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Active edible coating to preserve fresh figs

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The present study aimed to evaluate the effects of active edible coating (AEC) on fresh fruits to extend the shelf life (15 days) and maintain their nutraceutical content by minimizing the rate of respiration and reducing the water loss.
The coating efficacy was enhanced with the addition of active compounds extracted from agriculture by-products, such as pomegranate peel, olive leaf, and chestnut bark. In particular, a high efficiency in preserve the nutraceutical characteristics was obtained when figs were coated with the following active polysaccharide edible mixture: 70/30 Alginic acid sodium salt (viscosity 20-40 cps, 1% in H_2O)/ Pectin from the citrus peel (low degree of esterification), added with 0.5% Olive Leaf Extract (OLE), prepared as described (Volpe et al., 2014). The safeguard of nutraceutical content was monitored in the two weeks following the fruit harvest by measuring total phenols and flavonoids amounts and specific bioactive phenolic compounds. For comparison, figs in polypropylene containers, wrapped in the micro-perforated film (UN-FIG), as normally marketed, and figs treated with an edible coating (EC) without biomolecules were prepared and analyzed. The results showed a decrease of about 15% of phenols and flavonoids content in UN-FIG after 15 days to time 0, while AEC induced a slight but significant increase compared to UN-FIG and EC-FIG samples after 15 days.
The results obtained regarding the biochemical characterization demonstrated the ability of the active coating, particularly OLE, to contain the decay of the quality and functional characteristics of figs during storage.

1. Introduction

Ficus carica L., also known as the common fig, is a widespread tree native of southwest Asia and the eastern Mediterranean region, commonly grown in warm and dry climates. It belongs to the botanical Moraceae family and its fruit is one of the most important agricultural products of the Mediterranean regions. Indeed, fig fruit is an excellent source of nutrients and bioactive compounds that contribute to a healthy diet when properly inserted into human nutrition (Arvaniti et al., 2019). Several studies on fruits and derived products of Ficus carica highlighted the presence of numerous bioactive compounds including organic acids, amino acids, vitamins, phytosterols, triterpenoids, phenolic acids, flavonoids, and other classes of secondary metabolites (Veberic et al., 2008).
The bioactive compounds of fig fruits are responsible for biological activities to which beneficial effects on human health are attributed (Nunes et al., 2016; Solomon et al., 2006). However, the fruits are highly perishable after harvest resulting in limited shelf life, so the search for methods that retard fruit decay is of great interest to all the stakeholders from production to distribution of fresh fruits and so to preserve the functional properties.
Perishable fig nature has adversely affected their market in recent years so methods to increase the postharvest life for fresh fig fruit are continually being proposed (Kong et al., 2013). Post-harvest storage-life of fruits can be effectively increased by storage at low temperatures, modified atmosphere but more sustainable methods can be used (Antunes et al., 2012, Leneveu-Jenvrin et al., 2020, Cofelice, Lopez, and Cuomo 2019). Among the proposed methods, edible coating on fresh products can be a valid alternative to other storage techniques to reduce the physicochemical and nutritional changes during storage (Cofelice, Lopez, and Cuomo 2019, Paolucci et al., 2020) (Villalobos et al., 2016).
Natural polysaccharides are good candidates as base ingredients of both edible films and coating to provide a shelf-life extender (Hassan et al., 2018). Furthermore, active additives, such as antioxidant compounds and

antimicrobial agents can be included in the formulations of edible films and coatings to strengthen the coating efficacy and extend more the shelf-life of foods (Reyes-Avalos et al., 2016).
In this regard, olive leaf is a cheap and wide available olive oil production process by-product widespread in the Mediterranean region and its extract contains a large number of bioactive molecules as oleuropein, hydroxytyrosol, phenolic acids, and others (Flamminii et al., 2019). In particular, oleuropein possesses health-promoting properties, mainly mediated by its antioxidant characters, exerting several biological activities including antidiabetic, antihypertensive, anti-inflammatory, immune-stimulant, cardioprotective, hypotensive, and hepatoprotective actions (Ahamad et al., 2019, Zhang, Zhao, and Wang 2020).
The aim of this study has been to safeguard one of the most important issues of fruit consumption as the nutraceutical content during shelf life and the use of an active edible coating seemed to be a valid solution.
The alginic acid and agar have been used to make an edible coating, while Olive Leaf Extract (OLE) at a concentration of 0.5% was used as the bioactive agent. Other active components, including pomegranate peel extracts, chestnut peel extracts etc were explored in an initial screening; however OLE was preferred and selected for its easy commercial availability, solubility, and low cost.
To assess the effectiveness of the active coating, changes in the concentrations of total polyphenols, flavonoids and specific phenolic compounds were determined on fresh figs stored at 4 °C for 15 days.

2. Materials and methods

2.1. Chemicals

Alginic acid (AA) sodium salt (viscosity 20-40 cps, 1 % in H_2O) and Agar (Ag) fine powder (viscosity range 5-50 cps) were from Merck KGaA, Darmstadt, Germany. All reagents and solvents were of analytical or HPLC grade. Olive Leaf Extract (OLE) (6% oleuropein) was purchased from Epo S.r.l., Milano Italy.

2.2. Fruit samples

Samples of common figs (*Ficus carica* L.), free of physical damage and without microbiological contamination (data not shown), analysed in this study, derived from a fig population that is located in its origin areas of San Mango sul Calore (Avellino province, Campania region, Italy). Fruits were harvested in July 2019, placed in a 4°C refrigerated box for shipment, and directly subjected to analysis. Fruits were divided into five batches, about 500 g (5 or 6 fruits), before treatments were applied, while the control (UN-FIG) consisting of untreated samples was stored in a polypropylene container wrapped in micro-perforated film.

2.3. Sample preparation

AA and Ag were dissolved in demineralized water at 100 °C, under stirring. The solution concentration was 1.5% w/v, with the percentage ratio of the two polymers of 70/30 w/w (considered an optimal ratio of the two components based on the experiments that varied the percentage of the two in the range 90/10, 80/20, 70/30, 60/40, 50/50 and vice versa). After biopolymer solubilization, the solution was maintained at 35 °C until use. The fresh figs, after careful washing and drying with kitchen paper, were dipped in the coating solution and placed on a plastic grid until coating gelation. Thereafter, the figs were placed in cardboard containers with cover in micro-perforated polymeric material and stored at 4 °C for 15 days. For active coating, the solution AA/Ag (70/30) was added with 0.5% of OLE.

2.4. Fig preservation

Figs were divided into a control group and three groups subjected to the following treatments: Group 1: untreated figs used as control (UN-FIG); Groups 2: figs were preserved in a modified atmosphere with the following percentage of gas 2% O_2, 10% CO_2, 88% N_2 (MAP-FIG); Group 3: figs were coated with a polysaccharide edible coating (AA/Ag 70/30) (EC-FIG); Group 4: figs were coated with an active polysaccharide edible coating (AA/Ag 70/30 added with OLE 0.50%) (AEC-FIG). Analyses were performed in triplicate on all fig samples at 0 and 15 days.

2.5. Polar compound extraction

The full fig polar extracts were prepared from 5 g of sample with 50 mL of 80% aqueous methanol by triple extraction using an Ultra-Turrax Homogenizer. The methanol extracts obtained by centrifugation (2 min, 5000*xg*) were filtered on disposable syringe 0.45 µm filters (Millipore, Billerica, MA, USA) concentrated to dryness by rotary evaporation (30°C in a water bath) and the resulting residue was stored in a freezer (-20°C) for subsequent analyses.

2.6. Total polyphenol and flavonoids content

The total phenolic content (TPC) was determined by the Folin-Ciocalteu assay with minor modifications (Musci and Yao 2017). Briefly, aliquots of extract or standard (20-100 µg / mL of gallic acid) were brought at 1 mL final volume with distilled deionized water (ddH_2O). Folin-Ciocalteu's reagent (100 µL) was added to the mixture and after 5 min 100 µL of 7.5% Na_2CO_3 and 400 µL of ddH_2O were added. The absorbance was read at 750 nm after incubation in the dark for 90 min at RT. TPC was expressed as mg gallic acid equivalents (GAE)/100g fresh weight (FW).

Total Flavonoid Content (TFC) were measured by the aluminum chloride colorimetric assay (Khodaie et al. 2012). An aliquot of the extract was mixed with 1 mL of H_2O and 75 µL of 5% $NaNO_2$. After 5 min, 150 µL of 10% $AlCl_3$ were added, and after 10 min, 500 µL of 1 M NaOH were added. The final volume was adjusted to 2.5 mL with H_2O. The standard solution of catechin (20-100 µg/mL) was processed in the same way. The absorbance was measured at 510 nm and TF were expressed as mg catechin equivalents (CE)/100g FW.

2.7. HPLC analysis of specific phenolic compounds

Analysis of specific phenolic compounds (SPC) was performed by HPLC/UV-DAD. Lyophilized extracts were dissolved in 1 mL of methanol and filtered before HPLC analysis. The injected sample volume was 20 µL and HPLC was carried out by using a method previously described (Bennett et al., 2006). In brief, a C_{18} Hypersil column (250 x 4.6 mm, 5 µm, Thermo Fisher Scientific, USA) was used. The solvent flow rate was 0.9 ml min^{-1} and the gradient was 0-30 min, 10% B; 30-35 min, 55% B; 35-45 min, 100% B; where solvent A was 2% acetic acid in water and solvent B was 0.5% acetic acid in 50% acetonitrile. UV detection was carried out at 280 nm. The pure standards utilized were: (+)-catechin, (-)-epicatechin, quercetin, rutin, oleuropein, hydroxytyrosol, tyrosole and gallic, *trans*-cinnamic, *p*-coumaric, ferulic, chlorogenic, vanillic and protocatechuic acids. Peak identifications were achieved by retention times and direct comparison to standards. Results were expressed as mg of SPC /100g FW.

2.8. Statistical analysis

All tests were performed in triplicate and expressed as mean ± Standard Deviation (SD) calculated by Microsoft Excel 2013. Statistical analysis was carried out by GraphPad Prism (version 5). Significant differences were determined by two-way analysis of variance (ANOVA) with Bonferroni post-tests. Mean values were considered not significantly different at p e 0.05.

3. Results and discussion

The figs samples were analysed at time zero and after 15 days after harvest to determine total polyphenols and flavonoids content and specific components by HPLC analysis.

Total polyphenols content of fresh fig fruit preserved in different packaging systems in function of shelf life is reported in Table 1. The results highlight the capacity of active edible coating (AEC-FIG) to enhance the content of polyphenols comparable to the initial quantity while UN-FIG and MAP-FIG show a prominent decrease and EC-FIG was almost the same respect to the initial content. The initial total phenolic content was 97.1 mg GAE/100g FW while the total phenolic content after 15 days was 80.5 mg GAE/100g FW.

On the other hand, AEC-FIG increases by about 36% compared to UN-FIG after 15 days, going from 96.9 to 98.5. The explanation of this behaviour of the active edible coating (AEC-FIG) lies in the high content of bioactive molecules (polyphenols and others compounds) present in the olive leaf extract added to the coating mixture.

Table 1: TPC and TFC in fig fruit (mg GAE/100 g FW) and (mg CE/100g FW) respectively at 0 and 15 days of storage at 4°C in different preservation systems.

Sample	Treatment	TPC (mg GAE/100g FW)		TFC (mg CE/100g FW)	
		Day 0	Day 15	Day 0	Day 15
UN-FIG	Untreated (Control)	97.1 ± 2.3	80.5 ± 1.9	50.8 ± 1.1	40.2 ± 0.9
MAP-FIG	$O_2/CO_2/N_2$ 2/10/ 88 (%)	96.7 ± 2.0	86.1 ± 1.2	53.2 ± 1.2	45.0 ± 1.0
EC -FIG	AA/Ag 70/30 (%)	95.8 ± 1.5	91.4 ± 1.1	52.9 ± 1.5	51.8 ± 1.1
AEC-FIG	AA/Ag 70/30 (%) / 0.5% OLE	96.9 ± 1.8	110.2 ± 2.0	53.8 ± 1.0	55.8 ± 1.1

Total flavonoids content was determined in function of shelf life and results are reported in the same Table 1. TFC of UN-FIG decreased from 54.8 mg to 40.2 mg CE/100g FW after 15 days, showing a consistent loss of flavonoids during storage. Also in the MAP-FIG sample, the amount of flavonoids decreases in the same way.

TFC of EC-FIG sample remains almost constant, while AEC-FIG sample increases TFC at the end of the experimentation. Also in this case, the active component, OLE, plays a decisive role in the conservation of the initial content of flavonoids, ranging from 53.8 mg to 55.6 mg CE/100g FW, demonstrating that the active component in the coating was responsible for flavonoid content protection.

In Table 2, some of the specific components of fig fruit polyphenols are determined after 0 and 15 days in the untreated group (UN-FIG) and in the treated group.
During storage, a decrease of almost all the polyphenolic components is revealed in UN-FIG and MAP-FIG samples, while no change is recorded for the EC-FIG sample.
A modest increase of polyphenol and the presence of oleuropein and other phenolic compounds (hydroxytyrosol, tyrosole, vanillic acid) representative of olive leaf extract are found in the AEC-FIG sample. Particularly oleuropein is a phytochemical that can be found in abundance in olive trees (Benincasa et al., 2019) and it is easily extracted as part of the phenolic fraction of olive fruits, leaves, and seeds. Several studies demonstrated that OLE has the potential to be used in the food industry to inhibit the growth of foodborne pathogens either in foods, on food processing equipment, or in food packaging material (Liu et al., 2017; Borjan et al., 2020).
The hydrophilic nature of OLE extract allows the total dissolution in the coating and creates a network of physical bonds with the polysaccharide matrix, enhancing its barrier effect against humidity and oxygen to protect figs sample from decay.

Table 2: SCP in fig fruit at 0 and 15 days of storage at 4 °C in different preservation systems.

Compound (mg/100g FW)	Untreated (Control)		MAP-FIG		EC-FIG		AEC-FIG	
	D 0	D 15	D 0	D 15	D 0	D 15	D 0	D 15
Gallic acid	1.95±0.11	1.50± 0.09	1.95±0.11	1.75±0.09	1.95±0.11	1.90±0.08	1.95±0.10	2.4±0.12
(+) Catechin	2.4±0.08	2.0±0.09	2.4±0.1	2.2±0.11	2.4±0.15	2.3±0.12	2.4±0.14	2.6±0.11
Chlorogenic acid	0.75±0.05	0.62±0.0	0.75±0.02	0.68±0.0	0.75±0.03	0.72±0.04	0.75±0.03	0.78±0.05
Protocatecuic acid	0.12±0.0	0.15±0.0	0.12±0.0	0.12±0.0	0.12±0.0	0.14±0.0	0.12±0.0	0.15±0.0
(-) Epicatechin	0.85±0.03	0.70±0.02	0.85±0.04	0.76±0.03	0.85±0.03	0.80±0.02	0.85±0.04	0.89±05
Rutin	12.2±1.1	10.5±0.9	12.2±0.6	11.2±0.7	12.2±0.8	11.5±0.7	12.2±0.5	12.6±0.5
Quercetin	2.9±0.1	2.5±0.11	2.9±0.11	2.65±0.09	2.9±0.12	2.75±0.11	2.9±0.11	3.2±0.12
p-coumaric acid	0.35±0.01	0.25±0.0	0.35±0.02	0.30±0.02	0.35±0.02	0.32±0.02	0.35±0.02	0.40±0.03
trans cinnamic acid	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.15±0.01
Ferulic acid	0.1±0.0	nd	0.1±0.0	0.1±0.0	0.1±0.0	0.15±0.0	0.1±0.0	0.1±0.0
Oleuropein	nd	nd	nd	nd	nd	nd	nd	16,2±1.3
Hydroxytyrosol	nd	nd	nd	nd	nd	nd	nd	1.45±0.07
Tyrosol	nd	nd	nd	nd	nd	nd	nd	0.70±0.04
Vanillic acid	0.45±0.02	0.35±0.0	0.45±0.02	0.39±0.01	0.45±0.01	0.42±0.02	0.45±0.01	1.05±0.03

Results are expressed as mg of SPC/100g FW. D indicates day

Phenolic compounds are widely distributed in fruit and vegetables, including in figs. They represent important constituents of fig fruit quality contributing to the taste, flavor and nutraceutical properties and are the subject of increasing scientific interest because of their possible beneficial effects on human health and in preventing the development of human diseases (Cory et al., 2020; Veberic et al., 2008). The presence of polyphenols in food can be particularly important for consumer health, thus, it becomes very important to preserve their content during shelf life. The safeguard of the active components was the main objective of the present research. The formulation of the described edible active coating allowed the achievement of this goal.

4. Conclusions

This study highlighted the ability of an edible coating added with olive leaf extract to preserve the nutraceutical characteristic of fresh figs. The main innovation of the edible coating employed consisted in including in its formulation molecules possessing a high biological activity combined with features leading to increase food shelf life. Moreover, the biopolymers and the resources used for the production of the extract, the leaves of the olive tree, are widely available and inexpensive. Furthermore, the latter is recovered from waste during the process of pruning and harvesting of the olive, according with the concept of circular economy. In conclusion, the results of the present study can result of interest for both economic aspects and nutritional safeguard.

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