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.

KEYWORDS

Ready-to-eat fruit, Green tea, *Posidonia oceanica*, dipping, antimicrobials, antioxidants

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 (Jung 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. 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 (Guillen 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., 2014).

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 et al., 2007). Twenty-three phenolic compounds were identified in this species (Cuny et al., 1995; Agostini et al., 1997) 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 (Figure 1), was freeze dried and finally stored at -20°C until use.

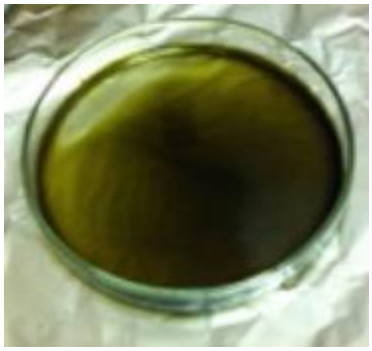


Figure 1. Extract of *P. oceanica*

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 as g gallic acid/100 g powder. Each formulation was analyzed in triplicate.

2.4 Antioxidant assay

Analysis of the antioxidant capacity of PO, GT, GS and GK extracts was carried out employing the DPPH assay, following the method of Brand-Williams et al. (1995) with some modifications. The DPPH solution was diluted in methanol to obtain 1.00 ± 0.03 absorbance units at 515 nm. The extracts samples were dissolved (20 g/L) in 70 % methanol (v/v) and, after centrifugation, they were serially diluted. The DPPH solution (2.94 mL) was placed in a cuvette where a 60 µL sample was added. The absorbance readings were carried out after incubation for 50 min at 20 ± 1°C. A calibration curve was prepared by adding increasing concentrations of Trolox ranged from 50 to 1000 µM; each concentration was assayed in triplicate. Results were expressed as mol Trolox per 100 g dry weight. Each formulation was analyzed in triplicate.

2.5 Microorganisms and culture conditions

Antimicrobial activity was analyzed by carrying out *in vitro* tests determining the Minimal Inhibitory Concentration (MIC) against strains belonging to official collections, i.e. *Escherichia coli* CECT 434 (Spanish Type Culture Collection), *Listeria innocua* DSM 20649 (Deutsche Sammlung von Mikroorganismen und Zellkulturen), *Pseudomonas putida* ATCC 12633 (American Type Culture Collection), *Staphylococcus aureus* ATCC 29213, *Aspergillus niger* NRRL 565 (Agricultural Research Service Culture Collection) and *Penicillium chrysogenum* CECT 2802. These microorganisms were selected among the most common spoilage and/or pathogen microorganisms that might be present in fresh food products (Mascheroni et al., 2014).

Bacterial strains were weekly maintained on TSB (Tryptic Soy Broth, Scharlau Chemie, Spain), incubated at 30°C for 24 h and then stored at 4°C, while moulds were maintained on MEA solid culture (MEB added with 15 g/L agar), incubated at 25°C for 5-7 d and then stored at 4°C until use.

2.6 Determination of antimicrobial activity *in vitro*

Qualitative determination of antimicrobial activity was performed as follows: 30 mL of soft TSA or MEA (TSB or MEB added with 8 g/L agar) were poured in a Petri Dish and inoculated with 300 µL of a microbial suspension prepared in sterile distilled water (OD 600nm: 0.300 ± 0.050); moulds were inoculated as spores suspension in sterile distilled water (OD 600 nm: 0.300 ± 0.050). Once solidified, holes were made by using a sterile tip and 150 µL of extracts were poured inside. Cultures were all incubated at each appropriate temperature for 24 h (up to 7 d for moulds). The presence of a growth inhibition halo around holes indicates an antimicrobial activity.

Quantitative determination of antimicrobial activity was performed only with PO and tea extracts. Ten mL of soft TSA or MEA were poured in a Petri Dish, to which aliquots of PO extract were added in order to obtain a final concentration of 0.5-1.0-1.5-2.0-2.5 and 3.0 g/L. Tea extract was tested at the following concentrations: 0.5-1.0-1.5-2.0 g/L. Once solidified, plates were all surface inoculated with aliquots (3 µL) of appropriately diluted (OD 600nm: 0.300 ± 0.050) overnight microbial cultures on TSB (bacteria). Moulds were inoculated as spore suspension in sterile distilled water (OD 600 nm: 0.300 ± 0.050). For each strain control trials were also prepared without the addition of extracts. In order to highlight any possible inhibitory effect of the solvent present in extracts, a set of solid cultures was also set up by adding ethanol (up to 3 g/L) to the culture media.

Cultures were all incubated at each appropriate temperature for 24 h or up to 7 d for moulds. MIC (Minimum Inhibitory Concentration) was determined as the lowest extract concentration (g/L) able to inhibit microbial growth. Trials were repeated twice for each extract.

2.7 Fresh-cut peach storage

Peaches (*Prunus persica* L. Batsch cv. 'Rich May') were purchased at the wholesale market (3 kg), 24 h after harvesting at commercial maturity and stored for 1 d at 4°C until use. Peaches were homogenous in weight (180 ± 5 g) and ripening (10.85 ± 0.07 °Brix). Fruits were pre-washed with distilled water, sanitized for 2 min in chlorinated water (150 mg/L sodium hypochlorite), rinsed with distilled water and gently dried by hand. Peaches with skin were cut into slices (8 slices per fruit) of about 1.5 cm thickness (15 ± 2 g each slice), using a sterile stainless-steel knife, and dipped for 3 min in the following solutions: PO extract (2% w/v), GT extract (1% w/v) and distilled water for control samples (CTRL). Slices were then left in the air for 2 min in order to drain off the excess solution. Three slices (45 ± 6 g) were placed into low-density polyethylene (LDPE) bags (22 x 15 cm, 25 µm thickness, bag volume 450 mL, ratio between fruit weight and container volume 100 g/L, surface film for each bag 660 cm^2, O_2 permeability 6200 $cm^3\ m^{-2}\ d^{-1}\ bar^{-1}$, CO_2 permeability 24000 $cm^3\ m^{-2}\ d^{-1}\ bar^{-1}$ at 10 °C). Bags were all stored at 4 ± 1°C up to 7 d. A total number of 48 bags were prepared, 24 used for color evaluation, and the remaining ones for the other analyses. Samples were then collected after 0, 3, 5 and 7 d. Each trial at each day was carried out in duplicate.

2.8 Colour evaluation

Flesh colour was evaluated using the CIE L*a*b* System by a Minolta CR-300 chromameter (Konica Minolta Sensing, Inc., Japan). Three measurements were performed on each side of slices. The instrument was calibrated using a standard white plate. The chroma (C) was calculated as follows (1):

$$C^* = \sqrt{a^{*2} + b^{*2}} \qquad (1)$$

2.9 Total soluble solids and titratable acidity

Total soluble solids (TSS, %) and titratable acidity (TA, g/L) were measured on the juice obtained from slices (30 g for each sample) by an electronic blender (Ariete, Italy). TSS were determined by

a digital refractometer (Atago Co., Ltd, Tokyo, Japan model PR-32), while TA was determined by titrating 1:10 diluted juice using sodium hydroxide 0.1 M by an automatic titrator (Compact 44-00, Crison Instruments, SA, Barcelona, Spain).

2.10 Antimicrobial activity of green tea and *P. oceanica* on peach slices

After 0, 3, 5 and 7 d, peach slices (15 g) were transferred aseptically into a Stomacher bag (400 mL PE, Barloworld, France) containing 135 mL of sterile peptoned water (10 g/L bacteriological peptone, Costantino, Italy) and blended in a Stomacher (Star Blender LB 400, Biosystem, Belgium) at high speed for 3 min. Ten-fold dilution series of the obtained suspensions were made of the same solution for plating. The following culture media were used: TSA (Merck, Germany) for mesophiles, *Pseudomonas* Agar base (Himedia, India) for *Pseudomonas spp.*, VRBLA (Violet Red Bile Agar, Merck, Germany) for *Enterobacteriaceae* and MEA for yeasts and fungi. Colonies were counted after incubation at 30°C for 24 h for mesophiles, 30°C for 5 d for yeasts and fungi and 25°C for 24 h for *Pseudomonas*. Counts were performed in triplicate and reported as logarithms of the number of colony forming units (log cfu/g peach), and means and standard deviations (SD) were calculated.

2.11 Statistical analysis

Statistical analysis was carried out using the STATISTICA 7.1 software package (Statsoft Inc., Tulsa, OK, USA). One-way analysis of variance (ANOVA) was performed on mean values and Tukey's test was carried out for the comparison of difference among treatments for each storage time and for each treatment during storage. Differences were considered significant at $p \leq 0.05$.

3. Results and discussion

3.1 *In vitro* evaluation of total phenolic index and antioxidant activity

243 Total phenol index (TPI) and antioxidant capacity (AC) were evaluated for each investigated extract
244 (Table 1). PO showed the highest TPI (711 mg/g) followed by the GS extract (526 mg/g). The
245 highest AC was found for GT extract (3.8 ± 0.11 mg/L EC_{50}).
246 In case of PO, the highest polyphenols content corresponded to the lowest antioxidant capacity.
247 Such a low value could be attributable not only to the extraction procedure but can also be related to
248 the type of polyphenols present in the extracted matrix (Berfad and Alnour, 2014). The choice of
249 the solvent to use (50% v/v ethanol-water solution) was based on the best results obtained by Berfad
250 and Alnour (2014), who investigated the extraction performance of different solvent mixtures on *P.*
251 *oceanica*, as well as also taking into account its food-grade nature. As reported in the literature, the
252 principal polyphenols present in PO are acetosyringone, ferulic acid and acetovanillone, while those
253 in GT are gallic acid, catechin gallate and epicatechin (Agostini et al., 1998). These last compounds
254 are characterized by the presence of three proximal hydroxyl groups, able to efficiently delocalize
255 radicals present in the aromatic ring thus acting as radical scavengers. On the contrary, the
256 polyphenols present in PO hydroxyl groups are not in close proximity and a methyl moiety is often
257 present, thus reducing its antioxidant capacity (Agostini et al., 1998).
258 Nevertheless, the polyphenols values obtained were comparable with those reported in the literature
259 for other plants (Mensor et al., 2001). The highest antioxidant capacity of the GT extract is not
260 surprising since GT is particularly rich in phenols, as proanthocyanidins, with low redox potential
261 and it does not contain bi-flavanols (Lee et al., 2014).
262
263 **3.2 *In vitro* determination of antimicrobial activity**
264 In qualitative trials performed employing 1 g/L extract solutions, all samples showed the highest
265 antimicrobial activity against *L. innocua*. GT proved to be the most powerful sample, while at the
266 tested concentration PO did not show any antibacterial activity but was the only extract possessing
267 an antifungal action against *Aspergillus niger* (Table 2).

Quantitative determination of antimicrobial performance found that MIC for GT and PO extracts were 1 and 2 g/L respectively against the two Gram positive strains *L. innocua* and *S. aureus*. The two Gram negative strains showed a reduction of microbial growth attributable to a bacteriostatic, rather than a bactericidal, effect (Figure 2). *Aspergillus* and *Penicillium* showed a marked reduction of hyphal growth (up to 30% for *A. niger* and to 70% for *P. chrysogenum* employing 3 g/L extract) and sporulation when grown in presence of PO, while no effect was evident with GT (Figure 3). Only in the control plate (no extract added) after 7 d incubation the two moulds showed an antagonistic effect, while in all the other plates the hyphal growth of each strain was not influenced by the presence of the other one. Note that ethanol, the solvent used to prepare extracts, was not found to inhibit microbial growth at the tested concentrations, thus confirming literature results (Dantigny et al., 2005).

Recently, Alkan and Yemenicioglu (2016) tested the *in vitro* antimicrobial activity of various plant phenolics, finding that clove extract was the most potent antimicrobial, with MIC values of 10.24 g/L against the plant pathogens *Erwinia amylovora*, *E. carotovora*, *Pseudomonas syringae* and *Xanthomonas vesicatoria*. The reported values are much higher than those found in the present research (MIC of 1-2 g/L), at least for PO, highlighting that the tested extracts are of actual interest.

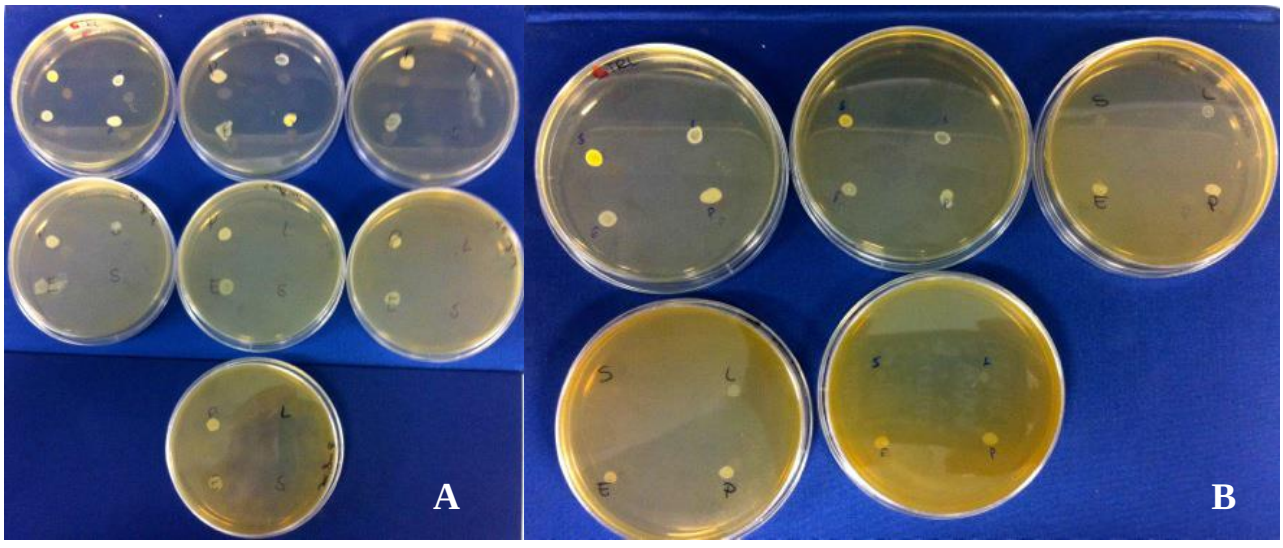


Figure 2. Growth of bacterial strains in presence of *P. oceanica* (A) and Green Tea (B) extracts. Concentrations used: 0-0.5-1-1.5-2-2.5-3 g/L PO; 0-0.5-1-1.5-2 g/L GT. Strains: L-*Listeria innocua*, S-*Staphylococcus aureus*, E-*Escherichia coli*, P-*Pseudomonas putida*.

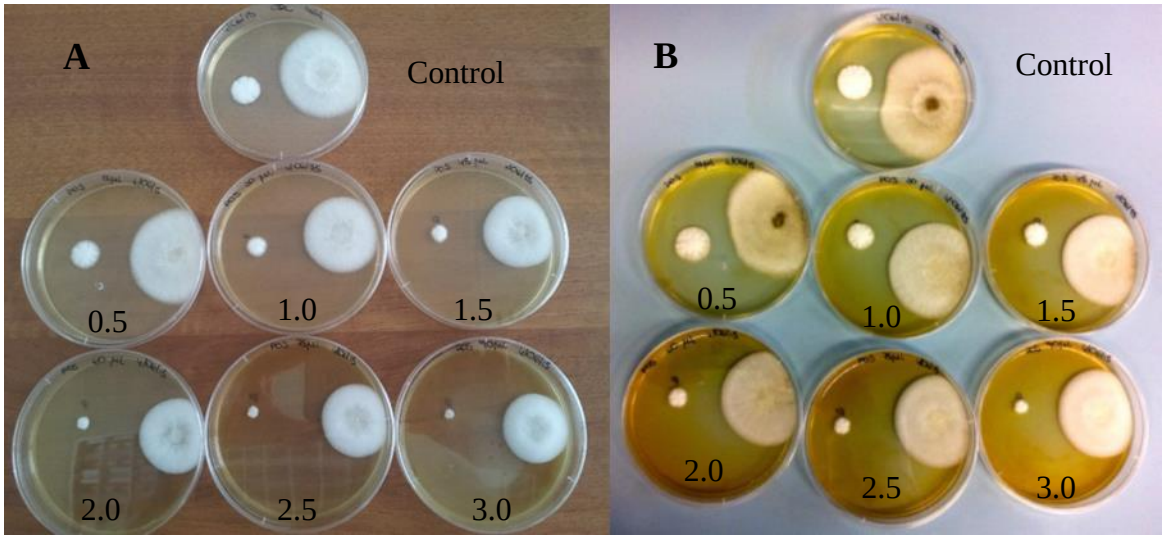


Figure 3. Growth after 2 days (A) or 7 days (B) incubation of *Penicillium chrysogenum* (left in each plate) and *Aspergillus niger* (right in each plate) in presence of different concentration of *P. oceanica* extract (from 0.5 to 3.0 g/L). Control: medium without extract.

3.2 Fresh-cut peach storage

GT and PO extracts were chosen for the dipping of fresh cut peach slices: the first of these had the most powerful antioxidant capacity, while the second was chosen for its high polyphenols content and antifungal activity. Fruits are usually quite acid and hence quite resistant to invasion by bacteria. Therefore spoilage of fruit and fruit products is often caused by fungi (Pitt and Hocking,

1999). However, recent studies have documented the exponential growth of bacteria on a variety of fresh-cut fruit (Alegre et al., 2010; John et al., 2013).

In terms of pomological traits, after 7 d storage, TSS content was found almost constant in treated samples of peach slices, while in CTRL samples this value sharply increased up to 11.8 %, mainly from 5 to 7 d (Figure 4A).

Differences of TA content were significant between t 0 and 3 d, then reduced with storage. CTRL samples evidenced a significant decrease from 3 to about 1 g/L. Peach slices dipped in the two extracts showed a TA decrease of only 17 % with respect to the initial value, but with different time courses: when PO was employed, a sharp decrease of TA in the last 3 d of shelf life was evident, while in samples treated with GT the decrease was evident in the first 3 d.

Note that the sharp decrease of TA in CTRL samples occurred at the same time with the TSS increase in the last 3 d of the shelf life. For the GT treatment, no significant changes were evident in TSS between 3 and 7 d (Figure 4B).

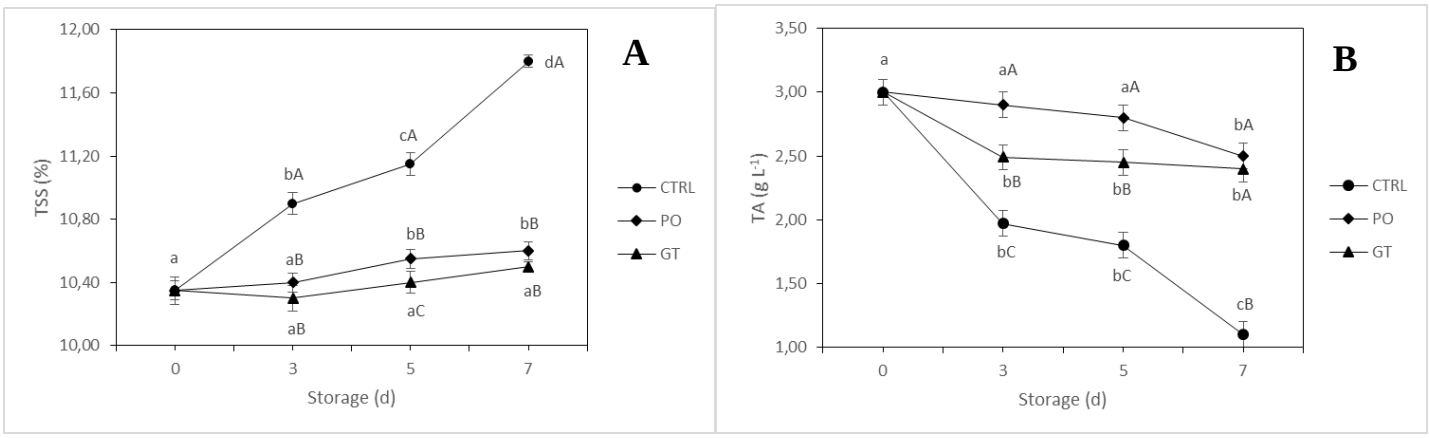


Figure 4. Evolution of total soluble solids (%) (A) and titratable acidity (g/L) (B) in peach (*Prunus persica*) cv. Rich May slices treated with *Posidonia oceanica* (PO), Green Tea (GT) and untreated (CTRL). Data are means ± SE. Minor and capital letters show significant differences ($p \leq 0.05$) for each treatment and among treatments for each storage time, respectively.

After three days, lightness (L*) of peach slices decreased in all treatments (Table 3). When prolonging the incubation time up to 7 d, lightness decreased much more in CTRL sample rather than in peach slices treated by GT or PO. Caceres et al. (2016) developed a flesh browning assessment methodology for fresh whole peaches stored for a long time, and reported that ΔL* values higher than 21 can be considered symptoms of extreme flesh browning. Even if our data relate to peach slices, ΔL* values calculated at 7 d storage were found higher than 21 only in the control sample.

An indicator of chlorophyll degradation is the a* value, which decreases when color changes from green to red (Martín-Diana et al., 2008). An increase of a* values was only found for PO treatment (Table 3). This behavior can be attributed to the greenish colour imparted by the PO extract, similarly to what was found by Martín-Diana et al. (2008), after a dipping treatment with natural extracts, which they also correlated to lightness lowering.

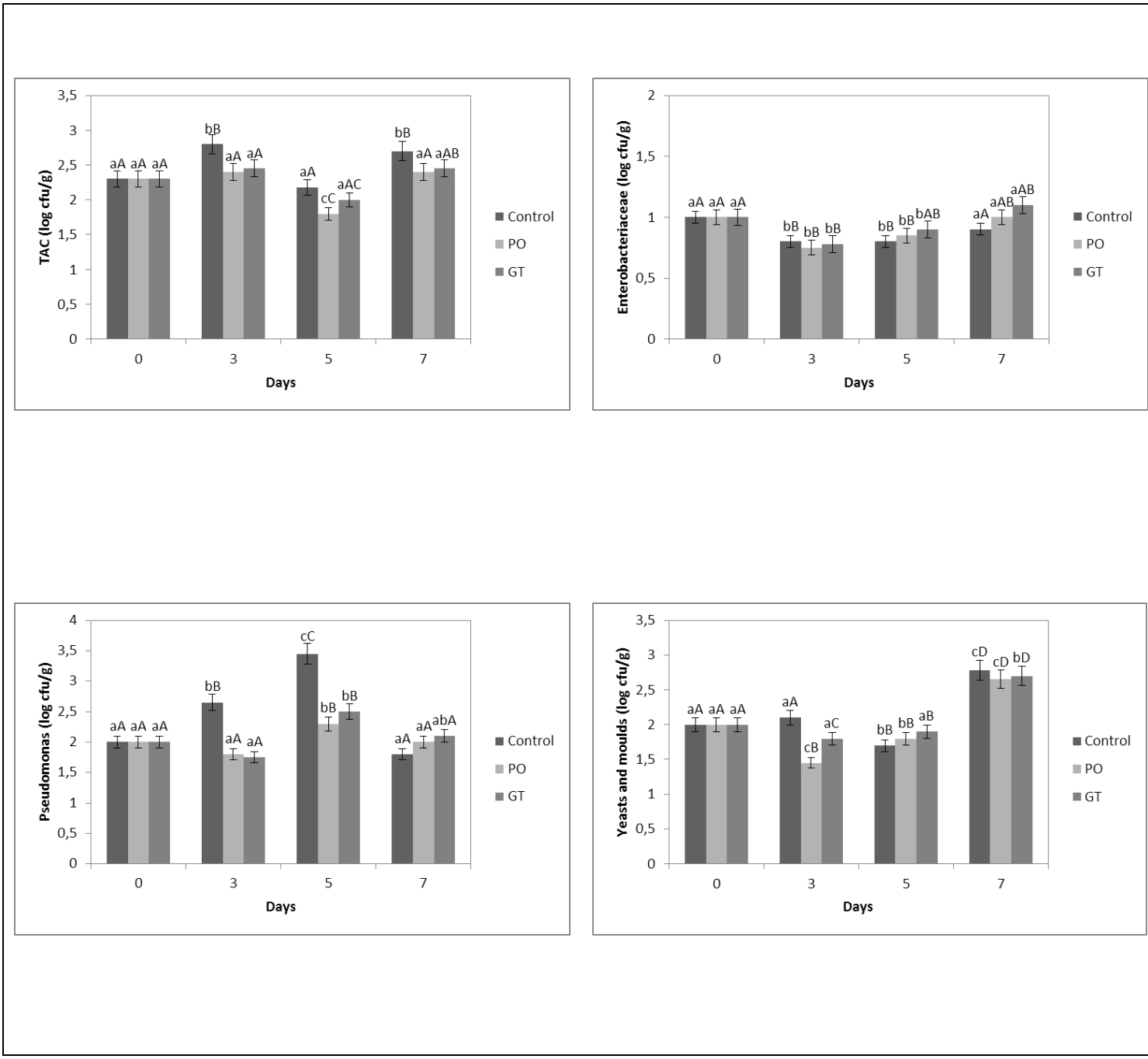
The b* parameter indicates the color changes from yellow to blue, and its values decreased in all samples during storage (Table 3) due to phenolic degradation taking part on tissues (Fuentes-Perez, 2014). However, after 7 d storage, treated samples showed higher values than the control.

Finally, the Chroma decreased after 3 and 5 d storage more rapidly in PO samples compared to GT ones (Table 3). After 7 d, GT showed a higher value compared with samples dipped in PO.

These results, in accordance with the findings reported by Oms-Oliu et al. (2010), highlighted that dipping treatment after peeling and/or cutting can represent an effective way to control browning phenomena in fresh-cut fruit, since it can affect enzyme activity or substrates availability for enzymatic degradation. In particular, the high polyphenols content present in both extracts can protect the cut surface of fruit products against oxidative rancidity, degradation and enzymatic browning, thus slowing their senescence process (Rojas-Grau et al., 2009).

From the microbiological point of view, the applied dipping treatments were found effective in lowering the total aerobic count (TAC) and *Pseudomonas* population present in peach slices of about 0.5 log cfu/g mainly up to 5 d (Figure 5). This behavior may also be favored by the fruit respiration process (Rojas-Grau et al., 2009). Yeasts and moulds were found significantly lower than the CTRL only at 3 d, with the best performance being shown by peach slices dipped in PO extract. No significant changes were found for the *Enterobacteriaceae* population. These results are in accordance with those reported by Siroli et al. (2014) for minimally processed apples dipped in different antimicrobials comparatively: shelf life of fresh cut fruit is limitedly affected by microbial growth: independently from the addition of natural antimicrobials, the end of shelf life is mainly determined by changes in colour.

Time course of CO_2 and O_2 during storage trials was not determined as it was assumed meaningless because of the high gas permeability and the geometry of the packaging system used. Assuming an oxygen respiration rate of the fruits of about 18 mg kg^{-1} h^{-1} at 10 °C and taking into account the permeable surface of the package (0.066 m^2), the headspace volume (450 cm^3), the amount of the product (0.045 kg) and the oxygen and carbon dioxide permeabilities (respectively 6200 and 24000 cm^3 m^{-2} d^{-1} bar^{-1}, at 10 °C) using a common model for forecasting atmosphere changes in ready-to-eat vegetables (Piergiovanni et al., 1999) we estimated after 7 d a maximum CO_2 concentration equal to 0.8% and a minimum O_2 concentration of 18%.



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377 **Figure 5.** Time course of Total Aerobic Count (TAC), *Enterobacteriaceae*, *Pseudomonas* and
378 yeasts and moulds presence (log cfu/g peach) in samples of peach slices subjected to dipping
379 treatment with *P. oceanica* (PO) and Green Tea (GT) extracts and then stored at 4 °C. CTRL: peach
380 slices dipped in sterile distilled water. Data are means ± SD. Minor and capital letters show

significant differences ($p \leq 0.05$) for each treatment and among treatments for each storage time, respectively.

4. Conclusions

In this study *Posidonia oceanica* (PO) and green tea (GT) extracts were applied by dipping on peach slices, once having shown their highest total phenolic content and antifungal activity, as well as the highest antioxidant activity, respectively. Results showed that these natural extracts limited microbial spoilage of fresh-cut peach, especially the *Pseudomonas* population, and maintained the pomological parameters during storage at 4°C while not modifying their characteristic taste.

Overall, polyphenolic extracts derived from PO and GT could provide an additional post-harvest benefit of fresh-cut produce. To the best of our knowledge, this paper represents the first report on the application of *P. oceanica* extract on fresh-cut fruit, even if this marine protected plant never should be collected from the sea for industrial application.

Although this work relates to the application of natural extracts directly on fresh-cut fruit in a traditional package, future trials will be aimed at setting up innovative active packaging solutions from which these extracts will be released. Further research will be also needed to complement antioxidant activity of plant or other extracts with digestion simulation to assess parameters such as bioaccessibility, bioavailability and *in vivo* antioxidant performance; sensorial analyses should also be performed on treated fruit.

These results can pave the way to the use of innovative natural extracts to be applied on ready-to-eat vegetables, thus improving the attractiveness for consumers of these healthy foods.

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Table 1: Total Phenolic Index (TPI) and Antioxidant Capacity (AC) of the extracts investigated in this study. Data are presented as mean ± SE.

Extract	**TPI**	**AC**
	mg gallic acid/g extract	mg/L EC_{50}
Posidonia oceanica	710.6 ± 20.1	72.42 ± 22.9
Green tea	526.3 ± 14.9	3.80 ± 0.11
Grape skin	398.2 ± 9.5	6.14 ± 0.78
Grape seeds	596.4 ± 15.6	4.10 ± 0.14

Table 2. Diameter (mm) of microbial growth inhibition halos around wells containing the analyzed extracts (1 g/L) after incubation.

Microorganism	GT	PO	GK	GS
Listeria innocua	12	n.p.*	4	6
Staphylococcus aureus	10	n.p.	2	4
Escherichia coli	6	n.p.	2	4
Pseudomonas putida	4	n.p.	n.p.	4
Aspergillus niger	n.p.	8	n.p.	n.p.
Penicillium chrysogenum	n.p.	n.p.	n.p.	n.p.

*n.p.: inhibition halo not present.

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538 **Table 3.** Color parameters changes during storage of fresh cut peach slices treated by *P. oceanica*
539 (PO), Green Tea (GT) and untreated. Data are presented as mean ± SE. Minor and capital letters
540 show significant differences ($p \leq 0.05$) for each treatment and among treatments for each storage
541 time, respectively.

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Treatment	Day	Lightness (L*)	a*	b*	C*
	0	64.07 ± 1.30^{a}	-2.22 ± 0.41^{a}	41.56 ± 0.40^{a}	41.58 ± 0.40^{a}
	3	59.63 ± 0.85^{bA}	-1.04 ± 0.18^{bB}	39.63 ± 0.55^{aA}	39.73 ± 0.54^{aA}
CTRL	5	50.01 ± 1.14^{cA}	0.40 ± 0.22^{cB}	29.60 ± 1.63^{bA}	29.61 ± 1.63^{bA}
	7	41.70 ± 0.89^{dB}	1.57 ± 0.27^{dB}	18.96 ± 0.28^{cB}	19.06 ± 0.27^{cB}
	3	57.11 ± 0.19^{bB}	-3.15 ± 0.19^{abA}	33.88 ± 0.72^{bB}	34.04 ± 0.71^{bB}
PO	5	48.69 ± 1.11^{cA}	-1.65 ± 0.40^{bA}	25.92 ± 0.84^{cA}	26.03 ± 0.84^{cA}
	7	42.99 ± 0.62^{dAB}	-0.28 ± 0.41^{cA}	20.05 ± 0.39^{dA}	20.12 ± 0.40^{dAB}
	3	55.19 ± 0.79^{bB}	-1.29 ± 0.24^{bB}	39.39 ± 0.45^{aA}	39.42 ± 0.45^{aA}
GT	5	48.56 ± 0.58^{cA}	0.43 ± 0.41^{cB}	29.02 ± 1.59^{bA}	29.07 ± 1.59^{bA}
	7	44.71 ± 0.68^{dA}	1.24 ± 0.09^{cB}	20.28 ± 0.44^{cA}	20.32 ± 0.44^{cA}

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