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ACCEPTED MANUSCRIPT

New active antioxidant multilayer food packaging films containing Algerian Sage and Bay leaves extracts and their application for oxidative stability of fried potatoes.

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ABSTRACT

The antioxidant activity of Sage leaf (SL) and Bay leaf (BL) extracts was studied. Both plants were extracted using water and ethanol at different concentration, and the antioxidant activity was measured by ABTS [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation scavenging and reducing power (RP) methods. In both cases 60 % and 80 % ethanolic extracts of Sage and Bay leaves showed the highest activity and were incorporated into multilayer films. The initial concentration for 60 % ethanolic extracts of Sage and Bay leaves to scavenge 50 % of free radical ABTS were 5.67 ± 0.26 $\mu g \times mL^{-1}$ and 18.68 ± 0.16 $\mu g \times mL^{-1}$ respectively, whereas for 80 % ethanolic extracts the concentrations were 7.96 ± 0.02 and 14.65 ± 0.59 $\mu g \times mL^{-1}$ respectively. The initial concentrations of ethanolic 60 %

extracts of Sage and Bay leaves to allow absorbance 0.5 for reducing power were 35.38 ± 0.19 µg × mL^{-1} and 91.43 ± 2.84 µg × mL^{-1} respectively, while for 80 % ethanolic extracts of Bay and Sage leaves were 46.01 ± 1.21 µg × mL^{-1} and 85.47 ± 0.9 µg × mL^{-1} respectively. Then, the multilayer films were exposed to a gas stream enriched with free radicals to evaluate the free radicals scavenging. The new packaging with 60 % ethanolic Sage extract exhibited the highest activity with low percentage of hydroxylation (69.64 ± 6.86 %) followed by that with 80 % ethanolic extract for both Bay (85.49 ± 5.3 %) and Sage (87.09 ± 3.93 %) leaves extracts. The ability of two active packaging built with 60 % ethanolic Sage extract and 80 % ethanolic Bay extract to inhibit lipid oxidation of fried potatoes was studied by measuring secondary lipid oxidation products using thiobarituric acid reactive substances (TBARS). Significant lower value of Malondialdehyde (MDA) was obtained for fried potatoes stored in active packaging built with ethanolic 60 % extract of Sage and 80 % ethanolic extract of Bay leaves (0.342 ± 0.01 and 0.392 ± 0.02 µg MDA × g^{-1} respectively) at 40 °C for 20 days compared to the control (0.568 ± 0.03 µg MDA × g^{-1}). Lipid oxidation decreased 40 % and 31 % for packaging with 60 % Sage and 80 % Bay ethanolic extracts respectively. The UPLC–MS–QTOF analysis of Sage and Bay leaves extracts revealed the presence of phenolic acids, tannins, flavonoids, and terpenoids. Migration tests from active materials demonstrated the absence of migration.

KEYWORDS

Bay leaf, Sage leaf extract, fried potatoes, antioxidant multilayer, lipid oxidation, non-migrating active packaging.

1. INTRODUCTION

Food deterioration is responsible for the loss of quality and safety and it can occur during production, distribution, processing or storage. Lipids are naturally found in most biological

materials consumed as food products and are also added as ingredients to many processed foods (López-De-Dicastillo, Gómez-Estaca, Catalá, Gavara, & Hernández-Muñoz, 2012). Lipid oxidation is the main cause of food deterioration, limiting considerably the shelf life of many products (Contini et al., 2014; Nerín, Tovar, & Salafranca, 2008). It is also responsible for the development of unpleasant odor, rancid taste and discoloration, generating compounds like aldehydes and derivatives, such as Malondialdehyde (MDA), which can be harmful to health. Malondialdehyde is the major product of lipid peroxidation, and has been used as an index marker of oxidative reaction (Guyon, Meynier, & de Lamballerie, 2016; Katja M. Fisch, Volker Böhm, Anthony D. Wright, *, & König†, 2003; Nakamura, Watanabe, Miyake, Kohno, & Osawa, 2003).Due to the great economic impact of this phenomenon, the food industry is constantly looking for new methods to reduce the effects of oxidation (López De Dicastillo et al., 2011). Antioxidants are the most efficient way to delay lipid oxidation (Roby, Sarhan, Selim, & Khalel, 2013; Shah, Bosco, & Mir, 2014).

According to the European Union Legislation ("Directive 2006/52/EC of the European Parliament and of the Council of 5 July 2006"), several antioxidants are authorized to be used in foods, most of them synthetic substances such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertbutylhydroquinone and propyl gallate, but their use was only authorized in processed food. Despite the wide use of BHA and BHT by the food industry to inhibit lipid oxidation (Mohdaly, Sarhan, Mahmoud, Ramadan, & Smetanska, 2010; Shah et al., 2014), concerns about the safety and adverse effects of synthetic antioxidants, together with a growing demand for healthier products, have promoted the interest in natural antioxidants (Shahidi & Zhong, 2010).

Hence, the use of natural antioxidants is emerging as an effective alternative for product preservation (Sabeena Farvin, Grejsen, & Jacobsen, 2012). To this end, many sources of natural antioxidants have been investigated, such as herbs, plants, fruits and apiculture

74 products (ARAI et al., 2002; Bandoniene, Murkovic, & Venskutonis, 2005; Karre, Lopez, &
75 Getty, 2013).

76 Food companies are constantly looking for plant extracts with high antioxidant power in order
77 to develop new natural products which can fulfil the growing demands of consumers. The
78 direct addition of natural antioxidants into product formulations is challenging because they
79 tend to be less potent than synthetic additives and therefore must be added in larger amounts,
80 which may change the organoleptic properties of the product, such as colour, flavour, taste or
81 viscosity. In order to overcome this challenge, researchers started to incorporate them into
82 packaging materials. The development of antioxidant active packaging systems is attracting
83 considerable attention as one of the preferred emerging technologies to reduce lipid per-
84 oxidation (Carrizo, Gullo, Bosetti, & Nerín, 2014; Echegoyen & Nerín, 2015; Nerín et al.,
85 2008; Nerín et al., 2006). These materials can work by scavenging free radicals from the
86 product or from the internal atmosphere. In both cases, this kind of packaging does not
87 require direct contact to the foodstuff to exhibit antioxidant properties (Nerín et al., 2006;
88 Roman, Decker, & Goddard, 2016; Wrona, Bentayeb, & Nerín, 2015). Some studies have
89 already demonstrated the potential of antioxidant food packaging containing rosemary or
90 oregano extracts, for instance to enhance the stability of both myoglobin of fresh meat (Nerín
91 et al., 2008; Nerín et al., 2006), to avoid the lipid oxidation of brined sardines achieved by a
92 tea extract active packaging (López-De-Dicastillo et al., 2012); or cut fresh nectarine in a
93 packaging containing green tea (Colon & Nerin, 2012; Colón & Nerín, 2015). Also, lipid
94 oxidation was reduced by a citrus active packaging in cooked turkey meat (Contini et al.,
95 2014), and by rosemary active packaging in pork patties (Bolumar, Lapeña, Skibsted, &
96 Orlien, 2016).

97 Sage (*Salvia officinalis* L.) is one of the most popular medicinal plant, a well-known spice
98 and flavouring agent, used not only in the food industry but also applicable in the area of

human health. The leaves of Sage are well known for their antioxidant properties (Zhang, Lin, Leng, Huang, & Zhou, 2013). Bay (*Laurus nobilis* L.) commonly known as laurel, sweet bay and bay, has attracted continuous and renewed interest because of its pharmacological and health beneficial properties related to several compounds present in the plant (Dall'Acqua et al., 2009). The antioxidant property of Bay has been demonstrated by several studies, mainly attributed to its phenolic compounds (Dall'Acqua et al., 2009; Muñiz-Márquez et al., 2013; Simić, Kundaković, & Kovacević, 2003).

The principal goal of this work was the evaluation of the antioxidant activity of new active packaging materials containing the extracts of Bay and Sage leaves. First, the bioactive molecules were identified and the antioxidant capacity of the extracts was measured. Secondly, the packaging materials were built. Afterwards, the antioxidant activity of active packaging was tested *in vitro* by the free radicals scavenging method. Then, the capacity of antioxidant active packaging to prevent lipid oxidation of fried potatoes was evaluated.

2. MATERIALS AND METHODS

2.1 Reagents

Hydrogen peroxide (30%, CAS 7722-84-1); sodium salicylate (>99.5%, CAS 54-21-7); 2,5-dihydroxybenzoic acid (>99%, CAS 490-79-9); acetic acid (≥99.8%, CAS 64-19-7); sodium acetate trihydrate (≥99.5%, CAS 6131-90-4); trichloroacetic acid (TCA, minimum 99%, CAS 76-03-9); thiobarbituric acid (TBA, 98%, CAS 504-17-6) ; malonaldehyde bis(diethyl acetal) 1,1,3,3-tetraethoxypropane (CAS 122-31-6); and ABTS^{TM} (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (CAS 30931-67-0) were supplied by Sigma-Aldrich (Germany). The plants were delipidated by using hexane (96%, CAS 110-54-3) from Scharlab (Spain) and filtered with a sintered glass (porosity 4). Ethanol absolute (>99,8%, CAS 64-17-5) and HCl (Hydrochloric acid solution) CAS 7647-01-0 were provided

123 by Sigma-Aldrich (Spain). Methanol (LC-MS, CAS 67-56-1); ethanol absolute (HPLC grade,
124 CAS 64-17-5); ortho-phosphoric acid (85% reagent grade, CAS 7664-38-2) and sodium
125 hydroxide (0.01 moL × L^{-1}; CAS 1310-73-2) were purchased from Scharlab (Spain).
126 Ultrapure water was obtained from a Millipore Milli-Q_{PLUS} 185 system (Madrid, Spain).
127 Compressed air was supplied by a Cecatto Bluair compressor (Brendola, Italy).

128 2.2. Plant Material and sample preparation

129 Bay Leaf (BL) and Sage Leaf (SL) were both randomly collected from Akfadou, Bejaia
130 (Algeria). The leaves of both plants were cleaned with tap water, then they were air dried for
131 5 weeks until the water content of the leaves was less than 10 %. Dried leaves were ground by
132 electric grinder and sieved (Retsch Analytical sieve shaker AS 200) to obtain a mean particle
133 size below 0.5 mm. The powder was stored in airtight glass containers in the dark until use.

134 2.3. Preparation of the extracts

135 Distilled water (W) and 60 %, 80 % and absolute ethanol were used as solvents for extraction
136 of Bay leaves (BL) and Sage leaves (SL). As a first step, Sage and Bay leaves were
137 delipidated with hexane using soxhlet apparatus. After this, the extraction was carried out
138 following the method described by (Oomah, Corbé, & Balasubramanian, 2010) but with some
139 modifications. Briefly, 1 g of the delipidated samples was extracted using 40 mL of water or
140 ethanol at different concentration (60 %, 80 % and absolute ethanol), leaving them at a
141 constant agitation for 2 h at room temperature. The extracts were then filtered using a sintered
142 glass under vacuum and centrifuged at 4500 g for 30 min. After that, the supernatant was
143 concentrated under vacuum at 40 °C using a rotary evaporator and lyophilized. The powdered
144 extracts of each plant were stored in a microtube at 4 °C until further use, and referred as
145 follows: BL60 and SL60 (Sage and Bay leaves extracted with 60 % ethanol), BL80 and SL80

(extracted with 80 % ethanol), BL100 and SL100 (extracted with absolute ethanol), and BLW and SLW (extracted with distilled water).

For the ABTS, reducing power and free radical scavenging tests the lyophilized extracts were dissolved in methanol, and filtered using a 0.22 μm nylon syringe filter.

2.4. Apparatus and equipment

A IEC HN-SII centrifuge, International Equipment Company (Needham Heights, MA, USA) was used after the plant extraction. The extracts were analyzed by an UPLC Acquity™ system coupled to an ESI probe to a Xevo G2 QTOF (Time-of-flight mass spectrometer) supplied by Waters (Milford, MA, USA). A UPLC BEH C18 column of 1.7 μm particle size (2.1 × 100 mm) also from Waters (Milford, MA, USA) was used for the separation of the compounds. Chromatographic and MS data were processed by MassLynx (v. 4.1) software (Waters).

Due to the fact that the experimental section was carried out in two different laboratories, two different spectrophotometers were used. Hence, a UV-920 (Biotech Engineering Management) was employed for the ABTS radical scavenging and the reducing power assay (RP), and a UV-1700 (Shimadzu Pharmaspec Ibérica, Madrid, Spain) for the rest of antioxidant methods.

In order to perform the multilayer active materials, a coating machine (KK coater, RK print) was used and afterwards, the packaging was passed through a laminator (Bio 330). These active materials were employed to form plastic bags, using for this purpose a thermo sealer (PFS-200, Zhejiang Dongfeng Packing Machine Co., Wenzhou, Zhejiang, China).

A free radical scavenging system using the method and device developed by Pezo, Salafranca, & Nerín, (2008) was used to measure the antioxidant properties of both the extracts and the packaging material. In short, the system generates an atmosphere enriched with free radicals

that passes through the packaging material, and afterwards bubbles into a salicylic acid (SA) solution. This solution is hydroxylated due to the free radicals, producing two fluorescent compounds. If the material scavenges the free radicals, these do not arrive at the SA solution and the fluorescent compounds are not formed. Then, hydroxylation percentage is a quantitative measurement of the scavenging properties in such a way that 100 % hydroxylation means that the material is not antioxidant, and the opposite happens with 0 % hydroxylation. The details and the description of the procedure and device can be read in Pezo *et al.* (2008). The final solution is analyzed by high-performance liquid chromatography (HPLC) (Alliance 2695 Separations Module (Waters, Milford, MA, USA) with a 474 Scanning Fluorescence Detector (Waters, Milford, MA, USA). A Waters reversed phase (RP) column (100 mm long, 4.6 mm i.d., 3 µm) Atlantis dC18 was used.

2.5. Antioxidant packaging material

A multilayer film composed by 12 µm of polyethylene terephthalate (PET) and 35 µm low density polyethylene (LDPE) was used. Both layers were attached by using an aqueous adhesive approved to be employed in food packaging materials. In this way, the extracts of Bay and Sage leaves were prepared at the same concentration by using isopropanol and then incorporated into the adhesive formula at 10 % (w/w). This adhesive formula (with or without extract) was spread on the PET sheet using the coating machine. After air dried of the solvent, the LDPE sheet was overlapped to the PET layer. Afterwards, the packaging materials (fig.1) were passed through a laminator and the final concentration of the extracts into the packaging was calculated (table 1).

Details about the adhesive formula cannot be disclosed here for confidentiality reason. The same kind of multilayer materials but without the active compounds was used as control packaging.

194 In all assays whether for extract or for active packaging, blank samples containing the same
195 solvent used in the extract samples was measured. By doing this, we can confirm that the
196 antioxidant activity achieved was due to the active substances.

197 2.6. Identification of bioactive molecules by UPLC–MS -QTOF

198 The constituents of different extracts of Bay and Sage leaves were analyzed by UPLC–MS -
199 QTOF. The different powdered extracts of both Bay and Sage were solubilized in ethanol
200 (60 %, 80 % and absolute ethanol) and ultrapure water at the initial conditions of the
201 extraction (WBL: 4.16 mg × mL^{-1}, BL60: 7.81 mg × mL^{-1}, BL80: 6.89 mg × mL^{-1}, BL100:
202 3.89 mg × mL^{-1}, SLW: 4.65 mg × mL^{-1}, SL60: 6.02 mg × mL^{-1}, SL80: 5.25 mg × $mL^{-1}$and
203 SL100: 2.01 mg × mL^{-1}). The samples were previously filtered through a 0.22 µm nylon
204 membranes (Millipore) and injected. The injection volume was 10 µL. Chromatography was
205 carried out at 0.4 mL × min^{-1} column flow and 40 °C column temperature. The mobile phase
206 was water with 0.1 % formic acid (phase A) and methanol with 0.1 % formic acid (phase B).
207 Chromatography started at 98/2 phase A/phase B (1 min), changed to 0/100 in 6 min and
208 stayed at 0/100 during 2 minutes. Electrospray probe (ESI) in positive ionization mode was
209 selected. Samples were injected by triplicate.

210 2.7. ABTS radical scavenging assay

211 The evaluation of the scavenging capacity of the radical ABTS by the extracts was carried out
212 according to the method of Re et al., (1999). To prepare the ABTS radical, a solution of
213 ABTS (7 mmol × L^{-1}) was mixed with a solution of potassium persulfate 2.45 mmol × L^{-1}
214 (final concentration). This mixture was allowed to react for 12-16 h in the dark, after which it
215 was diluted with ethanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm. A 100 µL aliquot
216 of the extracts at different concentrations (50-8000 µg × mL^{-1}) were allowed to react with
217 1000 µL of the ABTS solution for 7 min in the dark. The absorbance of blue/green ABTS

chromophore was measured at 734 nm. The results were expressed as initial concentration ($\mu g \times mL^{-1}$) of extract in the reaction medium, which reduces 50 % of ABTS, and IC50 was calculated from the graph, plotting the percentage of ABTS reduction against the extract concentration. The percentage of ABTS scavenging activity was calculated using the following formula:

$$\% \text{ ABTS scavenging activity} = \left[\frac{Ac - As}{Ac}\right] \times 100$$

Where Ac is the absorbance of the control and As is the absorbance of the sample.

Control: contain all reagents with solvent instead of the extract

2.8. Reducing power (RP) assay

The method described by Oyaizu (1986) was used to measure the reducing power of the extracts. Briefly, 200 µL of different concentrations (40-4000 $\mu g \times mL^{-1}$) of extracts were mixed with 500 µL of 0.2 M phosphate buffer (pH 6.6) and 500 µL of 1 % aqueous potassium ferricyanide solution [$K_3Fe(CN)_6$]. The mixture was incubated at 50 °C for 20 min, adding 500 µL of an aqueous solution of trichloroacetic acid (TCA at 10 %). After centrifugation (1000 g/10 min), a 1 mL aliquot of the supernatant was mixed with an equal amount of distilled water and 200 µL of ferric chloride $FeCl_3$ (0.1 %, w/v). The absorbance was read at 700 nm using a spectrophotometer, where a high absorbance indicates a high reducing power. The results were expressed as initial concentration ($\mu g \times mL^{-1}$) of the extract in the reaction medium, being the absorbance 0.5 for the reducing power (Jabri-Karoui et al., 2012) and IC50 was calculated from the 700 nm graphs versus the concentration of the extract.

2.9. Characterization of the packaging colour

The colour of the active packaging materials containing Bay and Sage leaves extracts, as well as the Control material was determined by performing a scanning in the visible range between

400-700 nm. For both plants, the packaging materials (2 cm × 3 cm) containing ethanolic extracts at 80 % were used (AP-BL80 and AP-SL80). The absorbance of each packaging was measured three times by using a spectrophotometer.

2.10. Free Radical scavenging assay

The antioxidant capacity not only of the selected extracts but also of the active packaging was determined. For this purpose, the same methodology applied in previous works was applied (Pezo, Salafranca, & Nerín, 2006; Pezo, Salafranca, & Nerín, 2008). The antioxidant capacity were measured in a different way according to the type of sample. That is, the pure extracts were evaluated by using Pasteur pipettes containing glass wool to which 10 µL (2000 µg × mL^{-1}) of each extract was added. As control, the same pipette with glass wool but without active substance (just with the solvent used for the extract) was employed. In the case of active materials, bags with internal dimensions of 15 cm × 15 cm were built from the multilayer active material and thermosealed at 180 °C for a short time needed to seal the bags under manual force, being the LDPE layer the inner side of the bag. The same material but without plant extract was used for the control bags. In both cases, the method was the same as the one followed by Pezo, Salafranca, & Nerín, (2008), but our work samples were kept 48 h instead of 24 h, after which the residual salicylic acid was measured by HPLC coupled to a fluorescence detector. The mobile phase was a mixture of aqueous acetate buffer (35 mmol × L^{-1}, pH 5.8, and 1.0 mL × min^{-1}) and methanol, 90:10 (v/v). The injection volume was 10 µL. Excitation and emission wavelengths were set at 324 and 448 nm, respectively.

The 2,5-DHB formed was quantified using a calibration curve of 2,5-hihydroxybenzoic acid and the results were expressed as ng × mL^{-1} (ppb) of 2,5-DHB. Radical scavenging activity was calculated as a percentage as follows: $OH\% = \left[\frac{A}{Ac}\right] \times 100$,

Where OH % is the percentage of hydroxylation, A (active film) the area value of 2,5-DHB using the active material, and Ac the area value of 2,5-DHB of the control material.

2.11. Specific migration analysis by UPLC-MS-QTOF

The migration test was carried out by using 10 % ethanol as food simulant. For the experiment, bags of 10 x 6 cm with the active materials described in previous sections were built and thermosealed. They were filled in with the simulant following the ratio 6 $dm^2 \times kg^{-1}$ foods, keeping the samples at 40 °C. After 10 days of incubation, the content of the bags was analyzed by UPLC-MS-QTOF. All samples were prepared in triplicate, and all the concentrations were calculated according to the ratio 6 dm^2 of packaging material per 1 kg of simulant, in accordance with the legislation for food contact materials EU/10/2011 ("Commission Regulation (EU) Nº 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food").

2.12. Effect of active packaging on lipid oxidation of fried potatoes

Due to the results obtained in the ABTS and the RP experiments, Sage leaf ethanolic extract at 60 % (AP-SL60) and Bay leaf ethanolic extract at 80 % (AP-BL80) were chosen to build the active packaging materials to study the lipid oxidation of fried potatoes. Unpackaged fried potatoes were bought in a corner shop. Bags of 4 cm × 4 cm were made with both the active and control materials, filled in with 10 g of fried potatoes then thermosealed (fig. 2) and incubated at 40 °C for 20 days, to accelerate the oxidation process.

The oxidation of fried potatoes was evaluated by the spectrophotometric method of thiobarbituric acid reactive substances (TBARS). Hence, analysis of TBARS was performed according to the procedure described by Pfalzgraf, Frigg, & Steinhart, (1995).

Ten grams of fried potatoes were homogenized with 40 mL of trichloroacetic acid (TCA 10 %) and then filtered through a Whatman N°.1 filter paper. Then, 2 mL of the filtrate was

introduced into a test tube, adding 2 mL of TBA (fresh solution 20 mM) and homogenized. Tubes were heated in a silicon bath at 97 °C for 20 min and then cooled at room temperature. The absorbance of the solution was measured at 531 nm against a blank containing 2 mL of TCA 10 % and 2 mL of TBA. The results were expressed as µg MDA × g^{-1} fried potatoes using a calibration curve prepared with 1, 1, 3, 3-tetramethoxypropane.

2.13. Statistical analysis

All extractions and determinations were carried out in triplicate. Data were expressed as mean ± standard deviation (SD). The means were compared by using the one-way and two way anova analysis of variance (ANOVA) with a post-hoc "LSD test", using STATISTICA 5.5 to determine significant differences. Significant differences were considered at $P < 0.05$ level. The IC50 value was calculated from the Prism dose–response curve by using Graphpad prism5.

3. RESULTS AND DISCUSSION

3.1. Identification of bioactive molecules by UPLC-MS-QTOF

The extracts of both plants obtained with the four solvents previously described were analyzed using UPLC-MS-QTOF. As can be seen in table 2, up to 68 chemical compounds were identified, due to the diverse composition found among both plants.

As expected, due to the fact of being herbs, the extracts of Bay leaf showed 28 compounds corresponding to phenolic compounds and terpenoids, indicating a considerable variation among them. Flavonols such as quercetin and kaempferol and phenylterpenoides such as nimbolinin D were identified in the ethanolic (BL100) and hydroethanolic (BL60, BL80) extracts, whereas 3-dodecyldihydro-2,5-furanedione was only found in the hydroethanolic (BL60, BL80) extracts. The cinnamtannin B1 (condensed tannin), (-)-andrographolide (diterpene lactone) was found in the water extract (BLW) and 60 % ethanolic extract (BL60).

Also, 2,6-di-tert-butyl-4-hydroxymethylphenol was found in water (BLW) and hydroethanolic (BL60, BL80) extracts of Bay leaf, and this compound was not previously identified in Bay. Phenacetin aromatic compound was found in water extract of bay leave and 4-Acetyl-4-(ethoxycarbonyl) heptanedioic acid was found only in water Bay leaf extract (BLW), whereas, nimbolinin D was obtained in the three ethanolic extracts (BL60, BL80, BL100).

Previous studies reported the content of phenolic acids, flavonoids and rutin in Bay leaves (Lu, Yuan, Zeng, & Chen, 2011). Muchuweti et al., (2007), confirmed the presence of caffeic, ferulic and vanillic acids by HPLC in *L. nobilis* extracts. The studies carried out by Dall'Acqua et al., (2009) revealed the presence of cinnamtannin B1, kaempferol-3-O-α-L-rhamnoside in Bay leaf extract (Emam, Mohamed, Diab, & Megally, 2010). Flavonoids such as quercetin, luteolin, apigenin, kaempferol and myrcetin, as well as flavan-3-ols were reported as the most abundant phenolic compounds found in Bay leaves (Dall'Acqua et al., 2009; Lu et al., 2011; Škerget et al., 2005).

In the case of Sage leaf extracts, different substances including phenolic compounds, terpenoids and flavonoids were obtained. As before, a great variation in the composition was seen among the different extracts, highlighting a high number of compounds in the case of 60 % ethanolic extract (SL60) compared to the others (SLW, SL80, SL100).

Several flavonoids were identified in the different extracts: apigenin-6-C-glucoside-7-O-glucoside (SL60, SL80), fluorescein (SL80), luteolin 4'-methyl ether (SL60, SL80), pectolinarigenin (SL60, SL80, SLA), quercetin-3-O-β-D-glucuronopyranoside (SL60, SL80) and pectolinarin (SL80). Some phenolics acids were also found: 4-{2-(Benzyloxy)-6-[(benzyloxy)carbonyl]benzoyl}-3,5-bis(methoxymethoxy) benzoic acid (SLW, S60) and ellagic acid-4-O-β-xyloside-3,3 (SLW, SL60). Also, some volatile and terpenoid compounds were obtained, such as (3,3'-{[3,5Bis(benzyloxy)phenyl]methylene}bis(2-hydroxy-4H-chromen-4-one (SLA), enoxolone (SL60, SL80, SLA), tretinoin (SL60, SL80, SLA),

337 isopropyl 5-(3,3-dimethyl-2-oxobutoxy)-2-methyl-1-benzofuran-3-carboxylate (SLA),
338 umbelliferone (SL60, SL80, SLA), cyclohexene-1,1-diylbis (methylene) bis{3-[4-hydroxy-
339 3,5-bis(2-methyl-2-propanyl)phenyl]propanoate(SL60), piceol (SLW), and anthraquinones
340 such as carminic acid (SLW, SL60) and scortechinone F (SL60, SL80, SL100).
341 These results are in agreement with other works, where rosmarinic acid, apigenine, luteoline,
342 quercetin-7-o glucoside, luteolin-7-o-rutinose, and luteolin 7-O-β-D-glucopyranoside were
343 obtained as the major compounds of Sage (Đorđević, Cakić, & Amr, 2000; Dragović-Uzelac,
344 Garofulić, Jukić, Penić, & Dent, 2012; Nagy, Solar, Sontag, & Koenig, 2016; Roby et al.,
345 2013; Wang et al., 1998).
346 For both plants the polarity of the solvent influenced the quantity and quality of bioactive
347 molecules identified by UPLC-MS-Q-TOF. Hence, more bioactive molecules were found
348 when using a hydroalcoholic solvent, since this solvent allows to extract both water and
349 ethanolic soluble molecules and a large number of molecules of different structure and nature
350 (Kim, Murthy, Hahn, Lee, & Paek, 2007; Naczk & Shahidi, 2006; Nawaz, Shi, Mittal, &
351 Kakuda, 2006; Turkmen, Sari, & Velioglu, 2006; Yang & Zhang, 2008).

352 3.2. ABTS radical scavenging assay

353 ABTS is widely used for screening the antioxidant activity in both lipophilic and hydrophilic
354 samples (Re et al., 1999).
355 In the present experiment, all tested extracts exhibited scavenging ability on ABTS free
356 radical (Table 3). The ABTS free radical is reduced with hydrogen in the presence of
357 hydrogen-donating antioxidants. Statistic analysis showed a significant influence ($P < 0.05$) of
358 both solvent and plant leaf on the ABTS antioxidant capacity. The capacity to scavenge the
359 ABTS free radical by different extracts of both plants follows this decreasing order: SL60 >
360 SL80 > SLA > BL80 > BLA > BL60 > BLW > SLW. As will be shown in the reducing
361 power assay, the extracts containing ethanol exhibited a stronger antiradical ABTS activity

compared to the water extract. Sage leaf water extract (SLW) achieved to scavenge 50 % of radical ABTS at the highest concentration (25.86 ± 1.14 µg × mL^{-1}). On the other hand, Sage leaf ethanolic 60 % extract (SL60) exhibited a strong scavenging capacity, reducing 50 % of radical ABTS with a concentration of 5.67 ± 0.26 µg × mL^{-1}. This tendency was followed by SL80 (7.96 ± 0.02 µg × mL^{-1}), SLA (11.50 ± 0.18 µg × mL^{-1}) and BL80 (14.65 ± 0.59 µg × mL^{-1}). As can be observed, ABTS antiradical capacity increased with ethanol concentration. However, when increasing the concentration of water, a significant ($P < 0.05$) decrease of the antiradical ABTS capacity was observed in both plants. Hence, the strongest activity was achieved by the ethanolic 60 % extract in the case of Sage, whereas for Bay the strongest was the ethanolic 80 % extract. In our work higher activity was obtained for Bay leaves compared to those shown by (Kivrak, Göktürk, & kivrak, 2017), where IC50 ABTS value for the water extract was 99.75 ± 1.41 µg × mL^{-1} and for the absolute ethanolic extract 43.74 ± 0.57 µg × mL^{-1}. This difference between our results and those from literature might be attributed either to the origin of the plant or to the extraction process. Similarly, our value for the sage leaf extract was lower than the one found by Garcia et al., (2016), where the ethanolic 80 % extract gave 1.57 µg × mL^{-1}. The antioxidant activity can be attributed to the phenolic and flavonoid compounds previously identified by UPLC in the extract. Wang *et al.* (1998) determined that the high antioxidant activity shown by Sage extracts was due to the presence of rosmarinic acid and lutéoléine-7-O-α-glucopyranoside. Phenolic compounds constitute one of the major groups of compounds in both plants, being well-known as free radical scavenging substances. Therefore, the antioxidant capacity of Bay and Sage leaves extracts could be due to the presence of these phenolic compounds. Finally, the lowest antioxidant activity for both plants was obtained with the water extract, which is in agreement with the results published by Kivrak, Göktürk, & Kivrak, (2017) and Lu et al., (2011).

3.3. Reducing power (RP) assay

The reducing capacity of a compound may serve as an interesting assay for the estimation of the antioxidant activity (Elmasta, Gulçin, et al., 2006). So, the initial concentration of plant extract needed to decrease the initial concentration of Fe^{3+} by 50% is referred to as IC50, a parameter widely used to measure the antioxidant activity. A lower IC50 correspond to the higher antioxidant power. As can be observed in table 3, all the extracts exhibited reducing power of Fe^{3+} due to the bioactive compounds previously identified (table 2). The ability to reduce 50 % Fe^{3+} varied in the range of 35.38 ± 0.19 μg × mL^{-1} - 238.36 ± 11.09 μg × mL^{-1} and 47.28 ± 1.08 - 171.74 ± 2.64 μg × mL^{-1} for Sage and Bay leaves respectively. The reducing Fe^{3+} capacity depends on both the solvent polarity and the plant leaves. The reduction of Fe^{3+} has often been used as an indicator of electron donor of a substance. One of the chemical properties of phenolic compounds is the reducing power, being able to act as electron and/or hydrogen donor, and then, to scavenge free radicals (Koşar, Dorman, & Hiltunen, 2005; Rice-Evans, Miller, & Paganga, 1997; Wong, Leong, & William Koh, 2006). As expected, the ethanolic extracts exhibited a stronger reducing power than the water extract because of the higher concentration of active compounds in the ethanolic extract. The reducing power of BL and SL extracts followed this order: SL80 ≥ SLA > SL60 ≥ BLA > BL80 ≥ BL60 > BLW > SLW. In both plants the water extract (BLW and SLW) gave the lower reducing power, as expected, requiring higher concentrations (171.74 ± 2.64 and 238.36 ± 11.09 μg × mL^{-1} respectively) to reduce 50 % of Fe^{3+}. Then, the incorporation of ethanol in the extraction solvent increased the reducing power and provided similar results to those published by Then, Vásárhelyi-Perédi, Szöllosy, & Szentmihályi, (2004); Elmasta, Gülçin, et al., (2006) and Muñiz-Márquez et al., (2013). Sage has been reported to be a radical scavenger, reducing metal ions and inhibiting lipid oxidation (Zhang et al., 2013).

In order to correlate the antioxidant capacity obtained with both ABTS and RP methods, a regression analysis was applied and a significant correlation between both parameters (P <

412 0.05, r = 0.839) was found. This result is similar to that found by Dudonné, Vitrac, Coutière,
413 Woillez, & Mérillon, (2009), and it might be due to the occurrence of a similar redox reaction
414 for ABTS and reducing power assays (Huang, Ou, & Prior, 2005).

415 3.4. Characterization of the packaging colour

416 Due to the previous results concerning ABTS and RP assays, ethanol 60 % and 80 % of both
417 plants (SL60, BL60, SL80, and BL80) were selected to perform the active packaging. To
418 study the effect of the extract on the final colour of the active packaging, a spectrum scanning
419 in the visible range 400-700 nm of AP-BL80, AP-SL80 and control was carried out. The
420 absorbance value of the control material was 0.268 ± 0.002, and for AP-BL80 and AP-SL80
421 were 0.269 ± 0.002 and 0.266 ± 0.002 respectively. All the spectra were overlapped, with
422 maximum absorbance at 500 nm. No significant differences were obtained ($P = 0.096 > 0.05$),
423 which can be due to the low concentration of the extract into the packaging. Hence, these
424 results showed that the extract did not affect the colour of the new active packaging prototype.

425 3.5. Free radical scavenging assay

426 The antioxidant capacity of both multilayer active film (AP-BL60, AP-BL80, AP-SL60, AP-
427 SL80) and the selected extracts (BL60, BL80, SL60, SL80) used to prepare the active
428 packaging was analyzed by the system developed by Pezo et al. (Pezo, Salafranca, & Nerín,
429 2006, Pezo, Salafranca, & Nerín, 2008). The results are presented as percentage of
430 hydroxylation (fig.3). Comparing both plants, higher antioxidant activity was observed with
431 Sage leaf 60 % extract (SL60) with hydroxylation of 73.86 ± 2.16 % and showing a
432 significant reduction ($P < 0.05$) compared to the control (fig. 3A). However, no significant
433 differences were observed between Bay leaf 60 % extract (BL60) with 91.07 ± 8.91
434 percentage of hydroxylation and the control. Finally, a significant reduction ($P < 0.05$) in the
435 percentage of hydroxylation was obtained for Sage 80 % (SL80) and Bay leaf 80 % (BL80)

436 with values of 85.49 ± 5.30 % and 87.09 ± 3.93 % respectively. The free radicals (OH) not
437 scavenged by the extracts were trapped into a solution of salicylic acid and then converted
438 into 2,5-dihydroxybenzoic acid (2,5-DHB). As we can see in table 4, the strongest reduction
439 in 2,5-DHB concentration compared to the control was obtained for SL60, showing again the
440 highest antioxidant activity. Besides, statistic analysis revealed significant differences ($P < 0.05$)
441 between the amount of 2,5-DHB formed in SL80, BL80 and the control. The
442 scavenging activity of Bay and Sage can be attributed to the flavonoids, phenolic compounds
443 and terpenoids previously identified (table 2). These results highlight once again the
444 antioxidant activity determined by ABTS radical scavenging and by reducing power assays.
445 In the case of flavonoids it is known that the hydroxyl groups attached to the ring structure
446 confer them antioxidant properties, acting as reducing agents, hydrogen donators, metal
447 chelators and radical scavengers (Carocho & Ferreira, 2013).
448 After the evaluation of the extracts, the active packaging materials were studied. The strongest
449 antioxidant activity was obtained from the packaging containing SL60 (AP-SL60), giving the
450 lowest percentage (69.64±6.86 %) of hydroxylation (fig. 3B). This confirms the previous
451 results where SL60 gave the highest free radical scavenging and consequently, the lowest
452 amount of 2,5-DHB formed after the reaction of the not scavenged free radicals with the
453 salycilic acid (table 5).
454 No significant differences ($P > 0.05$) of the percentage of hydroxylation was observed with
455 AP-BL60 and control, whereas AP-BL80 (85.49±5.3 %) and AP-SL80 (87.09±3.93 %)
456 showed a significant decrease ($P < 0.05$) of OH free radicals compared to the control (100%).
457 In both plants the 80% ethanolic extract reduced the amount of 2,5-DHB (table 5). The results
458 obtained confirm that the reactive free radicals are able to pass through the LDPE layer and
459 react with the antioxidant extract present between both LDPE and PET layers. Similar
460 conclusion has been already demonstrated by previous works studying the multilayer

antioxidant active packaging (Carrizo, Taborda, Nerín, & Bosetti, 2016; Colón & Nerín, 2015; Vera et al., 2016). Hence, this enhances the fact that the antioxidant does not require a direct contact with the food product or food simulant to exhibit antioxidant properties and the release of antioxidant is not necessary.

In light of the good results obtained, the multilayer active packagings containing SL60 and BL80 were chosen to test the specific migration and to study the efficiency of the antioxidant packaging on the lipid oxidation of fried potatoes.

3.6. Specific migration analysis by UPLC-MS/QTOF

To ensure the safety of the antioxidant active packaging, the possible migration of molecules from the antioxidant active multilayer AP-SL60 and AP-BL80 was evaluated versus ethanol 10% as food simulant (10 days at 40 °C), being the LDPE layer in contact with the simulant.

There was no migration of the compounds identified from the packaging containing the extracts of sage leaf (SL60) and bay leaf (BL80). These results could be expected, as probably the compounds are grafted in the adhesive behind the LDPE layer in contact with the simulant, as previous studies demonstrated (Carrizo et al., 2016). Thus, this new antioxidant packaging can be defined as safe according to the European Legislation ("Regulation (EC) Nº 1935/2004 of the European parliament and of the council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC"), which requires that components present in food contact material must not be transferred to the food in contact with it.

3.7. Effect of active packaging on lipid oxidation of fried potatoes

Fried potatoes were selected to evaluate the influence of the active packaging on their lipid oxidation. In order to accelerate the oxidation process, the fried potatoes were kept at 40 °C for 20 days. After this period, TBARS method was applied to both the control (using the same

material with blank adhesive and solvent) and the samples AP-SL60 and AP-BL80 to quantify the amount of Malondialdehyde (MDA). Malondialdehyde is one of the most relatively stable end product generated from secondary lipid oxidation of polyunsaturated fatty acids (PUFA).

As can be seen in figure 4, the fried potatoes stored in multilayer bags without antioxidant (control) showed high amount of MDA (0.568 ± 0.03 µg × g^{-1}), while both antioxidant active bags caused a significant decrease ($P < 0.05$) on the amount of MDA, which confirms the inhibition of lipid oxidation of fried potatoes. Among them, AP-SL60 was more efficient, showing a low amount of MDA (0.342 ± 0.01 µg × g^{-1}), that is, a 40 % reduction compared to the control. AP-BL80 was also antioxidant, showing significant differences ($P < 0.05$) compared to the control, with a 31% diminution of MDA content (0.392 ± 0.02 µg × g^{-1}). These results confirm again the antioxidant performance already measured in the material.

Once again, this demonstrates that this antioxidant approach does not imply a positive migration of antioxidants to food, but a real scavenging and non-migrating system that can take place without a direct contact between the antioxidant extract and the food product (Carrizo et al., 2016; Vera et al., 2016; Wrona et al., 2015) .

4. CONCLUSIONS

The study carried out demonstrates that both extracts from Sage and Bay have antioxidant compounds in their composition, which are responsible for the antioxidant properties of their ethanolic extracts. Ethanolic 60 % extract of the Sage and Bay leaves exhibited the strongest antioxidant activity. The extracts were incorporated into an adhesive and a multilayer LDPE-PET was built. The active multilayer showed again a strong antioxidant activity, either evaluated alone or as food packaging for fried potatoes. A significant improvement of the fried potatoes was achieved, which will extend their shelf life. Migration of antioxidants or direct contact between the antioxidant agents (extract of Sage and Bay) and the food were not

510 required. Also, the colour of the packaging was not affected by the incorporation of the Bay
511 and Sage extracts. It has been demonstrated that these extracts act as free radical scavengers,
512 where the free radicals across the LDPE layer and arrive at the adhesive where the Sage or
513 Bay leaf extracts are anchored.

514 Therefore, a multilayer LDPE/PET active film containing Bay and Sage leaf extract can be
515 used as a safe packaging material for food products.

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FIGURE CAPTIONS

Fig. 1 Picture of multilayer packaging film

Fig. 2 Picture of bags filled in with fried potatoes

Fig. 3 Free radical scavenging of the different extracts and the antioxidant packaging performed with them. The 60 % and 80 % ethanolic extracts of Bay leaves (BL) and Sage leaves (SL) compared to the control (A). The packaging performed with the different extracts (AP-BL and AP-SL) compared to the control material (B).

Fig. 4 Lipid oxidation of fried potatoes. Control samples compared to those kept into the active packaging materials containing 80% ethanolic extract of Bay leaves (AP-BL80) and 60% ethanolic extract of Sage leaves (AP-SL60).

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Table 1. Concentration of the different extracts of Bay leaves (BL) and Sage leaves (SL) in the active packaging materials.

Extract	Concentration in the packaging ($g \times m^{-2}$)
Control	_
BL80	0.029 ± 0.0017
SL80	0.027 ± 0.0014
BL60	0.034 ± 0.0026
SL60	0.025 ± 0.0007

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Table 2. Compounds identified in the extracts of Bay leaves (BL) and Sage leaves (SL) by UPLC-MS-QTOF using different solvents. The retention time (Tr) is also indicated.

Compounds	W- Extra		60%H-Extra.		80%H-Extra.		100%H-Extra.		Tr (min)	Molecular formula	formula ID-cod
	BL	SL	BL	SL	BL	SL	BL	SL			
Phenyl1-hydroxy-2-naphthoate	-	-	-	-	-	-	+	-	0.84	C17H12O3	60571
3-(4-Methylphenoxy)-4-oxo-4H-chromen-7-yl 2-oxo-2H-chromene-3-carboxylate	-	-	-	+	-	+	-	-	0.89	C26H16O7	2070736
(+)-a-Viniferin	-	-	-	+	-	-	-	-	0.92	C42H30O9	170167
L-valinol	-	-	-	+	-	+	-	-	0.95	C5H13NO	556322
2-Aminoheptanedioic acid (APM)	+	-	-	-	-	-	-	-	0.97	C7H13NO4	91360
Hexamethyl 1,1,2,2,4,4-cyclopentanehexacarboxylate	-	-	-	+	-	-	-	-	1.11	C17H22O12	2158790
4S)-5-Methoxy-4-({[(2-methyl-2-propanyl)oxy]carbonyl}amino)-5-oxopentanoic acid	-	-	-	-	-	-	-	+	1.04	C11H19NO6	5381728
Ditolylguanidine	+	-	-	-	-	-	-	-	1.26	C15H17N3	7056
2,3,4-Tri-O-acetylpentaric acid	-	+	-	-	-	-	-	-	1.61	C11H14O10	287015
Azodolen	+	-	-	-	-	-	-	-	1.8	C18H19N3O2	6494
Phenacetin	+	-	-	-	-	-	-		1.92	C10H13NO2	4590
4-Acetyl-4-(ethoxycarbonyl)heptanedioic acid	+	-	-	-	-	-	-	-	2.63	C12H18O7	2056378

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Tetramethyl (3E,7Z)-5-methoxy-2,9-dioxo-3,7-decadiene-1,3,8,10-tetracarboxylate	+	-	-	-	-	-	-	-	3.07	C19H24O11	4528833
Piceol	-	+	-	-	-	-	-	-	3.73	C8H8O2	7189
Diethyl 1,1-cyclobutanedicarboxylate	-	+	-	-	-	-	-	-	4.03	C10H16O4	69822
Phenyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside hydrate (1:1)	+	-	-	-	-	-	-	-	4.13	C20H26O11	17461141
Methyl(2-amyl-3-oxocyclopentyl)acetate	-	+	-	-	-	-	-	-	4.14	C13H22O3	92919
4,4'-Biphenyldiyl bis[4-(2-methyl-2-propanyl)benzoate]	-	+	-	-	-	-	-	-	4.32	C34H34O4	3520884
Cinnamtannin B1	+	-	+	-	-	-	-	-	4.35	C45H36O18	417255
Benzyl 6-O-[(2R,3R,4R)-3,4-dihydroxy-4-(hydroxymethyl)tetrahydro-2-furanyl]-β-D-glucopyranoside	-	+	-	-	-	-	-	-	4.68	C18H26O10	22913850
9-Dimethoxy-5-oxo-5H-furo[3,2-g]chromene-7-carboxylic acid	-	+	-	-	-	-	-	-	4.75	C14H10O7	652749
3ξ)-D-erythro-Pentitol- D-fructose (1:1)	+	-	-	-	-	-	-	-	4.76	C11H24O11	157903
4-Methyl-2-oxo-2H-chromen-7-yl β-D-xylopyranosyl-(1->4)-β-D-xylopyranosyl-(1->4)-β-D-xylopyranoside	-	+	-	+	-	-	-	-	5.04	C25H32O15	9390328
3,4-Dibutoxy-3-cyclobutene-1,2-dione	-	+	-	-	-	-	-	-	5.10	C12H18O4	58618
Luteolin7-O-β-D-diglucuronide	-	+	-	-	-	-	-	-	5.43	C27H26O18	4445350
3,6,9,12,15,18,21,24,27,30-Decaoxadotriacontane-1,32-diol	-	+	-	-	-	-	-	-	5.49	C22H46O12	73316

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Fmoc-L-alanine	-	-	+	-	+	-	-	-	5.55	C18H17NO4	
Quercetin-3-O-β-D-glucuronopyranoside	-	-	+	-	+	-	-	-	5.60	C21H18O13	4438874
Dipivefrin	+	-	+	-	+	-	-	-	5.75	C19H29NO5	2994
Apigenin-6-C-glucoside-7-O-glucoside	-	-	-	+	-	+	-	-	5.84	C27H30O15	390121
Scutellarin	-	+	-	-	-	-	-	-	5.92	C21H18O12	161366
Umbelliferone	-	-	-	+	-	+	-	+	5.95	$C_9H_6O_3$	4444774
Quercetin	-	-	+	-	+	-	+	-	6.01	C15H10O7	5381998
Carminic Acid	-	+	-	+	-	-	-	-	6.06	C22H20O13	8430568
4-{2-(Benzyloxy)-6-[(benzyloxy)carbonyl]benzoyl}-3,5-bis(methoxymethoxy)benzoic acid	-	+	-	+	-	-	-	-	6.19	C33H30O10	9170697
2-Ethyl-3-methyl-1-{[2-(4-morpholinyl)ethyl]amino} pyrido[1,2-a]benzimidazole-4-carbonitrile	+	-	+	-	+	-	-	-	6.24	C21H25N5O	853771
Diosmin	-	-	-	+	-	+	-	-	6.27	C28H32O15	4444932
Ellagic acid-4-O-β-xyloside-3,3', 4'-trimethyl ether	-	+	-	+	-	-	-	-	6.36	C22H20O12	9013603
2 - H-1-Benzopyran-2-one, 7-(β-D-galactopyranosyloxy)-4-methyl	-	-	-	+	-	+	-	-	6.51	C16H18O8	84473
Pectolinarin	-	-	-	+	-	+	-	-	6.59	C29H34O15	147700

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Fluorescein	-	-	-	-	-	+	-	-	6.99	C20H12O5	15968
Kaempferol	-	-	+	-	+	-	+	-	6.76	C15H10O6	4444395
2,6-Di-tert-butyl-4-hydroxymethylphenol	+	-	+	-	+	-	-	-	7.37	C15H24O2	6663
Luteolin 4'-methyl ether	-	-	-	+	-	+	-	-	7.40	C16H12O6	4444931
(-)-Andrographolide	+	-	+	-	-	-	-	-	7.52	C20H30O5	4477067
Pectolinarigenin	-	-	-	+	-	+	-	+	7.65	C17H14O6	4478521
3-Dodécyldihydro-2,5-Furanedione	-	-	+	-	+	-	-	-	7.86	C16H28O3	88579
14-Hydroxy-19-oxo-3-(pentopyranosyloxy) carda-4,20(22)-dienolide	-	-	-	-	+	-	-	-	8.12	C28H38O9	146228
Zeranol	-	-	-	+	-	-	-	-	8.26	C18H26O5	2271133
2R,3S,4S,5R,6R)-2-(Hydroxymethyl)-6-{[(3S,4R,5R)-3,4,5-trihydroxy-2-(hydroxymethyl)tetrahydro-2-furanyl]oxy}tetrahydro-2H-pyran-3,4,5-triol	-	-	-	+	-	-	-	-	8.35	C11H20O11	29399926
Methylprednisolone	-	-	-	+	-	-	-	-	8.51	C22H30O5	6485
Cimilactone B	-	+	-	-	-	-	-	-	8.53	C33H48O9	9299914
Cardinalin 5	-	-	-	-	+	-	-	-	9.04	C31H28O11	8730566

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3S,4S)-4-Methyl-5-methylene-4-[2-(trityloxy)ethyl]-3-[3-(trityloxy)propyl]dihydro-2(3H)-furanone	-	-	-	-	-	-	+	-	9.15	C49H46O4	9214944
Enoxolone	-	-	-	+	-	+	-	+	9.25	C30H46O4	9710
Tretinoin (Acid A Vit)	-	-	-	+	-	+	-	+	9.39	C20H28O2	392618
(+)-Betulonic acid	-	-	-	+	-	-	-	-	9.57	C30H46O3	109508
Octylα-D-mannopyranosyl-(1->2)-α-D-mannopyranosyl-(1->2)-α-D-arabinofuranoside	-	-	+	-	+	+	-	+	10.02	C25H46O15	9687437
3,3'-{[3,5-Bis(benzyloxy)phenyl]methylene}bis(2-hydroxy-4H-chromen-4-one	-	-	-	-	-	-	-	+	10.11	C39H28O8	345490
Scortechinone F	-	-	-	+	-	+	-	+	10.24	C34H40O9	10140412
3-(β-D-Glucopyranosyloxy)-4,6-dihydroxy-2-nonylphenylβ-D-glucopyranoside	-	-	+	-	+	-	+	-	10.26	C27H44O14	
2,4-Bis(diphenylmethyl)-5-methoxy-6-(4-pentylphenyl)-2,4,6-cycloheptatrien-1-one	-	-	-	+	-	-	-	-	10.30	C45H42O2	9170880
Erucic amid	-	-	+	-	+	-	-	-	10.43	C22H43NO	
Nimbolinin D	-	-	+	-	+	-	+	-	10.62	C36H44O9	9016869
Methyl 6-deoxy-3-O-[2,3,4-tris-O-(2,2-dimethylpropanoyl)-6-methyl-β-D-glucopyranuronosyl]-α-L-mannopyranoside	-	-	-	+	-	+	-	-	10.58	C29H48O14	9094457
26-acetamido-22-oxo-16,23-cyclochole sta-5,16(23)-dien-3-yl acetate	-	-	+	-	+	-	-	-	10.91	C31H45NO4	
Methyl (5S)-5-C-{(1S)-1-[(4R,6S)-6-{(2R,3S,5S)-7-(benzyloxy)-3-methoxy-5-[(4-methoxybenzyl)oxy]	-	-		-	+	-	-	-	11.06	C50H78O12	9030233

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3-Cyclohexene-1,1-diylbis(methylene) bis{3-[4-hydroxy-3,5-bis(2-methyl-2-propanyl)phenyl] propanoate}	-	-	-	+	-	+	-	-	11.31	C42H62O6	2124355

W- Extra: watter extract
60%H-Extra: Ethanol 60% extract
80%H-Extra: Ethanol 80% extract
100%H-Extra: Ethanol 100% extract
(+): presence
(-): absence

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Table 3. Antioxidant capacity of Bay leaves (BL) and Sage leaves (SL) extracts evaluated by ABTS scavenging and ferric reducing power (RP) assays.

Sample	Solvent	IC50 RP ($\mu g \times mL^{-1}$)	IC50 ABTS ($\mu g \times mL^{-1}$)
Bay leaves (BL)	Water	171.74 ± 2.64^{b}	20.64 ± 0.79^{b}
	Ethanol 60 %	91.43 ± 2.84^{c}	18.68 ± 0.16^{c}
	Ethanol 80 %	85.47 ± 0.9^{c}	14.65 ± 0.59^{e}
	Ethanol 100 %	47.28 ± 1.08^{d}	17.68 ± 0.47^{d}
Sage leaves (SL)	Water	238.36 ± 11.09^{a}	25.86 ± 1.14^{a}
	Ethanol 60 %	46.01 ± 1.21^{d}	5.67 ± 0.26^{h}
	Ethanol 80 %	35.38 ± 0.19^{e}	7.96 ± 0.02^{g}
	Ethanol 100 %	36.72 ± 0.78^{e}	11.50 ± 0.18^{f}

a-f: indicate a significant difference ($P < 0.05$)

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Table 4. Concentration of 2,5-dihydroxybenzoic acid (2,5-DHB) formed during free radical scavenging of Bay leaves (BL) and Sage leaves (SL) extracts.

Sample	2,5-DHB ($ng \times mL^{-1}$)
Control	$2.05 \times 10^2 \pm 3.06^a$
BL60	$1.95 \times 10^2 \pm 7.65^a$
SL60	$1.51 \times 10^2 \pm 4.42^b$
control	$1.75 \times 10^2 \pm 1.17^a$
BL80	$1.63 \times 10^2 \pm 0.61^b$
SL80	$1.58 \times 10^2 \pm 1.34^c$

a-c: different letters indicate a significant difference ($P < 0.05$)

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Table 5. Concentration of 2,5-dihydroxybenzoic acid (2,5-DHB) formed during free radical scavenging assay for the active packaging samples.

Sample	2,5-DHB ($ng \times mL^{-1}$)
Control	$1.57 \times 10^2 \pm 7.46^a$
AP-BL60	$1.51 \times 10^2 \pm 0.88^a$
AP-SL60	$1.09 \times 102 \pm 10.80^b$
Control	$2.39 \times 10^2 \pm 4.16^a$
AP-BL80	$2.04 \times 10^2 \pm 12.66^b$
AP-SL80	$2.08 \times 10^2 \pm 9.37^b$

a,b: different letters indicate a significant difference ($P < 0.05$)

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Fig.1

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Fig.2

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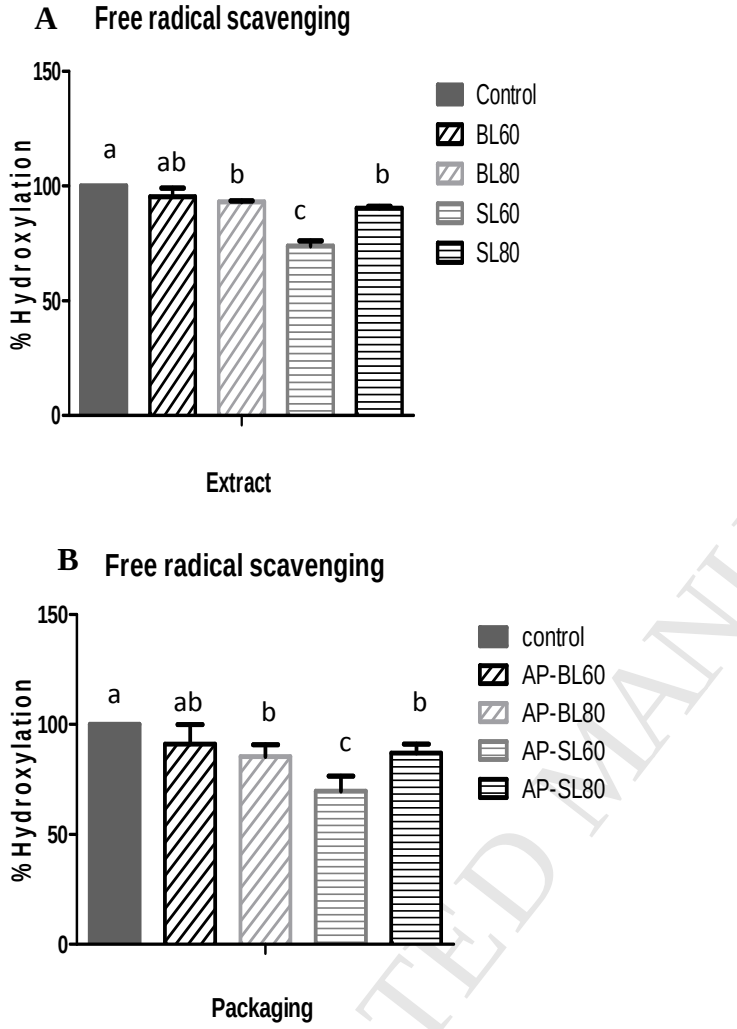
Fig.3



a-c: Different letters indicate a significant difference ($P<0.05$)

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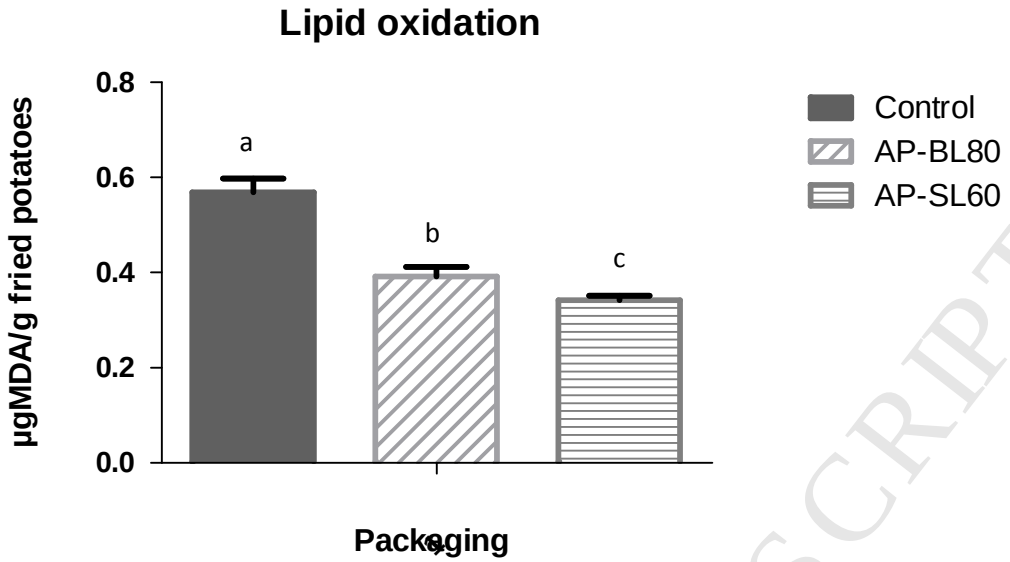
Fig.4



a-c: Different letters indicate a significant difference ($P<0.05$).

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HIGHLIGHTS

- Phenolic compounds and terpenoids were identified in Bay and Sage leaves extracts.
- Both Bay and Sage leaves extracts showed a high antioxidant activity.
- A multilayer antioxidant packaging was built from Sage and Bay
- The new packaging scavenged free radicals and delay oxidation of fried potatoes.

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