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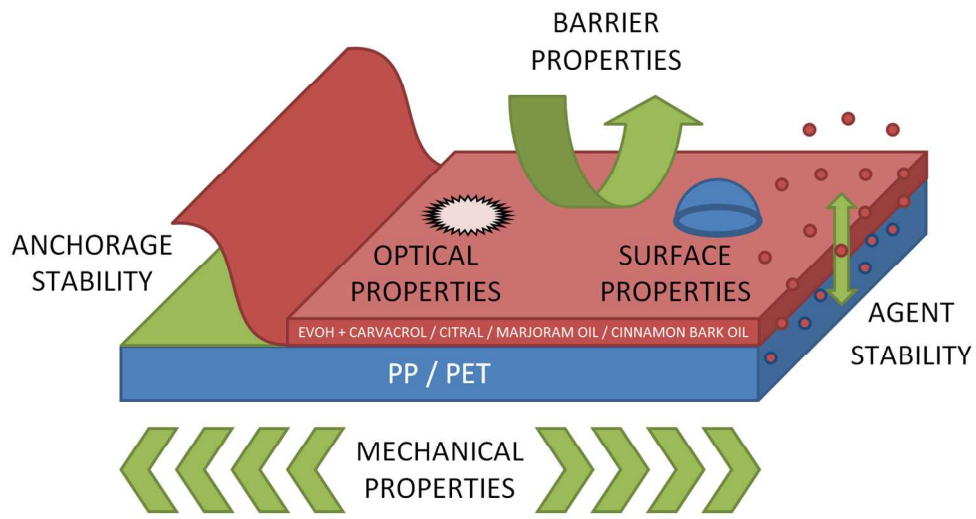


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2 1 Natural antimicrobial – containing EVOH coatings on PP and PET films: functional and active  
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4 2 properties characterization  
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**ABSTRACT**

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

1  
2 60 nature can strongly modify its barrier characteristics with the absorption of water vapor [27 – 29]  
3  
4 61 thus allowing for a high retention or protection of the embedded compounds in dry conditions  
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6 62 while triggering their activity when exposed to the humid environment generated by a packaged  
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8  
9 63 food product [25, 30]. The composite materials obtained were used as active layers in multilayer  
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11 64 packaging films, which were employed in the construction of active packages for fresh products  
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13 65 and studied by means of mathematical models. Results showed good chemical compatibility  
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15 66 between the active agents and the packaging materials, a gradual release of the embedded  
16  
17 67 compounds into the package headspace as a function of the water activity, and a significant  
18  
19 68 inhibition of numerous spoiling bacteria and microflora at the beginning of the storage period. In  
20  
21 69 addition, some EVOH matrix modifications with  $\beta$ -cyclodextrin [31, 32] or bentonite nanoclay [33,  
22  
23 70 34] were evaluated in order to increase the retention capacity or to reduce the active agents'  
24  
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28 71 release rate.

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30  
31 72 In this work, some selected films from the active materials developed in previous studies [23, 25,  
32  
33 73 26, 33] were subjected to several analyses with the aim of assessing their suitability for food  
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35 74 packaging applications. Firstly, the stability of EVOH coatings on pretreated polypropylene (PP)  
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37 75 and polyethylene terephthalate (PET) substrates was investigated by evaluating the results yielded  
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39 76 by four different anchorage technologies, namely UV radiation, corona treatment, and corona  
40  
41 77 treatment followed by the application of primers based on polyurethane (PU) and  
42  
43 78 polyethyleneimine (PEI), in essays of adhesion and thermosealability. Secondly, active multilayer  
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45 79 films consisting of EVOH coatings containing natural antimicrobials on PP and PET substrates were  
46  
47 80 manufactured and subsequently analyzed, together with their passive counterparts, for the  
48  
49 81 determination of several physical properties of relevance for food packaging applications, as well  
50  
51 82 as for the retention of the active agents in the coating matrices. In particular, films were assayed  
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53 83 for mechanical, optical, surface, and barrier properties, as well as for the partition coefficients of  
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1  
2 84 the active agents between the material layers, and for the efficiency of their incorporation. The  
3  
4 85 embedded compounds were carvacrol (main component of *Origanum vulgare* essential oil, b.p.:  
5  
6 86 236 °C), citral (main component of *Litsea cubeba* essential oil, b.p.: 229 °C), and marjoram  
7  
8 87 essential oil (extracted from *Thymus mastichina*, b.p.: 168 °C) in PP films, and citral and cinnamon  
9  
10 88 bark essential oil (extracted from *Cinnamomum zeylanicum*, b.p.: 249 °C) in PET films. The  
11  
12 89 improvements expected from an EVOH matrix modification on the retention capacity and on the  
13  
14 90 rate of release of the active agents were assessed by manufacturing two different active  
15  
16 91 multilayer films consisting of PP substrates coated by either neat EVOH or EVOH and bentonite  
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18 92 nanocomposites, both containing carvacrol as antimicrobial agent, and employing them in the  
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20 93 construction of active packages intended to preserve fresh-cut salad products, and by comparing  
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22 94 their performance in actual working conditions.  
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## 32 96 2. MATERIALS AND METHODS

### 33 34 35 97 2.1. MATERIALS

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37  
38 98 Ethylene-vinyl alcohol copolymer with a 29% ethylene molar content (EVOH-29) was kindly  
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40 99 supplied by The Nippon Synthetic Chemical Industry Co., Ltd. (Osaka, Japan). Food-contact grade  
41  
42 100 bi-oriented polypropylene (PP) and polyethylene terephthalate (PET) films 30 µm thick were kindly  
43  
44 101 supplied by Envaflex S.A. (Utebo, Spain).

45  
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47  
48 102 H-760-A primer, consisting of an aqueous solution of 12% polyethyleneimine (PEI), was purchased  
49  
50 103 from MICA Corp. (Shelton, CT, USA), and NAF-1 primer, consisting of an ethyl acetate solution of  
51  
52 104 43% polyurethane (PU), was kindly provided by Artibal S.A. (Sabiñánigo, Spain).

53  
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55  
56 105 Reagent-grade phosphorus pentoxide and sodium bentonite nanoclay were purchased from  
57  
58 106 Sigma-Aldrich Co. LLC. (St. Louis, MO, USA), as well as several food-grade essential oils and their  
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1  
2 107 constituents, concretely, carvacrol of at least 98% purity, citral of at least 96% purity, marjoram  
3  
4 108 essential oil, and cinnamon bark essential oil. Reagent-grade 1-propanol and high-vacuum silicone  
5  
6 109 were supplied by Panreac Química S.L.U. (Barcelona, Spain), and deionized water was obtained  
7  
8  
9 110 from a Milli-Q Plus purification system of EMD Millipore Corp. (Billerica, MA, USA).

111

## 112 2.2. PREPARATION OF THE ACTIVE MULTILAYER FILMS. CONSTRUCTION OF THE PACKAGES

113 All the multilayer films assayed in this work, active as well as passive, were manufactured by  
114 coating PP or PET substrates, previously treated with an anchorage technology, with  
115 hydroalcoholic solutions of EVOH-29. These solutions contained, depending on the case, an  
116 essential oil, such as marjoram or cinnamon bark oil, or one of its constituents, such as carvacrol  
117 or citral, as active antimicrobial agent, and / or an inorganic filling material, namely sodium  
118 bentonite nanoclay, as polymer matrix modifier. The preparation of EVOH-29 solutions with one or  
119 both incorporated additives is described in detail in previous works: [23, 33], respectively. In brief,  
120 active solutions with the neat polymer were prepared by dissolving a given amount of EVOH-29  
121 pellets in a 1:1 (w/w) mixture of deionized water and 1-propanol, at a concentration of 15 %  
122 (w/w), into a glass flask placed on a RTC basic magnetic stirrer with heater plate (IKA-Werke GmbH  
123 & Co. KG, Staufen, Germany), coupled to a constant reflux vapor system. Once the polymer was  
124 fully dissolved the antimicrobial compound was added to the mixture at a concentration of either  
125 7.5 or 10 g / 100 g EVOH-29, depending on the substance incorporated, and was let then  
126 homogenizing for at least 10 minutes more. Regarding the active solutions with EVOH-29  
127 nanocomposite, their preparation was analogous to the previous ones with just substituting  
128 deionized water by a hydrocolloidal dispersion of bentonite nanoparticles. Such dispersion was  
129 obtained by thoroughly mixing sodium bentonite in deionized water, at a concentration of 0.6 %  
130 (w/w), with the aid of an Ultra-Turrax T25 basic disperser (IKA-Werke GmbH & Co. KG, Staufen,



1  
2 131 Germany) and of an Ultrasons ultrasonic bath (J. P. Selecta S. A., Barcelona, Spain). Specifically, the  
3  
4 132 initial mixture was firstly stirred for 30 s at 24000 rpm, afterwards sonicated for 1h, left then  
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6 133 resting overnight, and finally sonicated again for at least 15 minutes more to obtain a stable  
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8  
9 134 colloidal suspension.

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11  
12 135 In the case of the films tested for coating stability, several sheets of PP and PET were subjected to  
13  
14 136 four different anchorage treatments, namely UV irradiation, corona discharge, and corona  
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17 137 discharge followed by the application of PU and PEI-based primers, prior to being coated by a  
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19 138 solution of neat EVOH-29. Sheets subjected to UV irradiation were placed under a VUV Xe excimer  
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21 139 lamp with 6 W at 172 nm (UV-Consulting Peschl España S.L., València, Spain) for 1 min in an air  
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23 140 atmosphere, whereas sheets subjected to corona discharge were passed through the plasma  
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26 141 region generated by the electrode of a BD-20AC high frequency corona surface treater three times  
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28 142 (Electro-Technic Products, Inc., Chicago, IL, USA). Both technologies were able to create ozone  
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30 143 molecules from air ionization, which could react with surface polymeric chains and break some of  
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32  
33 144 their covalent bonds. Hence, they were aimed at reducing the surface tension of the polymers,  
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35 145 and thus at improving their wettability with the primers or their adhesiveness with the coatings.  
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38 146 Primers, in turn, were casted on the polymer sheets with the aid of an Elcometer 4340 automatic  
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40 147 coating applicator (Elcometer Ltd., Manchester, UK), equipped with a Mayer rod of 10 µm deep  
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42 148 thread, and programmed to carry out a gradual deposition of the solutions at a temperature of 60  
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44  
45 149 °C and an application velocity of 8 cm/s. The same instrument and conditions were also used to  
46  
47 150 apply the neat EVOH-29 solution on the four pretreated PP and PET sheets to yield the final  
48  
49 151 multilayer films. After manufacturing, the deposited layers of primer and EVOH-29 were found to  
50  
51  
52 152 be homogeneous and transparent, and their thickness could be estimated by measuring both  
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54 153 coated and uncoated areas of the substrate sheets with an ABSOLUTE Digimatic Indicator ID-C  
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57 154 Series 543 digital micrometer (Mitutoyo America Corp., Aurora, IL, USA) and subtracting the  
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2 155 results obtained, yielding values of about 1 and 1.5  $\mu\text{m}$ , respectively.  
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5 156 With respect to the active films tested for various physicochemical properties, a coating  
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7 157 technology based on gravure printing was used at the facilities of Envaflex S.A. (Utebo, Spain). In  
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9 158 this case, the coating system was fed with reels of PP and PET films, which were pretreated with  
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11 159 corona discharge and subsequently coated with a PEI-based primer, followed by a layer of the  
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13 160 corresponding polymer solution. Production speed was about 60 m/min, and solvent evaporation  
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15 161 was rapidly performed in drying tunnels, fed by hot air at 40  $^{\circ}\text{C}$ , after the deposition of each layer.  
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17 162 The diverse active multilayer films finally produced were: PP / PEI / EVOH + 10% (w/w) citral (PP /  
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19 163 E + CI), PP / PEI / EVOH + 7.5% (w/w) marjoram essential oil (PP / E + MO), PET / PEI / EVOH + 10%  
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21 164 (w/w) citral (PET / E + CI), and PET / PEI / EVOH + 7.5% (w/w) cinnamon bark essential oil (PET / E +  
22  
23 165 CO). In addition, two passive multilayer films were also obtained to be used as controls in the tests  
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25 166 carried out: PP / PEI / EVOH (PP / E), and PET / PEI / EVOH (PET / E). In this case, the coated areas  
26  
27 167 of the manufactured films presented a homogeneous, translucent, and matte appearance, similar  
28  
29 168 to the materials previously produced at laboratory scale, and the thickness of PEI and EVOH layers  
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31 169 was estimated at about 0.8 and 1.15  $\mu\text{m}$ , respectively. In order to avoid potential activity losses all  
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33 170 the films were rolled in reels, packaged in hermetic aluminum-coated polyethylene bags, and  
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35 171 stored until utilization in thermostatic chambers at  $4 \pm 1$   $^{\circ}\text{C}$  of temperature.  
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43 172 Finally, in relation with the films tested for the effects of the matrix modification on the package  
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45 173 performance, as well as for some other physicochemical properties, several sheets of PP were  
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47 174 pretreated with corona discharge, and subsequently coated with three different EVOH solutions  
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49 175 by using the aforementioned automatic coating applicator, equipped and programmed as  
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51 176 previously described. In these conditions, one passive and two active multilayer films could be  
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53 177 obtained: PP / EVOH + 2% (w/w) sodium bentonite nanoclay (PP / E + B), PP / EVOH + 7.5% (w/w)  
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55 178 carvacrol (PP / E + CA), and PP / EVOH + 2% (w/w) sodium bentonite nanoclay + 7.5% (w/w)  
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2 179 carvacrol (PP / E + B + CA), presenting homogeneous and transparent coating layers of  
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4 180 approximately 2  $\mu\text{m}$  thick. These two latter materials were employed in the construction of active  
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6 181 packages intended to preserve fresh-cut salad products, by following the procedure described in a  
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8 182 previous work [23]. In brief, the active films were cut into pieces measuring 25 x 30 cm, and closed  
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10 183 in the form of bags with the aid of a 420 SBM impulse heat sealer (Audion Elektro B.V., Weesp,  
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12 184 The Netherlands). Prior to applying the final seal, bags were filled with five small cylindrical pieces  
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14 185 of gelified water of 10 mL volume, in order to generate and maintain inner relative humidity  
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16 186 constant and close to 100%, and thus to simulate the presence of a high water activity food  
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18 187 product. Once bags were hermetically closed, a septum was adhered to their outer face, and 1 L of  
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20 188 air at atmospheric pressure was injected into them with the aid of a syringe.  
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### 29 190 2.3. DETERMINATION OF THE STABILITY OF EVOH COATINGS ON PRETREATED PP AND PET 30 31 191 SUBSTRATES: ADHESION AND THERMOSEALABILITY TESTS 32 33

34  
35 192 The adhesiveness of EVOH coatings to PP and PET substrates subjected to different treatments for  
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37 193 surface modification was evaluated by means of the well-known Scotch tape test, a qualitative test  
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39 194 method based on the ASTM D3359 – 09e2 standard [35]. In detail, two identical pieces of Scotch  
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41 195 Magic adhesive tape (3M Corp., St. Paul, MN, USA), of 19 mm wide and 100 mm long, were firmly  
42  
43 196 applied to the front and back surfaces of the films in both transversal and machine directions, left  
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45 197 for about 20 s at room conditions (about 23  $^{\circ}\text{C}$  of temperature and 50% of relative humidity), and  
46  
47 198 stripped off in a 180 $^{\circ}$  angle with one quick peeling. In order to ensure the maximum strength for  
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49 199 the anchorages, and thus the optimal testing conditions for the films, essays were performed 24 h  
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51 200 after manufacturing, and, for each assayed sample, at least five parallel tests were carried out.  
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57 201 The thermosealability of the multilayer films is closely related to the sealing capabilities of their  
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2 202 material layers, just as to the force of adhesion between them. Because of that, several  
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4 203 thermosealing tests were performed in parallel with the previous adhesion tests in order to assess  
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6 204 the anchorage strength of the EVOH coatings to the diverse pretreated PP and PET substrates  
7  
8 205 more thoroughly. With this objective, several sheets of each manufactured material were placed  
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10 206 in pairs between the jaws of the impulse heat sealer described above, to be sealed by their coated  
11  
12 207 face. Immediately afterwards, the timer of the instrument was set to 10, and its jaws were closed  
13  
14 208 to provide a long, continuous, and homogeneous seal. Once sealing area was cooled, films were  
15  
16 209 removed from the instrument and visually examined for faults or imperfections. If no sealing  
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18 210 defects were observed, films were pulled apart by applying increasing tensile forces on the seal  
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20 211 until causing its detachment or failure. This way, the strength of the seal, and thus the  
21  
22 212 thermosealing characteristics of the assayed material, could be qualitatively evaluated by  
23  
24 213 estimating the magnitude of the tension forces required to break the union between the films,  
25  
26 214 and by the visual inspection of the ripped zones. Furthermore, films which provided the best  
27  
28 215 sealability results were additionally subjected to peeling tests, together with their corresponding  
29  
30 216 substrates, in order to quantitatively determine their sealing strength and to compare it with the  
31  
32 217 values yielded by the original uncoated materials. These essays were performed by following a  
33  
34 218 test procedure based on the ASTM D882 – 12 standard method [36] with the aid of a MultiTest 1-i  
35  
36 219 universal testing machine (Mecmesin Ltd., Slinfold, UK), equipped and programmed as described  
37  
38 220 below in section 2.7. In detail, the sealed films were cut into 1 in. wide strips, their ends were held  
39  
40 221 under 3 bars of pressure with two pneumatic plane grips, and an increasing load was applied on  
41  
42 222 their seal over time until causing its failure. The load force recorded at the breaking point was then  
43  
44 223 identified as the sealing strength of the assayed sample. Just as for the adhesion tests, both  
45  
46 224 sealing and peeling assays were performed at room conditions the day after film manufacturing,  
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48 225 and repeated at least five times for each material sample.  
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2 226 2.4. DETERMINATION OF THE RETENTION OF ACTIVE AGENTS IN THE COATING LAYERS OF  
3  
4 227 MULTILAYER FILMS: INCORPORATION EFFICIENCIES AND PARTITION COEFFICIENTS  
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7 228 Given that all the active agents incorporated in the multilayer films were volatile compounds, their  
8  
9 229 evaporation losses during the processes of preparation and application of the coating solutions,  
10  
11 230 and thus the retention capabilities of the polymer matrices forming the coating layers, were  
12  
13 231 investigated by measuring the concentrations of these substances remaining in the final active  
14  
15 232 films immediately after being produced at the industrial facilities. These concentrations were  
16  
17 233 determined by thermal desorption and GC analysis, using an 890 thermal tube desorber  
18  
19 234 (Dynatherm Analytical Instruments Inc., Kelton, PA, USA) connected in series to an HP 5890 Series  
20  
21 235 II Plus gas chromatograph (Hewlett Packard Co., Wilmington, DE, USA) equipped with a flame  
22  
23 236 ionization detector (FID) and an Agilent HP-1 semi-capillary column of 30 m length, 0.53 mm  
24  
25 237 internal diameter, and 2.65  $\mu\text{m}$  film thickness (Teknokroma S.C.L., Barcelona, Spain), and following  
26  
27 238 the procedure described in previous works [23, 33].  
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34 239 Since just after the film manufacturing all the retained active molecules were supposed to be  
35  
36 240 contained exclusively in the coating matrices, the amounts of substances detected by the  
37  
38 241 instruments were only attributed to the film mass fraction corresponding to those material layers.  
39  
40 242 This way, the values for the concentrations of each active compound  $i$  in the coating matrix of  
41  
42 243 each multilayer film  $F$  at the beginning of the material shelf life,  $C_{i_0}^F$ , were calculated, and then  
43  
44 244 introduced, together with the nominal concentrations detailed in the previous section 2.2,  $C_{i_n}^F$ , in  
45  
46 245 the following equation (1), in order to determine the percentage efficiency of the incorporation  
47  
48 246 process,  $E_i^F$ .  
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55 247 
$$E_i^F (\%) = \frac{C_{i_0}^F}{C_{i_n}^F} \cdot 100 \quad (1)$$
  
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1  
2 248 The retention of the active molecules within the coating layers of the multilayer films after  
3  
4 249 manufacture was also assessed by measuring their tendency to migrate to the substrate layers  
5  
6 250 over time. This tendency is determined by the thermodynamic equilibrium of each substance  
7  
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9 251 between both polymer matrices, which can be represented by the partition coefficient  
10  
11 252 corresponding to that system,  $K_i^{c/s}$ . This thermodynamic parameter can be defined as the ratio  
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13  
14 253 between the concentrations at equilibrium of an active compound  $i$  in the coating,  $C_i^c$ , and in the  
15  
16 254 substrate layers,  $C_i^s$ , as equation (2) shows:

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21 255 
$$K_i^{c/s} = \frac{C_i^c}{C_i^s} \quad (2)$$
  
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25 256 In order to properly evaluate these concentrations, the active films were analyzed 6 months after  
26  
27 257 manufacturing, when the thermodynamic equilibrium was assumed to have been reached in the  
28  
29 258 stored reels. All analyses were performed with the same equipment, conditions, and procedure as  
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31  
32 259 for the analyses of the initial concentrations described before. However, since in this case the  
33  
34 260 studied compound was distributed between two material phases, which did not allow physical  
35  
36 261 separation, the concentration of the active agent in the coating layer had to be estimated by first  
37  
38 262 measuring the average concentration in the entire film and in the substrate layer, and then by  
39  
40 263 introducing the values obtained in the following equation (3), deduced through a mass balance on  
41  
42 264 the two material layers constituting the complete multilayer film:  
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47 265 
$$C_i^c = \frac{C_i^f \cdot \rho_f \cdot L_f - C_i^s \cdot \rho_s \cdot L_s}{\rho_c \cdot L_c} \quad (3)$$
  
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51

52 266 where  $L_c$ ,  $L_s$ , and  $L_f$ , are the thicknesses of the coating and substrate layers, and of the entire  
53  
54 267 film respectively, whereas  $\rho_c$ ,  $\rho_s$ , and  $\rho_f$ , are their corresponding densities. Since the active films  
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56 268 were manufactured by a coating technology based on gravure printing, and the printed areas did  
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1  
2 269 not completely cover the entire film surface, the concentration of the active agent in the substrate  
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4 270 layer could be determined by analyzing polymer samples from the film zones that remained  
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6 271 uncoated.  
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#### 11 12 13 273 2.5. DETERMINATION OF THE SURFACE PROPERTIES OF THE MULTILAYER FILMS 14

15  
16 274 The surface properties of the coating and substrate layers of the manufactured films, and, in  
17  
18 275 particular, their wettability, were investigated by measuring static contact angles, in the diverse air  
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20 276 / water / polymer systems formed by the films, through the sessile drop method. The studied  
21  
22 277 systems were thus the passive coatings: E and E + B, the active coatings: E + Cl, E + MO, E + CO, E +  
23  
24 278 CA, and E + B + CA, and the substrate polymers: PP and PET, as control materials. With this  
25  
26 279 objective, an OCA 15EC goniometer (DataPhysics Instruments GmbH, Filderstadt, Germany) was  
27  
28 280 employed to deposit water droplets on the assayed film surfaces, and its SCA20 software was used  
29  
30 281 for data acquisition and image analysis purposes. Tests were carried out at room conditions in an  
31  
32 282 air atmosphere, by carefully dropping about 8  $\mu$ L of deionized water with the aid of a dispenser  
33  
34 283 syringe. The static contact angles formed between the water droplets, the film surfaces, and the  
35  
36 284 air atmosphere, were measured 60 s after their deposition, by analyzing the images obtained, and  
37  
38 285 by fitting their shape equations to the Young - Laplace model. Assays were performed on, at least,  
39  
40 286 five different locations of each film sample, and in triplicate for each studied material, in order to  
41  
42 287 ensure the reproducibility of the values obtained and to allow their proper averaging.  
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#### 51 52 53 289 2.6. DETERMINATION OF THE OPTICAL PROPERTIES OF THE MULTILAYER FILMS 54

55  
56 290 The optical properties of the produced films were determined by measuring the material color  
57  
58 291 with a CM-3500D spectrophotometer (Konica Minolta Optics Inc., Tokyo, Japan). The instrument  
59  
60

1  
2 292 was set to D65 illuminant / 10° observer, equipped with an 8 mm aperture target mask, calibrated  
3  
4 293 with a white calibration plate and a zero calibration box, and programmed to perform three shots  
5  
6 294 in each measurement and subsequently yield the averaged results. The essayed materials were, in  
7  
8 295 this case, the developed active and passive multilayer films, as well as the polymers constituting  
9  
10 296 their substrate layers. Measurements were performed on eight different locations of each film  
11  
12 297 sample, and in triplicate for each studied material; and they were carried out at room conditions  
13  
14 298 of temperature and humidity by placing the film samples onto the device lens and against the  
15  
16 299 surface of a standard white tile. The instrument's software SpectraMagic NX was employed to  
17  
18 300 acquire the color data and to display them in the CIELAB color space. The color coordinates of  
19  
20 301 each film sample:  $L^*$  (lightness),  $a^*$  (greenness – redness), and  $b^*$  (blueness – yellowness), could  
21  
22 302 therefore be obtained, and the corresponding polar coordinates:  $C_{ab}^*$  (chroma or saturation  
23  
24 303 index), and  $h_{ab}$  (hue or angle), could easily be estimated from the latter two parameters by  
25  
26 304 introducing them in the following equations:  
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$$305 \quad C_{ab}^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (4)$$

$$306 \quad h_{ab} = \arctan\left(\frac{b^*}{a^*}\right) \quad (5)$$

## 307 308 2.7. DETERMINATION OF THE MECHANICAL PROPERTIES OF THE MULTILAYER FILMS

309 The mechanical properties of the diverse manufactured films, and of their substrate polymers,  
310 were evaluated by following a test procedure based on the ASTM D882 – 12 standard method [36]  
311 with the aid of a MultiTest 1-i universal testing machine (Mecmesin Ltd., Slinfold, UK), equipped  
312 with a 100 N static load cell and with small pneumatic plane grips. In detail, the studied materials



1  
2 313 were cut into strips of 25.4 mm wide and 140 mm long, and the obtained samples were  
3  
4 314 conditioned in climatic chambers at  $23 \pm 1$  °C temperature and  $0 \pm 5$  % of relative humidity for at  
5  
6 315 least 48 h prior to testing. Immediately afterwards, at least 10 specimens of each assayed film  
7  
8 316 were successively extracted from the chambers, inserted between the grips, and firmly held in  
9  
10 317 place with 3 bars of pressure. The instrument's software Emperor was then used to set the grip  
11  
12 318 separation at exactly 100 mm, the cross-head speed at 25 mm/min, and the data sampling rate at  
13  
14 319 1 kHz. In these test conditions, an increasing load was applied on the film samples over time,  
15  
16 320 causing their elongation, and the corresponding force ( $F_t$ ) – displacement ( $\Delta L_t$ ) curves were thus  
17  
18 321 obtained. These plots were standardized by transforming them into stress ( $\sigma_t$ ) – strain ( $\varepsilon_t$ ) curves  
19  
20 322 with the application of the corresponding definition equations:  
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24  
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$$26 \quad 323 \quad \sigma_t = \frac{F_t}{A_0} = \frac{F_t}{w_0 \cdot t_0} \quad (6)$$

$$27 \quad 324 \quad \varepsilon_t = \frac{\Delta L_t}{L_0} \cdot 100 \quad (7)$$

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29  
30  
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35  
36 325 where  $A_0$  is the original cross-sectional area of the tested sample, product of its original width  
37  
38 326 ( $w_0$ ) and thickness ( $t_0$ ), and  $L_0$  is the original grip separation. This way, the mechanical properties  
39  
40 327 of the studied materials, namely the ultimate tensile strength, the deformation at break, and the  
41  
42 328 Young's modulus, could be estimated through the graphical assessment of the newly obtained  
43  
44 329 curves.  
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## 52 331 2.8. DETERMINATION OF THE BARRIER PROPERTIES OF THE MULTILAYER FILMS

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54  
55 332 The barrier properties of the produced passive films, and of their corresponding substrates, were  
56  
57 333 investigated by measuring their oxygen and carbon dioxide permeances as a function of the  
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1  
2 334 relative humidity, following the procedures described in a previous work [33], based in the ASTM  
3  
4 335 D1434 – 82(2009)e1 standard method [37]. Oxygen transport was studied by means of an OX-  
5  
6 336 TRAN model 2/21 ML (Paul Lippke Handels – GmbH, Neuwied, Germany) programmed to measure  
7  
8 337 the oxygen permeance at  $23 \pm 2$  °C and at 0, 35, 50, 75, and  $90 \pm 0.01$  % RH, whereas for carbon  
9  
10 338 dioxide transport, an isostatic permeation apparatus, described in this previous work, was used to  
11  
12 339 measure the gas permeance at  $23 \pm 2$  °C and at 0, 30, 45, 75, 85, and  $100 \pm 0.1$  % RH. In both  
13  
14 340 cases, between two and four film samples were placed in the instrument cells, held in place with  
15  
16 341 high-vacuum silicone, and conditioned for 12 h prior to the permeation tests. The gas permeance  
17  
18 342 was measured every 45 min until constant, and, from that moment, at least three more points  
19  
20 343 were recorded in order to obtain an average value.  
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## 29 345 2.9. DETERMINATION AND SIMULATION OF THE PERFORMANCE OF THE ACTIVE PACKAGES

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31  
32 346 Differences in performance between the packages constructed with neat EVOH and those  
33  
34 347 comprising an EVOH / clay nanocomposite were assessed by studying their activity over time in  
35  
36 348 actual working conditions. With this objective, three packages of each type were placed in a  
37  
38 349 climatic room, at  $23 \pm 1$  °C and  $30 \pm 5$  % RH, for one week, and the concentration of carvacrol in  
39  
40 350 their headspace was periodically evaluated. Analyses were performed on a daily basis, by  
41  
42 351 extracting at least three gas samples of 500  $\mu$ L from their inner atmosphere with the aid of a 1750  
43  
44 352 Gastight precision syringe (Hamilton Co., Reno, NV, USA), and by injecting them in the inlet port of  
45  
46 353 an HP 5890 Series II Plus gas chromatograph (Hewlett Packard Co., Wilmington, DE, USA). The  
47  
48 354 instrument was equipped with a flame ionization detector (FID) and a 30 m, 0.32 mm, 0.25  $\mu$ m  
49  
50 355 Agilent HP-5 capillary column (Teknokroma S.C.L., Barcelona, Spain), programmed in accordance  
51  
52 356 with the procedure described in previous work [23], and calibrated by injecting carvacrol in known  
53  
54 357 concentrations. After averaging the results acquired in each assay period, two experimental curves  
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1  
2 358 for the evolution in time of the concentration of carvacrol in the headspace of both studied  
3  
4 359 packages could be obtained.  
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6  
7 360 In order to assess the effects of the EVOH matrix modification on the package performance more  
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9  
10 361 thoroughly, as well as to validate the experimental procedure carried out, a mathematical model  
11  
12 362 based on the finite element method was applied to the assayed packages, and the simulation of  
13  
14 363 their activity over time was therefore performed. This model had already been developed  
15  
16 364 specifically for those systems in prior work [23] with the aid of the Chemical Transport of Diluted  
17  
18 365 Species physics interface of the COMSOL Multiphysics 4.2 modeling suite (COMSOL AB, Stockholm,  
19  
20  
21 366 Sweden). In the present case, the model was fed with the fundamental hypotheses and  
22  
23 367 physicochemical properties gathered in the previous studies [23, 33], as well as with the geometric  
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25 368 characteristics and integration conditions corresponding to the systems investigated here, further  
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27  
28 369 detailed throughout this paper. As a result, two theoretical curves for the evolution in time of the  
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30 370 concentration of carvacrol in the headspace of both packages could be obtained, and were then  
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32 371 subsequently compared with their experimental counterparts.  
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#### 37 373 2.10. DATA ANALYSIS

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42 374 The values for all the parameters and coefficients presented in this work are expressed as " $(\bar{x} \pm \varepsilon)$   
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44 375 units," where  $\bar{x}$  stands for the sample mean of parameter  $x$ , and  $\varepsilon$  stands for its absolute error.  
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46 376 This absolute error is equal to the sample standard deviation for the measured variables, and was  
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48 377 determined by propagation of uncertainty (partial derivatives method) for the calculated  
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50 378 variables.  
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56 379 Statistical analysis of the results obtained for mechanical properties was performed with the aid of  
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58 380 IBM SPSS Statistics 21 commercial software (IBM Corp., Armonk, NY, USA). Concretely, a one-way  
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2 381 analysis of variance (ANOVA) was carried out, and differences found between mean values for the  
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4 382 studied materials were assessed by means of confidence intervals using Tukey's test at a  $p \leq 0.05$   
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6 383 level of significance.  
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2 385 **3. RESULTS AND DISCUSSION**  
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7

8 387 3.1. ASSESSMENT OF THE STABILITY OF EVOH COATINGS ON PRETREATED PP AND PET  
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10 388 SUBSTRATES  
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12  
13 389 Although both PP and PET substrates can easily be coated by hydroalcoholic EVOH solutions,  
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15 390 yielding highly transparent and homogeneous multilayer films, the large differences in chemical  
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17 391 structure and polarity existing between both materials, and thus their low chemical compatibility,  
18  
19 392 prevent them from developing a sufficient anchorage mechanism in their contact interface. This  
20  
21 393 lack of coating adhesiveness leads to film delamination when slight tensile forces are applied  
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23 394 between the material layers, such as during the manufacturing of the films or during the formation  
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25 395 of the packages, or when external compounds, such as water or plant essential oils, are scalped  
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27 396 from the preserved food products [38].  
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33 397 Since in this work all the manufactured materials were intended to be employed in the  
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35 398 construction of active packages for fresh-cut salad or similar products, containing essential oils or  
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37 399 their constituents as natural antimicrobials, the application of an effective anchorage treatment  
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39 400 on the PP and PET substrates was indispensable to ensure good stability of the deposited EVOH  
40  
41 401 coatings. With this objective, four different technologies for surface modification, namely UV  
42  
43 402 irradiation, corona discharge, and corona discharge followed by the application of PU or PEI-based  
44  
45 403 primers, were implemented in the manufacture process of the developed films, and qualitatively  
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47 404 evaluated by means of Scotch tape adhesion tests. The results obtained are displayed in **Table 1**  
48  
49 405 and, as shown, for every applied treatment no distinction is made between both assayed  
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51 406 substrates, because no relevant differences were found in their adhesiveness behavior.  
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58 407 With respect to the diverse technologies investigated, UV irradiation failed to produce a  
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2 408 satisfactory adhesion on either PP or PET substrates, thus yielding easily delaminable films,  
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4 409 although in the former material some peeling resistance was observed during the progression of  
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6 410 the tests (weak adhesion). The corona discharge, by contrast, produced reasonably good results in  
7  
8 411 both materials, complicating or even impeding their delamination, although, according to  
9  
10 412 observation during the tests, the surface treatment had not been applied uniformly, and, in  
11  
12 413 consequence, the points of anchorage between layers were heterogeneously distributed  
13  
14 414 (moderate adhesion). Finally, the casting of primers after corona pretreatment yielded very  
15  
16 415 opposite results depending on the substance applied. In this sense, whereas the PEI-based primer  
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18 416 produced an excellent adhesion with both the EVOH coatings and the PP or PET substrates,  
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20 417 making their detachment absolutely impossible (strong adhesion), the PU-based primer not only  
21  
22 418 failed in providing some adhesion between layers, but, owing to its obstructive location, also  
23  
24 419 prevented the corona-treated surfaces of the substrates from interacting with the coatings, and  
25  
26 420 thus from generating some anchorage points between both (none adhesion). These facts led the  
27  
28 421 corona discharge treatment to reach worse results when followed by a PU-based primer, or better  
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30 422 when followed by PEI-based one, than when applied alone.  
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38 423 In addition to the adhesion tests, the stability of the EVOH coatings on the pretreated PP or PET  
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40 424 substrates was also assessed by subjecting the manufactured films to thermosealing tests, carried  
41  
42 425 out with the aid of a manual impulse heat sealing machine. The performance of these parallel  
43  
44 426 assays was motivated by two main reasons. Firstly, the thermosealing properties of the studied  
45  
46 427 materials were closely related to the hermeticity attainable in the final targeted packages, key  
47  
48 428 characteristic of their structural design. Secondly, the sealing capabilities of the developed films  
49  
50 429 were also dependent upon the force of adhesion between their material layers and thus their  
51  
52 430 evaluation could contribute to corroborating the results obtained in the former tests. Just as for  
53  
54 431 those essays, the qualitative results of the thermosealability tests are displayed in **Table 1**, and for  
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1  
2 432 every applied treatment no distinction is made between both studied substrates either.  
3  
4  
5 433 As this table shows, there was a good correlation between the results yielded by both tests for the  
6  
7 434 four different treatments applied on the multilayer films. According to this, materials  
8  
9 435 manufactured with UV-irradiated substrates were not sealable, owing to the low strength of the  
10  
11 436 anchorage points created between layers, and thus of the final seal formed between films.  
12  
13 437 Materials subjected to corona discharge, in turn, were only sealable to a limited extent, because,  
14  
15 438 although the obtained seal presented quite good appearance and seemed to attach both films  
16  
17 439 firmly, when slight tensile forces ( $\leq 1$  N) were applied on the joint it promptly failed and the films  
18  
19 440 were easily detached again. This phenomenon could be product of an irregular application of the  
20  
21 441 anchorage treatment, as explained above in the results of the adhesion tests. Lastly, films formed  
22  
23 442 through primers application yielded extreme results depending upon the substance employed. In  
24  
25 443 agreement with the adhesion essays, materials containing a PEI-based primer were perfectly  
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27 444 sealable, because very strong tensile forces had to be applied to separate the films, and also films  
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29 445 were not detached by seal failure but by matrix failure, while the sealing area remained intact. On  
30  
31 446 the contrary, materials containing a PU-based primer could not be assayed because of the  
32  
33 447 absolute lack of adhesion between their layers, as reported before in the previous tests.  
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41 448 Finally, since the substrates coated through the application of a PEI-based primer yielded the best  
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43 449 results in both stability tests, the quantitative analysis for the determination of the sealing  
44  
45 450 strength was only carried out on these materials. The values found are also included in **Table 1**,  
46  
47 451 and compared with those obtained for the uncoated substrates. As can be seen, the EVOH-coated  
48  
49 452 films presented the highest peel strengths, thus confirming the excellent sealability properties of  
50  
51 453 EVOH copolymers, as published elsewhere [30]. Also, the improvement observed with respect to  
52  
53 454 the uncoated films is probably due to the lower melting temperature of EVOH with respect to the  
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57 455 substrate polymers, especially to the PET film. Hence, since all the sealing tests were carried out  
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1  
2 456 under the same testing conditions, the higher percentage of polymer melted during the essays  
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4 457 would be responsible for the stronger sealings.  
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7 458 To conclude, the application of corona discharge followed by a PEI-based primer was definitely the  
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9  
10 459 best anchorage technology available to bond EVOH coatings with PP or PET substrates, and it was  
11  
12 460 therefore implemented in the manufacturing process of the active multilayer films. Obviously, the  
13  
14 461 physicochemical assays described below were only performed on such films.  
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18 462

### 20 463 3.2. ASSESSMENT OF THE RETENTION OF ACTIVE AGENTS IN THE COATING LAYERS OF 21 22 23 464 MULTILAYER FILMS

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25  
26 465 The retention of a chemical compound in a polymeric matrix is a function of the thermodynamic  
27  
28 466 equilibrium of the substance between the system phases involved, in this case: air, coating and  
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30 467 substrate, and of its kinetics within them. Since some of the active agents studied in this work are  
31  
32  
33 468 known to show volatility as well as a considerable solubility in other polymeric materials, and thus  
34  
35 469 to undergo a substantial migration to the adjacent phases over time, especially in highly hot  
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37  
38 470 and/or humid conditions [23, 25, 26], the evaluation of their remaining concentration in the  
39  
40 471 coating and substrate layers of the multilayer films, immediately after their manufacturing and  
41  
42 472 after long-term storage in reels, was essential to assess their retention in the original carrier  
43  
44 473 matrices. For this reason, all the concentrations involved were measured at the mentioned  
45  
46 474 moments by thermal desorption and GC analysis, and subsequently introduced in the previous  
47  
48 475 equations (1) and (2), to finally estimate the percentage efficiency of the incorporation process for  
49  
50 476 the active agent,  $E_i^F$ , and its partition coefficient between the film layers,  $K_i^{C/S}$ . Since both  
51  
52 477 parameters quantify the tendency of the embedded compounds to transfer over time to other  
53  
54 478 system phases, i.e. air or substrates, respectively, they are considered good indicators of their  
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1  
2 479 retention in the coating layers.  
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5 480 The results for the incorporation efficiencies of the active agents in the coating layers of the  
6  
7 481 multilayer films, together with their corresponding initial and nominal concentrations, are  
8  
9 482 collected in **Table 2**, whereas the values estimated for the partition coefficients are detailed in  
10  
11 483 **Table 3**. As **Table 2** shows, the efficiencies of the incorporation procedure range, in general terms,  
12  
13 484 between 40 and 60% for all the materials and compounds essayed. These values mean important  
14  
15 485 losses of activity during the manufacture of the films, probably produced by evaporation during  
16  
17 486 the processes of preparation and application of the coating solutions. This hypothesis can also be  
18  
19 487 reinforced by the fact that all the reported efficiencies show quite high correlation with the  
20  
21 488 volatility of the embedded compounds. In fact, citral, the most volatile substance among the  
22  
23 489 active agents studied in this work, yielded very low efficiencies in both PP and PET-based films, on  
24  
25 490 which grounds it was incorporated with the highest nominal concentration. However, and as can  
26  
27 491 be seen in this table 2, the performance of a matrix modification in the EVOH coating, by  
28  
29 492 embedding a 2% of bentonite nanoclay in its structure, resulted in a great enhancement of its  
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31 493 retention capacity, with an increase of about 22% with respect to the original value.  
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39 494 Finally, the partition coefficients of the active agents between the coating and substrate layers, as  
40  
41 495 **Table 3** shows, are all noticeably higher than the unity, up to three orders of magnitude in some  
42  
43 496 case, and are thus in agreement with the value reported in a previous work for the partition of  
44  
45 497 carvacrol between EVOH and PP, which, at room temperature and dry conditions (23 °C and 0%  
46  
47 498 RH), was of about 10000 [23]. These results mean that all the investigated compounds, that is,  
48  
49 499 essential oils and their constituents, are far more soluble in EVOH than in PP or PET matrices,  
50  
51 500 which is very advantageous for the retention of the active agents in the coating layers. However,  
52  
53 501 considering that the substrate layers are about 26 times thicker than their corresponding coatings  
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55 502 in all cases, the amount of substance finally transferred to the former material after 6 months of  
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1  
2 503 storage can become significant in some. In this context, citral is worth mentioning, since it shows  
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4 504 extreme behaviors depending upon the substrate employed. Indeed, it undergoes an intense  
5  
6 505 migration towards the PP layer but its lower affinity to the PET matrix makes it attain a high  
7  
8 506 retention in the EVOH coating, which must be taken into account when it comes to employing  
9  
10 507 these materials in the construction of active packages. In order to better illustrate such behaviors,  
11  
12 508 the expected concentrations at equilibrium of the active agents in the coating,  $C_{i\infty}^C$ , and substrate  
13  
14 509 layers,  $C_{i\infty}^S$ , of the multilayer films, assuming a value of 100% for the incorporation efficiency, have  
15  
16 510 been estimated from the reported values of the nominal concentrations and the partition  
17  
18 511 coefficients by introducing them in the previous equations (2) and (3). The results obtained are  
19  
20 512 also displayed in **Table 3**, and, as shown, in those films presenting lower values of the partition  
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22 513 coefficients the greater thickness of the substrate layers with respect to their coatings can lead to  
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24 514 the virtual exhaustion of the active agents in the latter materials.  
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### 35 516 3.3. ASSESSMENT OF THE SURFACE PROPERTIES OF THE MULTILAYER FILMS

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38 517 Since all the multilayer films developed in this work were intended to be used in the construction  
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40 518 of active packages for minimally processed vegetables, good optical characteristics, such as high  
41  
42 519 gloss or transparency, were a strong requirement for them. However, the high water content of  
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44 520 this foodstuff could saturate their headspace atmosphere with humidity, and subsequently  
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46 521 promote its condensation on their inner surfaces, giving them a foggy appearance and thus a  
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48 522 limited transparency. The formation of small water droplets on the packaging films, responsible  
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50 523 for the light scattering phenomena, is a function of their surface properties, and, in particular, of  
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52 524 their wettability. Hence, this property was investigated on the manufactured materials by  
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54 525 measuring static contact angles of water droplets on their surface through the sessile drop  
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2 526 method with the aid of a goniometer, and the results obtained were plotted in **Figure 1**.  
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5 527 As this figure shows, contact angles of untreated PP and PET substrates are quite high, of about 98  
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7 528 and 72° respectively, which proves their hydrophobic nature in their original conditions, especially  
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10 529 in the former material, owing to its lower polarity. Surprisingly, coatings of neat EVOH, a highly  
11  
12 530 hydrophilic polymer, also show a high value for this parameter, around 99°, very similar to that  
13  
14 531 reported for PP. This result means that, despite its hydrophilicity, EVOH shows a great  
15  
16 532 hydrophobic behavior when its surface chains are not given enough time to interact with the  
17  
18 533 water molecules. In any case, all three polymers seem to be naturally hydrophobic under those  
19  
20 534 experimental conditions, which could pose some difficulties when it comes to fixing inks or to  
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22 535 removing fogging in the final packages. However, when an active agent is embedded into the  
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24 536 EVOH matrix, the contact angles drop dramatically, between 33 and 46% from the original value,  
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26 537 reaching a minimum at about 53° for the marjoram oil. This phenomenon can be due to the  
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28 538 presence of active molecules on the material surface, which, owing to their greater polarity, could  
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30 539 substantially increase its interactions with water, and thus its wettability. In addition, the  
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32 540 incorporation of only 2% of bentonite nanoclay in its structure is also capable, by itself, of reducing  
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34 541 its contact angle by 37%, and, as a result, of reaching even lower values, up to 57°, when  
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36 542 combined with carvacrol as active agent. In this case, the presence of numerous nanometric  
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38 543 particles of bentonite, a highly hydrophilic material which can absorb several times its weight in  
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40 544 water, in the EVOH surface could also multiply its interactions with the water molecules and,  
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42 545 consequently, further enhance its wettability. This result is also in agreement with the findings in a  
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44 546 previous study [33], where 2% of bentonite was reported to increase the hydrophilic behavior of  
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46 547 EVOH by 17%.  
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## 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,

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2 574 therefore yielding highly light scattering polymer coatings. However, when an active compound  
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4 575 was embedded in those matrices lightness decreased again by absorption phenomena, just as  
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6 576 occurred in the substrate materials. Finally, the effect of the incorporation of a 2% of bentonite  
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8 577 nanoclay in the EVOH coatings was an increase of about a 0.2% in their lightness, probably due to  
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10 578 the additional scattering phenomena introduced by the dispersed particles. In any case, it must be  
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12 579 noticed that all the values reported in the graph range only between 96 and 99, so it can be  
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14 580 concluded that the lightness of the studied materials can barely be modified by the presence of  
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16 581 either active compounds or inorganic fillers.  
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21 582 Regarding the chroma and hue parameters, whereas PP substrates showed almost no coloration,  
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23 583 with a chroma value near zero, PET substrates showed a substantial chroma, of about 0.7, with a  
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25 584 slight reddish / yellowish tonality. In addition, when both polymers were analyzed after absorbing  
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27 585 active compounds from their EVOH coatings, very opposite results were found, unlike for the  
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29 586 lightness study. In detail, although both materials reduced their chroma in similar proportions,  
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31 587 whereas PP practically conserved its hue, PET substrates moved sharply from a reddish to a  
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33 588 greenish tonality, while maintaining their yellow intensity. Furthermore, when both substrates  
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35 589 were coated by neat EVOH different behaviors were also observed, concretely, the chroma value  
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37 590 was slightly reduced for PET while doubled for PP, although both materials shared a noticeable  
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39 591 displacement in the green direction. This movement was further sharpened with the incorporation  
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41 592 of active agents to the EVOH coatings, especially in the case of the PET-based films, which reached  
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43 593 a deeper yellowish tonality while changing their redness to greenness, thus attaining a higher  
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45 594 value for their chroma, between 0.9 and 1.5, depending on the compound embedded. Finally, the  
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47 595 addition of a 2% of bentonite nanoclay to the EVOH matrices of PP-based films again doubled their  
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49 596 chroma value, up to 0.45 approximately, also moving them on the way towards the greenish  
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51 597 region. To summarize, it can be stated that in general terms, the coating of PP or PET substrates  
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2 598 with a neat EVOH layer does not significantly modify their color, but when natural substances,  
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4 599 such as active compounds or inorganic fillers, are also present in the polymers, they will produce a  
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6 600 considerable increase of the chroma and a substantial displacement to the greenish and yellowish  
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8 601 regions in most cases, probably due to their characteristic and predominant colorations. However,  
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10 602 and just as occurred for lightness, it must be remarked that no studied material presented chroma  
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12 603 values greater than 1.5, which is quite a low amount. Hence, it can be concluded that all the  
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14 604 changes observed in the coloration of the films will not be macroscopically perceptible to the  
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16 605 naked eye, and thus will virtually not affect their optical properties in a significant way either.  
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### 24 607 3.5. ASSESSMENT OF THE MECHANICAL PROPERTIES OF THE MULTILAYER FILMS

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28 608 Among the physical properties of relevance for food packaging applications, the mechanical ones  
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30 609 are probably the most important since films must undergo numerous and different strains during  
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32 610 their manufacturing, as well as during the filling, transportation, and commercialization of the final  
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34 611 packages. In addition, the potential scalping of diverse organic substances from the preserved  
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36 612 food products by the packaging materials, such as water or plant essential oils, can deteriorate  
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38 613 their polymeric structure and thus reduce their mechanical performance. Because of this, some  
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40 614 mechanical properties, namely the ultimate tensile strength, the deformation at break, and the  
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42 615 Young's modulus, were determined on the diverse developed active and passive films, by  
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44 616 elongating them under increasing load forces in a universal testing machine, and by converting the  
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46 617 obtained force – displacement curves into stress – strain curves through the application of  
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48 618 equations (6) and (7). The values finally found for the three mentioned parameters are plotted in  
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50 619 bar graphs in **Figure 4**, together with their corresponding subset tags, according to the grouping of  
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52 620 means yielded by the post-hoc statistical test.  
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2 621 In relation to the ultimate tensile strength, as this figure depicts, PP substrates resisted a  
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4 622 considerable level of stress, around  $130 \text{ N/mm}^2$ , whereas PET materials almost doubled this  
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6 623 number, up to  $230 \text{ N/mm}^2$ , when tested in their original conditions. However, when coated with a  
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8 624 layer of EVOH these values decreased significantly, by about 7%. Furthermore, if this material, in  
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10 625 turn, contained an active compound, a further reduction of about 1.6 and 5%, respectively, was  
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12 626 also observed in both types of films with respect to those comprising the neat polymer, although  
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14 627 according to the ANOVA test, the differences found were not significant. This slight worsening of  
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16 628 the tensile strength in both materials can be attributed to two different facts. Firstly, given that  
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18 629 the EVOH coatings were not deposited on the substrates as single layers but as small individual  
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20 630 droplets, as explained in the previous section 3.4, they would only increase the thickness of the  
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22 631 final films, but could not provide any additional noticeable resistance to the load forces applied.  
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24 632 Hence, and according to equation (6), the tensile stresses finally obtained would always be lower  
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26 633 for them than for their corresponding substrates. Secondly, the scalping of organic substances  
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28 634 from these coatings by their substrate materials, such as water or 1-propanol, could also have  
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30 635 plasticized them, therefore reducing their ultimate tensile strength, but this phenomenon could  
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32 636 even have been aggravated with the absorption of active compounds, since they are known to be  
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34 637 good plasticizers of such polymers, and, in addition, their activity could also have promoted some  
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36 638 reaction with them, thus somewhat degrading their molecular structure. To end, the presence of  
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38 639 2% of bentonite nanoparticles in the EVOH matrix produced a slight increase of the tensile  
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40 640 strength of about 0.5% with respect to the unfilled film, which, despite being statistically  
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42 641 insignificant, can be indicative of some improvement in its mechanical performance, in agreement  
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44 642 with the results reported elsewhere for other clay nanocomposites.  
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46 643 Regarding the deformation at break, in this case both substrates reached high values, around  
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48 644 380% for PP and 300% for PET, which were also slightly lower for their EVOH-coated counterparts,  
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2 645 by about 0.2 and 0.7% respectively, although without statistical significance ( $p > 0.05$ ). Equally, in  
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4 646 their active equivalents further reductions were also observed with respect to the passive films, up  
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6 647 to 3.9 and 12.8% respectively, which were significant only for the PET-based materials. These  
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8 648 results could also be explained by the scalping effect mentioned above, through which the studied  
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10 649 substrates would absorb diverse organic substances from their EVOH coatings, and, especially,  
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12 650 from the active ones, and consequently undergo some matrix deterioration, such as a weakening  
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14 651 or perhaps breaking of its molecular bonds, which, besides leading them to premature failure,  
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16 652 would also prevent them from reaching such large deformations. Concerning the modification of  
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18 653 the EVOH structure by the addition of bentonite, in this case no differences were found with  
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20 654 respect to the neat polymer.  
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26 655 The assessment of the Young's modulus, in turn, yielded similar results to the other two  
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28 656 parameters, thus contributing to corroborating the hypotheses posed above. In the case here, PP  
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30 657 substrates showed a moderate value for this property, around  $760 \text{ N/mm}^2$ , whereas PET materials  
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32 658 could attain a far higher number, of about  $1820 \text{ N/mm}^2$ , owing to their greater stiffness. However,  
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34 659 the values reported for both polymers were significantly reduced when an EVOH coating was  
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36 660 applied, by 13.5 and 2.8% respectively, and further decreases were observed again with respect to  
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38 661 these new materials when active substances were also incorporated, up to 2% more, depending  
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40 662 upon the compound embedded. In addition, and just as for the ultimate tensile strength, the  
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42 663 presence of 2% of bentonite nanoclay increased the value of this parameter by approximately  
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44 664 0.75%, although this difference once more lacked statistical significance, as the previous results.  
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50 665 In summary, it can be stated that all the variations reported for the three studied parameters are  
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52 666 indicative that, in effect, some scalping phenomenon is occurring in the developed multilayer  
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54 667 films, especially in the active ones, which, by either plasticization or reaction mechanisms, could  
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56 668 be responsible for the softening or degradation of the structure of their substrates, and thus of the  
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2 669 lower values found for their ultimate tensile strength, deformation at break, and Young's modulus.  
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4 670 This behavior can be partially counteracted by the presence of bentonite nanoparticles in the  
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6 671 coating matrix, although, according to the values detailed, only to a low extent. In any case, it  
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8 672 must be remarked that none of the properties assessed in the manufactured films was observed  
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10 673 to vary by more than 15% with respect to their corresponding substrates, therefore it can be  
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12 674 concluded that their mechanical performance will not become substantially deteriorated in the  
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14 675 final packages.  
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### 24 677 3.6. ASSESSMENT OF THE BARRIER PROPERTIES OF THE MULTILAYER FILMS

25 678 Given that the food packages aimed in this work were sealed bags for the preservation of fresh-cut  
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27 679 salad or similar products, as mentioned above, all the developed multilayer films had to allow an  
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29 680 adequate gas exchange between their inner and outer atmospheres as to properly maintain the  
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31 681 quality and sensory characteristics of the contained foodstuffs. However, the simple application of  
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33 682 a thin coating of EVOH, a high-barrier polymer, on the original PP films, for example, could lead to  
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35 683 a substantial decrease of the gas transmission rate through these packaging materials.  
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37 684 Consequently, it could put the preserved food products at risk of developing strange flavors or  
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39 685 smells throughout their shelf life, by effect of the excessive accumulation of carbon dioxide in the  
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41 686 package headspace, and thus at risk of becoming inedible. For this reason, the barrier properties  
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43 687 of all the manufactured passive films and substrates were studied by measuring their oxygen and  
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45 688 carbon dioxide permeances as a function of the relative humidity, in both isostatic permeation  
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47 689 instruments, yielding as a result the values collected in **Table 4**.  
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54 690 As this table shows, for the substrate polymers no distinction is made between the diverse relative  
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56 691 humidities assayed in the permeation tests, because, although five or six different conditions were  
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2 692 considered, depending upon the compound studied, no relevant differences were found in the gas  
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4 693 transmission rates, according to the well-known hydrophobic behavior of those materials. With  
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6 694 respect to the values displayed, the oxygen permeance was quite high in both polymers, especially  
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8 695 in the PP film, although the transport of carbon dioxide yielded even higher figures for this  
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10 696 parameter, about 10 times more on average for both substrates. However, when a thin coating of  
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12 697 EVOH was applied on those materials, their permeance dropped dramatically, being able to reach,  
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14 698 in dry conditions, about a 1/160 of its original value for the PP-based films, and about a 1/6 for the  
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16 699 PET ones, as expected from the high barrier characteristics of such a polymer. Nevertheless, owing  
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18 700 to its highly hydrophilic nature, as reported elsewhere [27 – 29], this barrier effect gradually  
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20 701 dissipated as the relative humidity increased, undergoing a sharper change at 75% RH, probably  
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22 702 due to the proximity of the glass transition point [33], and attaining permeance values at 100% RH  
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24 703 somewhat comparable to those reported for the uncoated substrates, mostly between 70 and  
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26 704 80% of the original values. Finally, the incorporation of 2% of bentonite nanoclay to the EVOH  
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28 705 coatings produced a further decrease of the films' permeances to both gases when they were  
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30 706 assayed at moderate relative humidities, up to 76% with respect to the unfilled materials, whereas  
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32 707 at extreme ambient conditions the differences found did not exceed a 5%. As a conclusion, it could  
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34 708 be asserted that, given the highly humid conditions expected in the package headspace during the  
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36 709 product preservation time, the presence of a thin coating layer of EVOH or bentonite-filled EVOH  
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38 710 on the packaging materials will not affect their gas transport in a substantial way, and thus will  
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40 711 neither modify the respiration rate of the contained foodstuffs so much as to significantly  
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42 712 deteriorate their quality or sensory characteristics.  
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### 55 714 3.7. ASSESSMENT OF THE PERFORMANCE OF THE ACTIVE PACKAGES

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58 715 In previous works, food packages consisting of EVOH-coated PP films containing essential oils or  
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2 716 their constituents as active antimicrobial agents were successfully developed and tested for the  
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4 717 preservation of fresh-cut salad and fish products. However, the assays carried out in both systems  
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6 718 revealed an unconstrained free release of the active compounds to the headspace of the  
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9 719 packages, which induced an early peak of antimicrobial activity at the beginning of the product  
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11 720 shelf-life, followed by a rapid decay in a few days. For this reason, a matrix modification based on  
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13 721 the incorporation of bentonite nanoclay was assessed on EVOH active films in a subsequent study  
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16 722 [33], yielding substantial improvements in the retention capacity and rate of release of the active  
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18 723 agents. Hence, following this line, the effects of such modification on the performance of active  
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20 724 packages, assayed in actual working conditions, were investigated in the present work, by  
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22 725 determining their activity over time with a gas chromatograph, and by simulating it through the  
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24 726 application of a mathematical model. Both experimental and theoretical results are displayed in  
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27 727 **Figure 5**, where the evolution in time of the concentration of carvacrol in the headspace of the  
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29 728 packages has been plotted for both original and nanocomposite active films.

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33 729 As can be seen in the graph, both theoretical curves describe the experimental behavior of the  
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35 730 assayed packages very accurately, meaning that both the test procedure carried out and the  
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37 731 mathematical model applied, were perfectly valid for the systems and conditions studied in this  
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39 732 work. On another matter, the evolutions in time of the carvacrol concentration described by both  
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41 733 curves are analogous to those reported in a previous work for a similar package [23], since they  
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43 734 exhibit a maximum value immediately after the beginning of the storage period, followed by a  
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45 735 gradual and continuous decline for the remaining days. In this case, neither the initial overshoot  
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47 736 nor the subsequent drop of antimicrobial activity are as pronounced as in the previous study,  
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49 737 probably owing to the lower relative humidity existing in the storage room (30 % vs. 70 %),  
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51 738 although the performance of the active packages still diminishes quite rapidly with the course of  
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53 739 the preservation time, in agreement with what was observed in the mentioned work.  
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2 740 Nevertheless, the addition of only 2% of bentonite nanoparticles into the EVOH active layer  
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4 741 noticeably improved both the release rate of the embedded compound and the retention capacity  
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6 742 of the carrier matrix. In effect, as **Figure 5** shows, the peak concentration of carvacrol in the  
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8 743 headspace of the packages, which mainly depends upon the amount of substance finally retained  
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10 744 in their active layer, is about 21% higher in those containing the nanocomposite material than in  
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12 745 the original ones. In addition, since this difference is maintained or even increased during the  
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14 746 preservation time, up to 39% at the end of the storage period, it means that the release rate of the  
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16 747 active compound must also be slower in those systems. This effect can be visualized better in the  
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18 748 inset graph, where the theoretical curves for the evolution in time of the carvacrol concentration  
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20 749 in the package atmosphere have been normalized to their maximum values, in order to become  
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22 750 comparable from a kinetic point of view. As a result, an average reduction of about 23% in the  
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24 751 slope of the nanocomposite curve is finally observed, demonstrating that the incorporation of  
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26 752 bentonite nanoclay to the carrier matrix not only increases antimicrobial activity in the package  
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28 753 headspace, by reducing its losses to the external atmosphere, but also contributes to maintaining  
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30 754 it more constant and stable throughout the product's shelf life.  
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2 756 **4. CONCLUSIONS**  
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5 757 In this work, diverse passive, active, and nanocomposite multilayer films, selected from the  
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7 758 materials developed in previous studies, were subjected to several physicochemical tests in order  
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10 759 to demonstrate their suitability for food packaging applications. In detail, the properties  
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12 760 investigated were: the stability of EVOH coatings on PP and PET substrates pretreated with  
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14 761 different anchorage technologies; the mechanical, optical, surface, and barrier properties of active  
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16 762 multilayer films, consisting of EVOH coatings on PP and PET substrates containing essential oils or  
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18 763 their constituents as natural antimicrobials, as well as the retention of these compounds in their  
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20 764 carrier matrices; and lastly, the effects of a matrix modification, based on the addition of a 2% of  
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22 765 bentonite nanoclay into an EVOH active layer, on the performance of PP / EVOH packages  
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25 766 containing carvacrol as active agent, and assayed in actual working conditions.

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29 767 The results obtained indicated the application of corona discharge followed by a PEI-based primer  
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31 768 as the best anchorage technology available to bond EVOH coatings with PP or PET substrates. With  
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33 769 respect to the active agents incorporated, their retention into EVOH matrices ranged from low to  
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35 770 moderate, depending upon the substance included and upon the substrate employed.  
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38 771 Nevertheless, the final manufactured films showed a substantial improvement in their wettability  
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40 772 characteristics, and no noticeable or important differences in their mechanical, optical, or barrier  
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42 773 properties. The inclusion of bentonite nanoparticles into EVOH active coatings, in turn,  
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44 774 considerably enhanced the performance of the preservation system, by increasing its retention  
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46 775 capacity for the embedded compounds, while reducing their release rate to the package  
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48 776 headspace. In addition, the surface and mechanical properties of these nanocomposite materials  
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51 777 were also slightly improved, whereas their optical appearance stayed practically the same.  
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56 778 In conclusion, all the developed active films are perfectly suitable for food packaging applications,  
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58 779 and the incorporation of bentonite nanoclay to their carrier layers is strongly recommended, as to  
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2 780 improve their physical properties as much as to increase the performance of the targeted  
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4 781 packages, although some additional assays might be then necessary to confirm that the current  
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6 782 legal thresholds for the migration of inorganic nanoparticles were never exceeded.  
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13 784 **ACKNOWLEDGMENTS**

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2 883 **Table 1. Qualitative results of the adhesion and thermosealability tests for the four anchorage**  
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4 884 **technologies applied on the multilayer films, and sealing strength on uncoated substrates and**  
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6 885 **on those coated with EVOH through the application of corona discharge + PEI-based primer**  
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Anchorage technology	Adhesion	Thermosealability
UV radiation	weak	none
Corona discharge	moderate	poor
Corona discharge + PU-based primer	none	none
Corona discharge + PEI-based primer	strong	good

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Sealed films	Sealing strength (N)	Type of failure
PET // PET	5.7 ± 0.9	Seal opening
PET / E // E / PET	12.1 ± 2.4	Seal opening + delamination
PP // PP	8.0 ± 1.0	Seal opening
PP / E // E / PP	21.6 ± 3.0	Seal opening

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1  
2 888 **Table 2. Initial and nominal concentrations of the active agents in the coating layers of the**  
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4 889 **multilayer films, and efficiencies of the incorporation process**  
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Active multilayer film	$C_{i_0}^F$ (%)	$C_{i_n}^F$ (%)	$E_i^F$ (%)
PP / E + CI	$4.3 \pm 0.4$	10	$43 \pm 4$
PP / E + MO	$4.6 \pm 0.2$	7.5	$62 \pm 3$
PP / E + CA	$3.8 \pm 0.1$	7.5	$50 \pm 1$
PP / E + B + CA	$4.6 \pm 0.2$	7.5	$61 \pm 3$
PET / E + CI	$4.6 \pm 0.3$	10	$46 \pm 3$
PET / E + CO	$3.0 \pm 0.6$	7.5	$40 \pm 8$

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2 891 **Table 3. Equilibrium concentrations of the active agents in the coating and substrate layers of**  
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4 892 **the multilayer films, as derived from the mass balance depicted in equation (3), and their**  
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6 893 **partition coefficients between both materials**  
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Active multilayer film	$K_i^{C/S}$	$C_{i\infty}^C$ (%)	$C_{i\infty}^S$ (%)
PP / E + Cl	$1.5 \pm 2.1$	0.71	0.47
PP / E + MO	$42.4 \pm 2.9$	5.10	0.12
PET / E + Cl	$1260 \pm 240$	9.76	0.01
PET / E + CO	$6.0 \pm 5.6$	1.22	0.20

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895 **Table 4. Oxygen and carbon dioxide permeances in the passive multilayer films and their**  
 896 **substrates as a function of the relative humidity**

Films	$\rho_{O_2}^F \cdot 10^{15} \text{ (m}^3 \text{ (STP) / (m}^2 \cdot \text{s} \cdot \text{Pa))}$				
	0% RH	35% RH	50% RH	75% RH	90% RH
PP	$148 \pm 2$				
PP / E	$0.85 \pm 0.03$	$1.49 \pm 0.08$	$3.30 \pm 0.13$	$34.8 \pm 0.2$	$112 \pm 2$
PP / E + B	$0.86 \pm 0.05$	$0.76 \pm 0.06$	$0.79 \pm 0.08$	$12.5 \pm 0.1$	$111 \pm 1$
PET	$4.8 \pm 0.2$				
PET / E	$0.76 \pm 0.07$	$0.84 \pm 0.09$	$1.20 \pm 0.09$	$2.98 \pm 0.06$	$3.83 \pm 0.03$

Films	$\rho_{CO_2}^F \cdot 10^{15} \text{ (m}^3 \text{ (STP) / (m}^2 \cdot \text{s} \cdot \text{Pa))}$					
	0% RH	30% RH	45% RH	75% RH	85% RH	100% RH
PP	$1170 \pm 40$					
PP / E	$7.8 \pm 0.2$	$9.83 \pm 0.08$	$11.4 \pm 0.1$	$48.8 \pm 0.5$	$146 \pm 2$	$247 \pm 2$
PP / E + B	$7.61 \pm 0.19$	$7.71 \pm 0.17$	$11.0 \pm 0.3$	$30.5 \pm 1.0$	$129 \pm 2$	$234 \pm 15$
PET	$56.3 \pm 0.8$					
PET / E	$9.36 \pm 0.18$	$10.2 \pm 0.9$	$11.2 \pm 0.2$	$30.7 \pm 0.4$	$35.2 \pm 0.5$	$39.4 \pm 0.3$

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2 899 **FIGURE CAPTIONS**  
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5 900 **Figure 1.** Static contact angles of water droplets on the surfaces of the multilayer films.  
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8 901 **Figure 2.** Lightness of the multilayer films on the CIELAB color space.  
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11 902 **Figure 3.** Chroma and hue of the multilayer films on the CIELAB color space.  
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14 903 **Figure 4.** Ultimate tensile strength, deformation at break, and Young's modulus of the multilayer  
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17 904 films. Letters correspond to homogeneous subsets of means with significant differences.  
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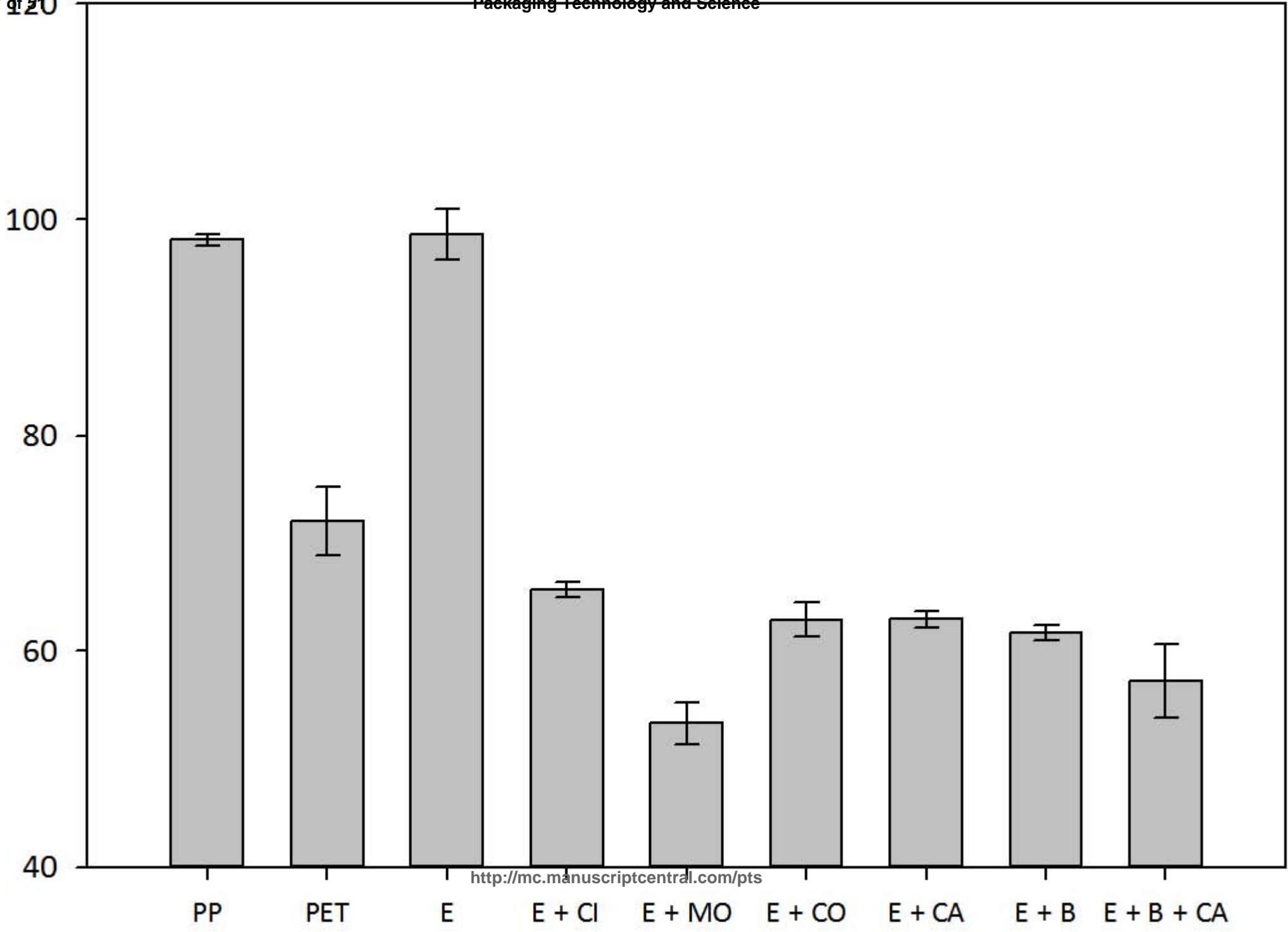
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20 905 **Figure 5.** Evolution in time of the carvacrol concentration in the headspace of packages containing  
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23 906 a neat or nanocomposite EVOH active layer. Symbols correspond to experimental values and  
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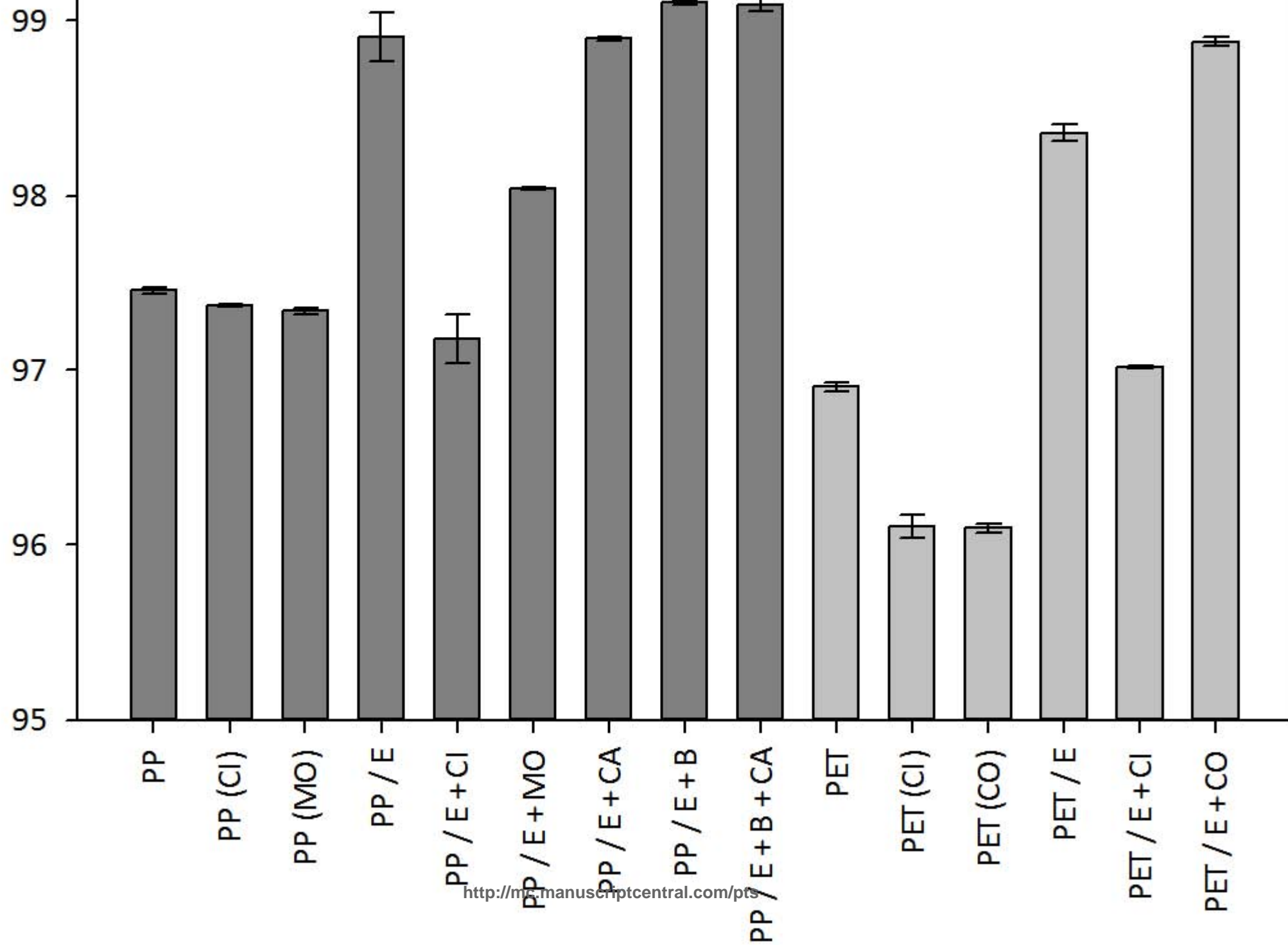
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26 907 curves correspond to theoretical performances predicted by the mathematical model. **Inset graph:**  
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29 908 normalized curves of the theoretical performances of the active packages in full time scale.  
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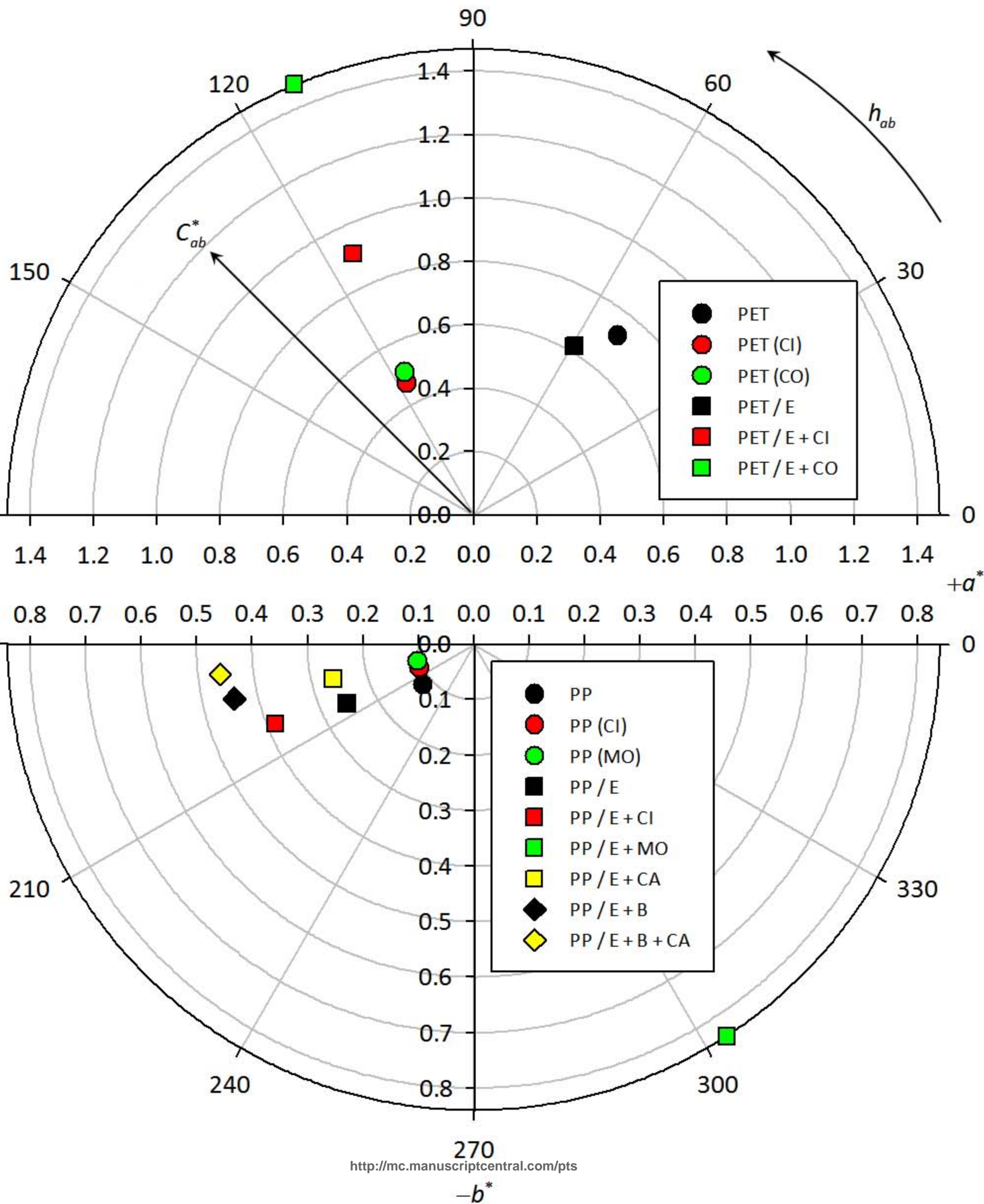
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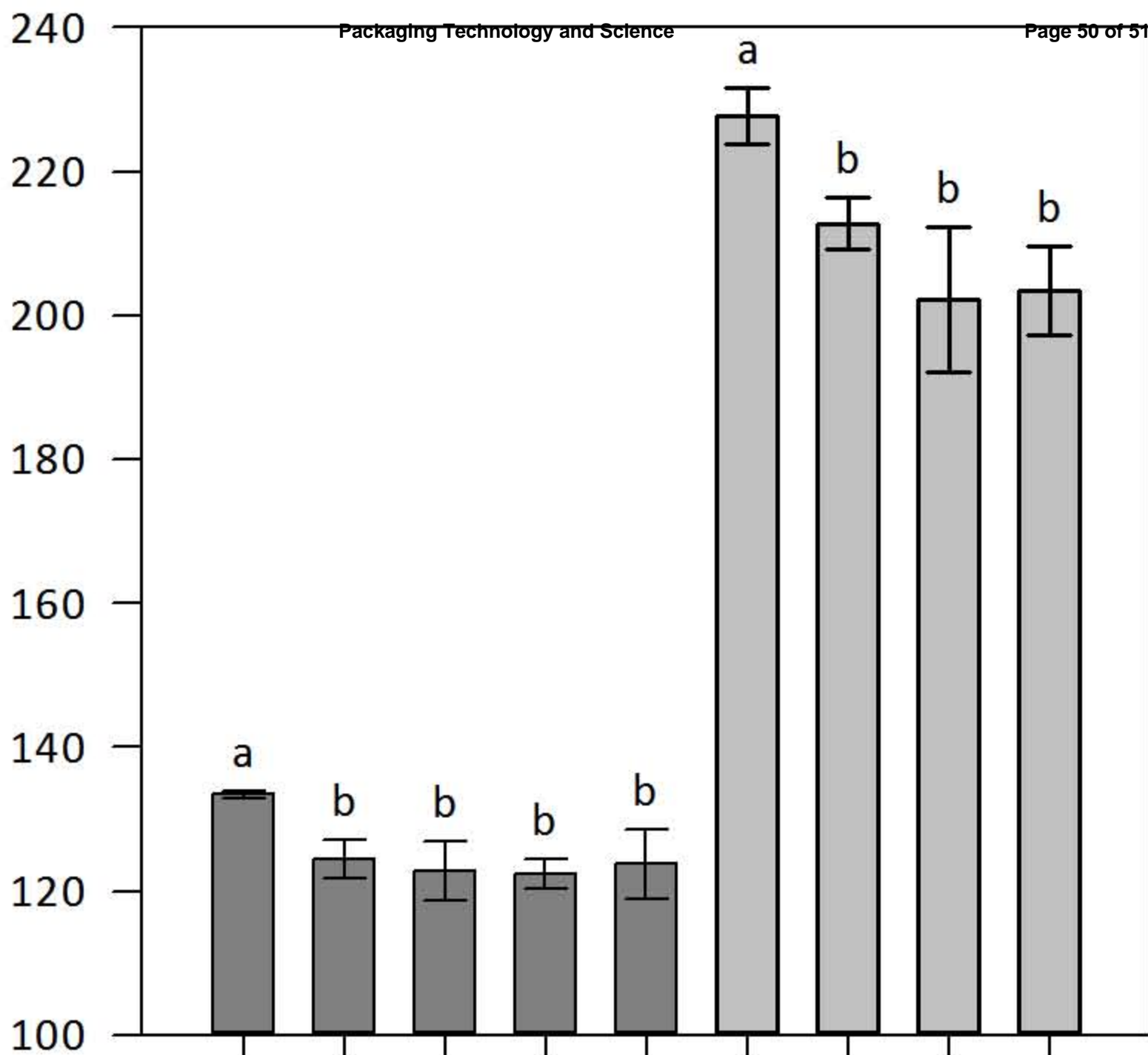




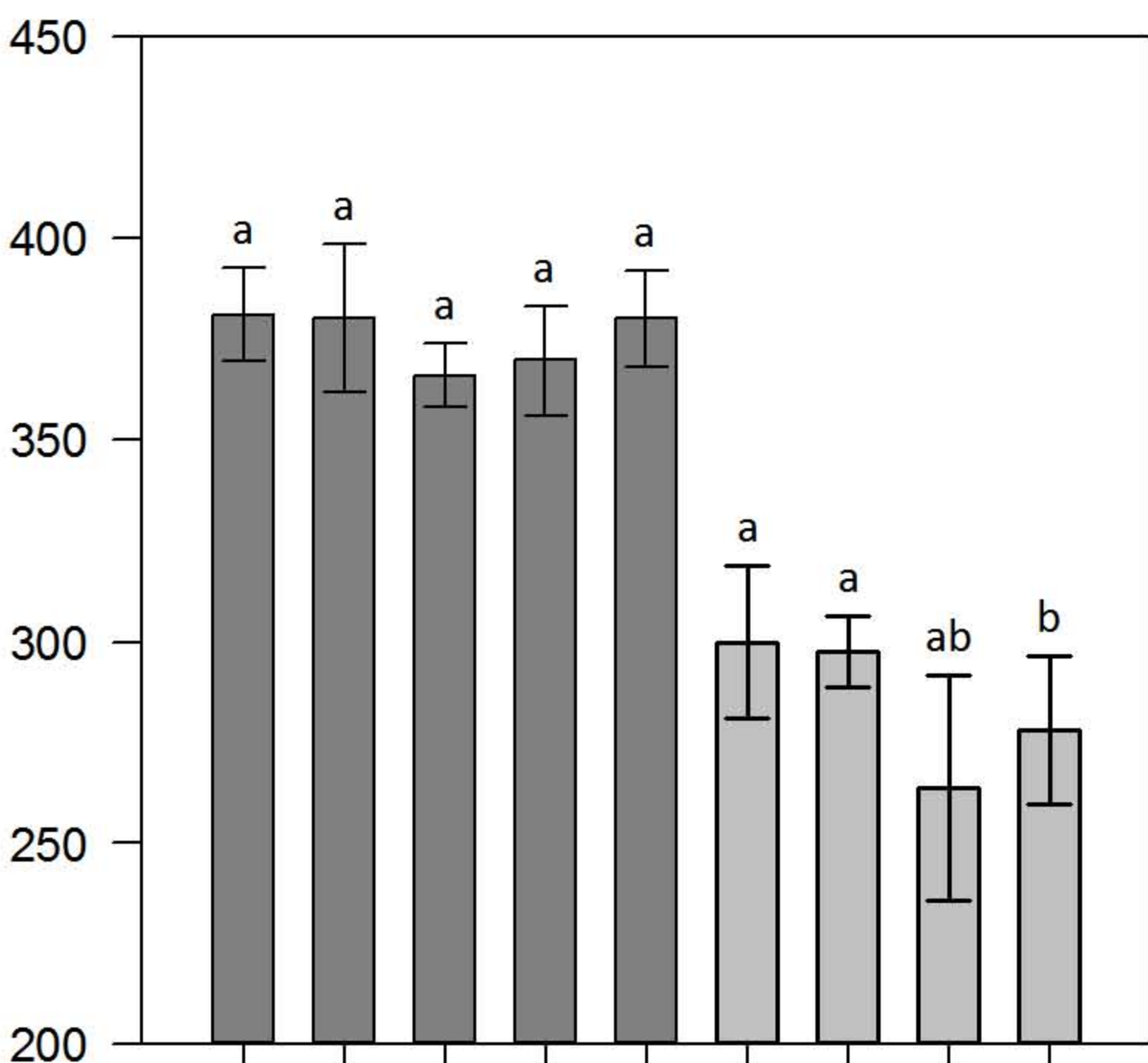
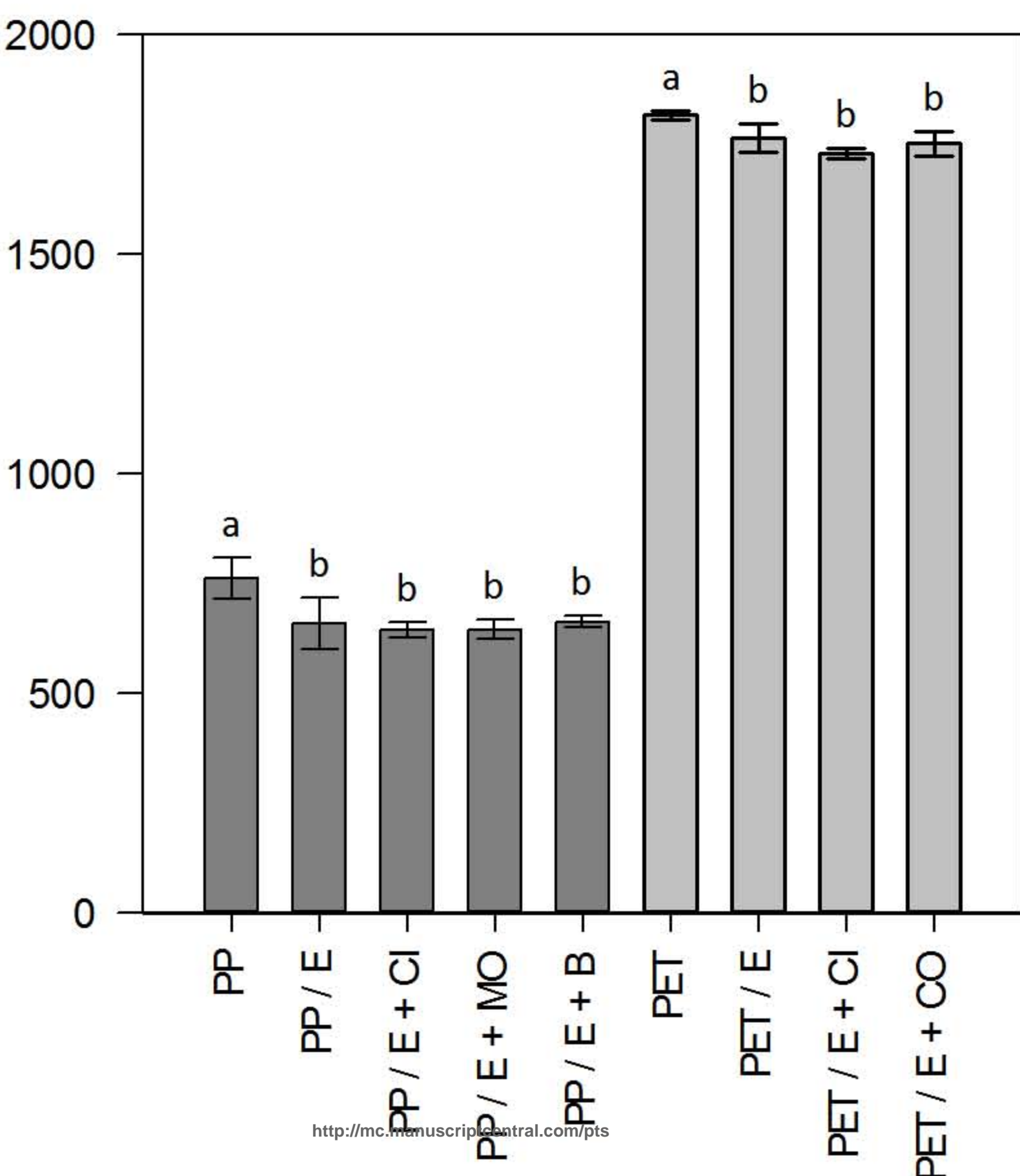
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60Tensile strength (N / mm<sup>2</sup>)

Deformation at break (%)

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