CHEMICAL ENGINEERING TRANSACTIONS

VOL. 87, 2021

A publication of
AIDIC
The Italian Association
of Chemical Engineering
Online at www.cetjournal.it

Guest Editors: Laura Piazza, Mauro Moresi, Francesco Donsì

ISBN 978-88-95608-85-3; **ISSN** 2283-9216

DOI: 10.3303/CET2187015

Biodegradable Films Based on Poly(lactic acid) Coatings and Natural Olive-Wastewater Extracts for Active Food Packaging

Annalisa Apicella*, Giuseppina Adiletta, Donatella Albanese, Marisa Di Matteo, Loredana Incarnato

Dipartimento di Ingegneria Industriale, Università degli Studi di Salerno
anapicella@unisa.it

The goal of this work was to develop innovative, 100% biodegradable films with antioxidant effectiveness, based on poly(lactic acid) (PLA) and a natural olive wastewater extract (OWE). Active PLA coatings were realized by spreading a PLA/OWE coating solution, with an OWE amount up to 20 wt%, on a Poly(lactic acid)/Poly(butylene adipate-co-terephthalate) substrate. The study of active films antioxidant activity and release kinetics in foods with high lipid content was accomplished using 95% ethanol as food simulant. Preliminary shelf-life tests on a sensitive greasy food matrix (i.e. avocado fruits) were also carried out, in order to qualitatively examine the films potential in limiting fruit oxidative/browning phenomena. Finally, the effect of the OWE on the films color and photo-oxidative stability under natural light exposure was also examined. The results pointed out the influence of the morphology and distribution of the active agent on the coatings release rate, and the antioxidant activity and equilibrium time increased by increasing the antioxidant concentration. The shelf-life tests highlighted the promising perspectives for the films in retarding the oxidation/browning reactions of short shelf-life foods, and pave the way to more in-depth investigations on the most appropriate strategies to prevent the transfer of the OWE brown color while keeping intact the benefits of the antioxidant packaging.

1. Introduction

In recent years, the growing concern towards ecological issues due to the persistence of plastic waste in ecosystems has pushed scientific and industrial research towards the development of new materials and new processes that represent effective eco-compatible solutions. The flexible packaging field has recently been identified as a key sector to face the challenge of global sustainability, thanks the reduced weights and raw materials employment, yielding reduced waste and disposal issues (Apicella et al., 2018). In terms of raw materials, the use of biodegradable and/or compostable materials aims at minimizing the environmental impact induced by post-consumer synthetic plastic waste (Apicella et al., 2019a). The biodegradable polymers, thanks to the undoubted ecological advantages and their functional characteristics, are ideal for the realization of short life-cycle products such as food packaging. In a circular economy perspective, these materials can be incorporated with natural, bioactive molecules deriving from revaluation of agri-food waste, obtaining active packaging capable of significantly improving the shelf-life of sensitive foods (Apicella et al., 2019c) and reducing food waste (Guillard et al., 2018).

To this aim, recent innovative research has focused on the addition of active agents from olive wastes and by-products (i.e. olive leaves, pomace and mill wastewaters) within biodegradable films (Martiny et al., 2020; Apicella et al., 2019b). Olive mill wastewaters (OW) represent the liquid fraction of residues in the three phase oil extraction system, which involves the use of a large amount of water (Nunes et al., 2016). Different organic compounds can be found in olive wastewaters as sugars, phenolic compounds, polyalcohols, lipids and pectins. Hydroxytyrosol is the main phenolic compound of OW, demonstrating several benefits such as inhibition of low-density lipo-protein oxidation, free radical scavenging activity and *in-vitro* antimicrobial activity (De Marco et al., 2007). Several extraction and membrane separation processes have been described to obtain olive wastewater extracts (OWEs) yielding to different phenolic composition and antioxidant

Paper Received: 12 October 2020; Revised: 21 January 2021; Accepted: 24 April 2021
Please cite this article as: Apicella A., Adiletta G., Albanese D., Di Matteo M., Incarnato L., 2021, Biodegradable Films Based on Poly(lactic Acid) Coatings and Natural Olive-wastewater Extracts for Active Food Packaging, Chemical Engineering Transactions, 87, 85-90 DOI:10.3303/CET2187015

effectiveness (Sabatini, 2010). For this reason, previous research published by Apicella et al. (2019b) addressed the study of antioxidant activity, chemical-physical properties, and compatibility with the polymer matrix of several OWEs, obtained from different separation treatments. The most suitable OWE was selected to preliminary realize poly(lactic acid) (PLA) coated antioxidant films. In this study, new biodegradable coatings were produced with higher load of OWE (up to 20 wt%) and a detailed analysis on the release rate and *in vitro* antioxidant activity of the active films was conducted. The influence of films composition on the mass diffusive transport and polyphenols release in fatty food simulant was discriminated, evaluating the possibility to modulate films performance according to the food requirements. Moreover, preliminary shelf-life tests on avocado fruits were carried out, in order to qualitatively examine the potential of the active packaging in limiting modifications of the visual quality characteristics due to oxidative phenomena. Finally, the effect of the OWE on the films color and photo-oxidative stability under natural light exposure was also examined.

2. Experimental

2.1 Materials and preparation of the active coated films

Active coated films were produced using as substrate a biodegradable blown film based on a mixture of Poly(lactic acid) and Poly(butylene adipate-co-terephthalate) (Euromaster, Pistoia, Italy) hereafter referred as BS (i.e. Biodegradable Substrate). The substrate had a thickness of 22 ± 1.0 μm. The coating solution, hereafter referred as PLA, mainly comprised PLA4060 of Natureworks (Minnetonka, USA), having a D-lactide content of 12 wt% which confers an amorphous morphology to the polymer. The PLA coating solution was incorporated with the olive wastewater extract (named OWE), donated by Fangiano Farming Company (Nocera Terinese, CZ, Italy). The active agent was added at 0, 5, 10 and 20 wt% based on PLA content. Details on the chemical-physical properties of the OWE and on the preparation of the PLA active coatings are reported elsewhere (Apicella et al., 2019b). The PLA dry coating thickness was 7 ± 0.8 μm, leading to a total structure (BS/PLA) thickness of 29 ± 1.8 μm. DPPH (2,2-diphenyl-1-picrylhydrazyl) and Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) were obtained from Sigma Chemical Co. (St. Louis, Mo., USA). All organic solvents used were analytical grade.

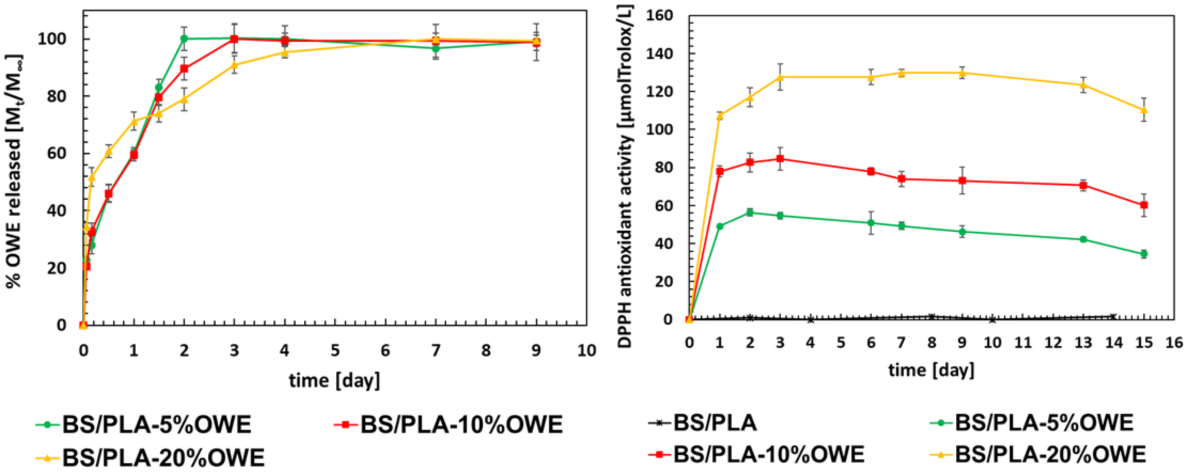
2.2 Characterization of the biodegradable active films

The release kinetic of the antioxidant films was evaluated by total immersion method as reported by Apicella et al., 2019b. 95% v/v Ethanol was selected as fatty foods simulant (D2) according to the Regulation (EU) 10/2011. Film samples were cut in rectangles of surface area equal to 1 dm^2 and immersed in 100 mL of release medium. The flasks were kept in the dark under magnetic stirring, at room conditions up to 15 days. An aliquot of the simulant was periodically sampled for measurements and then reinserted. The concentration of antioxidants released into the simulant was quantified using a UV-Vis spectrophotometer (Lambda Bio 40, Perkin Elmer, Waltham, MA, USA) at 280 nm, on the basis of a OWE Concentration vs. Absorbance curve in the range 0-600 ppm). The selected wavelength was the one at which the maximum absorbance of the OWE was found. To eliminate the influence of other polymer additives, release studies were also conducted on the control films (BS and BS/PLA) and no significant absorbance at 280 nm was observed. Results were expressed as percent ratio of M_t/M_∞ (M_t is the concentration of antioxidant (mg/mL) diffused at time t, and M_∞ represents the concentration of antioxidant diffused at equilibrium). The antioxidant activity released into the simulant solution was also measured by DPPH method, as reported by Adiletta et al. 2017 with some modifications. 50 μL of the simulant was withdrawn from the flask and mixed with 1.95 mL of DPPH methanolic solution (6×10^{-5} M) in a capped cuvette. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 20 min, then the absorbance was measured at 517 nm. The blank was conducted using the pure release medium. The obtained values were expressed as μmolTrolox/L, based on the standard curve of Trolox, and the maximum antioxidant activity was also expressed per unit volume of the coating, in mmolTrolox/dm^3. All analyses were performed in triplicate. The potential of the active films in preserving vegetables with high lipid content was investigated by sensory evaluation, in particular analysing colour and texture changes over time, of packaged fresh-cut avocado (cv. Bacon) fruit. The fruit samples were cut in slices of ca. 0.5 cm thickness, mixed thoroughly in order to allow a random selection and divided into single bags, each one measuring 15 x 10 cm^2 in size and approximatively of the same weight (ca. 40 g). The bags were sealed and a good contact among the active coating and the food surface was ensured. Then, the packages were stored in a refrigerator at 6°C up to 9 d. Three bags were prepared for each testing day and were opened to better evaluate the color changes on the avocado surface. Finally, colour measurements were carried out on the films by using a colorimeter CIE-Lab (Chroma Meter II Reflectance CR-300, Minolta, Japan) and the results were expressed according to colour coordinates L* (darkness/lightness), a* (greenness/redness), b* (blueness/yellowness). The chromatic parameters were evaluated soon after the coatings production (i.e. at time 0) and at regular time intervals (up to 73 d) exposing films to natural light, in order to evaluate possible effects due to photo-oxidation phenomena. The colour variation of the films over time was evaluated by Δa^* and Δb^* parameters, and by means of the colour-difference equation CIELAB $\Delta E^*_{ab} = [(L^*-L_0^*)^2 + (a^*-a_0^*)^2 + (b^*-b_0^*)^2]^{1/2}$, based on the coordinates L*, a* and b*, where the subscript 0 refers to the BS sample.

3. Results and discussion

3.1 Study of films release kinetic, antioxidant activity and inhibition of food oxidation phenomena.

The characterization of the release behavior of OWE from PLA coatings and the comparison among the release curves for all the films investigated are reported in Figure 1: Figure 1(a) depicts the %OWE released as percent ratio of M_t/M_∞, where measured M_∞ was 74, 100 and 154 mg/L for films at 5%, 10% and 20% OWE, respectively; Figure 1(b) shows the antioxidant activity in the food simulant during the time, expressed as µmolTrolox/L; Table 1 reports the maximum antioxidant activity per unit volume of the coating, expressed as mmolTrolox/dm^3 and the time at which it was reached. As can be observed from Figure 1(a), the films at 5% and 10% OWE follow almost the same release kinetic within the first 36 h. The percentage of antioxidant released is equal to ~20%, ~30%, 46%, 60% and ~80% after 1, 3, 12, 24 and 36 h, respectively, suggesting for these samples similar morphology and mass transport interactions regulating the diffusion mechanisms. The equilibrium was reached after 48 h and 72 h, respectively. By contrast, the BS/PLA-20%OWE film shows a different release behavior at short (t < 24 h) and long (t > 24 h) times. For t < 24 h, the release rate is the highest and the % OWE released is equal to ~34%, 52%, 61% and 71% after 1, 3, 12 and 24 h, respectively. After 24 h, a slower kinetic is established, and the system reaches the equilibrium after ca. 7 d of test. This behavior suggests an uneven distribution of the active phase within the coating thickness, being more concentrated on the coating surface and leading to a faster initial release of the antioxidant. The resistance to diffusive transport increases when the diffusion involves the inner side the coating and the concentration of the OWE increases. A less than linear increase of antioxidant activity (Figure 1(b)) was observed by increasing OWE content, with a maximum equal to 28.21 ± 1.93, 42.31 ± 2.2 and 64.86 ± 1.2 mmolTrolox/dm^3 for BS/PLA-5%OWE, BS/PLA-10%OWE and BS/PLA-20%OWE samples, respectively (Table 1). These outcomes confirmed the effectiveness of the produced films as carriers for antioxidants release, with the possibility to tune the release kinetic with a proper design of the systems composition and to tailor the films performance on the preservation requirements of the target food.



(a) (b)

Figure 1: (a) OWE release rate (Mt/M∞) from active coated films in 95% Ethanol, at 23°C and (b) DPPH antioxidant activity (expressed in µmolTrolox/L) released from active coated films in Ethanol 95%, at 23°C.

Table 1: Time at which the maximum antioxidant activity is reached, and average maximum antioxidant activity (expressed as mmol Trolox/dm3) for the active coated films. Values followed by different letters within the same column were significantly different according to Duncan's test (P < 0.05).

Sample Film	Time at max antioxidant activity [d]	Average max. antioxidant activity [mmolTrolox/dm^3]
BS/PLA-5%OWE	2	28.21 ± 1.93^a
BS/PLA-10%OWE	3	42.31 ± 2.2^b
BS/PLA-20%OWE	7	64.86 ± 1.2^c

The results, coupled with the measured low barrier performance of the films (Apicella et al., 2019b) suggested their possible application to preserve sensitive foods with short shelf life as fresh-cut horticultural products. To this aim, the efficacy of the active films in inhibiting oxidation/browning phenomena of sensitive foods was evaluated by preliminary shelf life tests on fresh-cut avocado slices. Figure 2 shows the comparison among the pictures of the samples stored at 6°C up to 9 d without package (first row), in BS/PLA film (second row) and BS/PLA-20%OWE film (third row), taken as example. Avocado is a fruit of unusually high oil content (15% to 30% depending on the variety) and its shelf-life is severely determined by oxidative processes, which affect both lipidic and aqueous fractions. The decay is produced by enzyme mediated oxidative reactions, with the formation of dark compounds, as well as changes in lipids due to auto-oxidation (Elez-Martinez et al., 2005). In order to better evaluate the colour changes undergone by the packaged fruits, different bags were opened at fixed times. As observable in Figure 2 (b), (c) and (d), unpackaged avocado displayed the fastest and most severe dehydration and blackening of the tissues. Fruit samples packaged in BS/PLA film (Figure 2 (f), (g) and (h)) also showed a progressive darkening attributable to oxidation phenomena, which was mainly concentrated on the fruit edges and became more consistent by increasing the storage time. The pulp also exhibited an ongoing loss of firmness which was qualitatively evaluated to the touch.

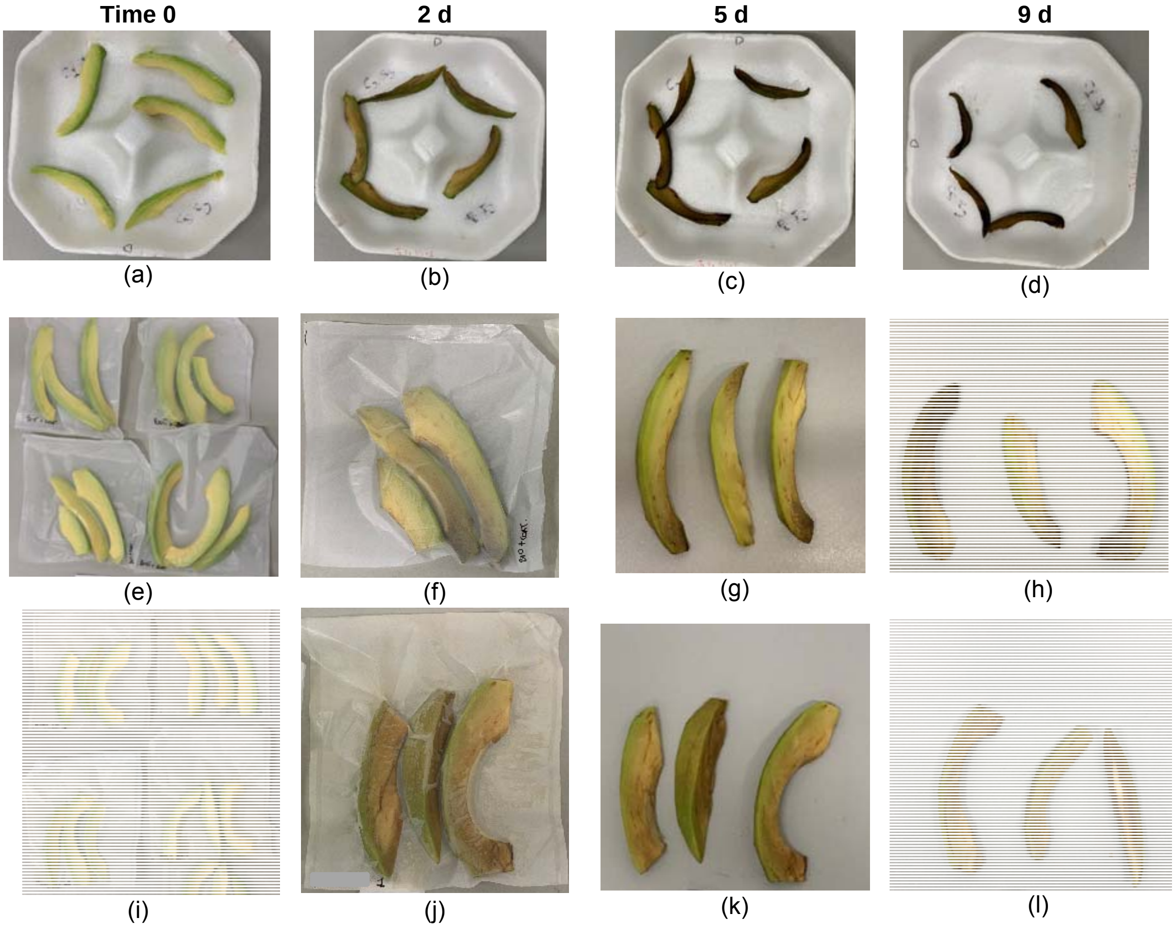


Figure 2 Pictures of avocado slices unpackaged (first row), packaged in BS/PLA (second row) and BS/PLA-20%OWE (third row) films. From left to right: slices after 0, 2, 5 and 9 storage days at 6°C.

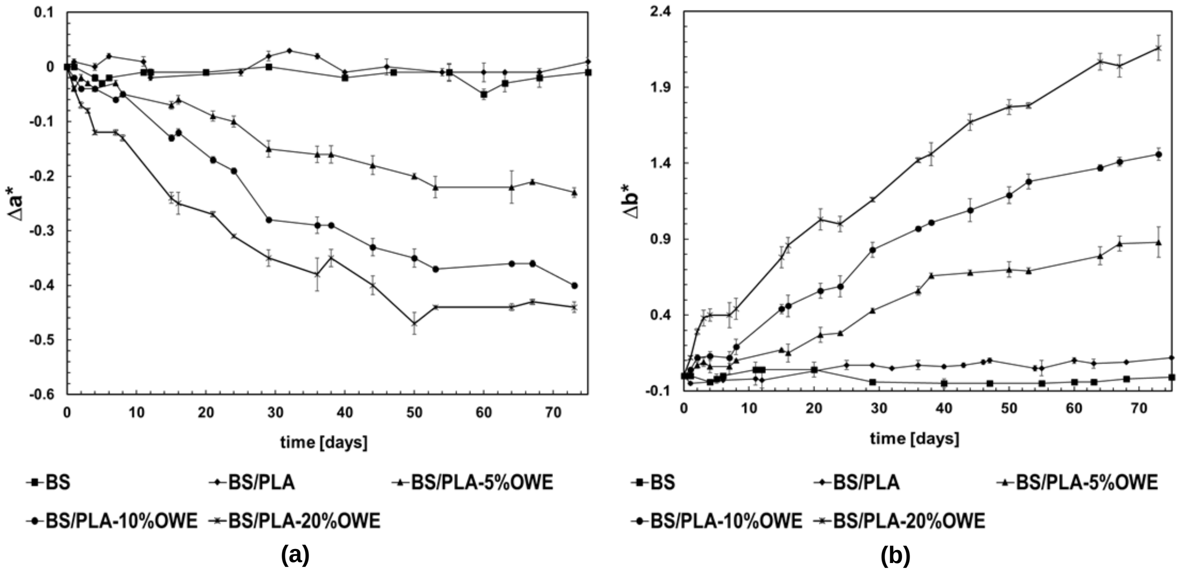
The different avocado slices stored in the active film at 20%OWE (Figure 2 (j), (k) and (l)), on the other hand, experienced a rapid color variation from yellow to brown. However, the homogeneous distribution of the brown color over the surface of the fruit, which remained almost unaltered over the time, could be attributable to the OWE migration from the film to the avocado pulp. In fact, it is well known that the OWs are characterized by a typical dark brown color, due to the polymerization of tannins and to low molecular weight phenolic compounds (Apicella et al., 2019b; Otles and Semih, 2012). Moreover, no appearance of oxidation black spots focused on the edges was detected, and a good preservation of the pulp texture was observed. These exploratory results underlined the potential helpful role of the OWE in retarding the oxidation/browning reactions of sensitive foods, and pave the way to more in-depth, quantitative shelf-life studies on the most appropriate strategies to prevent/limit the transfer of the OWE brown color while keeping intact the benefits of the antioxidant packaging.

3.2 Evaluation of optical properties and photo-oxidative stability

The effect of the OWE addition on the optical properties and color stability of the PLA coatings was evaluated by colorimetric analyses. Table 2 reports the CieLab color coordinates L*, a*, b* and the chromatic variation ΔE for all the films soon after their production. No significant differences ($P > 0.05$) were measured among BS and BS/PLA samples, thanks to PLA excellent optical properties (Apicella et al., 2019a). Instrumental color analyses confirmed that the incorporation of the OWE gave a colored brown taint to the films, with an increase in greenness (a*) and yellowness (b*) values by increasing the antioxidant concentration.

Table 2: CieLab color coordinates L, a* and b* for the neat biodegradable substrate (BS) and for the PLA coated films at 0, 5, 10 and 20% OWE concentration. Chromatic variation ΔE is also reported. Values followed by different letters within the same column were significantly different according to Duncan's test ($P < 0.05$).*

Sample Film	L*	a*	b*	ΔE
BS	96.9 ± 0.2^{b}	-0.74 ± 0.12^{c}	2.28 ± 0.20^{a}	-
BS/PLA	97.3 ± 0.8^{b}	-0.80 ± 0.25^{c}	2.27 ± 0.26^{a}	0.40
BS/PLA-5%OWE	96.7 ± 0.3^{b}	-1.04 ± 0.30^{b,c}	3.98 ± 0.11^{b}	1.73
BS/PLA-10%OWE	96.2 ± 1.5^{b}	-1.51 ± 0.33^{a,b}	6.02 ± 0.44^{c}	3.80
BS/PLA-20%OWE	93.9 ± 0.8^{a}	-1.80 ± 0.56^{a}	11.9 ± 0.63^{d}	10.1



(a) (b)

Figure 3: Change in redness ($\Delta a^*=a|_t - a|_{t=0}$, (a)) and yellowness ($\Delta b^*=b|_t - b|_{t=0}$, (b)) during the time, for the BS substrate and BS/PLA coated films at 0, 5, 10 and 20% OWE concentration.

The total color change of the active films (ΔE*) with respect to the neat BS and BS/PLA films was also highlighted. Similar outcomes were also found by other authors (Marcos et al., 2014; Manzanarez-López et al., 2011) reporting increased yellowness in PLA films containing olive leaves extract or α-tocopherol. In order to analyze the photo-oxidative stability of the films on long storage time, the changes of a* and b* values with respect to time 0 was measured over 73 d. Δa* and Δb* trends are shown in Figure 3 (a) and (b), respectively. No significant color change for the BS substrate and the BS/PLA films were observed, while the BS/PLA-OWE coatings undergo an increase both in redness and yellowness of the samples, which became more significant by increasing the antioxidant content. In particular, the total increase in Δa* and Δb* parameters, after 73 d, was equal to -0.23, -0.4, -0.44 and 0.88, 1.46, 2.16 for the films at 5%, 10% and 20% OWE, respectively. The progressive browning is attributable to the oxidation of the active phase over the time. Polyphenols are very unstable and susceptible to degradation because of high temperatures, light, oxygen, solvents, enzymes, metallic ions, or association with other food constituents (Volf et al., 2014). However, only minor changes in the photo-oxidative stability of the films occurred considering short shelf-life packaging application times.

4. Conclusions

In this work, innovative biodegradable antioxidant films based on olive mill wastewaters were developed through a conventional technique commonly applied in packaging industry. The analysis on the release rate and *in vitro* antioxidant activity proved that the films are able to be used as carriers for the controlled release of the antioxidant agent, with the possibility to modulate the release performance by properly designing the films composition. An increasing antioxidant activity (from 28.21 to 64.86 mmolTrolox/dm^2) and release time (from 2 to 7 d) was found by increasing OWE concentration up to 20%. Preliminary shelf-life tests endorsed the perspectives to use films as 100% green alternative for preserving O_2-sensitive foods with high respiration rates, such as fresh-cut avocado. However, appropriate strategies to prevent/limit the transfer of the natural OWE brown color to the food have to be implemented for films application. Finally, only minor changes in the photo-oxidative stability of the films occurred if considering short shelf-life packaging applications times.

Acknowledgements

The authors gratefully acknowledge Fangiano Farming Company for gently supplying of olive wastewater extract used in this research.

References

Adiletta G., Liguori L., Albanese D., Russo P., Di Matteo M., Crescitelli A., 2017, Soft-Seeded pomegranate (Punica granatum L.) varieties: preliminary characterization and quality changes of minimally processed arils during storage. Food Bioprocess Technology, 10, 1631–1641.

Apicella A., Scarfato P., Di Maio L., Incarnato L., 2019a, Sustainable Active PET Films by Functionalization With Antimicrobial Bio-Coatings, Frontiers in Materials, 6:243.

Apicella A., Adiletta G., Di Matteo M., Incarnato L., 2019b, Valorization of Olive Industry Waste Products for Development of New Eco-sustainable, Multilayer Antioxidant Packaging for Food Preservation., Chemical Engineering Transactions, 75, 85-90.

Apicella A., Scarfato P., Di Maio L., Incarnato L., 2019c, Antioxidant activity of bio-coatings for food packaging, Italian Journal of Food Science, 31,198-203.

Apicella A., Scarfato P., Di Maio L., Incarnato, L., 2018, Transport properties of multilayer active PET films with different layers configuration. Reactive and Functional Polymers, 127, 29–37.

De Marco E., Savarese M., Paduano A., Sacchi R., 2007, Characterization and fractionation of phenolic compounds extracted from olive oil mill wastewaters, Food Chemistry, 104, 858-867.

Elez-Martinez P., Soliva-Fortuny R. C., Gorinstein S., Martin-Belloso O., 2005, Natural Antioxidants Preserve the Lipid Oxidative Stability of Minimally Processed Avocado Purée, Journal of Food Science, 70, 325–329.

Guillard V., Gaucel S., Fornaciari C., Angellier-Coussy H., Buche P., Gontard, N., 2018, The Next Generation of Sustainable Food Packaging to Preserve Our Environment in a Circular Economy Context. Frontiers in Nutrition, 5, 121.

Marcos B., Sárraga C., Castellari M., Kappen F., Schennink G., Arnau, J., 2014. Development of biodegradable films with antioxidant properties based on polyesters containing α-tocopherol and olive leaf extract for food packaging applications, Food Packaging and Shelf Life, 1, 140–150.

Manzanarez-López F., Soto-Valdez H., Auras R., Peralta E. 2011, Release of α-Tocopherol from Poly(lactic acid) films, and its effect on the oxidative stability of soybean oil, Journal of Food Engineering, 104, 508–517.

Martiny T.R., Raghavan V., Moraes, C.C., Rosa G.S., Dotto G.L., 2020, Bio-Based Active Packaging: Carrageenan Film with Olive Leaf Extract for Lamb Meat Preservation, *Foods* , *9*, 1759.

Nunes M.A., Pimentel F.B., Costa A.S.G., Alves R.C., Oliveira M.B.P.P., 2016, Olive by-products for functional and food applications: Challenging opportunities to face environmental constraints. Innovative Food Science and Emerging Technologies, 35, 139–148.

Otles S., Semih I., 2012, Treatment of Olive Mill Wastewater and the Use of Polyphenols Obtained After Treatment, Italian Journal of Food Safety, 1, 85-100.

Sabatini N., 2010, Recent Patents in Olive Oil Industry: New Technologies for the Recovery of Phenols Compounds from Olive Oil, Olive Oil Industrial by-Products and Waste Waters, Recent Patents on Food, Nutrition and Agriculture, 2, 154-159.

Volf I., Ignat I., Neamtu M., Popa V., 2014, Thermal stability, antioxidant activity, and photo-oxidation of natural polyphenols, Chemical Papers, 68, 121–129.