino 7, 46980 Paterna, SPAIN, Phone: +34-963900022, Fax: +34-963636301, e-mail: rgavara@iata.csic.es

*Corresponding author
ABSTRACT

Natural antimicrobials are currently being tested by many researchers for active packaging applications as a response to consumer demands for safer food products. In previous works, several packaging materials consisting of EVOH-coated PP films containing essential oils or their constituents as active agents were successfully developed and tested for antimicrobial activity. In this work, selected films from those materials, namely EVOH coatings with carvacrol, citral, marjoram essential oil, or cinnamon bark essential oil, on PP and PET substrates, were subjected to diverse physicochemical analysis in order to assess their suitability for food packaging applications. Concretely, the investigated properties were: the stability of EVOH coatings on PP and PET substrates, the retainability of EVOH matrices for active compounds, the mechanical, optical, surface, and barrier properties of the final active films, and the effects of a matrix modification based on the addition of bentonite nanoclay on the performance of PP/EVOH active packages studied in actual working conditions.

Results showed that the application of corona discharge followed by a PEI-based primer was the best anchorage treatment available to stabilize EVOH coatings on PP and PET substrates. Furthermore, they demonstrated that the retention of active agents into EVOH matrices ranged from low to moderate, depending on the embedded substance, and that their presence into an EVOH coating in the final multilayer films did not noticeably affect their mechanical, optical, or barrier properties, although it considerably improved their wettability. They also indicated that the inclusion of bentonite nanoparticles into their carrier layers substantially enhanced the performance of the final packages, while maintaining or slightly improving their other physical properties. Hence, as a conclusion, all the assayed multilayer films were considered perfectly valid for food packaging applications, and the incorporation of bentonite nanoclay to their carrier layers was also highly recommended.

Keywords: active packaging, natural antimicrobial, EVOH, essential oil, bentonite nanoclay, modeling
1. INTRODUCTION

Consumption habits in our society have recently been changing towards healthier, fresher, and more natural, additive free, food products, preserved in safer, more convenient, and eco-friendly food packages which could extend their shelf life while maintaining quality and sensory characteristics. These present demands are leading manufacturers to introducing novel food products in the market, such as fresh-cut vegetables and ready-to-eat meals, and researchers to developing novel technologies, such as modified-atmosphere packaging or active packaging, in order to preserve them while satisfying both consumer and manufacturer expectations [1 – 9].

The main hazard to face in the preservation of these kinds of foodstuffs is the potential contamination and growth of pathogenic or spoiling microorganisms, and antimicrobial active packaging has proven to be a quite effective technique in preventing their deterioration. In some applications of this technology, a volatile antimicrobial agent embedded in the packaging materials is progressively released into the package headspace to be concentrated on the food surfaces and thus inhibit bacterial proliferation [10 – 17]. However, current consumer concerns about the use of chemical preservatives in food products are making researchers direct their efforts towards alternative compounds of natural origin, such as vegetal extracts or essential oils of herbs and spices. These latter substances, in particular, are already recognized as food additives by the EFSA and as GRAS (Generally Recognized As Safe) substances by the US FDA, and have been the object of numerous studies demonstrating their effectiveness against a wide spectrum of microorganisms, as well as their advantages in matters of consumer acceptance, antioxidant activity, and biodegradable or edible capabilities in recent years [18 – 22].

In previous works [23 – 26] some essential oils and their constituents were successfully incorporated into polymeric matrices and tested for antimicrobial activity. The packaging material carrying the active agents was EVOH, an ethylene-vinyl alcohol copolymer whose hydrophilic
nature can strongly modify its barrier characteristics with the absorption of water vapor \[27 – 29\] thus allowing for a high retention or protection of the embedded compounds in dry conditions while triggering their activity when exposed to the humid environment generated by a packaged food product \[25, 30\]. The composite materials obtained were used as active layers in multilayer packaging films, which were employed in the construction of active packages for fresh products and studied by means of mathematical models. Results showed good chemical compatibility between the active agents and the packaging materials, a gradual release of the embedded compounds into the package headspace as a function of the water activity, and a significant inhibition of numerous spoiling bacteria and microflora at the beginning of the storage period. In addition, some EVOH matrix modifications with β-cyclodextrin \[31, 32\] or bentonite nanoclay \[33, 34\] were evaluated in order to increase the retention capacity or to reduce the active agents’ release rate.

In this work, some selected films from the active materials developed in previous studies \[23, 25, 26, 33\] were subjected to several analyses with the aim of assessing their suitability for food packaging applications. Firstly, the stability of EVOH coatings on pretreated polypropylene (PP) and polyethylene terephthalate (PET) substrates was investigated by evaluating the results yielded by four different anchorage technologies, namely UV radiation, corona treatment, and corona treatment followed by the application of primers based on polyurethane (PU) and polyethyleneimine (PEI), in essays of adhesion and thermosealability. Secondly, active multilayer films consisting of EVOH coatings containing natural antimicrobials on PP and PET substrates were manufactured and subsequently analyzed, together with their passive counterparts, for the determination of several physical properties of relevance for food packaging applications, as well as for the retention of the active agents in the coating matrices. In particular, films were assayed for mechanical, optical, surface, and barrier properties, as well as for the partition coefficients of
the active agents between the material layers, and for the efficiency of their incorporation. The embedded compounds were carvacrol (main component of *Origanum vulgare* essential oil, b.p.: 236 °C), citral (main component of *Litsea cubeba* essential oil, b.p.: 229 °C), and marjoram essential oil (extracted from *Thymus mastichina*, b.p.: 168 °C) in PP films, and citral and cinnamon bark essential oil (extracted from *Cinnamomon zeylanicum*, b.p.: 249 °C) in PET films. The improvements expected from an EVOH matrix modification on the retention capacity and on the rate of release of the active agents were assessed by manufacturing two different active multilayer films consisting of PP substrates coated by either neat EVOH or EVOH and bentonite nanocomposites, both containing carvacrol as antimicrobial agent, and employing them in the construction of active packages intended to preserve fresh-cut salad products, and by comparing their performance in actual working conditions.

### 2. MATERIALS AND METHODS

#### 2.1. MATERIALS

Ethylene-vinyl alcohol copolymer with a 29% ethylene molar content (EVOH-29) was kindly supplied by The Nippon Synthetic Chemical Industry Co., Ltd. (Osaka, Japan). Food-contact grade bi-oriented polypropylene (PP) and polyethylene terephthalate (PET) films 30 µm thick were kindly supplied by Envaflex S.A. (Utebo, Spain).

H-760-A primer, consisting of an aqueous solution of 12% polyethyleneimine (PEI), was purchased from MICA Corp. (Shelton, CT, USA), and NAF-1 primer, consisting of an ethyl acetate solution of 43% polyurethane (PU), was kindly provided by Artibal S.A. (Sabiñánigo, Spain).

Reagent-grade phosphorus pentoxide and sodium bentonite nanoclay were purchased from Sigma-Aldrich Co. LLC. (St. Louis, MO, USA), as well as several food-grade essential oils and their
constituents, concretely, carvacrol of at least 98% purity, citral of at least 96% purity, marjoram essential oil, and cinnamon bark essential oil. Reagent-grade 1-propanol and high-vacuum silicone were supplied by Panreac Química S.L.U. (Barcelona, Spain), and deionized water was obtained from a Milli-Q Plus purification system of EMD Millipore Corp. (Billerica, MA, USA).

2.2. PREPARATION OF THE ACTIVE MULTILAYER FILMS. CONSTRUCTION OF THE PACKAGES

All the multilayer films assayed in this work, active as well as passive, were manufactured by coating PP or PET substrates, previously treated with an anchorage technology, with hydroalcoholic solutions of EVOH-29. These solutions contained, depending on the case, an essential oil, such as marjoram or cinnamon bark oil, or one of its constituents, such as carvacrol or citral, as active antimicrobial agent, and / or an inorganic filling material, namely sodium bentonite nanoclay, as polymer matrix modifier. The preparation of EVOH-29 solutions with one or both incorporated additives is described in detail in previous works: [23, 33], respectively. In brief, active solutions with the neat polymer were prepared by dissolving a given amount of EVOH-29 pellets in a 1:1 (w/w) mixture of deionized water and 1-propanol, at a concentration of 15 % (w/w), into a glass flask placed on a RTC basic magnetic stirrer with heater plate (IKA-Werke GmbH & Co. KG, Staufen, Germany), coupled to a constant reflux vapor system. Once the polymer was fully dissolved the antimicrobial compound was added to the mixture at a concentration of either 7.5 or 10 g / 100 g EVOH-29, depending on the substance incorporated, and was let then homogenizing for at least 10 minutes more. Regarding the active solutions with EVOH-29 nanocomposite, their preparation was analogous to the previous ones with just substituting deionized water by a hydrocolloidal dispersion of bentonite nanoparticles. Such dispersion was obtained by thoroughly mixing sodium bentonite in deionized water, at a concentration of 0.6 % (w/w), with the aid of an Ultra-Turrax T25 basic disperser (IKA-Werke GmbH & Co. KG, Staufen,
Germany) and of an Ultrasons ultrasonic bath (J. P. Selecta S. A., Barcelona, Spain). Specifically, the initial mixture was firstly stirred for 30 s at 24000 rpm, afterwards sonicated for 1h, left then resting overnight, and finally sonicated again for at least 15 minutes more to obtain a stable colloidal suspension.

In the case of the films tested for coating stability, several sheets of PP and PET were subjected to four different anchorage treatments, namely UV irradiation, corona discharge, and corona discharge followed by the application of PU and PEI-based primers, prior to being coated by a solution of neat EVOH-29. Sheets subjected to UV irradiation were placed under a VUV Xe excimer lamp with 6 W at 172 nm (UV-Consulting Peschl España S.L., València, Spain) for 1 min in an air atmosphere, whereas sheets subjected to corona discharge were passed through the plasma region generated by the electrode of a BD-20AC high frequency corona surface treater three times (Electro-Technic Products, Inc., Chicago, IL, USA). Both technologies were able to create ozone molecules from air ionization, which could react with surface polymeric chains and break some of their covalent bonds. Hence, they were aimed at reducing the surface tension of the polymers, and thus at improving their wettability with the primers or their adhesiveness with the coatings.

Primers, in turn, were casted on the polymer sheets with the aid of an Elcometer 4340 automatic coating applicator (Elcometer Ltd., Manchester, UK), equipped with a Mayer rod of 10 µm deep thread, and programmed to carry out a gradual deposition of the solutions at a temperature of 60 °C and an application velocity of 8 cm/s. The same instrument and conditions were also used to apply the neat EVOH-29 solution on the four pretreated PP and PET sheets to yield the final multilayer films. After manufacturing, the deposited layers of primer and EVOH-29 were found to be homogeneous and transparent, and their thickness could be estimated by measuring both coated and uncoated areas of the substrate sheets with an ABSOLUTE Digimatic Indicator ID-C Series 543 digital micrometer (Mitutoyo America Corp., Aurora, IL, USA) and subtracting the
results obtained, yielding values of about 1 and 1.5 µm, respectively. With respect to the active films tested for various physicochemical properties, a coating technology based on gravure printing was used at the facilities of Envaflex S.A. (Utebo, Spain). In this case, the coating system was fed with reels of PP and PET films, which were pretreated with corona discharge and subsequently coated with a PEI-based primer, followed by a layer of the corresponding polymer solution. Production speed was about 60 m/min, and solvent evaporation was rapidly performed in drying tunnels, fed by hot air at 40 ºC, after the deposition of each layer. The diverse active multilayer films finally produced were: PP / PEI / EVOH + 10% (w/w) citral (PP / E + CI), PP / PEI / EVOH + 7.5% (w/w) marjoram essential oil (PP / E + MO), PET / PEI / EVOH + 10% (w/w) citral (PET / E + CI), and PET / PEI / EVOH + 7.5% (w/w) cinnamon bark essential oil (PET / E + CO). In addition, two passive multilayer films were also obtained to be used as controls in the tests carried out: PP / PEI / EVOH (PP / E), and PET / PEI / EVOH (PET / E). In this case, the coated areas of the manufactured films presented a homogeneous, translucent, and matte appearance, similar to the materials previously produced at laboratory scale, and the thickness of PEI and EVOH layers was estimated at about 0.8 and 1.15 µm, respectively. In order to avoid potential activity losses all the films were rolled in reels, packaged in hermetic aluminum-coated polyethylene bags, and stored until utilization in thermostatic chambers at 4 ± 1 ºC of temperature. Finally, in relation with the films tested for the effects of the matrix modification on the package performance, as well as for some other physicochemical properties, several sheets of PP were pretreated with corona discharge, and subsequently coated with three different EVOH solutions by using the aforementioned automatic coating applicator, equipped and programmed as previously described. In these conditions, one passive and two active multilayer films could be obtained: PP / EVOH + 2% (w/w) sodium bentonite nanoclay (PP / E + B), PP / EVOH + 7.5% (w/w) carvacrol (PP / E + CA), and PP / EVOH + 2% (w/w) sodium bentonite nanoclay + 7.5% (w/w)
carvacrol (PP / E + B + CA), presenting homogeneous and transparent coating layers of approximately 2 µm thick. These two latter materials were employed in the construction of active packages intended to preserve fresh-cut salad products, by following the procedure described in a previous work [23]. In brief, the active films were cut into pieces measuring 25 x 30 cm, and closed in the form of bags with the aid of a 420 SBM impulse heat sealer (Audion Elektro B.V., Weesp, The Netherlands). Prior to applying the final seal, bags were filled with five small cylindrical pieces of gelified water of 10 mL volume, in order to generate and maintain inner relative humidity constant and close to 100%, and thus to simulate the presence of a high water activity food product. Once bags were hermetically closed, a septum was adhered to their outer face, and 1 L of air at atmospheric pressure was injected into them with the aid of a syringe.

2.3. DETERMINATION OF THE STABILITY OF EVOH COATINGS ON PRETREATED PP AND PET SUBSTRATES: ADHESION AND THERMOSEALABILITY TESTS

The adhesiveness of EVOH coatings to PP and PET substrates subjected to different treatments for surface modification was evaluated by means of the well-known Scotch tape test, a qualitative test method based on the ASTM D3359 – 09e2 standard [35]. In detail, two identical pieces of Scotch Magic adhesive tape (3M Corp., St. Paul, MN, USA), of 19 mm wide and 100 mm long, were firmly applied to the front and back surfaces of the films in both transversal and machine directions, left for about 20 s at room conditions (about 23 ºC of temperature and 50% of relative humidity), and stripped off in a 180º angle with one quick peeling. In order to ensure the maximum strength for the anchorages, and thus the optimal testing conditions for the films, essays were performed 24 h after manufacturing, and, for each assayed sample, at least five parallel tests were carried out.

The thermosealability of the multilayer films is closely related to the sealing capabilities of their
material layers, just as to the force of adhesion between them. Because of that, several thermosealing tests were performed in parallel with the previous adhesion tests in order to assess the anchorage strength of the EVOH coatings to the diverse pretreated PP and PET substrates more thoroughly. With this objective, several sheets of each manufactured material were placed in pairs between the jaws of the impulse heat sealer described above, to be sealed by their coated face. Immediately afterwards, the timer of the instrument was set to 10, and its jaws were closed to provide a long, continuous, and homogeneous seal. Once sealing area was cooled, films were removed from the instrument and visually examined for faults or imperfections. If no sealing defects were observed, films were pulled apart by applying increasing tensile forces on the seal until causing its detachment or failure. This way, the strength of the seal, and thus the thermosealing characteristics of the assayed material, could be qualitatively evaluated by estimating the magnitude of the tension forces required to break the union between the films, and by the visual inspection of the ripped zones. Furthermore, films which provided the best sealability results were additionally subjected to peeling tests, together with their corresponding substrates, in order to quantitatively determine their sealing strength and to compare it with the values yielded by the original uncoated materials. These essays were performed by following a test procedure based on the ASTM D882 – 12 standard method [36] with the aid of a MultiTest 1-i universal testing machine (Mecmesin Ltd., Slinfold, UK), equipped and programmed as described below in section 2.7. In detail, the sealed films were cut into 1 in. wide strips, their ends were held under 3 bars of pressure with two pneumatic plane grips, and an increasing load was applied on their seal over time until causing its failure. The load force recorded at the breaking point was then identified as the sealing strength of the assayed sample. Just as for the adhesion tests, both sealing and peeling assays were performed at room conditions the day after film manufacturing, and repeated at least five times for each material sample.
2.4. DETERMINATION OF THE RETENTION OF ACTIVE AGENTS IN THE COATING LAYERS OF MULTILAYER FILMS: INCORPORATION EFFICIENCIES AND PARTITION COEFFICIENTS

Given that all the active agents incorporated in the multilayer films were volatile compounds, their evaporation losses during the processes of preparation and application of the coating solutions, and thus the retention capabilities of the polymer matrices forming the coating layers, were investigated by measuring the concentrations of these substances remaining in the final active films immediately after being produced at the industrial facilities. These concentrations were determined by thermal desorption and GC analysis, using an 890 thermal tube desorber (Dynatherm Analytical Instruments Inc., Kelton, PA, USA) connected in series to an HP 5890 Series II Plus gas chromatograph (Hewlett Packard Co., Wilmington, DE, USA) equipped with a flame ionization detector (FID) and an Agilent HP-1 semi-capillary column of 30 m length, 0.53 mm internal diameter, and 2.65 µm film thickness (Teknokroma S.C.L., Barcelona, Spain), and following the procedure described in previous works [23, 33].

Since just after the film manufacturing all the retained active molecules were supposed to be contained exclusively in the coating matrices, the amounts of substances detected by the instruments were only attributed to the film mass fraction corresponding to those material layers. This way, the values for the concentrations of each active compound \( i \) in the coating matrix of each multilayer film \( F \) at the beginning of the material shelf life, \( C_{i0}^F \), were calculated, and then introduced, together with the nominal concentrations detailed in the previous section 2.2, \( C_{i0}^n \), in the following equation (1), in order to determine the percentage efficiency of the incorporation process, \( E_i^F \).

\[
E_i^F (\%) = \frac{C_{i0}^F}{C_{i0}^n} \cdot 100
\]  

(1)
The retention of the active molecules within the coating layers of the multilayer films after manufacture was also assessed by measuring their tendency to migrate to the substrate layers over time. This tendency is determined by the thermodynamic equilibrium of each substance between both polymer matrices, which can be represented by the partition coefficient corresponding to that system, $K_{ic}^{cs}$. This thermodynamic parameter can be defined as the ratio between the concentrations at equilibrium of an active compound $i$ in the coating, $C_i^c$, and in the substrate layers, $C_i^s$, as equation (2) shows:

$$K_{ic}^{cs} = \frac{C_i^c}{C_i^s}$$

(2)

In order to properly evaluate these concentrations, the active films were analyzed 6 months after manufacturing, when the thermodynamic equilibrium was assumed to have been reached in the stored reels. All analyses were performed with the same equipment, conditions, and procedure as for the analyses of the initial concentrations described before. However, since in this case the studied compound was distributed between two material phases, which did not allow physical separation, the concentration of the active agent in the coating layer had to be estimated by first measuring the average concentration in the entire film and in the substrate layer, and then by introducing the values obtained in the following equation (3), deduced through a mass balance on the two material layers constituting the complete multilayer film:

$$C_i^c = \frac{C_i^c \cdot \rho_s \cdot L_s - C_i^s \cdot \rho_c \cdot L_c}{\rho_c \cdot L_c}$$

(3)

where $L_c$, $L_s$, and $L_f$, are the thicknesses of the coating and substrate layers, and of the entire film respectively, whereas $\rho_c$, $\rho_s$, and $\rho_f$, are their corresponding densities. Since the active films were manufactured by a coating technology based on gravure printing, and the printed areas did
not completely cover the entire film surface, the concentration of the active agent in the substrate
layer could be determined by analyzing polymer samples from the film zones that remained
uncoated.

2.5. DETERMINATION OF THE SURFACE PROPERTIES OF THE MULTILAYER FILMS

The surface properties of the coating and substrate layers of the manufactured films, and, in
particular, their wettability, were investigated by measuring static contact angles, in the diverse air/
water/polymer systems formed by the films, through the sessile drop method. The studied
systems were thus the passive coatings: E and E + B, the active coatings: E + Cl, E + MO, E + CO, E +
CA, and E + B + CA, and the substrate polymers: PP and PET, as control materials. With this
objective, an OCA 15EC goniometer (DataPhysics Instruments GmbH, Filderstadt, Germany) was
employed to deposit water droplets on the assayed film surfaces, and its SCA20 software was used
for data acquisition and image analysis purposes. Tests were carried out at room conditions in an
air atmosphere, by carefully dropping about 8 µL of deionized water with the aid of a dispenser
syringe. The static contact angles formed between the water droplets, the film surfaces, and the
air atmosphere, were measured 60 s after their deposition, by analyzing the images obtained, and
by fitting their shape equations to the Young-Laplace model. Assays were performed on, at least,
five different locations of each film sample, and in triplicate for each studied material, in order to
ensure the reproducibility of the values obtained and to allow their proper averaging.

2.6. DETERMINATION OF THE OPTICAL PROPERTIES OF THE MULTILAYER FILMS

The optical properties of the produced films were determined by measuring the material color
with a CM-3500D spectrophotometer (Konica Minolta Optics Inc., Tokyo, Japan). The instrument
was set to D65 illuminant / $10^\circ$ observer, equipped with an 8 mm aperture target mask, calibrated
with a white calibration plate and a zero calibration box, and programmed to perform three shots
in each measurement and subsequently yield the averaged results. The essayed materials were, in
this case, the developed active and passive multilayer films, as well as the polymers constituting
their substrate layers. Measurements were performed on eight different locations of each film
sample, and in triplicate for each studied material; and they were carried out at room conditions
of temperature and humidity by placing the film samples onto the device lens and against the
surface of a standard white tile. The instrument’s software SpectraMagic NX was employed to
acquire the color data and to display them in the CIELAB color space. The color coordinates of
each film sample: $L^*$ (lightness), $a^*$ (greenness – redness), and $b^*$ (blueness – yellowness), could
therefore be obtained, and the corresponding polar coordinates: $C_{ab}^*$ (chroma or saturation
index), and $h_{ab}$ (hue or angle), could easily be estimated from the latter two parameters by
introducing them in the following equations:

$$C_{ab}^* = \sqrt{(a^*)^2 + (b^*)^2}$$

$$h_{ab} = \arctan\left(\frac{b^*}{a^*}\right)$$

2.7. DETERMINATION OF THE MECHANICAL PROPERTIES OF THE MULTILAYER FILMS

The mechanical properties of the diverse manufactured films, and of their substrate polymers,
were evaluated by following a test procedure based on the ASTM D882 – 12 standard method [36]
with the aid of a MultiTest 1-i universal testing machine (Mecmesin Ltd., Slinfold, UK), equipped
with a 100 N static load cell and with small pneumatic plane grips. In detail, the studied materials
were cut into strips of 25.4 mm wide and 140 mm long, and the obtained samples were conditioned in climatic chambers at 23 ± 1 °C temperature and 0 ± 5 % of relative humidity for at least 48 h prior to testing. Immediately afterwards, at least 10 specimens of each assayed film were successively extracted from the chambers, inserted between the grips, and firmly held in place with 3 bars of pressure. The instrument’s software Emperor was then used to set the grip separation at exactly 100 mm, the cross-head speed at 25 mm/min, and the data sampling rate at 1 kHz. In these test conditions, an increasing load was applied on the film samples over time, causing their elongation, and the corresponding force ($F_i$) – displacement ($\Delta L_i$) curves were thus obtained. These plots were standardized by transforming them into stress ($\sigma_i$) – strain ($\varepsilon_i$) curves with the application of the corresponding definition equations:

$$\sigma_i = \frac{F_i}{A_0} = \frac{F_i}{w_0 \cdot t_0}$$  \hspace{1cm} (6)$$

$$\varepsilon_i = \frac{\Delta L_i}{L_0} \cdot 100$$  \hspace{1cm} (7)$$

where $A_0$ is the original cross-sectional area of the tested sample, product of its original width ($w_0$) and thickness ($t_0$), and $L_0$ is the original grip separation. This way, the mechanical properties of the studied materials, namely the ultimate tensile strength, the deformation at break, and the Young’s modulus, could be estimated through the graphical assessment of the newly obtained curves.

### 2.8. DETERMINATION OF THE BARRIER PROPERTIES OF THE MULTILAYER FILMS

The barrier properties of the produced passive films, and of their corresponding substrates, were investigated by measuring their oxygen and carbon dioxide permeances as a function of the
relative humidity, following the procedures described in a previous work [33], based in the ASTM D1434 – 82(2009)e1 standard method [37]. Oxygen transport was studied by means of an OXTRAN model 2/21 ML (Paul Lippke Handels – Gmbh, Neuwied, Germany) programmed to measure the oxygen permeance at 23 ± 2 °C and at 0, 35, 50, 75, and 90 ± 0.01 % RH, whereas for carbon dioxide transport, an isostatic permeation apparatus, described in this previous work, was used to measure the gas permeance at 23 ± 2 °C and at 0, 30, 45, 75, 85, and 100 ± 0.1 % RH. In both cases, between two and four film samples were placed in the instrument cells, held in place with high-vacuum silicone, and conditioned for 12 h prior to the permeation tests. The gas permeance was measured every 45 min until constant, and, from that moment, at least three more points were recorded in order to obtain an average value.

2.9. DETERMINATION AND SIMULATION OF THE PERFORMANCE OF THE ACTIVE PACKAGES

Differences in performance between the packages constructed with neat EVOH and those comprising an EVOH / clay nanocomposite were assessed by studying their activity over time in actual working conditions. With this objective, three packages of each type were placed in a climatic room, at 23 ± 1 °C and 30 ± 5 % RH, for one week, and the concentration of carvacrol in their headspace was periodically evaluated. Analyses were performed on a daily basis, by extracting at least three gas samples of 500 µL from their inner atmosphere with the aid of a 1750 Gastight precision syringe (Hamilton Co., Reno, NV, USA), and by injecting them in the inlet port of an HP 5890 Series II Plus gas chromatograph (Hewlett Packard Co., Wilmington, DE, USA). The instrument was equipped with a flame ionization detector (FID) and a 30 m, 0.32 mm, 0.25 µm Agilent HP-5 capillary column (Teknokroma S.C.L., Barcelona, Spain), programmed in accordance with the procedure described in previous work [23], and calibrated by injecting carvacrol in known concentrations. After averaging the results acquired in each assay period, two experimental curves...
for the evolution in time of the concentration of carvacrol in the headspace of both studied packages could be obtained.

In order to assess the effects of the EVOH matrix modification on the package performance more thoroughly, as well as to validate the experimental procedure carried out, a mathematical model based on the finite element method was applied to the assayed packages, and the simulation of their activity over time was therefore performed. This model had already been developed specifically for those systems in prior work [23] with the aid of the Chemical Transport of Diluted Species physics interface of the COMSOL Multiphysics 4.2 modeling suite (COMSOL AB, Stockholm, Sweden). In the present case, the model was fed with the fundamental hypotheses and physicochemical properties gathered in the previous studies [23, 33], as well as with the geometric characteristics and integration conditions corresponding to the systems investigated here, further detailed throughout this paper. As a result, two theoretical curves for the evolution in time of the concentration of carvacrol in the headspace of both packages could be obtained, and were then subsequently compared with their experimental counterparts.

2.10. DATA ANALYSIS

The values for all the parameters and coefficients presented in this work are expressed as \( \bar{x} \pm \varepsilon \), where \( \bar{x} \) stands for the sample mean of parameter \( x \), and \( \varepsilon \) stands for its absolute error. This absolute error is equal to the sample standard deviation for the measured variables, and was determined by propagation of uncertainty (partial derivatives method) for the calculated variables.

Statistical analysis of the results obtained for mechanical properties was performed with the aid of IBM SPSS Statistics 21 commercial software (IBM Corp., Armonk, NY, USA). Concretely, a one-way
analysis of variance (ANOVA) was carried out, and differences found between mean values for the studied materials were assessed by means of confidence intervals using Tukey’s test at a $p \leq 0.05$ level of significance.
3. RESULTS AND DISCUSSION

3.1. ASSESSMENT OF THE STABILITY OF EVOH COATINGS ON PRETREATED PP AND PET SUBSTRATES

Although both PP and PET substrates can easily be coated by hydroalcoholic EVOH solutions, yielding highly transparent and homogeneous multilayer films, the large differences in chemical structure and polarity existing between both materials, and thus their low chemical compatibility, prevent them from developing a sufficient anchorage mechanism in their contact interface. This lack of coating adhesiveness leads to film delamination when slight tensile forces are applied between the material layers, such as during the manufacturing of the films or during the formation of the packages, or when external compounds, such as water or plant essential oils, are scalped from the preserved food products [38].

Since in this work all the manufactured materials were intended to be employed in the construction of active packages for fresh-cut salad or similar products, containing essential oils or their constituents as natural antimicrobials, the application of an effective anchorage treatment on the PP and PET substrates was indispensable to ensure good stability of the deposited EVOH coatings. With this objective, four different technologies for surface modification, namely UV irradiation, corona discharge, and corona discharge followed by the application of PU or PEI-based primers, were implemented in the manufacture process of the developed films, and qualitatively evaluated by means of Scotch tape adhesion tests. The results obtained are displayed in Table 1 and, as shown, for every applied treatment no distinction is made between both assayed substrates, because no relevant differences were found in their adhesiveness behavior.

With respect to the diverse technologies investigated, UV irradiation failed to produce a
satisfactory adhesion on either PP or PET substrates, thus yielding easily delaminable films, although in the former material some peeling resistance was observed during the progression of the tests (weak adhesion). The corona discharge, by contrast, produced reasonably good results in both materials, complicating or even impeding their delamination, although, according to observation during the tests, the surface treatment had not been applied uniformly, and, in consequence, the points of anchorage between layers were heterogeneously distributed (moderate adhesion). Finally, the casting of primers after corona pretreatment yielded very opposite results depending on the substance applied. In this sense, whereas the PEI-based primer produced an excellent adhesion with both the EVOH coatings and the PP or PET substrates, making their detachment absolutely impossible (strong adhesion), the PU-based primer not only failed in providing some adhesion between layers, but, owing to its obstructive location, also prevented the corona-treated surfaces of the substrates from interacting with the coatings, and thus from generating some anchorage points between both (none adhesion). These facts led the corona discharge treatment to reach worse results when followed by a PU-based primer, or better when followed by PEI-based one, than when applied alone.

In addition to the adhesion tests, the stability of the EVOH coatings on the pretreated PP or PET substrates was also assessed by subjecting the manufactured films to thermosealing tests, carried out with the aid of a manual impulse heat sealing machine. The performance of these parallel assays was motivated by two main reasons. Firstly, the thermosealing properties of the studied materials were closely related to the hermeticity attainable in the final targeted packages, key characteristic of their structural design. Secondly, the sealing capabilities of the developed films were also dependent upon the force of adhesion between their material layers and thus their evaluation could contribute to corroborating the results obtained in the former tests. Just as for those essays, the qualitative results of the thermosealability tests are displayed in Table 1, and for
every applied treatment no distinction is made between both studied substrates either.

As this table shows, there was a good correlation between the results yielded by both tests for the
four different treatments applied on the multilayer films. According to this, materials
manufactured with UV-irradiated substrates were not sealable, owing to the low strength of the
anchorage points created between layers, and thus of the final seal formed between films.

Materials subjected to corona discharge, in turn, were only sealable to a limited extent, because,
although the obtained seal presented quite good appearance and seemed to attach both films
firmly, when slight tensile forces (≤ 1 N) were applied on the joint it promptly failed and the films
were easily detached again. This phenomenon could be product of an irregular application of the
anchorage treatment, as explained above in the results of the adhesion tests. Lastly, films formed
through primers application yielded extreme results depending upon the substance employed. In
agreement with the adhesion essays, materials containing a PEI-based primer were perfectly
sealable, because very strong tensile forces had to be applied to separate the films, and also films
were not detached by seal failure but by matrix failure, while the sealing area remained intact. On
the contrary, materials containing a PU-based primer could not be assayed because of the
absolute lack of adhesion between their layers, as reported before in the previous tests.

Finally, since the substrates coated through the application of a PEI-based primer yielded the best
results in both stability tests, the quantitative analysis for the determination of the sealing
strength was only carried out on these materials. The values found are also included in Table 1,
and compared with those obtained for the uncoated substrates. As can be seen, the EVOH-coated
films presented the highest peel strengths, thus confirming the excellent sealability properties of
EVOH copolymers, as published elsewhere [30]. Also, the improvement observed with respect to
the uncoated films is probably due to the lower melting temperature of EVOH with respect to the
substrate polymers, especially to the PET film. Hence, since all the sealing tests were carried out
under the same testing conditions, the higher percentage of polymer melted during the essays would be responsible for the stronger sealings.

To conclude, the application of corona discharge followed by a PEI-based primer was definitely the best anchorage technology available to bond EVOH coatings with PP or PET substrates, and it was therefore implemented in the manufacturing process of the active multilayer films. Obviously, the physicochemical assays described below were only performed on such films.

3.2. ASSESSMENT OF THE RETENTION OF ACTIVE AGENTS IN THE COATING LAYERS OF MULTILAYER FILMS

The retention of a chemical compound in a polymeric matrix is a function of the thermodynamic equilibrium of the substance between the system phases involved, in this case: air, coating and substrate, and of its kinetics within them. Since some of the active agents studied in this work are known to show volatility as well as a considerable solubility in other polymeric materials, and thus to undergo a substantial migration to the adjacent phases over time, especially in highly hot and/or humid conditions [23, 25, 26], the evaluation of their remaining concentration in the coating and substrate layers of the multilayer films, immediately after their manufacturing and after long-term storage in reels, was essential to assess their retention in the original carrier matrices. For this reason, all the concentrations involved were measured at the mentioned moments by thermal desorption and GC analysis, and subsequently introduced in the previous equations (1) and (2), to finally estimate the percentage efficiency of the incorporation process for the active agent, $E_i^f$, and its partition coefficient between the film layers, $K_i^{C/S}$. Since both parameters quantify the tendency of the embedded compounds to transfer over time to other system phases, i.e. air or substrates, respectively, they are considered good indicators of their
The results for the incorporation efficiencies of the active agents in the coating layers of the multilayer films, together with their corresponding initial and nominal concentrations, are collected in Table 2, whereas the values estimated for the partition coefficients are detailed in Table 3. As Table 2 shows, the efficiencies of the incorporation procedure range, in general terms, between 40 and 60% for all the materials and compounds essayed. These values mean important losses of activity during the manufacture of the films, probably produced by evaporation during the processes of preparation and application of the coating solutions. This hypothesis can also be reinforced by the fact that all the reported efficiencies show quite high correlation with the volatility of the embedded compounds. In fact, citral, the most volatile substance among the active agents studied in this work, yielded very low efficiencies in both PP and PET-based films, on which grounds it was incorporated with the highest nominal concentration. However, and as can be seen in this table 2, the performance of a matrix modification in the EVOH coating, by embedding a 2% of bentonite nanoclay in its structure, resulted in a great enhancement of its retention capacity, with an increase of about 22% with respect to the original value.

Finally, the partition coefficients of the active agents between the coating and substrate layers, as Table 3 shows, are all noticeably higher than the unity, up to three orders of magnitude in some case, and are thus in agreement with the value reported in a previous work for the partition of carvacrol between EVOH and PP, which, at room temperature and dry conditions (23 ºC and 0% RH), was of about 10000 [23]. These results mean that all the investigated compounds, that is, essential oils and their constituents, are far more soluble in EVOH than in PP or PET matrices, which is very advantageous for the retention of the active agents in the coating layers. However, considering that the substrate layers are about 26 times thicker than their corresponding coatings in all cases, the amount of substance finally transferred to the former material after 6 months of
storage can become significant in some. In this context, citral is worth mentioning, since it shows extreme behaviors depending upon the substrate employed. Indeed, it undergoes an intense migration towards the PP layer but its lower affinity to the PET matrix makes it attain a high retention in the EVOH coating, which must be taken into account when it comes to employing these materials in the construction of active packages. In order to better illustrate such behaviors, the expected concentrations at equilibrium of the active agents in the coating, $C_i^C$, and substrate layers, $C_i^S$, of the multilayer films, assuming a value of 100% for the incorporation efficiency, have been estimated from the reported values of the nominal concentrations and the partition coefficients by introducing them in the previous equations (2) and (3). The results obtained are also displayed in Table 3, and, as shown, in those films presenting lower values of the partition coefficients the greater thickness of the substrate layers with respect to their coatings can lead to the virtual exhaustion of the active agents in the latter materials.

3.3. ASSESSMENT OF THE SURFACE PROPERTIES OF THE MULTILAYER FILMS

Since all the multilayer films developed in this work were intended to be used in the construction of active packages for minimally processed vegetables, good optical characteristics, such as high gloss or transparency, were a strong requirement for them. However, the high water content of this foodstuff could saturate their headspace atmosphere with humidity, and subsequently promote its condensation on their inner surfaces, giving them a foggy appearance and thus a limited transparency. The formation of small water droplets on the packaging films, responsible for the light scattering phenomena, is a function of their surface properties, and, in particular, of their wettability. Hence, this property was investigated on the manufactured materials by measuring static contact angles of water droplets on their surface through the sessile drop
method with the aid of a goniometer, and the results obtained were plotted in Figure 1.

As this figure shows, contact angles of untreated PP and PET substrates are quite high, of about 98 and 72º respectively, which proves their hydrophobic nature in their original conditions, especially in the former material, owing to its lower polarity. Surprisingly, coatings of neat EVOH, a highly hydrophilic polymer, also show a high value for this parameter, around 99º, very similar to that reported for PP. This result means that, despite its hydrophilicity, EVOH shows a great hydrophobic behavior when its surface chains are not given enough time to interact with the water molecules. In any case, all three polymers seem to be naturally hydrophobic under those experimental conditions, which could pose some difficulties when it comes to fixing inks or to removing fogging in the final packages. However, when an active agent is embedded into the EVOH matrix, the contact angles drop dramatically, between 33 and 46% from the original value, reaching a minimum at about 53º for the marjoram oil. This phenomenon can be due to the presence of active molecules on the material surface, which, owing to their greater polarity, could substantially increase its interactions with water, and thus its wettability. In addition, the incorporation of only 2% of bentonite nanoclay in its structure is also capable, by itself, of reducing its contact angle by 37%, and, as a result, of reaching even lower values, up to 57º, when combined with carvacrol as active agent. In this case, the presence of numerous nanometric particles of bentonite, a highly hydrophilic material which can absorb several times its weight in water, in the EVOH surface could also multiply its interactions with the water molecules and, consequently, further enhance its wettability. This result is also in agreement with the findings in a previous study [33], where 2% of bentonite was reported to increase the hydrophilic behavior of EVOH by 17%.
3.4. ASSESSMENT OF THE OPTICAL PROPERTIES OF THE MULTILAYER FILMS

Just as explained in the last section, since the target packages of the materials studied in this paper were hermetic bags intended to preserve ready-to-eat food products, their optical properties had to be as good as possible in order to maintain the appearance of quality of the contained foodstuffs. However, given that all the developed films comprised active compounds or inorganic fillers of natural origin, they could become slightly opacified or colorized by them, thus losing their original aspect. For this reason, the optical properties of all the manufactured active and passive films were studied with a spectrophotometer, by measuring their color coordinates and subsequently displaying the acquired data in the CIELAB color space. The values found for their lightness were plotted in a bar graph in Figure 2, whereas the values obtained for their other color parameters were converted into chroma and hue parameters, through equations (4) and (5), and plotted in a polar graph in Figure 3.

With respect to lightness, both PP and PET substrates showed very high values, of about 97, owing to their high purity and homogeneity qualities. However, after being stored for six months in direct contact with active materials their lightness became slightly reduced, probably due to the absorption of active compounds migrating from their EVOH coatings. On the opposite, when both substrates were coated by neat EVOH their lightness increased significantly, by about a 1.5%, although when those coatings also included an active agent in their structure the value of this parameter decreased again, between 0.01 and 1.75%, depending upon the substance incorporated. From a macroscopic inspection of the manufactured films it could be stated that all the EVOH coatings showed a whitish appearance, which could contribute to the higher lightness observed in those materials, and this phenomenon could be due to the fact that, since a coating technology based on gravure printing was employed to apply the EVOH solutions on the substrate polymers, they were not casted as a single film but rather deposited as small individual droplets,
therefore yielding highly light scattering polymer coatings. However, when an active compound was embedded in those matrices lightness decreased again by absorption phenomena, just as occurred in the substrate materials. Finally, the effect of the incorporation of a 2% of bentonite nanoclay in the EVOH coatings was an increase of about a 0.2% in their lightness, probably due to the additional scattering phenomena introduced by the dispersed particles. In any case, it must be noticed that all the values reported in the graph range only between 96 and 99, so it can be concluded that the lightness of the studied materials can barely be modified by the presence of either active compounds or inorganic fillers.

Regarding the chroma and hue parameters, whereas PP substrates showed almost no coloration, with a chroma value near zero, PET substrates showed a substantial chroma, of about 0.7, with a slight reddish / yellowish tonality. In addition, when both polymers were analyzed after absorbing active compounds from their EVOH coatings, very opposite results were found, unlike for the lightness study. In detail, although both materials reduced their chroma in similar proportions, whereas PP practically conserved its hue, PET substrates moved sharply from a reddish to a greenish tonality, while maintaining their yellow intensity. Furthermore, when both substrates were coated by neat EVOH different behaviors were also observed, concretely, the chroma value was slightly reduced for PET while doubled for PP, although both materials shared a noticeable displacement in the green direction. This movement was further sharpened with the incorporation of active agents to the EVOH coatings, especially in the case of the PET-based films, which reached a deeper yellowish tonality while changing their redness to greenness, thus attaining a higher value for their chroma, between 0.9 and 1.5, depending on the compound embedded. Finally, the addition of a 2% of bentonite nanoclay to the EVOH matrices of PP-based films again doubled their chroma value, up to 0.45 approximately, also moving them on the way towards the greenish region. To summarize, it can be stated that in general terms, the coating of PP or PET substrates
with a neat EVOH layer does not significantly modify their color, but when natural substances, such as active compounds or inorganic fillers, are also present in the polymers, they will produce a considerable increase of the chroma and a substantial displacement to the greenish and yellowish regions in most cases, probably due to their characteristic and predominant colorations. However, and just as occurred for lightness, it must be remarked that no studied material presented chroma values greater than 1.5, which is quite a low amount. Hence, it can be concluded that all the changes observed in the coloration of the films will not be macroscopically perceptible to the naked eye, and thus will virtually not affect their optical properties in a significant way either.

3.5. ASSESSMENT OF THE MECHANICAL PROPERTIES OF THE MULTILAYER FILMS

Among the physical properties of relevance for food packaging applications, the mechanical ones are probably the most important since films must undergo numerous and different strains during their manufacturing, as well as during the filling, transportation, and commercialization of the final packages. In addition, the potential scalping of diverse organic substances from the preserved food products by the packaging materials, such as water or plant essential oils, can deteriorate their polymeric structure and thus reduce their mechanical performance. Because of this, some mechanical properties, namely the ultimate tensile strength, the deformation at break, and the Young’s modulus, were determined on the diverse developed active and passive films, by elongating them under increasing load forces in a universal testing machine, and by converting the obtained force – displacement curves into stress – strain curves through the application of equations (6) and (7). The values finally found for the three mentioned parameters are plotted in bar graphs in Figure 4, together with their corresponding subset tags, according to the grouping of means yielded by the post-hoc statistical test.
In relation to the ultimate tensile strength, as this figure depicts, PP substrates resisted a considerable level of stress, around 130 N/mm$^2$, whereas PET materials almost doubled this number, up to 230 N/mm$^2$, when tested in their original conditions. However, when coated with a layer of EVOH these values decreased significantly, by about 7%. Furthermore, if this material, in turn, contained an active compound, a further reduction of about 1.6 and 5%, respectively, was also observed in both types of films with respect to those comprising the neat polymer, although according to the ANOVA test, the differences found were not significant. This slight worsening of the tensile strength in both materials can be attributed to two different facts. Firstly, given that the EVOH coatings were not deposited on the substrates as single layers but as small individual droplets, as explained in the previous section 3.4, they would only increase the thickness of the final films, but could not provide any additional noticeable resistance to the load forces applied. Hence, and according to equation (6), the tensile stresses finally obtained would always be lower for them than for their corresponding substrates. Secondly, the scalping of organic substances from these coatings by their substrate materials, such as water or 1-propanol, could also have plasticized them, therefore reducing their ultimate tensile strength, but this phenomenon could even have been aggravated with the absorption of active compounds, since they are known to be good plasticizers of such polymers, and, in addition, their activity could also have promoted some reaction with them, thus somewhat degrading their molecular structure. To end, the presence of 2% of bentonite nanoparticles in the EVOH matrix produced a slight increase of the tensile strength of about 0.5% with respect to the unfilled film, which, despite being statistically insignificant, can be indicative of some improvement in its mechanical performance, in agreement with the results reported elsewhere for other clay nanocomposites.

Regarding the deformation at break, in this case both substrates reached high values, around 380% for PP and 300% for PET, which were also slightly lower for their EVOH-coated counterparts,
by about 0.2 and 0.7% respectively, although without statistical significance (p > 0.05). Equally, in
their active equivalents further reductions were also observed with respect to the passive films, up
to 3.9 and 12.8% respectively, which were significant only for the PET-based materials. These
results could also be explained by the scalping effect mentioned above, through which the studied
substrates would absorb diverse organic substances from their EVOH coatings, and, especially,
from the active ones, and consequently undergo some matrix deterioration, such as a weakening
or perhaps breaking of its molecular bonds, which, besides leading them to premature failure,
would also prevent them from reaching such large deformations. Concerning the modification of
the EVOH structure by the addition of bentonite, in this case no differences were found with
respect to the neat polymer.

The assessment of the Young’s modulus, in turn, yielded similar results to the other two
parameters, thus contributing to corroborating the hypotheses posed above. In the case here, PP
substrates showed a moderate value for this property, around 760 N/mm², whereas PET materials
could attain a far higher number, of about 1820 N/mm², owing to their greater stiffness. However,
the values reported for both polymers were significantly reduced when an EVOH coating was
applied, by 13.5 and 2.8% respectively, and further decreases were observed again with respect to
these new materials when active substances were also incorporated, up to 2% more, depending
upon the compound embedded. In addition, and just as for the ultimate tensile strength, the
presence of 2% of bentonite nanoclay increased the value of this parameter by approximately
0.75%, although this difference once more lacked statistical significance, as the previous results.

In summary, it can be stated that all the variations reported for the three studied parameters are
indicative that, in effect, some scalping phenomenon is occurring in the developed multilayer
films, especially in the active ones, which, by either plasticization or reaction mechanisms, could
be responsible for the softening or degradation of the structure of their substrates, and thus of the
lower values found for their ultimate tensile strength, deformation at break, and Young’s modulus. This behavior can be partially counteracted by the presence of bentonite nanoparticles in the coating matrix, although, according to the values detailed, only to a low extent. In any case, it must be remarked that none of the properties assessed in the manufactured films was observed to vary by more than 15% with respect to their corresponding substrates, therefore it can be concluded that their mechanical performance will not become substantially deteriorated in the final packages.

3.6. ASSESSMENT OF THE BARRIER PROPERTIES OF THE MULTILAYER FILMS

Given that the food packages aimed in this work were sealed bags for the preservation of fresh-cut salad or similar products, as mentioned above, all the developed multilayer films had to allow an adequate gas exchange between their inner and outer atmospheres as to properly maintain the quality and sensory characteristics of the contained foodstuffs. However, the simple application of a thin coating of EVOH, a high-barrier polymer, on the original PP films, for example, could lead to a substantial decrease of the gas transmission rate through these packaging materials. Consequently, it could put the preserved food products at risk of developing strange flavors or smells throughout their shelf life, by effect of the excessive accumulation of carbon dioxide in the package headspace, and thus at risk of becoming inedible. For this reason, the barrier properties of all the manufactured passive films and substrates were studied by measuring their oxygen and carbon dioxide permeances as a function of the relative humidity, in both isostatic permeation instruments, yielding as a result the values collected in Table 4.

As this table shows, for the substrate polymers no distinction is made between the diverse relative humidities assayed in the permeation tests, because, although five or six different conditions were
considered, depending upon the compound studied, no relevant differences were found in the gas
transmission rates, according to the well-known hydrophobic behavior of those materials. With
respect to the values displayed, the oxygen permeance was quite high in both polymers, especially
in the PP film, although the transport of carbon dioxide yielded even higher figures for this
parameter, about 10 times more on average for both substrates. However, when a thin coating of
EVOH was applied on those materials, their permeance dropped dramatically, being able to reach,
in dry conditions, about a 1/160 of its original value for the PP-based films, and about a 1/6 for the
PET ones, as expected from the high barrier characteristics of such a polymer. Nevertheless, owing
to its highly hydrophilic nature, as reported elsewhere [27 – 29], this barrier effect gradually
dissipated as the relative humidity increased, undergoing a sharper change at 75% RH, probably
due to the proximity of the glass transition point [33], and attaining permeance values at 100% RH
somewhat comparable to those reported for the uncoated substrates, mostly between 70 and
80% of the original values. Finally, the incorporation of 2% of bentonite nanoclay to the EVOH
coatings produced a further decrease of the films’ permeances to both gases when they were
assayed at moderate relative humidities, up to 76% with respect to the unfilled materials, whereas
at extreme ambient conditions the differences found did not exceed a 5%. As a conclusion, it could
be asserted that, given the highly humid conditions expected in the package headspace during the
product preservation time, the presence of a thin coating layer of EVOH or bentonite-filled EVOH
on the packaging materials will not affect their gas transport in a substantial way, and thus will
neither modify the respiration rate of the contained foodstuffs so much as to significantly
deteriorate their quality or sensory characteristics.

3.7. ASSESSMENT OF THE PERFORMANCE OF THE ACTIVE PACKAGES

In previous works, food packages consisting of EVOH-coated PP films containing essential oils or
their constituents as active antimicrobial agents were successfully developed and tested for the
preservation of fresh-cut salad and fish products. However, the assays carried out in both systems
revealed an unconstrained free release of the active compounds to the headspace of the
packages, which induced an early peak of antimicrobial activity at the beginning of the product
shelf-life, followed by a rapid decay in a few days. For this reason, a matrix modification based on
the incorporation of bentonite nanoclay was assessed on EVOH active films in a subsequent study
[33], yielding substantial improvements in the retention capacity and rate of release of the active
agents. Hence, following this line, the effects of such modification on the performance of active
packages, assayed in actual working conditions, were investigated in the present work, by
determining their activity over time with a gas chromatograph, and by simulating it through the
application of a mathematical model. Both experimental and theoretical results are displayed in

Figure 5, where the evolution in time of the concentration of carvacrol in the headspace of the
packages has been plotted for both original and nanocomposite active films.

As can be seen in the graph, both theoretical curves describe the experimental behavior of the
assayed packages very accurately, meaning that both the test procedure carried out and the
mathematical model applied, were perfectly valid for the systems and conditions studied in this
work. On another matter, the evolutions in time of the carvacrol concentration described by both
curves are analogous to those reported in a previous work for a similar package [23], since they
exhibit a maximum value immediately after the beginning of the storage period, followed by a
gradual and continuous decline for the remaining days. In this case, neither the initial overshoot
nor the subsequent drop of antimicrobial activity are as pronounced as in the previous study,
probably owing to the lower relative humidity existing in the storage room (30 % vs. 70 %),
although the performance of the active packages still diminishes quite rapidly with the course of
the preservation time, in agreement with what was observed in the mentioned work.
Nevertheless, the addition of only 2% of bentonite nanoparticles into the EVOH active layer noticeably improved both the release rate of the embedded compound and the retention capacity of the carrier matrix. In effect, as Figure 5 shows, the peak concentration of carvacrol in the headspace of the packages, which mainly depends upon the amount of substance finally retained in their active layer, is about 21% higher in those containing the nanocomposite material than in the original ones. In addition, since this difference is maintained or even increased during the preservation time, up to 39% at the end of the storage period, it means that the release rate of the active compound must also be slower in those systems. This effect can be visualized better in the inset graph, where the theoretical curves for the evolution in time of the carvacrol concentration in the package atmosphere have been normalized to their maximum values, in order to become comparable from a kinetic point of view. As a result, an average reduction of about 23% in the slope of the nanocomposite curve is finally observed, demonstrating that the incorporation of bentonite nanoclay to the carrier matrix not only increases antimicrobial activity in the package headspace, by reducing its losses to the external atmosphere, but also contributes to maintaining it more constant and stable throughout the product’s shelf life.
4. CONCLUSIONS

In this work, diverse passive, active, and nanocomposite multilayer films, selected from the materials developed in previous studies, were subjected to several physicochemical tests in order to demonstrate their suitability for food packaging applications. In detail, the properties investigated were: the stability of EVOH coatings on PP and PET substrates pretreated with different anchorage technologies; the mechanical, optical, surface, and barrier properties of active multilayer films, consisting of EVOH coatings on PP and PET substrates containing essential oils or their constituents as natural antimicrobials, as well as the retention of these compounds in their carrier matrices; and lastly, the effects of a matrix modification, based on the addition of a 2% of bentonite nanoclay into an EVOH active layer, on the performance of PP / EVOH packages containing carvacrol as active agent, and assayed in actual working conditions.

The results obtained indicated the application of corona discharge followed by a PEI-based primer as the best anchorage technology available to bond EVOH coatings with PP or PET substrates. With respect to the active agents incorporated, their retention into EVOH matrices ranged from low to moderate, depending upon the substance included and upon the substrate employed. Nevertheless, the final manufactured films showed a substantial improvement in their wettability characteristics, and no noticeable or important differences in their mechanical, optical, or barrier properties. The inclusion of bentonite nanoparticles into EVOH active coatings, in turn, considerably enhanced the performance of the preservation system, by increasing its retention capacity for the embedded compounds, while reducing their release rate to the package headspace. In addition, the surface and mechanical properties of these nanocomposite materials were also slightly improved, whereas their optical appearance stayed practically the same.

In conclusion, all the developed active films are perfectly suitable for food packaging applications, and the incorporation of bentonite nanoclay to their carrier layers is strongly recommended, as to
improve their physical properties as much as to increase the performance of the targeted packages, although some additional assays might be then necessary to confirm that the current legal thresholds for the migration of inorganic nanoparticles were never exceeded.

ACKNOWLEDGMENTS

The authors thank the Spanish Ministry of Science and Innovation (projects AGL2009-08776 and AGL2012-39920-C03-01), European Comission (Nafispack project 212544), and Generalitat Valenciana (J.P.C. fellowship) for financial support, ITENE (Associated Unit of CSIC) for scientific collaboration and Mr. Tim Swillens for correction services.

REFERENCES


[38] Pieper, G., Petersén, K. Free fatty acids from orange juice absorption into laminated cartons and their effects on adhesion. *Food Science*, 1995, 60 (5), 1088 – 1091.
Table 1. Qualitative results of the adhesion and thermosealability tests for the four anchorage technologies applied on the multilayer films, and sealing strength on uncoated substrates and on those coated with EVOH through the application of corona discharge + PEI-based primer

<table>
<thead>
<tr>
<th>Anchorage technology</th>
<th>Adhesion</th>
<th>Thermosealability</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV radiation</td>
<td>weak</td>
<td>none</td>
</tr>
<tr>
<td>Corona discharge</td>
<td>moderate</td>
<td>poor</td>
</tr>
<tr>
<td>Corona discharge + PU-based primer</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>Corona discharge + PEI-based primer</td>
<td>strong</td>
<td>good</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sealed films</th>
<th>Sealing strength (N)</th>
<th>Type of failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>PET // PET</td>
<td>5.7 ± 0.9</td>
<td>Seal opening</td>
</tr>
<tr>
<td>PET / E // E / PET</td>
<td>12.1 ± 2.4</td>
<td>Seal opening + delamination</td>
</tr>
<tr>
<td>PP // PP</td>
<td>8.0 ± 1.0</td>
<td>Seal opening</td>
</tr>
<tr>
<td>PP / E // E / PP</td>
<td>21.6 ± 3.0</td>
<td>Seal opening</td>
</tr>
</tbody>
</table>
Table 2. Initial and nominal concentrations of the active agents in the coating layers of the multilayer films, and efficiencies of the incorporation process

<table>
<thead>
<tr>
<th>Active multilayer film</th>
<th>$C_{i0}$ (%)</th>
<th>$C_{iN}$ (%)</th>
<th>$E_i$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP / E + CI</td>
<td>4.3 ± 0.4</td>
<td>10</td>
<td>43 ± 4</td>
</tr>
<tr>
<td>PP / E + MO</td>
<td>4.6 ± 0.2</td>
<td>7.5</td>
<td>62 ± 3</td>
</tr>
<tr>
<td>PP / E + CA</td>
<td>3.8 ± 0.1</td>
<td>7.5</td>
<td>50 ± 1</td>
</tr>
<tr>
<td>PP / E + B + CA</td>
<td>4.6 ± 0.2</td>
<td>7.5</td>
<td>61 ± 3</td>
</tr>
<tr>
<td>PET / E + CI</td>
<td>4.6 ± 0.3</td>
<td>10</td>
<td>46 ± 3</td>
</tr>
<tr>
<td>PET / E + CO</td>
<td>3.0 ± 0.6</td>
<td>7.5</td>
<td>40 ± 8</td>
</tr>
</tbody>
</table>
Table 3. Equilibrium concentrations of the active agents in the coating and substrate layers of the multilayer films, as derived from the mass balance depicted in equation (3), and their partition coefficients between both materials.

<table>
<thead>
<tr>
<th>Active multilayer film</th>
<th>$K_{C/S}$ ($%$)</th>
<th>$C_{i_{\alpha}}^C$ (%)</th>
<th>$C_{i_{\alpha}}^S$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP / E + CI</td>
<td>1.5 ± 2.1</td>
<td>0.71</td>
<td>0.47</td>
</tr>
<tr>
<td>PP / E + MO</td>
<td>42.4 ± 2.9</td>
<td>5.10</td>
<td>0.12</td>
</tr>
<tr>
<td>PET / E + CI</td>
<td><strong>1260 ± 240</strong></td>
<td>9.76</td>
<td>0.01</td>
</tr>
<tr>
<td>PET / E + CO</td>
<td>6.0 ± 5.6</td>
<td>1.22</td>
<td>0.20</td>
</tr>
</tbody>
</table>
Table 4. Oxygen and carbon dioxide permeances in the passive multilayer films and their substrates as a function of the relative humidity

<table>
<thead>
<tr>
<th>Films</th>
<th>$\Phi_{\text{O}_2} \cdot 10^{15}$ (m$^3$ (STP) / (m$^2$ · s · Pa))</th>
<th>% RH</th>
<th>35% RH</th>
<th>50% RH</th>
<th>75% RH</th>
<th>90% RH</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP</td>
<td>148 ± 2</td>
<td>50% RH</td>
<td>3.30 ± 0.13</td>
<td>34.8 ± 0.2</td>
<td>112 ± 2</td>
<td></td>
</tr>
<tr>
<td>PP / E</td>
<td>0.85 ± 0.03</td>
<td>35% RH</td>
<td>1.49 ± 0.08</td>
<td>4.8 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP / E + B</td>
<td>0.86 ± 0.05</td>
<td>50% RH</td>
<td>0.76 ± 0.06</td>
<td>12.5 ± 0.1</td>
<td>111 ± 1</td>
<td></td>
</tr>
<tr>
<td>PET</td>
<td>4.8 ± 0.2</td>
<td>75% RH</td>
<td>0.79 ± 0.08</td>
<td>3.30 ± 0.13</td>
<td>111 ± 1</td>
<td></td>
</tr>
<tr>
<td>PET / E</td>
<td>0.76 ± 0.07</td>
<td>90% RH</td>
<td>1.20 ± 0.09</td>
<td>2.98 ± 0.06</td>
<td>3.83 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Films</td>
<td>$\Phi_{\text{CO}_2} \cdot 10^{15}$ (m$^3$ (STP) / (m$^2$ · s · Pa))</td>
<td>% RH</td>
<td>30% RH</td>
<td>45% RH</td>
<td>75% RH</td>
<td>85% RH</td>
</tr>
<tr>
<td>PP</td>
<td>1170 ± 40</td>
<td>50% RH</td>
<td>48.8 ± 0.5</td>
<td>146 ± 2</td>
<td>247 ± 2</td>
<td></td>
</tr>
<tr>
<td>PP / E</td>
<td>7.8 ± 0.2</td>
<td>30% RH</td>
<td>9.83 ± 0.08</td>
<td>11.4 ± 0.1</td>
<td>48.8 ± 0.5</td>
<td>146 ± 2</td>
</tr>
<tr>
<td>PP / E + B</td>
<td>7.61 ± 0.19</td>
<td>45% RH</td>
<td>11.0 ± 0.3</td>
<td>30.5 ± 1.0</td>
<td>129 ± 2</td>
<td>234 ± 15</td>
</tr>
<tr>
<td>PET</td>
<td>56.3 ± 0.8</td>
<td>75% RH</td>
<td>11.0 ± 0.3</td>
<td>30.5 ± 1.0</td>
<td>129 ± 2</td>
<td>234 ± 15</td>
</tr>
<tr>
<td>PET / E</td>
<td>9.36 ± 0.18</td>
<td>85% RH</td>
<td>11.2 ± 0.2</td>
<td>30.7 ± 0.4</td>
<td>35.2 ± 0.5</td>
<td>39.4 ± 0.3</td>
</tr>
</tbody>
</table>
FIGURE CAPTIONS

Figure 1. Static contact angles of water droplets on the surfaces of the multilayer films.

Figure 2. Lightness of the multilayer films on the CIELAB color space.

Figure 3. Chroma and hue of the multilayer films on the CIELAB color space.

Figure 4. Ultimate tensile strength, deformation at break, and Young’s modulus of the multilayer films. Letters correspond to homogeneous subsets of means with significant differences.

Figure 5. Evolution in time of the carvacrol concentration in the headspace of packages containing a neat or nanocomposite EVOH active layer. Symbols correspond to experimental values and curves correspond to theoretical performances predicted by the mathematical model. Inset graph: normalized curves of the theoretical performances of the active packages in full time scale.
The graph illustrates the concentration of a substance (CA) over time (t) for two different samples: PP/E + CA and PP/E + B + CA. The concentration is measured in kg/m³. The data points are accompanied by error bars indicating variability. The samples show a decreasing trend in concentration over time, with the PP/E + CA sample having a slightly higher initial concentration compared to the PP/E + B + CA sample. The inset graph provides a detailed view of the concentration ratio (C/Cₘₐₓ) over time, showing the exponential decay characteristic of the diffusion process.