

Valorization of Olive Industry Waste Products for Development of New Eco-sustainable, Multilayer Antioxidant Packaging for Food Preservation

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This work deals with the design, production and evaluation of the effectiveness of novel multilayer active films, based on biopolymers and natural antioxidants, suitable for food packaging applications.

Three phenolic olive extracts (named OEs), deriving from olives milling wastewaters, were analyzed in order to assess their suitability to develop antioxidant polymeric systems. The most performing extract was then selected to produce the active biodegradable films, spreading a polylactic acid (PLA) coating layer, at different percentages of OE (0-3% w/w), on a biodegradable substrate. The produced multilayer structures were then characterized by several techniques, in order to evaluate the interaction of the antioxidant phase with the polymer matrix, and its effect on the physical and functional performance of the active systems. Finally, the antioxidant capacity by ,2-diphenyl-1-picrylhydrazyl (DPPH) spectroscopic method was carried out, suggesting that the formulated active bio-films could be considered an innovative and promising solution for antioxidant packaging of sensitive food products.

1. Introduction

Nowadays, the increasing demand for fresh, safe and minimally processed food products have pushed the industrial and scientific research towards the development of innovative, active packages, through the incorporation of active molecules capable to increase the food shelf-life (Di Maio et al., 2017). This challenging approach allows the continuous modification of the environment inside the packaging through the release or the absorption of specific substances (Apicella et al., 2018a), and overcomes many of the issues of traditional approaches related to the direct addition of antimicrobials or antioxidants to the food matrix.

Oxidative phenomena are among the most important causes of food degradation, loss of precious nutrients and generation of toxic compounds (Apicella et al., 2018b). Synthetic antioxidants such as butylhydroxytoluene (BHT) and butyl hydroxyanisole (BHA) were widely used for the stabilization of foods containing oils and fats; however, these molecules can accumulate in the human tissues, increasing the risk of toxicity to the consumer. An innovative approach concerns the use of natural antioxidants, such as plant and fruits extracts, rich in nutritive properties (Adiletta et al., 2015), or essential oils derived from herbs or spices, which can be successfully used not only in pharmaceuticals, nutritional or cosmetics uses (Sillero et al., 2018), but also the production of active packaging. At the same time, the growing attention to sustainability and the rational use of resources has prompted the interest towards the development of eco-compatible, eco-sustainable packaging, through the use of biodegradable and/or compostable materials as unique active packaging options for the protection of food products (Scarfato et al., 2015), as well as natural and bioactive compounds, coming from the valorization of industrial waste materials. For these reasons, a growing interest is nowadays dedicated to the use of antioxidant extracts from olive oil industry wastes. The olive oil industry produces a large quantity of waste and by-products, such as pomace, vegetation waters and leaves. These products are rich in phenolic compounds, including oleuropein, luteolin, tyrosol, hydroxytyrosol, verbascoside, capable to interfere with the oxidation of lipids and other molecules by rapid donation of a hydrogen atom to radicals (Ajila et al. 2010), thus showing a strong antioxidant activity which makes them very attractive for the active packaging industry. In addition, the high quantity of phenolic compounds in the vegetation waters

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makes them resistant to microbial degradation, generating phytotoxicity and pollution of the groundwaters. Therefore, investigating new fields of applications to reuse these products is not only a way to restore their economic and commercial dignity, but also solves a burdensome problem of environmental impact. All these considerations inspired the aim of this work, which deals with the design, realization and verification of the effectiveness of biodegradable multilayer active films with antioxidant properties, based on phenolic extracts deriving from olives milling waste waters, properly subjected to separation pre-treatments. The active extracts were characterized in terms of the main chemical-physical properties (dry matter content, colour, reducing sugars) and antioxidant capacity. Then, the most performing and suitable extract was selected to produce active multilayer biodegradable films, spreading a PLA coating layer at different percentages of active phase (ranging from 0 to 3%), on a biodegradable substrate film. The barrier and surface properties of the produced systems were then evaluated, in order to obtain information on the interaction of the active phase with the polymer matrix and its effect on the functional performance, and surface wettability of the films. Moreover, release tests in a selected food simulant and DPPH radical scavenging measurements were carried out to investigate the effectiveness of the produced systems as antioxidant carriers and as potential new packaging for extending foods shelf-life.

2. Experimental

2.1 Materials

Three phenolic olive extracts deriving from olives milling wastewaters (named OE1, OE2 and OE3) and subjected to different pre-treatments (data non disclosed), were donated by Fangiano Farming Company (Nocera Terinese, CZ, Italy). A commercial biodegradable blown film, named Biopolymer (Euromaster, Pistoia, Italy), made by Poly(lactic acid) and Poly(butylene adipate-co-terephthalate) blend, having a thickness of $22 \pm 1.0 \mu\text{m}$, was used as substrate for the production of the active coatings. PLA4060 (Natureworks, Minnetonka, USA), characterized by a D-lactide content of 12 wt%, that confers an amorphous morphology to the material, was used for the coating layer. DPPH (2,2-diphenyl-1-picrylhydrazyl), Trolox ((\pm)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), Tween 80 surfactant and Fehling reagents were obtained from Sigma Chemical Co. (St. Louis, Mo., USA). All organic solvents used were analytical grade.

2.2 Characterization of the phenolic extracts

The dry matter content of the phenolic extracts was determined by means of Sartorius Moisture Analyzer MA100 (Sartorius AG, Göttingen, Germany). Reducing sugars were evaluated by Fehling assay, according to the method reported by Adiletta et al., 2018. A mixed Fehling's solution and methylene blue as indicator were used in titration. The results were expressed in g of reducing sugars in 100 g of phenolic extract (dry basis). The antioxidant activity of OEs was analyzed using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as previously reported (Adiletta et al., 2017). 100 μL of extracts at different dilutions were mixed with 3.9 mL of a methanolic solution of DPPH ($6 \times 10^{-5} \text{ M}$) in a capped cuvette. The mixture was shaken vigorously at room temperature and allowed to stand at room temperature in the dark for 30 min. The absorbance of the solution was measured at 517 nm with a UV-Vis spectrophotometer (Lambda Bio 40, Perkin Elmer, Waltham, MA, USA). The control was conducted in the same manner, but distilled water was used instead of the sample. All analyses were performed in triplicate, and the obtained values were reported using a calibration curve of Trolox as TEAC (Trolox Equivalent Antioxidant Capacity). Color measurements were carried out on the extracts by using a colorimeter CIE-Lab (Chroma Meter II Reflectance CR-300, Minolta, Japan), equipped with a CIE standard D65 illuminant (Fratanni et al. 2018). The results were expressed according to color coordinates L^* (darkness/lightness), a^* (greenness/redness), b^* (blueness/yellowness), hue value ($\tan^{-1} b^*/a^*$) and saturation index, chroma ($(a^2 + b^2)^{1/2}$). Chroma indicates the dullness/vividness of a product, while the hue angle is how an object's color is perceived by human eye: red, orange, green or blue (CIE $L^*a^*b^*$ colour system, 1986).

2.3 Preparation of the active systems

The preliminary investigations on the available phenolic extracts allowed to select the best candidate for the production of the active packaging. Then, antioxidant multilayer films were realized by precipitation, induced by solvent evaporation, of a PLA4060/Acetone coating solution (with mass ratio 20:80), incorporated at different percentages of the selected active phase (0, 1, 3% w/ w_{PLA}) under magnetic stirring (Apicella et al., 2018c). Non-ionic surfactant Tween 80 (HLB value=15) was previously added to the water-based extract (1% w/ w_{OE}), in order to enhance solubilization with PLA solution and avoid precipitation. The casting mixture was then spread on the Biopolymer substrate by means of a K Hand Coater (RK, Printocoat Instruments Ltd., Litlington, UK), equipped with stainless steel close wound rod, with wire diameter equal to 0.64 mm (Barbaro, 2015), yielding final coatings with comparable thicknesses, as listed in Table 1.

Table 1: List of the prepared films with their composition and thicknesses.

Sample	Olive extract [OE] concentration [%wt]	Total Thickness [μm]	Coating layer thickness [μm]
Biopolymer	0	22 ± 1.0	0
Biopolymer/PLA	0	29 ± 1.4	7 ± 0.4
Biopolymer/PLA-1%OE	1	29 ± 1.3	7 ± 0.3
Biopolymer/PLA-3%OE	3	29 ± 1.6	7 ± 0.6

Solvent was evaporated at room conditions overnight, then the coated films were stored under vacuum sealing in aluminium bags before analysis. The coating technology allowed to avoid thermal stresses to the thermo-sensitive active phase, while PLA coating allowed to obtain all eco-compatible, 100% biodegradable multilayer films. The coating layer thickness was evaluated as difference among the total thickness of the coated films and the Biopolymer substrate thickness.

2.4 Characterization of the coated films

Oxygen permeability tests were performed by means of a gas permeabilimeter (GDP-C, Brugger, Munchen Germany). The tests were carried out at 23 °C and 50% R.H., with an oxygen flow rate of 80 mL/min (ISO 15105–1). Results were expressed as P_{O_2} ($\text{cm}^3 \text{ cm}/(\text{m}^2 \text{ d bar})$), according to ASTM Standard D 1434 – 82, and average mean values were calculated from three measurements per each sample.

Water vapor permeability was measured by M7002 Water Vapor Permeation Analyzer (Systech Instruments Ltd, Oxfordshire, UK) according to the standard ASTM F 1249. Films were tested at 23°C and 50% R.H., and the results, performed in duplicate, were expressed as P_{WV} ($\text{g ml}/(\text{m}^2 \text{ Pa s})$), calculated as (Li et al., 2015):

$$P_{WV} = \frac{WVTR \times L}{\Delta P} \quad (1)$$

where WVTR is the water vapor transmission rate ($\text{g}/\text{m}^2\text{s}$) measured through the film, L is the average film thickness (m), and ΔP is the partial water vapor pressure difference (Pa) across the two sides of the film.

Static contact angle measurements were performed with a First Ten Angstrom Analyzer System 32.0 mod. FTA 1000 (First Ten Angstroms, Inc., Portsmouth, VA, USA), according to the standard test method ASTM D5946, using distilled water and ethanol/water 95:5 v/v as test liquids. The drop volume was taken within the range where the contact angle did not change with the variation of the volume ($2 \pm 0.5 \mu\text{L}$). Each reported value of the θ angle is the average of five replicate measurements. The release of antioxidants was evaluated by total immersion method as suggested by Chen et al. 2012, with some modifications. Film samples were cut in squares of 1 dm^2 area and immersed in 100 mL of 95% v/v ethanol release medium. The flask was kept in the dark under magnetic stirring, to minimize mass transfer resistance of antioxidants from the film, at room conditions for 14 days, and 100 microliters of the medium were periodically withdrawn for analysis. The antioxidant activity released into the simulant solution was quantified by DPPH method, as reported in Paragraph 2.3, and the results were expressed as follows:

$$\text{Antioxidant activity (\%)} = \left(1 - \frac{A_{\text{sample}}}{A_{\text{control}}}\right) \times 100 \quad (2)$$

where A_{sample} is the absorbance of sample and A_{control} is the absorbance of the control at initial time $t=0$, using 100 μL of fresh release medium.

3. Results and discussion

3.1 Characterization of the phenolic extracts

All active extracts are liquid samples, with a density comparable to that of an aqueous solution, more or less concentrated. Table 2 shows the dry matter, reducing sugars and antioxidant activity of the olive extracts under investigation. The results are expressed over dry matter in order to better compare the physico-chemical properties and the antioxidant activity of the phenolic extracts. The content of reducing sugars has been evaluated because the polar nature of the aldehyde and ketone groups can interfere with the nonpolar nature of the polymeric PLA coating mixture, inducing a precipitation of the active compound and consequently entailing the addition of a higher content of surfactant to stabilize the emulsion. In particular, the reducing sugars content was found proportional to the dry matter of the samples, with values equal to 10.67, 12.57 and 8.68 g/100g of DM for the OE1, OE2 and OE3, respectively.

Table 2: Dry matter, reducing sugars and antioxidant activity (expressed as TEAC) of the olive extracts under investigation.

Sample	Dry Matter [% w/wt]	Reducing sugars [g/100g of DM]	TEAC*10 ⁻³ [μmol Trolox/g of DM]
OE1	61.4 ± 0.3 ^b	10.67 ± 0.5 ^b	733.13 ± 15.41 ^a
OE2	68.7 ± 0.3 ^c	12.57 ± 0.6 ^c	793.53 ± 22.39 ^b
OE3	53.0 ± 0.3 ^a	8.68 ± 0.6 ^a	937.20 ± 25.15 ^c

Values followed by the same letter within the same column were not significantly different according to Duncan's test ($P < 0.05$)

Regarding the antioxidant activity, the results highlighted the higher antioxidant activity of OE3 sample (937.20 x10³ μmol Trolox/g of DM) with respect to OE1 and OE2 (equal to 733.13 x10³ and 793.53 x10³, respectively). In all cases, the antioxidant activity of the extracts analyzed was much more performing than the results reported in the literature on olive leaves extract (Amaro-Blanco et al., 2017) and pomace (De Moraes Crizel et al., 2017). In particular, De Moraes Crizel et al. (2017) found an antioxidant activity equal to 143.51 ± 13.56 μmol TE/g dry matter for olive pomace flour, while Amaro-Blanco et al. (2017) found an antioxidant activity on olive leaves extract equal to 234.32 ± 22.39 mg TE/g dry matter, corresponding to ca. 930 μmol TE/g dry matter. Moreover, the highest antioxidant activity of the extract with lower dry matter content suggests a better preservation of the bioactive components in the OE3 extract, and highlights the critical role of the pretreatment conditions in preserving the effectiveness of the phenolic components. The color of the samples was then evaluated by colorimetric analysis, and the associated parameters are shown in Table 3. All the extracts were characterized by a dark brown color, as confirmed by high Hue angle (ranging from 322.50 to 324.20 °) and a saturation value (C*_{ab}) always lower than 13. The typical color was due to polymerization of tannins and low molecular weight phenolic compounds, as also reported by Otles and Semih, 2012.

3.2 Characterization of the coated films

The results obtained from characterization of phenolic extracts pointed out the highest antioxidant activity and the lowest reducing sugars content of the OE3 sample, which was then selected as the most suitable for the production of the active multilayer films. The films obtained were characterized in terms of oxygen and water vapor transport properties, as well as wettability to water and 95% ethanol, and the data are reported in Table 4. From the comparison between Biopolymer and Biopolymer/PLA samples, it was possible to observe that the PLA coating layer addition resulted in a slight reduction in the permeability of the substrate to both oxygen and water vapor (from 5.15 to 4.07 cm³ cm/(m² d bar) and from 9.19 to 8.31 g m/(m² Pa s), respectively). The further addition of 1% and 3% w/w of active agent did not significantly affect the oxygen permeability of the multilayer films, while a slight increase in the P_{WV} values was observed. This behavior could be attributed to an increased affinity of the active coating to the permeating water vapor molecules, due to the inherent high polarity of the active phase. However, the differences among the water vapor permeabilities of the multilayer samples are negligible, in order to assess the performances of the films in the real working conditions.

Surface wettability of the films was then evaluated, as the knowledge of active packaging surface properties and the affinity with the target food matrix is essential for the success of technological operations such as the controlled release of active molecules during time. To this aim, distilled water was selected as aqueous food simulant, while 95% v/v ethanol is recommended by the European Commission as simulant for fats, oil and fatty foods owing to its similar hydrophobicity (Reg.(EU) No 10/2011). Results display a higher wetting tendency to both water and 95% ethanol for all the multilayer films, with respect to the Biopolymer substrate. However, the much lower CA_E values for all the coated samples, approaching to complete wetting, suggest a stronger affinity of the polymer matrix with the organic solvent, thus facilitating the swelling of PLA macromolecules during the release tests. On the basis of these considerations, 95% ethanol was selected as target food simulant for the release tests. Measurements were conducted on the active multilayer sample at the highest phenolic concentration (Biopolymer/PLA-3%OE), and DPPH analysis was carried out on withdrawals at 2, 4, 8, 10 and 14 days. Release test and antioxidant activity was also evaluated on Biopolymer and Biopolymer/PLA films, considered as reference samples, and the results are displayed in Figure 1. As expected, control films had no significant radical scavenging activity. On the other hand, the obtained DPPH% profiles for the Biopolymer/PLA-3%OE pointed out the effectiveness of the active film in releasing the antioxidant molecules, exhibiting an initial faster kinetic occurring in the first days, followed by a sustained release, with a typical Fick's curve. The maximum antioxidant activity displayed was equal to 8.56% after 4 days. The slight decrease in the DPPH% occurring after 6 days could be due to the degradation of the phenolic components reacting with the oxygen dissolved in the medium, leading to the formation of superoxide radicals (Martelli et al., 2017).

Table 3: Cielab color parameters of the olive extracts.

Sample	L*	a*	b*	h_{ab} [°]	C* _{ab}
OE1	19.8 ± 0.02 ^D	9.31 ± 0.18 ^a	-6.71 ± 0.31 ^D	324.20 ± 0.75 ^a	11.48 ± 0.32 ^a
OE2	20.3 ± 0.06 ^C	8.58 ± 0.32 ^a	-6.42 ± 0.37 ^D	323.21 ± 1.14 ^a	10.72 ± 0.45 ^a
OE3	18.5 ± 0.24 ^a	10.35 ± 0.75 ^D	-7.93 ± 0.27 ^a	322.50 ± 1.27 ^a	13.04 ± 0.75 ^b

Values followed by the same letter within the same column were not significantly different according to Duncan's test ($P < 0.05$)

Table 4: Oxygen permeability, water vapor permeability and static water and 95% v/v ethanol contact angle (CA_w and CA_E , respectively), for all the films.

Sample	P_{O_2} [cm ³ cm/(m ² d bar)]	$P_{WV} \cdot 10^{12}$ [g m/(m ² Pa s)]	CA_w [deg]	CA_E [deg]
Biopolymer	5.15 ± 0.35 ^b	9.19 ± 0.37 ^{a,b}	80.64 ± 1.59 ^b	31.07 ± 3.61 ^b
Biopolymer/PLA	4.07 ± 0.09 ^a	8.31 ± 0.21 ^a	66.50 ± 1.77 ^a	10.30 ± 0.35 ^a
Biopolymer/PLA-1%OE3	4.08 ± 0.25 ^a	8.76 ± 0.67 ^{a,b}	66.30 ± 1.09 ^a	10.20 ± 0.22 ^a
Biopolymer/PLA-3%OE3	4.02 ± 0.39 ^a	9.26 ± 0.43 ^b	65.84 ± 1.65 ^a	10.36 ± 0.06 ^a

Values followed by the same letter within the same column were not significantly different according to Duncan's test ($P < 0.05$)

It is known that several factors affect the release of the migrant from solid polymer phase into simulant liquid: the polymer morphology and molecular weight distribution, the polymer–antioxidant interactions, the simulant polarity, the temperature, as well as the antioxidant solubility in the simulant and its distribution within the polymer matrix. However, the results are in compliance with other studies conducted on the release of phenolic compounds from poly(lactic acid) films (Jamshidian et al., 2013), which underlined the effectiveness of ethanol as an aggressive solvent, able to penetrate the PLA chains and promote antioxidant diffusion (Manzanarez-Lopez et al., 2011). Moreover, these preliminary studies pave the way to a more complex investigation on migration kinetics with different release media and different concentration of the active agent.

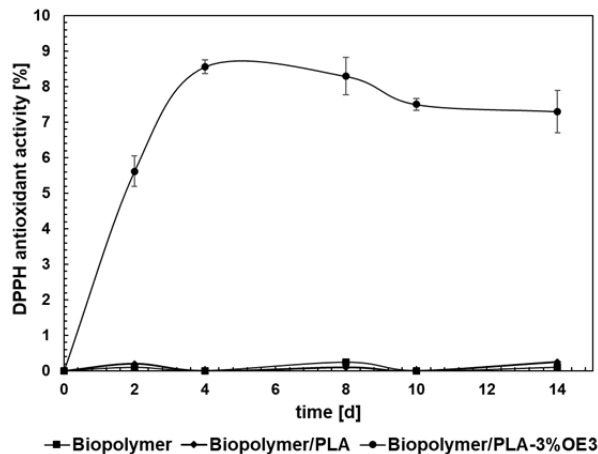


Figure 1: DPPH antioxidant activity [%] evaluated on the release medium after contact with Biopolymer, Biopolymer/PLA and Biopolymer/PLA-3%OE3 samples. Bars represent the standard deviation from triplicate determinations.

4. Conclusions

In this research, novel eco-sustainable multilayer antioxidant films, based on olive milling wastewater extracts, were successfully produced by coating technique. The results of the preliminary investigation, carried out on the available phenolic extracts, identified the OE3 as the best candidate for the development of the active bio-coatings, thanks to its highest antioxidant activity and lowest content of polar functional groups, which allow an easier incorporation within the PLA non-polar coating solution. The incorporation of the olive extract within 1-3% w/w range into the multilayer films did not significantly affect the barrier performance of the systems, required for food packaging applications in real working conditions. Surface wettability measurements pointed out the stronger affinity of the polymer matrix with the 95% ethanol solvent, which was then selected as target

food simulant for the release tests. Finally, DPPH antioxidant activity performed on the food simulant pointed out the potential of the produced films to be used as carriers for the controlled release of the antioxidant agent. Therefore, these active films represent an innovative and advantageous alternative for the preservation of oxidative-sensitive food products.

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