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Evaluation of the antioxidant/antimicrobial performance of *Posidonia oceanica* in comparison with three commercial natural extracts and as a treatment on fresh-cut peaches (*Prunus persica* Batsch)

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ABSTRACT

This research aimed at extending the choice of natural antimicrobials/antioxidants for food applications. Four plant extracts, *Posidonia oceanica* (PO), Green Tea (GT), Grape seeds (GS) and Grape skin (GK), were analyzed to determine their total phenolic content, antioxidant activity and *in vitro* antimicrobial performance. PO extract showed the highest total phenolic content (711 mg gallic acid/g extract) and antifungal activity against *Aspergillus niger* and *Penicillium chrysogenum*. The highest antioxidant (3.81 mg/L EC_{50}) and antibacterial activities (bactericidal against Gram positives and bacteriostatic against Gram negatives) were found for GT extract.

The best performing extracts (PO and GT) were applied by dipping on peach slices in storage trials. Microbiological and pomological parameters were evaluated during 7 d storage. Total aerobic count, *Pseudomonas* as well as yeasts and moulds populations, were reduced by about 0.5 log cfu/g, mainly up to 5 d in all treated samples compared to the control. Total soluble solids, titratable acidity and colour ($L^*a^*b^*$) changes were also delayed in treated fruit.

1. Introduction

One of the most important research areas as rated by a large majority of food companies is the development of healthy foods, and the introduction of fresh cut produce onto the market, in order facilitate fruit consumption, is rapidly growing (Lung and Zhao, 2016).

Nevertheless, the high perishability of minimally processed fruit may lead to an increase in food waste and economic losses (Amani and Gadde, 2015). Throughout production process, cell breakage takes place causing juice leakage and leading to microbial contamination and growth. Moreover, the contact between enzymes and cell juice under oxygen exposure increases cell respiration and activation of fruit senescence. Specifically, minimally processed fruit, and peaches in particular, are very susceptible to flesh browning (Denoya et al., 2016). Therefore one of the current challenges for the agro-food companies is to lengthen cut fruit shelf life, consequently improving attractiveness to customers as well as food safety.

The food industry has been increasingly employing polyphenols to limit enzymatic oxidation which affects the shelf life of ready-to-eat fruit (Gyawali and Ibrahim, 2014). The beneficial properties of polyphenols on human health also have to be taken into account (Pandey and Rizvi, 2009).

As sources of polyphenols, several trials have been carried out using plant extracts from common or endemic species (Perumalla and Hettiarachchy, 2011) or alternatively from by-products of the agro-food industry (Balasundram et al., 2006). Nowadays, exploitation of by-products and/or residues represents one of the environmental and economic priorities. Several substances discarded from agro-food production can find alternative applications in different contexts. As examples, grape skin (GK) and seeds (GS) are the main wastes from the wine industry, nevertheless they are appreciated for their high phenolic content which includes flavonoids, phenolic acids and non-flavonoid compounds (Poudel et al., 2008). The hydroxyl groups of gallic acid, present in grape by-products, showed antimicrobial activity against *Bacillus cereus*, *B.*

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subtilis, *B. coagulans*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*; also all the substituents of the benzene rings were found effective against *S. aureus* (evaluated by Minimal Inhibitory Concentration assay) (Jayaprakasha et al., 2003).

The antioxidant capacity of polyphenols can also be used to prevent or slow down enzymatic oxidation of vitamins and pigments contained in ready-to-eat fruit and vegetables (*e.g.* enzymatic browning), thus preventing the loss of nutritional elements and increasing attractiveness to consumers due to the maintenance of their sensorial characteristics (Rojas-Grau et al., 2009). Moreover, they can be added as antimicrobials thus increasing product shelf life (Guillén et al., 2013).

Flavonoids from plants have high antioxidant capacity and they are widely used substances, including catechin, epicatechin, gallocatechin, epigallocatechin, catechin gallate, epigallocatechin-3-gallate (the most abundant and biologically active compound in green tea), gallocatechin gallate and epicatechin gallate (Sutherland et al., 2006). The hydroxyl groups in the ring structure of catechin can be easily oxidized (Janeiro and Brett, 2004).

Green tea (GT) is one of the plant extracts with high antioxidant and antibacterial activities, and with anti-tumor effects due to its catechin content. GT catechin showed antimicrobial activity against Gram positive and Gram negative bacteria including certain pathogens of the gastrointestinal tract such as *S. aureus, S. epidermis* and *Plesiomonas shigelloides*, but it was not effective against *E. coli, Pseudomonas aeruginosa* and *Aeromonas hydrophila* (Kusmita et al., 2015).

Posidonia oceanica (PO) is a marine endemic plant of the Mediterranean sea protected by the EU (92/43 EEC Habitat Managerial and Community Board 97/62/EEC). It is an important species in coastal waters defence, forming extensive marine grasslands (Foden and Brazier, 2007). Twenty-three phenolic compounds were identified in this species (Cuny et al., 1995; Agostini et al., 1998) and several studies showed that PO extract is able to inhibit the growth of both Gram positive and Gram negative bacteria, and it was particularly effective against *P. aeruginosa* and *S. aureus* (Berfad and Alnour, 2014), as well as yeasts. PO extract was also assayed in the biomedical field, proving its high anti-diabetic and anti-oxidant effects (Gokce and Haznedaroglu, 2008). However, some reports found evidence for the transfer of toxins originating from toxic dinoflagellates which live as epiphytes on PO leaves (Bellassoued et al., 2012).

The present research is aimed at extending the choice of natural antimicrobials/antioxidants for food applications, derived from PO, GT, GS and GK. These extracts were analyzed to determine their total phenolic content and antioxidant activity as well as *in vitro* antimicrobial performance. The two best performing extracts were also used to set up fresh-cut storage trials on peach slices, applying the dipping procedure. Peach (*Prunus persica* L. Batsch) is a climacteric fruit that contains carbohydrates, organic acids, pigments, phenolics, vitamins, volatiles, antioxidants and trace amounts of proteins and lipids, which make it very attractive to consumers (Kader and Mitchell, 1989). However, peaches are susceptible to physiological disorders (internal breakdown and chilling injury), pathogen (moulds) and processing manipulation (browning of tissues) (Caceres et al., 2016).

2. Materials and methods

2.1. Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) 97%, Ethanol (≥99.8%), Ethyl acetate (anhydrous, 99.8%), Folin-Ciocalteu's phenol reagent, hydrochloric acid, sodium hydroxide and sodium sulfate (≥99.0%, anhydrous), were purchased from Sigma–Aldrich (Gallarate, MI, Italy).

Green tea, grape skin (*Vitis vinifera* L., Chardonnay variety) and seed extracts for oenological use (antioxidants) were obtained from DAL CIN GILDO S.p.A. (Concorezzo, MB, Italy).

2.2. Posidonia oceanica *extract*

Posidonia oceanica (L.) Delile was collected by scuba diving from Palermo (Sicily, Italy), Tyrrhenian Sea, in October 2014. Note that as this is a protected marine plant, for use in any industrial application, it should be sourced from aquaculture systems under controlled growing conditions. The epiphytes on the leaves were removed with paper towels without damaging the organs, as reported by Gokce and Haznedaroglu (2008). Leaves were dried in the dark at 20 ± 1 °C and then stored at 4 ± 1 °C before use. The extract was obtained according to the method of Gokce and Haznedaroglu (2008). Briefly, homogenized tissues were infused in 50% (v/v) ethanol-water solution for 3 h in a water bath at 40 °C with a reflux system in the dark. The homogenate was filtered and acidified at pH 3 with hydrochloric acid 2 N. After evaporation of ethanol under vacuum at 45 °C, the aqueous residue was extracted with ethyl acetate. The organic phase was filtered and evaporated under vacuum. The extract obtained, which was a green viscous material (Fig. 1), was freeze dried and finally stored at −20 °C until use.

2.3. Total phenolic content

Total phenols (TP) levels of the PO, GT, GS and GK extracts were estimated colorimetrically by the Folin-Ciocalteau method (Scalbert et al., 1989). Extracts (1 g/L) were dissolved in 50% (v/v) methanol/water and appropriately diluted (1:2.5, 1:5 and 1:10 v/v) in the same solvent. The Folin-Ciocalteau reagent was 10-fold diluted in water (v/v) and 2.5 mL were added to each 0.5 mL sample. Two milliliters of 75 g/L sodium carbonate solution were added and tubes kept for 1 h at 20 ± 1 °C in the dark. In the meanwhile, the calibration curve for gallic acid (5–100 mg/L) dissolved in 50% (v/v) methanol/water was achieved. The absorbance at 765 nm was measured and results were expressed

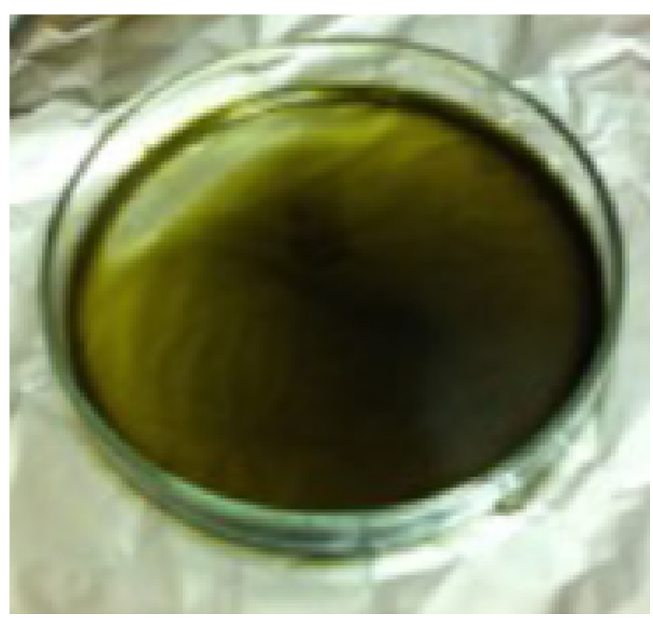

Fig. 1. Extract of *P. oceanica.* (For interpretation of the references to colour in the text, the reader is referred to the web version of this article.)