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Application of ozone in fresh-cut iceberg lettuce refrigeration

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Key words: environmental sustainability, fresh-cut vegetables, fresh vegetable quality, minimally fresh processed leaf vegetables, shelf-life.

Abstract: Recently, technological innovations have been geared to supporting environmental sustainability, also in the fruit and vegetable industry. The application of ozone in the cold storage of fruits and vegetables is a sustainable technology used to improve product quality and its antimicrobial effect, simple use, and characteristic of not leaving any residue, makes this treatment suitable for many applications in this field. The aim of this work was to evaluate the effect of refrigeration at 4°C, associated with ozonization treatment at a concentration of 0.2 ppm on the shelf life of fresh-cut lettuce, compared to a lettuce control stored only at 4°C. Lettuce quality throughout the storage period (7 days) was determined by means of color and microbiological indexes, such as total bacterial count, total coliforms, *Escherichia coli, Pseudomonadaceae,* yeasts and molds. The lettuce used in the experiment was found to have a low microbiological load. Microbiological results obtained at different storage times have shown that the use of ozone is effective in containing microbial growth during chilling storage of the raw material compared to the refrigerated control. In particular, the positive effects of ozonation were appreciable after the third day of storage. Furthermore, the ozone treatment did not affect the color of the product.

1. Introduction

It is well known that disinfection represents one of the most critical processing steps influencing quality, safety and shelf-life of fresh-cut fruits and vegetables. In the fresh-cut industry, chlorine is generally used as a product sanitizer; however, there is a trend in eliminating this substance due to the environmental and health risks associated with the formation of carcinogenic halogenated disinfection by-products (Gil *et al*., 2009).

Ozonation represents a sustainable technology able to improve the quality of the product; ozone is useful in diminishing the microbial load and the level of toxic organic compounds. Furthermore, this gas has a stronger and more rapid antimicrobial action against spores, faecal and pathogenic microorganisms and viruses with respect to chlorine (Artés and Allende, 2005). The antimicrobial effect of ozone and its relatively simple application, in addition to its characteristic of not leaving any residue or forming carcinogenic trihalomethanes (such as chlorination), make this treatment suitable for various applications in the food industry (Gil *et al*., 2009; Alexopoulos *et al*., 2013). Moreover, products treated with ozone do not modify their sensory characteristics. After it gained GRAS (Generally Recognized As Safe) status in 1997, the use of this gas has been approved as a disinfectant or sanitizer in foods and food processing in Europe and in the US (Ölmez and Kretzschmar, 2009).

The primary systems for ozone application include gaseous phase storage and ozonated dips (Artés *et al*., 2009). When ozone is used as a gas, the exposure time is longer (1-4 h) than ozone dissolved in water (1-10 min) (Carletti *et al*., 2013).

In recent years the use of this gas as a pretreatment to preserve fruit and vegetable quality has received great attention for its antimicrobial activity against bacteria, fungi, viruses, and bacterial and fungal spores. Several studies have focused on the effect of ozone treatment on the safety and quality of iceberg lettuce (Rico *et al*., 2006; Yuk *et al*., 2006; Hassenberg *et al*., 2007). In addition, various applications have been proposed concerned the sanitizing effect of ozone dissolved in water used for washing on the quality and safety of minimally processed fruits and vegetables (Seydim *et al*., 2004; Zhang *et al*., 2004; Selma *et al*., 2008; Artes *et al*., 2009; Ölmez and Kretzschmar, 2009), but there is scant literature about ozone applied to fresh-cut iceberg lettuce (Beltran *et al*., 2005; Ölmez and Akbas, 2009).

The aim of this work was to evaluate the effect of refrigeration at 4°C, associated with ozonization treatment

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at a concentration of 0.2 ppm on the shelf life of fresh-cut lettuce, compared to a lettuce control stored only for 7 days at 4°C.

2. Materials and Methods

Sampling and ozone treatment

Iceberg lettuce (*Lactuca sativa L.*) was obtained from Società Agricola "Rago" (Battipaglia, SA, Italy) and, immediately after harvesting, it was stored at 4°C and submitted to a continuous ozone exposure of 0.2 ppm. A control sample was stored at 4°C without ozone treatment. Ozone gas was generated using a corona discharge ozone generator (Model OXY MET TWIN, MET, San Lazzaro di Savena, BO, Italy) from purified oxygen gas. Functioning of this equipment takes place through sensors placed inside the storage compartment/chamber that reveal the exact concentration present in the refrigeration cell and it is reported on the display of the instrument. The tests lasted up to 7 days and the ozonated and non-ozonated product was sampled at 1, 2, 3, 5 and 7 days for analyses.

Colorimetric analysis

Color was measured on the lettuce surface using a Minolta Chroma meter CR-300 (Minolta Camera Co. Ltd., Osaka, Japan), with a D 65 illuminant, using CIELAB L*, a*, b* values. The data reported are the average of a set of four determinations carried out on the lower and upper surface of two leaves of lettuce.

Microbiological analysis

At each treatment time, 9 g of ozonated or control product were placed in a stomacher bag containing 81 ml of Tryptone Water (Liofilchem, Teramo, Italia) and homogenized for 2 min. Samples were then serially diluted in tripton water and surface plated in duplicate on Compact-Dry EC (PBI International, Milano, Italy) for the recovery of surviving total coliforms and *Escherichia coli*, on Compact-Dry YM (PBI International, Milano, Italy) for yeasts and moulds and on Cetrimide Agar (Liofilchem, Teramo, Italy) for the enumeration of *Pseudomonas aeruginosa* and *Pseudomonas* spp. Total mesophilic bacteria were enumerated on Plate Count Agar (Liofilchem, Teramo, Italy) using the pour plate technique. All the inoculated media were incubated at 30°C for 48 h.

Statistical analysis

In order to study the effect of ozone treatment and storage time on fresh-cut iceberg lettuce quality characteristics, data were processed by two-way analysis of variance (ANOVA). Moreover, a Student test was performed to compare the mean values between the samples treated with ozone and control samples for each time of storage. All statistical procedures were computed using the statistical package SYSTAT for Windows (ver. 10, 2003) (Systat Software, Chicago, USA).

3. Results and Discussion

Ozone can be used to disinfect fruit and vegetables by dissolving it in the water used for washing (Gil *et al*., 2009), or by keeping a constant ozone concentration, through the use of an appropriate generator, during storage of the product in a cooling room at 4°C.

In this paper we evaluated the effect of cold storage at 4°C, accompanied by ozonization at a concentration of 0.2 ppm on the shelf life of the fresh-cut lettuce, compared to a control subjected only to cold storage. Preliminary tests showed that the use of ozone at low concentrations, such as 0.2 ppm, did not determine sensory alterations; for this reason, the effect of treatment on both perceptible sensory parameters, such as color stability, and microbiological parameters, for safety of the product, has been evaluated. A two-way analysis (time, treatment) showed a significant effect of both factors on the colorimetric parameters ($p= 0.000$). In particular, total bacterial count and coliforms increased throughout the storage period, being significantly lower for ozonated samples than control samples. Therefore, the microbiological results obtained at different storage times show that the use of ozone is effective in containing microbial growth during storage in a cooling room of raw material compared to the untreated control. In particular, the positive effects of ozonation were appreciable after the third day of storage, as shown by the trend of the total bacterial count and total coliforms of the chilled and ozonized product compared to the refrigerated only product (Fig. 1 and 2). In particular, ozone was able to inhibit the proliferation of total coliforms, which were not detectable even after 7 days of storage in the ozonized samples, while in control samples these microorganisms reached 1.6×10^3 cfu/g at the same storage time. The growth of colonies on the plates inoculated for the detection of other microorganisms was sporadic and not significant for both the experimental theses considered. In the literature a reduction of 3-4 log units of *Escherichia coli* O157: H7 inoculated in lettuce and carrots, by sequential washes with aqueous solutions of Cl_2O/ozonated water, has been reported (Singh *et al*., 2002). Various experiments carried out by treating vegetables with ozonated water have shown the ability of ozone to reduce the growth of different microorganisms, including pathogens, and also to reduce enzymatic activities, including enzymatic browning (Artes *et al*., 2009). Alexopoulos *et al*. (2013) have highlighted the importance of keeping the concentration of ozone constant during treatment by continuous generation. In fact, the immersion of vegetables in water presaturated with ozone determined the reduction of 0.5 log of CBT after 15 min, whereas the immersion for the same time in continuously ozonated water involved the reduction of 2 log of CBT, showing in this latter case a greater effectiveness of ozonization with respect to chlorination. In fact, the reduction from 1.5 to 2.5 log units of the microflora of the lettuce was obtained with ozonated water with 1.5-3 ppm; this effect was comparable to the use of 100 ppm of chlorine

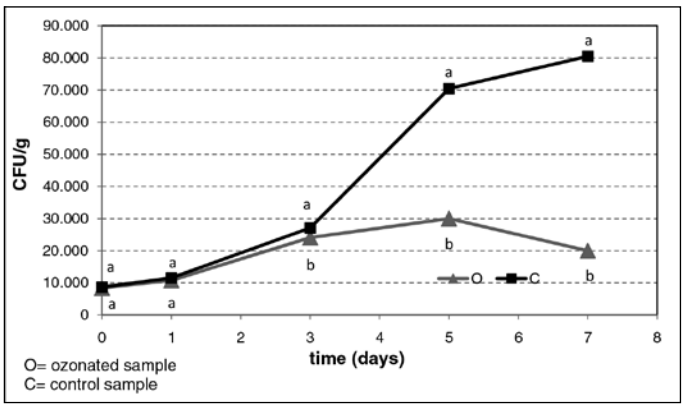

Fig. 1 - Evolution of total mesophilic bacteria during storage at 4°C. For each storage time, data followed by different letters are significantly different (t- Student test at p≤0.05).

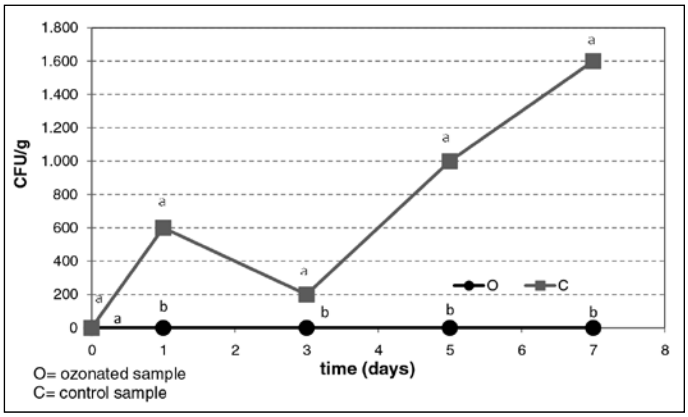

Fig. 2 - Evolution of total coliforms during storage at 4°C. For each storage time, data followed by different letters are significantly different (t- Student test at p≤0.05).

(Ölmez and Kretzschma, 2009).

Furthermore, ozone treatment did not adversely affect product color (Fig. 3 and 4), as also confirmed by two-way analysis of variance; in fact, a non significant effect of ozone treatment and storage time was recorded for the colorimetric indexes. It is well known that ozone is a strong oxidizing agent, which can induce damage to the structure and may influence the color of the products depending on the time of contact, the concentrations applied, and the type of product (Horvitz and Cantalejo, 2014; Olmez *et al*., 2009). However, in the present study, at concentrations of 0.2 ppm of gaseous ozone on lettuce stored at 4°C, no difference was detected relative to colorimetric parameters L *, a *, and b * between the control and the product treated with ozone has (Fig. 5 and 6).

Fig. 3 - Lettuce not treated with ozone after 3 days of storage at 4°C.

Fig. 4 - Lettuce treated with ozone after 3 days of storage at 4°C.

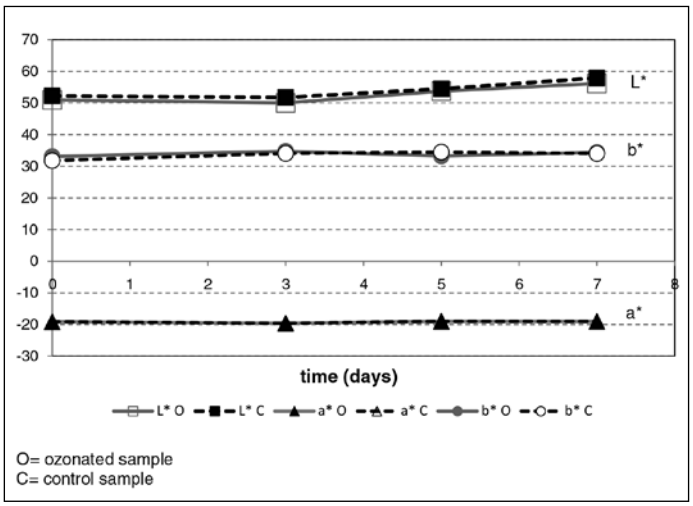

Fig. 5 - L*, a* and b* values determined on the upper leaf surface.

4. Conclusions

The microbiological results obtained at different storage times have shown that the use of ozone is effective in containing microbial growth during storage of the raw material compared to the untreated control. In particular, the positive effects of ozonation were appreciable after the third day of storage. Moreover, ozone treatment did not affect the color of the product. In order to better assess the

treatment effect of ozonation on lettuce, studies considering material with a higher degree of microbial contamination, or inoculated with microorganisms that have not been detected even in the thesis used as a control in this experiment, should be undertaken.

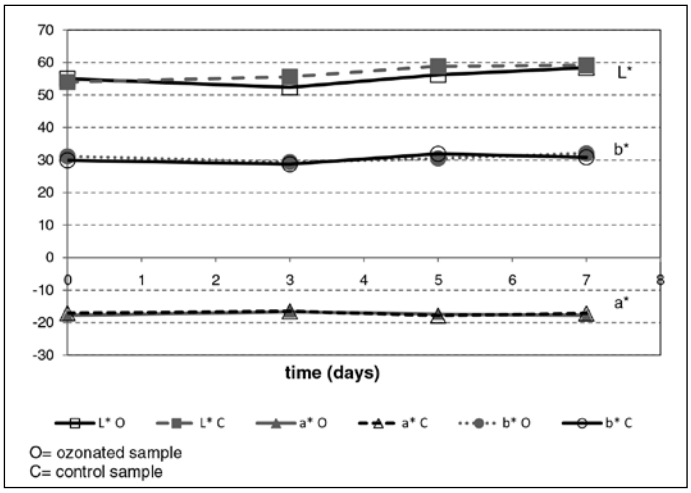

Fig. 6 - L*, a* and b* values determined on the lower leaf surface.

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Influence of edible coatings on postharvest physiology and quality of Honeydew melon fruit (*Cucumis melo L. inodorus*)

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Key words: electronic nose, ethane, ethylene, fermentative metabolites, firmness, internal oxygen, total soluble solids.

Abstract: Several techniques have been developed to preserve the quality of horticultural products throughout the supply chain. Edible coatings represent a promising technology as they can improve quality and extend shelf life of fruit and vegetables by changing gases and moisture permeabilties, enhancing fruit appearance, and reducing microbial contamination. The aim of this work was to assess the effectiveness of two kinds of novel coatings on the shelf life extension of Honeydew winter melons during retail. Sixty melons were used: 24 were uncoated as control; 18 were treated with a cellulose polymer coating (F1) and 18 with a synthetic polymer (F2) coating. Upon arrival, and after 6, 9 and 13 days at 13°C, six melons/treatment were individually analyzed for internal O_2, ethylene and ethane concentrations, fermentative metabolites, quality parameters, and aroma pattern. Already after six days, internal O_2 levels in coated fruit fell to ~1% in F1 and ~3% in F2 melons, triggering fermentative pathways as shown by the increased productions, mainly in F1 fruit, of acetaldehyde, ethanol, ethyl acetate, and ethane. This pattern caused changes in the responses of electronic nose sensors which were able to distinguish the three treatments. Coating did not influence fruit firmness and internal ethylene concentration. F1 coating reduced soluble solids content, strongly enhanced skin glossiness, and delayed yellowing, but it was not able to prevent moisture losses. In contrast, F2 coating significantly reduced weight loss and showed a slight positive effect on fruit appearance.

1. Introduction

Fresh fruit and vegetables undergo major quality and quantity losses after harvest throughout the supply chain up to consumers. The shelf life extension of a fruit depends on the control of phenomena related to ripening and senescence, water loss, and decay development. Several techniques have been developed in order to preserve the quality of horticultural products and to reduce quantity losses. They involve the management of temperatures at harvest, during transportation and storage, modification of the atmosphere composition in the storage rooms, and the application of chemical treatments.

Edible coating technology is a promising method to preserve the quality of fresh fruits and vegetables (Dahl, 2013) and meets the consumer requests to have safe food without any chemical treatments.

Edible coatings are applied in thin layers to the surface of the fresh produce and act as a semi-permeable barrier to respiratory gases and water vapor between the fruit and the surrounding atmosphere, thereby establishing a modified atmosphere around the product, which slows down respiration, senescence, and enzymatic oxidation. Edible coatings are effective in preserving food quality if they are water-resistant and stable during cold storage, do not cause excessive O_2 reduction or CO_2 accumulation, are minimally permeable to water vapor, improve fruit gloss and appearance and do not impart off-flavors and changes in aroma, taste, texture and appearance; they also must have low viscosity, be translucent and economical to use (Dhall, 2013; Mahaian *et al*., 2014).

Edible coatings are composed of polysaccharides, proteins, and lipids, alone or in combination, whose presence and abundance determine the barrier properties of the material. However, none of the three constituents can provide the needed protection by themselves and so they are usually used in a combination in order to obtain the best results (Valencia-Chamorro *et al*., 2010; Dhall, 2013; Mahaian *et al*., 2014).

They can be applied on whole or on fresh-cut fruits and vegetables. When applied on whole fruit, different and not always successful results are obtained. Arnon *et al*. (2014) found that in citrus fruit the application of edible natural biodegradable coatings enhanced fruit gloss, slightly increased fruit firmness, but was mostly not effective in preventing water loss and decreased the flavor acceptability in mandarins due to off-flavor development. Plums treated with a coating material based on carbohydrate plus sorbi-

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tol showed an extended shelf life period due to weight loss decrease and delayed changes in firmness, color, pH and acidity (Eum *et al*., 2009). Strawberries coated with edible coatings showed significant delays in the changes of weight loss, decay incidence, acidity, pH and soluble solids, and ascorbic acid contents and maintained higher concentration of total phenolics and anthocyanins in comparison to control fruit (Gol *et al.*, 2013). Positive effects of coating were also observed in mangoes treated with a nanomultilayer coating of pectin and chitosan: coated fruit presented a better external appearance, a less dehydrated surface apparently without fungal growth and lower mass loss after 45 days of storage (Medeiros *et al*., 2012). Carboxymethyl cellulose (CMC) coatings alone and in combination with gamma irradiation were tested for maintaining the storage quality and extending shelf life of pears. CMC alone was effective in extending shelf life of pears by six days, following 45 days of refrigeration, while the combinatory treatment maintained the storage quality and delayed pear decay, prolonging the shelf life period up to 12 days (Hussain *et al*., 2013). Fisk *et al*. (2008) found that coatings improved the surface appearance of kiwifruit without impairing ripening, and fruits were well liked by consumers even if no benefit was observed on weight loss.

As for melons, the majority of investigations on edible coatings have concerned their applications on fresh-cut rather than on the whole fruit (Oms-Onliu *et al*., 2008 a, b; Amaro *et al*., 2012).

Fallik *et al*. (2005) evaluated the external, internal and sensory traits of 'Galia'-type melon fruit coated with three polyethylene-based waxes. The best results were obtained by using waxes that contain no, or very low amounts, of shellac as these waxes reduced water loss, improved the general appearance, and maintained pleasant, sweet and fruity aroma notes of the fruit even after prolonged storage, while control fruit suffered from high-decay incidence and soft texture. When shellac was present in high amounts, off-flavor significantly increased in the melon fruit due to high internal levels of CO_2, ethanol, acetaldehyde and ethyl acetate. Cong *et al.* (2007) found that the bilayer coating of chitosan and polyethylene wax microemulsion containing natamycin extended the shelf life of 'Hami' melon by reducing weight loss, fruit decay, and the decrease of ascorbic acid content during storage at ambient temperature even if some doubts remained regarding the sensory quality of coated fruit.

The aim of the present work was to assess the effectiveness of different kinds of novel coatings (under patent) on shelf life extension of winter melon during retail by studying internal gas concentrations coupled to off-flavor development and fruit quality.

2. Materials and Methods

The experiment was carried out in 2014 on winter melons (*Cucumis melo* L. *inodorus* type Honeydew cultivar Natal) assigned for a large retail chain. Fruits were harvested in a commercial orchard in the Rio Grande do Norte Region on 21 January 2014 and arrived in Milan on 11 February, when the trial began. Natal melons are characterized by a yellow skin and white flesh. Sixty melons were selected: 24 uncoated fruits were used as control (T); 36 fruits were treated with two coatings (F1 and F2) currently under patent. F1 coating is a complex based on a cellulose polymer, it is water soluble, with a concentration of 25±2°Bx. F2 coating is a synthetic polymer, also water soluble, with a concentration of 15±1°Bx; F2 was used after dilution 1:2 (w/w) with tap water.

Fruits were dipped in the coating solutions for 30 s, dried for 24 h at room temperature and then put at 13°C together with uncoated melons. Upon arrival in the laboratory of CRA-IAA in Milan (d0), and after 6 (d6), 9 (d9) and 13 (d13) days at 13°C, six melons/treatment were individually analyzed for internal O_2, ethylene and ethane concentrations, fermentative metabolites, skin and pulp color, flesh firmness, soluble solid content (SSC), weight loss and aroma pattern by a commercial electronic nose (E-nose).

Internal oxygen

Internal oxygen was measured in the seed cavity by using a fluorescence-based optical sensor system (Neofox Fosfor-R, Ocean Optics). This Fiber Optic Oxygen Sensor uses the fluorescence of a chemical complex in a sol-gel to measure the partial pressure of oxygen. The pulsed blue LED sends light, at ~475 nm, to an optical fiber. The optical fiber carries the light to the probe. The distal end of the probe tip consists of a thin layer of a hydrophobic sol-gel material. A sensor formulation is trapped in the sol-gel matrix, effectively immobilized and protected from water. The light from the LED excites the formulation complex at the probe tip. The excited complex fluoresces, emitting energy at ~600 nm. If the excited complex encounters an oxygen molecule, the excess energy is transferred to the oxygen molecule in a non-radiative transfer, decreasing or quenching the fluorescence signal. The degree of quenching correlates to the level of oxygen concentration or to partial pressure of oxygen on the film, which is in dynamic equilibrium with the oxygen in the sample. The energy is collected by the probe and carried through the optical fiber to the spectrometer and the data are then displayed in the OOISensors Software.

Internal ethylene and ethane

Internal ethylene and ethane concentrations were measured by withdrawing 1 mL samples of internal gas from the seed cavity of each melon using a syringe equipped with 15 cm long, 15-gauge needle. The sample was injected in a DANI GS 86.10 gas chromatograph equipped with a deactivated aluminum oxide F1 (80-100 mesh) column (1/8 in. ×200 cm) and a flame ionization detector according to Rizzolo *et al*. (2005). Quantitative data were obtained by relating the peak of each hydrocarbon to that of its external standard and were expressed as ppm.

Fermentative metabolites

Fermentative metabolites (ethanol, acetaldehyde and ethyl acetate) were determined on the fruit pulp by means of static HS-GC. For each fruit, 10 g of homogenized pulp (two replications) were put into 25 mL vials tightly closed with an aluminum cap with a silicone-Teflon rubber septum; then samples were immediately frozen and kept at -20°C until analysis. After a 60 min thawing at room temperature and E-nose analysis, each vial was heated at 80°C for 30 min, and 0.5 mL of the headspace gas was sampled and injected using the automatic headspace sampler HSS 86.50 DANI fitted to a gas chromatograph DANI 8521, equipped with a PTV injector port operating in splitless mode, a FID detector, and a DB-1 column (60 m × 0.53 µm i.d., 1 µm film thickness). The following GC conditions were used: helium carrier gas flow rate, 1.6 mL min^{-1}; hydrogen flow rate, 66 mL min^{-1}; air flow rate, 146 mL min^{-1}; oven temperature program, 10 min at 50°C, 4°C min^{-1} to 100°C, injector port and detector temperatures, 200 and 250°C, respectively. Fermentative metabolites were quantified by relating the peak area of each one to that of external standards and were expressed as mg kg^{-1}.

Skin and pulp color

Skin and pulp color were measured on two opposite sides in the equatorial region of the fruit with a Spectrophotometer CM-2600d (Minolta Co, Japan), using the primary illuminant D65 and 10° observer in the *L**, *a**, *b** color space. The Gloss Index was also calculated by the SpectraMagic acquisition program by using the SCI and SCE numerical gloss control. Color readings were averaged for each fruit.

Flesh firmness

Flesh firmness was measured on the fruit flesh after having cut the melon into two parts along the longitudinal axis; measurements were carried out on one part in six opposite areas at the top, mid, and bottom positions of each fruit using an 8 mm diameter plunger mounted on an Instron Universal Testing Machine (model 4301, Instron Ltd, Great Britain) with the crosshead speed at 200 mm min^{-1}. The six measurements were averaged for each fruit.

Soluble solids content

Soluble solids content (SSC) was determined on the juice that came out during plunging at the same positions of firmness measurements; SSC was measured using an automatic refractometer (RFM81, Bellingham-Stanley Ltd., England) and the six readings were averaged for each fruit.

Aroma pattern

Aroma pattern was determined by a commercial E-nose on the same samples used for the fermentative metabolite analysis, soon after the 60 min thawing at room temperature. A PEN3 portable electronic nose (Win Muster Airsense Analytics Inc., Germany) was used (Rizzolo *et al.*, 2013). The PEN3 E-nose consists of a sampling section, a detector unit containing the array of sensors, and a pattern recognition software (Win Muster v. 3.0) for data recording and elaboration. The sensor array is composed of ten metal oxide semiconductor (MOS) type chemical sensors: W1C (aromatic), W5S (broad range), W3C (aromatic), W6S (hydrogen), W5C (aromatic aliphatics), W1S (broad), W1W (sulfur organic), W2S (broad alcohol), W2W (sulfur chlorinate), and W3S (methane aliphatics). The sensor response is given by the ratio of the conductivity response of the sensors to the sample gas (G) relative to the carrier gas (G_0) over time (G/G_0). The headspace gas was pumped over the sensor surfaces for 60 s (injection time) at a flow rate of 45 mL min^{-1}, and during this time the sensor signals were recorded. After sample analysis, the system was purged for 120 s with filtered air prior to the next sample injection to allow re-establishment of the instrument baseline. Each sample was evaluated three times. For each E-nose run, the conductivity G/G_0 of the 10 sensors at the time corresponding to the normalized maximum of all signals was taken as the vector of sensors signal. The average of the runs of each replicate was used for statistical analysis.

Statistical analysis

Data were submitted to analysis of variance (Statgraphics ver.7, Manugistic Inc., Rockville, MD, USA) considering coating and day at 13°C as a sources of variation, and means were compared by Tukey's test at P≤0.05%. E-nose data were also submitted to Principal component analysis. The principal component (PC) scores were then subjected to ANOVA and means were compared by Tukey's test at P≤0.05% considering as factors coating and day at 13°C. Correlations between PCs and internal O_2, ethylene and ethane concentrations and fermentative metabolites were also analyzed.

3. Results

Internal oxygen, ethylene, and ethane

Upon arrival in the laboratory, melons showed an internal O_2 amount of about 16.3%. Already after 6 days at 13°C O_2 levels fell to 1.3% in F1-coated melons and to 3.2% in F2 ones (Fig. 1) and remained at about these percentages up to 13 days. In contrast, in control fruit, internal O_2 levels decreased to about 14% at d6, slightly decreased at d9, falling to about 5% at d13. F2 melons showed higher variability in internal O_2 amounts in comparison to F1 ones, as in F2 fruit the internal O_2 ranged from 1 to 6.8%, and in F1 fruit from 0.8 to 1.8%.

Ethylene amount was 0.14±0.07 ppm at d0, increased at d6 to about 0.55 ppm and 0.78 ppm in control and F1 melons, respectively, then it decreased up to d9, to a greater extent in control fruit than in F1 ones, and remained constant up to the end of the storage period (Fig. 1). In F2 melons, ethylene was constant up to d6 and increased at d9, remaining at this level up to d13 (Fig. 1). F2 fruit showed the highest ethylene amounts from d9 to d13, while control fruit developed the least ethylene quantity in the same period.

Ethane was absent at d0; in control fruit it was produced only at d13 in very low amounts (about 8 ppm), while in F1-coated melons ethane levels steeply increased to about 60 ppm already after 6 days at 13°C, then decreased in the subsequent three days maintaining a high amount (30 ppm) up to d13 (Fig. 1). F2-coated melons showed a slight increased in ethane production, reaching about 13 ppm at d13 (Fig. 1).

Fermentative metabolites

At d0, fruit had acetaldehyde=12.3±0.6 mg kg^{-1}, ethanol=24.2±3.2 mg kg^{-1} and ethyl acetate= 1.7±0.5 mg kg^{-1}.

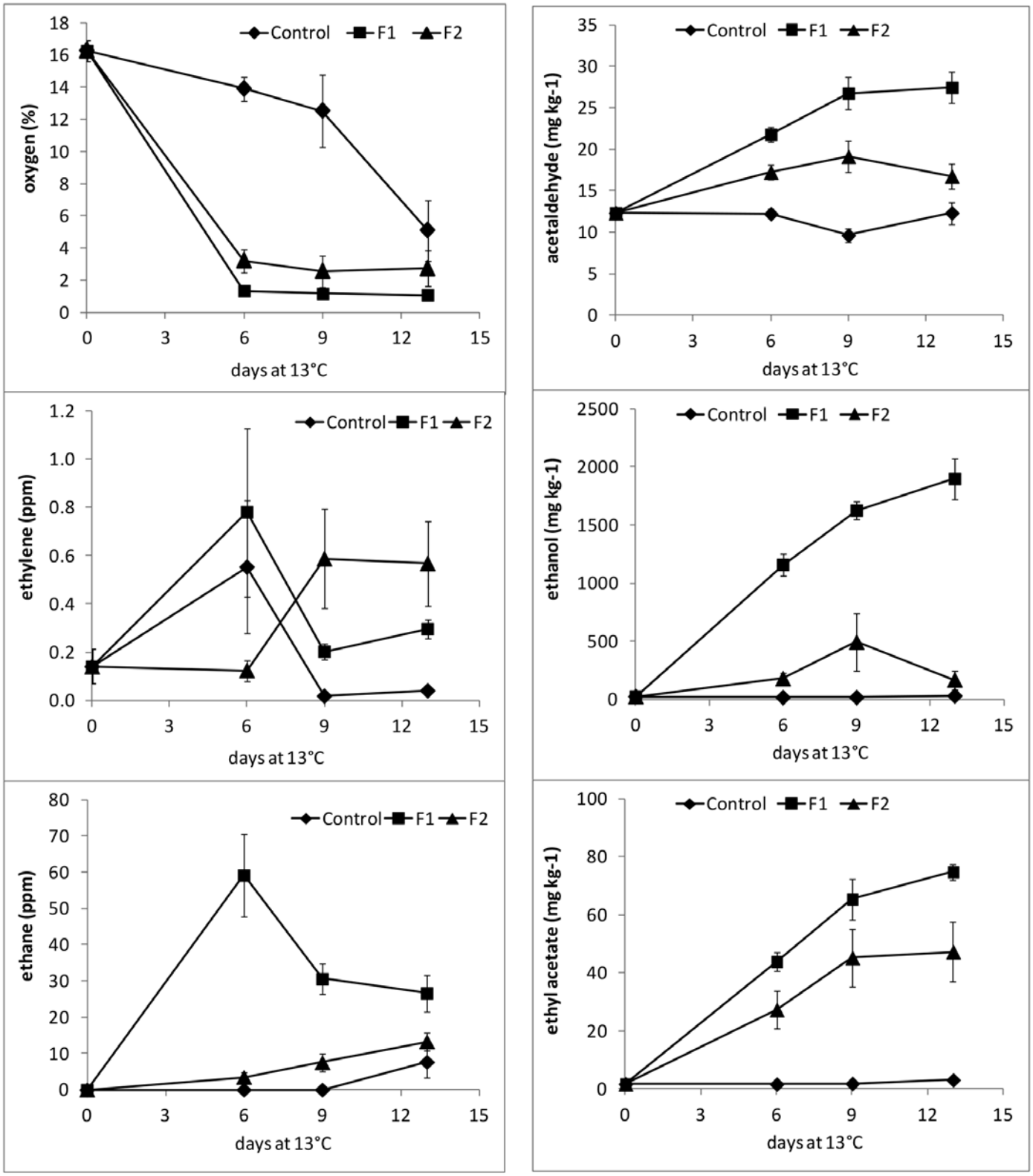

Fig. 1 - Internal oxygen, ethylene, and ethane concentrations (left) and fermentative metabolites (acetaldehyde, ethanol, ethyl acetate - right) amounts of control and coated (F1, F2) melon fruit during storage at 13°C. Bars refer to standard error of the mean (n=6).

All three fermentative metabolites dramatically increased in F1 fruit already after 6 days at 13°C (Fig. 1), increased further up to d9, showing a slight but not significant increase up to d13, with the exception of acetaldehyde which did not change from d9 to d13. Also in F2 fruit, fermentative metabolites significantly increased from d0 to d6 but to a lesser extent than in F1 melons; then they slightly increased up to d9, maintaining this amount up to 13 days. In contrast, in control melons, fermentative metabolites did not show any changes during storage at 13°C.

Skin and pulp color

The application of the coatings significantly affected *b** and Gloss Index in the fruit skin and *a** and *b** of the pulp (Table 1). Skin *b** was higher in control fruit than in coated fruit, and no difference was found between the two kinds of coating. Gloss Index showed the highest values in F1 melons, intermediate in F2 ones and the lowest in control fruit. As for pulp color, *a** was highest in control fruit and lowest in F2 melons, with F1 fruit showing intermediate values, whereas *b** had the lowest values in control fruit at d6, when F1 fruit had the highest values; no difference was found at d9 among the treatments, while at d13 control fruit still had lower *b** than both types of coated fruit. No changes were observed in skin and pulp color parameters during storage at 13°C, except for skin *L** and Gloss Index and for pulp *b** which significantly decreased during storage at 13°C.

Firmness, Soluble solids and weight loss

Firmness did not change with coating treatments and days at 13°C, while SSC, on average, were lower in F1 fruit and did not change with days at 13°C (Fig. 2).

Weight loss showed the lowest values in F2 fruit (Fig. 3). A different trend in weight loss increase during storage at 13°C was observed according to the treatment; a sharp increase was observed in F1 melon from d6 to d13, while in F2 fruit weight loss increased from d6 to d9 and then remained constant up to d13; in control melons weight loss dramatically increased up to d6, then it continued to increase but to a lesser extent up to d9 and then steeply increased up to d13.

Aroma pattern

The behavior of the signals generated by the sensor array is reported in figure 4. Each line represents the average signal variation of replicated samples for one sensor of the array, linking the conductance increase or decrease experienced by the sensors to the evolution of the coating type during the storage time. The responses of the 10 MOS sensors significantly changed with coating presence, with the exception of W1W, W2W and W3S sensors. The sensors W1C, W3C and W5C showed the lowest responses in F1 fruit and the highest in control melons; the opposite behavior was observed for W5S, W6S, W1S and W2S sensors. No sensors changed in control fruit with storage days, while the responses of W1C, W3C and W5C sensors decreased with storage time and those of W5S, W1S and

Table 1 - Skin and pulp color (mean ± standard error; n=6) of control and coated (F1, F2) melon fruit during storage at 13°C and ANOVA results

Storage		Skin color				Pulp color		
		*L**	*a**	*b**	Gloss Index	*L**	*a**	*b**
day 0		75.1±1.0	3.8±0.8	75.1±0.9	3.7±1.0	73.1±1.1	-3.0±0.3	15.3±0.6
day 6	Control	74.5±1.1	5.7±1.4	76.6±1.1	5.0±0.5	74.7±0.9	-1.9±0.4	12.7±0.4
	F1	74.6±0.8	3.7±1.3	72.8±1.2	21.5±4.5	76.4±0.6	-2.2±0.2	14.0±0.3
	F2	74.0±0.8	4.7±0.9	73.2±1.3	12.8±2.2	72.9±0.4	-3.3±0.2	15.5±0.2
day 9	Control	75.7±0.5	4.2±1.2	77.3±0.8	1.7±0.6	73.7±0.8	-1.9±0.8	14.6±1.1
	F1	73.6±0.2	7.1±0.7	73.7±0.4	20.5±4.5	74.1±0.8	-2.2±0.2	13.8±0.1
	F2	73.5±0.3	6.6±0.5	73.9±0.5	14.7±2.6	75.1±0.7	-2.4±0.3	13.2±0.4
day 13	Control	73.9±0.8	5.8±0.6	75.4±1.2	0.0±0.0	73.5±1.2	-1.6±0.2	12.0±0.5
	F1	72.3±0.5	6.9±0.9	71.7±0.6	10.5±3.3	74.7±0.3	-2.1±0.1	13.4±0.3
	F2	72.9±0.7	6.8±0.8	73.3±0.4	5.8±1.1	72.5±1.2	-2.5±0.2	13.6±0.8
					ANOVA			
coating (A)		NS	NS	***	***	NS	**	NS
days at 13°C (B)		*	NS	NS	**	NS	NS	*
A x B		NS	NS	NS	NS	NS	NS	**

***, P<0.001; **, P<0.01; *, P<0.05. NS= not significant.

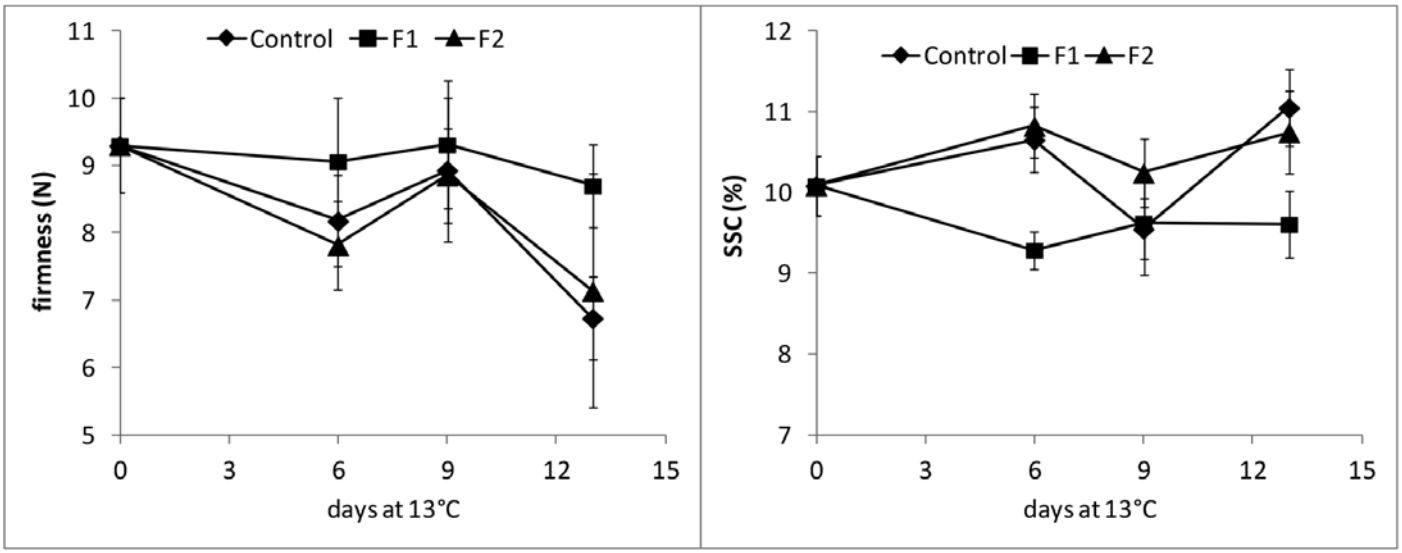

Fig. 2 - Firmness (left) and soluble solids content (SSC, right) of control and coated (F1, F2) melon fruit during storage at 13°C. Bars refer to standard error of the mean (n=6).

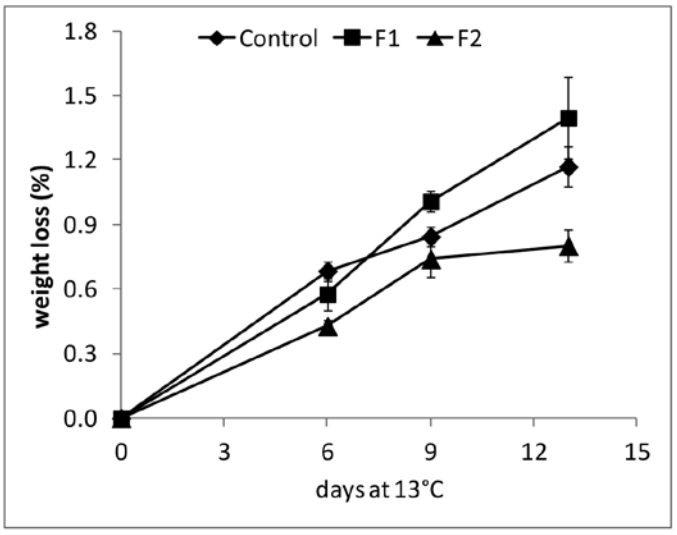

Fig. 3 - Weight loss of control and coated (F1, F2) melon fruit during storage at 13 °C. Bars refer to standard error of the mean (n=6).

W2S decreased in coated fruit. In F2 melons, the E-nose sensor responses were always intermediate between control and F1 melons.

To see whether the sensor array was able to distinguish the different kinds of coating, PCA was applied to the E-nose measurements. Two functions were extracted, explaining about 89% of the variability (Fig. 5). PC1 grouped W1S, W2S and W5S sensors opposite to W1C, W3C and W5 ones, while in PC2 W1W and W2W sensors were opposed to W3S ones. PC1 scores showed the lowest values for control melons, intermediate for F1 ones and highest for F2 (Fig. 6). The PC1 score was also lower at d0 in comparison to the other days at 13°C. PC2 scores didn't change, neither with coating nor with storage time (Fig. 6).

To compare the E-nose patterns with composition data, the correlations between PC1 and PC2 scores with internal O_2, ethylene, ethane, and fermentative metabolites were studied (Table 2). High and positive correlations were found between PC1 fermentative metabolites and ethane while a negative correlation was observed between PC1 and internal O_2 levels. No correlation was found for PC1 with ethylene amount and for PC2 with all compounds.

4. Discussion and Conclusions

Edible coatings have been used in order to retain quality and to extend shelf life of fresh fruits and vegetables. Most fruits and vegetables possess a natural waxy layer on the surface (cuticle) which generally has a low permeability to water vapor. Applying an external coating could enhance this natural barrier as a semi-permeable membrane is formed on the fruit surface and thus it will be possible to obtain a better control of gas diffusion and moisture loss, delaying ripening and senescence.

Table 2 - Linear correlation coefficients (r) of PC1 and PC2 scores, obtained from Principal Component Analysis, with oxygen level, ethylene, and ethane amounts and fermentative metabolites

Correlation coefficient	PC1	PC2
Oxygen	- 0.668***	- 0.040
Ethylene	0.211	- 0.090
Ethane	0.702***	0.034
Acetaldehyde	0.844***	- 0.034
Ethanol	0.836***	- 0.059
Ethyl acetate	0.882***	- 0.101

Significance of *r*: *, P ≤ 0.05; **, P ≤ 0.01; and ***, P ≤ 0.001)

The quality of fruits and vegetables depends on their internal O_2 and CO_2 concentrations which in turn are affected by the environmental concentrations of these gases (Hagenmaier, 2005). A reduced O_2 level is desirable for slowing down respiration and preventing exchange of food aroma and flavor compounds with the environment. The coatings applied to fruit form barriers to the diffusion of O_2 and CO_2 through the fruit peel. When the supply of O_2 needed for respiration or the release of CO_2 is blocked, fruit and vegetables quickly become inedible and rotten. Such blockage lowers and raises the interior O_2 and CO_2 concentrations, respectively, causing off-flavor development due to anaerobic fermentation. Thus, the selection of appropriate coating materials and formulations with a proper gas permeability represents a crucial point to obtain successful results.

Our results showed that already after 6 days at 13 °C, internal O_2 levels in coated fruit fell to about 1% in F1 melons and to 3% in F2 ones. This O_2 drop led to a remarkable increase in ethane, acetaldehyde, ethanol, and ethyl acetate amounts, mainly in F1 fruit. This means that F1

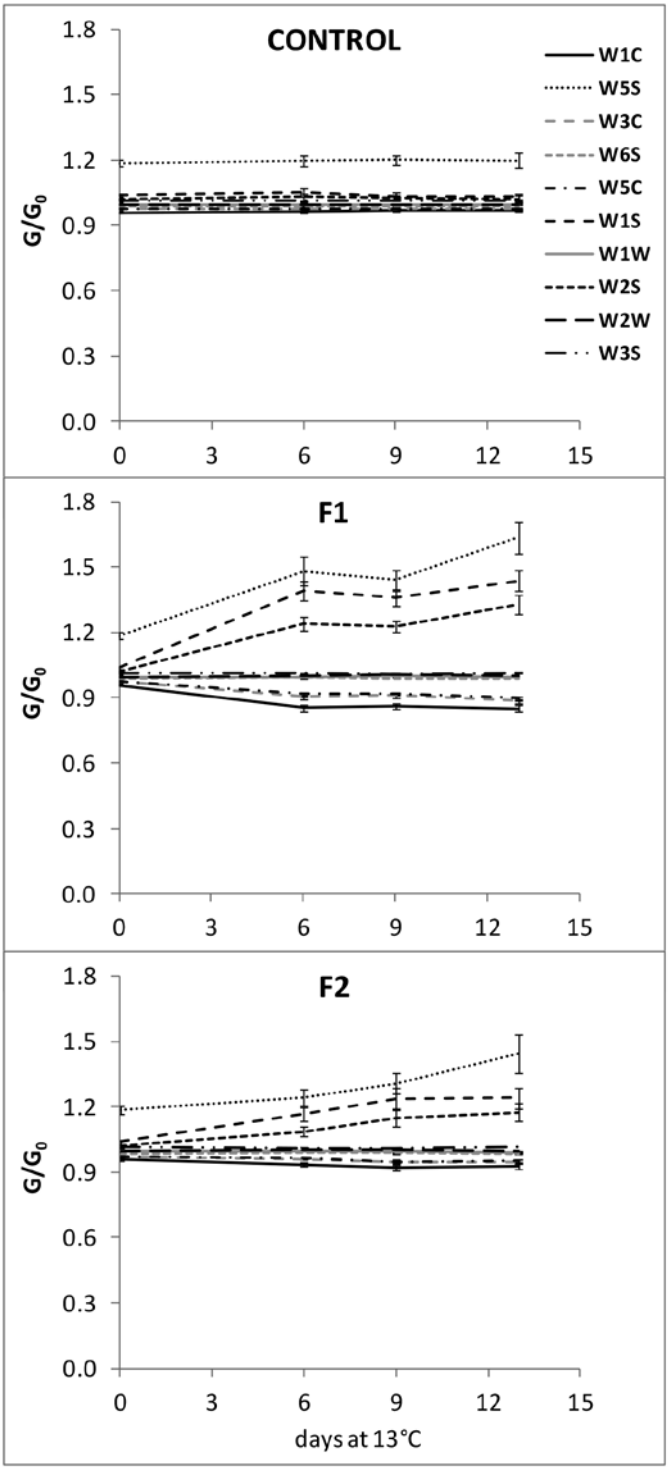

Fig. 4 - Relative conductivity (G/G_0) of each sensor in control and coated (F1, F2) melon fruit during storage at 13°C. Bars refer to standard error of the mean (n=6).

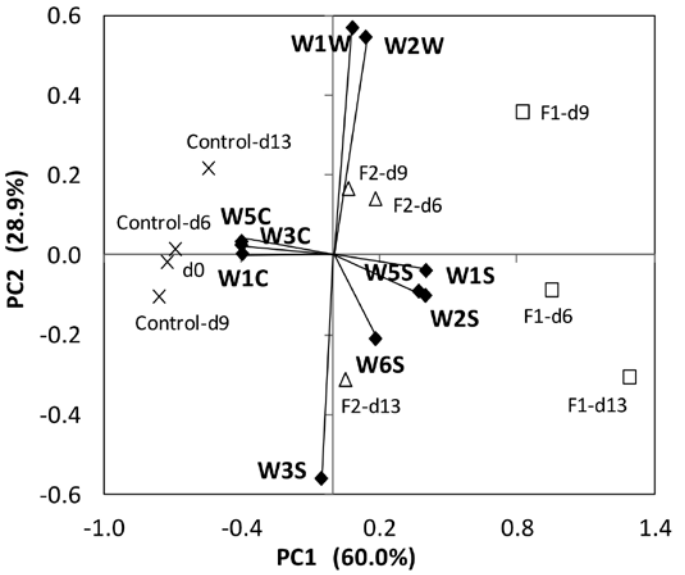

Fig. 5 - PCA of E-nose data: loadings and scores of PC1 versus PC2 according to coating treatment (control; coated=F1, F2) and day at 13°C (d0, d6, d9, d13 refers to arrival, after 6, 9 and 13 days at 13°C, respectively).

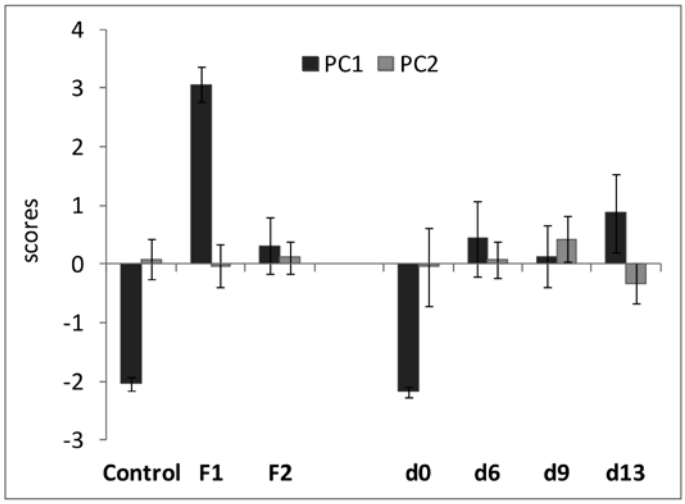

Fig. 6 - PCA scores of PC1 and PC2 according to coating treatment (control; coated=F1, F2) and days at 13°C (d0, d6, d9, d13 refers to arrival, after 6, 9 and 13 days at 13°C, respectively). Bars refer to standard error of the mean (n=18).

melons initiated anaerobic respiration, by which glucose is converted to pyruvate by glycolysis and then, pyruvate metabolized to acetaldehyde and acetaldehyde to ethanol.

The recommended percentage of O_2 in a modified atmosphere for fruits and vegetables for both safety and quality falls between 1 and 5% (Sandhya, 2010). It has been established that at a 2% O_2 level anaerobic respiration may result in the development of off-flavors and off-odors. Fruits exposed to such low O_2 levels may also lose their ability to attain uniform ripeness upon removal from the modified atmosphere packaging. The minimum O_2 concentration tolerated in controlled atmosphere storage of whole cantaloupe melons is 2% and impaired ripening, off-flavors and odors could develop when O_2 falls to 1% and CO_2 goes up to 20% (Kader *et al*., 1989).

Oms-Oliu *et al.* (2008 a, b), studying low oxygen modified atmospheres on shelf life extension of fresh-cut melons, found that fermentative pathways were triggered under a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere. This atmosphere caused a rapid reduction in the O_2 levels below 1 kPa and an accumulation of CO_2 so the initial respiratory quotient (RQ-ratio of CO_2 produced to O_2 consumed) of 1.2 increased above 1.3 after 10-14 days of storage. When RQ is higher than 1, anaerobic respiration takes place and fermentative products are developed (Fonseca *et al*., 2002). In fact, fresh-cut melons produced acetaldehyde and ethanol mainly inside package when O_2 concentrations drop below 2 kPa level (Oms-Oliu *et al.*, 2008 a, b).

Whole melon fruit coated with waxes characterized by high amounts of shellac developed off-flavors when internal O_2 and CO_2 levels reached about 3% and 20%, respectively. These fruits developed higher amounts of acetaldehyde, ethanol, and ethyl acetate than uncoated ones and were characterized by a 'bad flavor' as fruity-pleasant notes (due to butyl acetate and 2-methyl-propyl acetate) were very low, while 'ethyl acetate note' (which causes a solvent like smell) and the 'ethanol-like' note were high.

In our work, when melons were cut for analysis, a fermentative smell was perceived for F1-coated fruit, as the amounts of acetaldehyde and ethanol were higher than their detection thresholds of 25 μg L^{-1} and 990.000 μg L^{-1}, respectively (Czerny *et al.,* 2008). Flores *et al.* (2004) considered inedible a melon fruit when ethanol was about 64 μmol kg^{-1}, as found in fruit packed in modified atmospheres due to higher CO_2 levels.

In contrast, when oxygen levels remained at about 4% and CO_2 levels at about 10%, fresh-cut cantaloupe cubes retained salable quality for 9 days at 5 °C and fruit showed better color retention, reduced translucency, respiration rate and microbial populations (Bai *et al.*, 2001).

Hagenmaier (2005) found a rather wide range of internal CO_2 and O_2 values when individual coated oranges and apples were considered, in comparison with uncoated fruit showing a rather tight cluster of values. The internal gas values were particularly scattered for fruit with shellac and resin coatings, which caused the greatest reduction in peel permeance. This means that low-permeance coatings result in fruit with higher variation in product quality. On the contrary, in our work a large variation in internal O_2 levels was found for uncoated fruit followed by F2-coated fruit, while F1 melons which had the lowest O_2 levels also had the lowest variability in O_2 levels.

F1 melons also exhibited a dramatic increase in ethane levels already after 6 days at 13°C when O_2 fell from 16% to 1%. Similarly, Rizzolo *et al.* (2008) found that ethane production was maximum in pears under 0.1 kPa O_2 and absent in fruit stored at $O_2 \geq 2$ kPa. In our work a slight ethane production was also detected in F2-coated fruit where the O_2 level was about 3%. Ethane production depends also on CO_2 levels, as it was high in pears stored under 5 kPa CO_2 whatever the pO_2 (Rizzolo *et al*., 2008). Ethane is usually considered as a marker of lipid peroxidation in the cell membranes and it is released in pears affected by core browning while it was not detectable in healthy fruit (Veltman *et al*., 1999; Larrigaudière *et al*., 2001).

The aroma pattern as revealed by E-nose reflected the different O_2 and ethane levels as well as the different amounts of fermentative metabolites found in melon fruit according to coating treatment.

By using an E-nose it was possible to distinguish control fruit from F1- and F2-coated ones. W1S, W2S, W5S and W6S sensors showed higher responses for coated melons in comparison with control fruit, which in turn had the highest responses for W1C, W3C and W5C sensors. W1S, W2S, W5S and W6S sensors were grouped in PC1 and were positively related to acetaldehyde, ethanol, ethyl acetate, and ethane and negatively related to O_2 levels. PC1 had the highest scores in F1 fruit which were characterized by the lowest O_2 levels and by the highest production of ethane and fermentative metabolites and showed the lowest values in control fruit at day 0 when no fermentative metabolism occurred. The same relationships between MOS sensors and fermentative metabolites were found by Riva *et al.* (2005) in strawberries as a consequence of the formation of a peripheral layer of sugar with reduction of tissue porosity occurring during the osmodehydration process. Differently from that found for cold-stored peaches for which a high and significant correlation was found between ethylene production and E-nose pattern (Rizzolo *et al*., 2013), no correlation was found between E-nose pattern and internal ethylene. Ethylene production was very low as expected for a honeydew-type melon and slight changes were observed in relation to coating treatments and to days at 13°C in agreement with Barreiro *et al.* (2001).

Considering quality parameters, coating treatments had no influence on fruit firmness, while reduced SSC content was found when F1 coating was applied on fruit, and this decrease may be due to the fermentative pathway of accumulations of acetaldehyde and ethanol catalyzed by the enzymes pyruvate decarboxylase and alcohol dehydrogenase, respectively (Kader, 1995). Coating also affected skin and pulp color of melon fruit as F1 coating enhanced skin glossiness and delayed skin yellowing in compari-

son both to control and F2-coated fruit, while F2 melons showed a white-slightly greener pulp than control fruit.

In our experiment, weight loss was quite low, reaching the maximum values of about 1.5 % in F1-coated fruit and was lower in F2 fruit than in control and F1 ones. The inability of F1 coating to control weight loss could be due to the fact that this type of coating is based on a cellulose polymer and it is well known that the hydrophilicity of this material does not provide a sufficient moisture barrier (Lin and Zhao, 2007; Falguera *et al*., 2011; Dhall, 2013).

Application of coating to whole winter melons caused the onset of fermentation processes especially in fruit coated with the cellulose-based polymer (F1) where the O_2 level dropped to 1% which is the threshold value causing anaerobic metabolism and development of off-flavors and off-odors. Probably this kind of coating strongly decreased permeance of the skin to gas exchanges while it had no effect on water loss. The coating (F2) based on a water-soluble synthetic polymer showed a higher permeance to O_2 exchanges as O_2 levels were maintained at about 3% and therefore the development of fermentative metabolites was limited; this coating was also able to prevent fruit weight loss but no other positive effect was observed on fruit quality as firmness and soluble solids content were similar to those of uncoated melons. However, the cellulose based polymer coating improved fruit appearance, strongly enhancing fruit gloss and delaying skin yellowing, but these positive effects are of secondary importance in comparison to the anaerobic metabolism induced by this kind of coating.

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Assessment of antioxidant activity of carotenoid-enriched extracts from peach fruits using the new LOX/RNO method

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Abbreviations: AA, antioxidant activity; AAPH, 2,2'-azobis(2-amidinopropane); ABTS, 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonate); AUC, area under curve; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical; FRAP, Ferric Reducing Antioxidant Power; fluorescein, 3',6'-dihydroxyspiro[isobenzofuran-1[3*H*], 9'[9*H*]-xanthen]-3-one; f.w., fresh weight; LOX, lipoxygenase (linoleate: oxygen oxidoreductase, EC 1.13.11.12); ORAC, Oxygen Radical Absorbance Capacity; RNO, 4-nitroso-*N,N*-dimethylaniline; TEAC, Trolox Equivalent Antioxidant Capacity; Trolox, (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid.

Key words: antioxidant activity, carotenoids, LOX/RNO method, peach.

Abstract: Peach (*Prunus persica* L.) fruits contain several health-promoting phytochemicals. Among these, carotenoids, in addition to being involved in determining flesh color, play a relevant role in cell protection against oxidative stress. Nevertheless, antioxidant activity (AA) of peach carotenoids so far has not been investigated in as much detail as phenols. In the present study, for the first time, AA of peach carotenoid extracts was evaluated using the innovative lipoxygenase/4-nitroso-*N,N*-dimethylaniline (LOX/RNO) method, able to simultaneously detect different antioxidant mechanisms and synergistic antioxidant interactions, as well as using the well-known ORAC and TEAC assays. In particular, extracts were obtained from fruits collected in S4 development stage from two yellow-fleshed (Armking and Redhaven) and three white-fleshed (Silverking, Caldesi 2000, IFF331) varieties. The LOX/RNO method gave high AA values (10-150 µmol eq. Trolox/g f.w.), about 85-1900-fold higher than ORAC and TEAC methods. Moreover, the ratio between AA values, measured by the LOX/RNO method, of yellow- and white-fleshed peaches resulted equal to 14, but only 2.6 and 3.6 for ORAC and TEAC, respectively. Results of this study indicate that the LOX/RNO method, measuring high AA values and easily discriminating among samples, is an advisable tool to assess the AA of the carotenoid component in peach.

1. Introduction

Peach (*Prunus persica* L.), a member of the Rosaceae family, is one of the most economically important fruit species in the world, with Italy being the second world producer after China and the first in Europe (FAOSTAT, 2012). The flesh color of the fruit, white or yellow, is an important characteristic driving consumer choice and thus represents a key breeding trait for this crop. From a genetic point of view, it is controlled by a single *locus* and the white phenotype is fully dominant over the yellow (Connors, 1920; Falchi *et al*., 2013). From a biochemical point of view, yellow flesh color is determined by the accumulation of carotenoids in chromoplasts. In particular, in the early stages of the fruit developmental cycle, both white- and yellow-fleshed peaches have very high concentrations of total carotenoids, although masked by the green due to the presence of chlorophyll (Ma *et al*., 2014). In the later stages of the fruit cycle, carotenoids are massively degraded in the white-fleshed, but not in the yellow-fleshed fruits, which keep accumulating these compounds and whose content peaks at full fruit ripening (Ma *et al*., 2014). Recent studies carried out on peach chimeric mutants for flesh color showed a strongly reduced expression of *ccd4*, the gene codifying for dioxygenase in the yellow-fleshed genotype compared to its white-fleshed mutant,

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suggesting a key role of this gene in controlling carotenoid accumulation, hence flesh color in peach (Brandi *et al*., 2011; Adami *et al*., 2013).

In addition to their role in peach flesh color, carotenoids also represent relevant biologically active compounds, responsible for some beneficial health properties associated to increased consumption of fruit and vegetables (Liu, 2003 and references therein; Schreiner and Huyskens-Keil, 2006). In particular, β-carotene, α-carotene and β-cryptoxanthin have an essential function in human nutrition as they are a primary dietary source of provitamin A, which humans are unable to synthesize *de novo*. Moreover, the antioxidant properties of carotenoids, that may act as free-radical scavengers, singlet oxygen quenchers, and lipid antioxidants, have been reported to be highly involved in reducing the risk of cardiovascular disease, cancer, diabetes and other degenerative diseases associated with oxidative stress (Rao and Rao, 2007; Jomova and Valko, 2013).

In recent years several studies have been performed to investigate the antioxidant properties of peach fruit due to carotenoids, as well as anthocyanins and other phenolic compounds; nevertheless, the role of carotenoids so far has not been completely clarified. Peach carotenoid content has been quantified in these reports, as well as antioxidant activity (AA) evaluated using assays measuring *i*) the peroxyl radical scavenging capacity (Oxygen Radical Absorbance Capacity, ORAC) (Campbell and Padilla-Zakour, 2013), *ii*) the 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) radical cation scavenging capacity (Trolox Equivalent Antioxidant Capacity, TEAC) (Dalla Valle *et al*., 2007; Di Vaio *et al*., 2008; Legua *et al*., 2011; García-Parra *et al*., 2011; Oliveira *et al*., 2012), *iii*) the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (Gil *et al*., 2002; Lavelli *et al*., 2009; Puerta-Gomez and Cisneros-Zevallos, 2011; Zielinski *et al*., 2014), and *iv*) the Fe^{3+} ion reducing capacity (Ferric Reducing Antioxidant Power, FRAP) (Gil *et al*., 2002; Remorini *et al*., 2008; Tavarini *et al*., 2008; Durst and Weaver, 2013; Zielinski *et al*., 2014). In most of these studies, AA was evaluated by studying methanol extracts. Only in very few reports, the AA of both water- and fat-soluble antioxidant fractions (obtained from fruit extraction using water and acetone or THF or ethyl acetate, respectively) was investigated separately (Dalla Valle *et al*., 2007; Di Vaio *et al*., 2008; Legua *et al*., 2011). This was made possible by using only the TEAC assay, measuring mainly the antioxidant reducing capacity; interestingly, generally lower AA values of the lipophilic antioxidant fraction compared to those of the hydrophilic component were obtained, positively correlated to total carotenoid content (Legua *et al*., 2011).

In the present study, AA of extracts highly enriched in carotenoids obtained from the flesh of peach fruits was evaluated for the first time using the advanced and innovative lipoxygenase/4-nitroso-*N,N*-dimethylaniline (LOX/RNO) method (Pastore *et al*., 2009). This is a recently developed AA assay based on the RNO bleaching reaction due to some radical species generated by secondary anaerobic reactions catalyzed by soybean LOX-1 isoenzyme, occurring when the main aerobic cycle of linoleic acid (LH) hydroperoxidation has consumed oxygen (Pastore *et al*., 2000 a) (Fig. 1). With respect to the majority of AA assays, the LOX/RNO method is able to detect the scavenging capacity of antioxidants towards different physiological and biologically relevant radical species, such as alkoxyl ($LO^{\bullet}$), peroxyl ($LOO^{\bullet}$), hydroxyl ($^{\bullet}OH$) and perhaps alkylic ($L^{\bullet}$) radicals, as well as singlet oxygen ($^1O_2$), but this latter only in the presence of imidazole (Pastore *et al*., 2000 a; 2009). Moreover, other important antioxidant functions may be simultaneously detected, including chelating or reducing activities of iron ions essential for the catalysis and generation of radical species, as well as any possible inhibition of the apo-enzyme (Pastore *et al*., 2009) (Fig. 1). Consistently, the LOX/RNO method has been found to highlight very well synergistic effects among antioxidants (Pastore *et al*., 2009; Laus *et al*., 2012 b; Laus *et al*., 2013 b). To date, the LOX/RNO method has been applied to assess AA of food-grade antioxidants (Laus *et al*., 2013 b) and of whole flour of some cereals (Flagella *et al*., 2006; Pastore *et al*., 2009; Laus *et al*., 2012 b), pseudocereals (Laus *et al*., 2012 a), and grain-derived products (Laus *et al*., 2013 a), showing a much higher performance with respect to other commonly used AA assays.

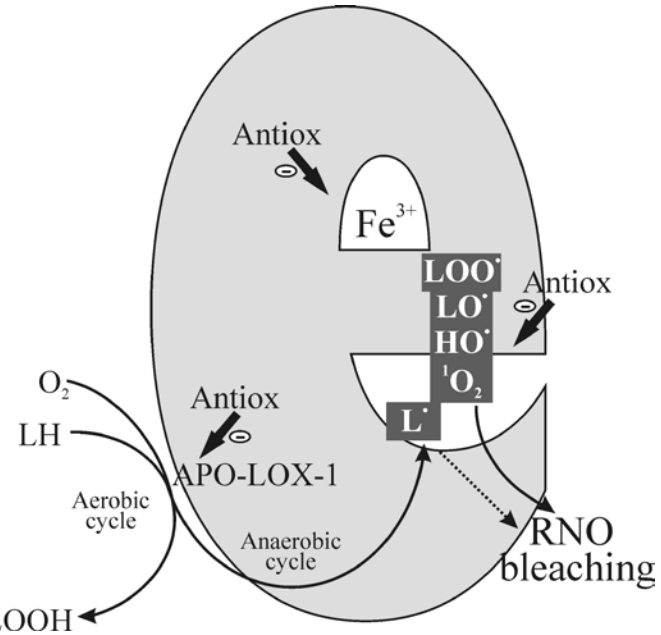

Fig. 1 - Schematic representation of the aerobic and anaerobic cycles catalyzed by the soybean lipoxygenase (LOX)-1 isoenzyme involved in the bleaching of the 4-nitroso-*N,N*-dimethylaniline (RNO). The soybean LOX-1 apoenzyme is represented as e-shaped and Fe^{3+} indicates non-heme iron atom essential for the LOX-1 catalysis. The aerobic cycle of soybean LOX-1-mediated hydroperoxidation of linoleic acid (LH) is schematized, as well as secondary anaerobic reactions involving generation of radical species, able to induce the RNO bleaching. The short black arrows indicate some different mechanisms by which antioxidant compounds may inhibit the LOX-1-dependent RNO bleaching. Antiox, antioxidant compounds; $LO^{\bullet}$, $LOO^{\bullet}$, $L^{\bullet}$, alkoxyl, peroxyl and alkylic radicals of LH; LOOH, 13-hydroperoxy derivative of LH; $^1O_2$, singlet oxygen; $^{\bullet}OH$, hydroxyl radical.

The objective of this study was to evaluate the performance of the LOX/RNO method with respect to AA assessment of the carotenoid fraction extracted from the flesh of peach fruits of some yellow and white varieties. To do this, the LOX/RNO method was compared with two well-established methodologies for AA measurement: the TEAC (Re *et al*., 1999) and ORAC (Ou *et al*., 2001) assays.

2. Materials and Methods

Chemicals

All reagents at the highest commercially available purity were purchased from Sigma-Aldrich Corp. (St. Louis, Mo., U.S.A.).

Plant material

Fruit from yellow-fleshed (Armking and Redhaven) and white-fleshed (Silverking, Caldesi 2000 and IFF331) cultivars/breeding selections was harvested from trees grown in the experimental farm of CRA-FRU (Roma, Italy) and CRA-FRF (Forlì, Italy). Replicates of three representative fruits at stage S4 (full ripening phase) of fruit development were sampled, peeled, cut into 0.5-cm slices and the mesocarp was immediately frozen in liquid nitrogen and stored at -80°C.

Preparation of aqueous solutions of linoleate and β-carotene

The sodium linoleate solution was prepared as described in Pastore *et al*. (2000 b; 2009) and the exact linoleate concentration was determined by means of the LOX assay (Pastore *et al*., 2000 b), by using a Perkin-Elmer λ45 UV-Vis Spectrophotometer (Perkin-Elmer, Wellesley, MA), managed by UV WINLAB software (Perkin Elmer version 2.85.04). The β-carotene solution was prepared according to the method reported by Pastore *et al*. (2000 b; 2009), and the concentration was spectrophotometrically determined as described in Pastore *et al*. (2000 b).

Extraction of carotenoids from peach fruit

Extraction was carried out according to the procedure described in Fraser *et al*. (2000) with some modifications. All manipulations were carried out on ice and shielded from strong light. Freeze-dried material (about 3.5 and 5 g for white- and yellow-fleshed fruits, respectively) was finely ground into a powder with a mortar and pestle using liquid nitrogen. Methanol was added according to a (v/w) ratio equal to 7.5 mL/g of ground tissue; the suspension was mixed by inversion and incubated for 5 min in an ice-water bath. Then, 50 mM Tris-HCl buffer pH 7.5 (containing 1 M NaCl) was added to the mixture according to a (v/w) ratio equal to 7.5 mL/g of homogenized tissue and incubated in an ice-water bath for 10 min. Chloroform at a (v/w) ratio equal to 2 mL/g of tissue was added to the mixture, incubated on ice for 10 min and then centrifuged at 5000x*g* for 5 min at 4°C. The aqueous phase was removed and the chloroform hypophase was pooled. Determination of total carotenoid content was performed on chloroform extract as described in the next section. Then, the chloroform extract was partitioned in two volumes. For AA measurements with the TEAC and ORAC methods, an aliquot was evaporated to dryness under vacuum at 40°C using a Buchi evaporator and the dry residue was reconstituted in absolute ethanol. As for the LOX/RNO assay, the second aliquot of the chloroform extract was added with Tween 80 according to a ratio equal to 0.4 µL Tween 80/µg of carotenoids; the mixture was dried under vacuum at 40°C and the dry residue was reconstituted in 100 mM sodium borate buffer pH 9.0.

Spectrophotometric determination of total carotenoids of extracts from peach fruits

Quantification of total carotenoids was carried out according to the procedure described by Lichtenthaler (1987) and by Lichtenthaler and Wellburn (1983). Briefly, the absorbance spectra of appropriate dilutions in 80% (v/v) acetone of the chloroform extract (see previous section) was recorded in the VIS region. Carotenoid concentration was calculated by means of a proper equation using a specific absorption coefficient at 470 nm of 198 $mL \cdot mg^{-1} \cdot cm^{-1}$, and involving a correction for chlorophyll a and b content based on absorbance measurements at 663 and 647 nm.

Determination of Antioxidant Activity (AA) by the LOX/RNO, ORAC and TEAC methods

The LOX/RNO reaction was spectrophotometrically monitored, as described in Pastore *et al*. (2000 a; 2009), by measuring the RNO absorbance decrease at 440 nm and 25°C in a reaction mixture (2 mL) containing 100 mM sodium borate buffer pH 9.0, 1 mM sodium linoleate, 1.5 µL Tween 20/µmol linoleate and 15 µM RNO; the reaction was started by adding 0.3 Enzymatic Units (EU) of soybean LOX-1. Since carotenoid extracts reconstituted in sodium borate buffer containing 0.4 µL Tween 80/µg carotenoids were analyzed, all LOX/RNO measurements were carried out in the presence of a constant volume (0.5 µL/mL) of Tween 80 in the assay mixture. The LOX/RNO reaction was measured both in the absence (control) and presence of carotenoid extract (or ±-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, Trolox, used as a standard antioxidant). The (%) decrease of the rate of RNO bleaching measured in the presence of extract (or Trolox) with respect to the rate of the control reaction was used to quantify AA. This was done by means of a dose-response curve derived for Trolox by plotting the (%) decrease of the rate of RNO bleaching as a function of standard antioxidant concentration. In particular, to calculate the rate of the LOX/RNO reaction in the presence of extract, the LOX-1-depedent bleaching of carotenoids was also monitored by measuring the absorbance decrease at 440 nm in the above reported assay mixture lacking RNO. Then, a new trace was built, representing the difference between the trace of the LOX/RNO reaction in the presence of carotenoid extract and that relative to carotenoid bleaching

(for details see Results).

The ORAC protocol, described in Ou *et al*. (2001) and modified as in Pastore *et al*. (2009) and Laus *et al*. (2012 b), was applied. Fluorescence intensity decay due to 3',6'-dihydroxyspiro[isobenzofuran-1[3*H*], 9'[9*H*]-xanthen]-3-one (fluorescein) oxidation by peroxyl radicals generated by 2,2'-azobis(2-amidinopropane) (AAPH) thermal decomposition was continuously monitored at 37°C at excitation and emission wavelengths of 485 and 515 nm, respectively. To quantify AA, the area under the fluorescence decay kinetic curve (area under curve, AUC) was used and, in particular, the net AUC (AUC_{net}), obtained by subtracting AUC of the blank from that of the sample. AA was calculated by means of a proper dose-response curve prepared with Trolox by plotting the AUC_{net} as a function of standard antioxidant concentration. Since carotenoid extracts reconstituted in ethanol were used, all ORAC measurements were carried out in the presence of a constant volume of ethanol in the assay mixture.

The TEAC protocol, reported in Re *et al*. (1999) and modified as in Pastore *et al*. (2009) and Laus *et al*. (2012 b), was used. The coloured radical cation $ABTS^{\bullet+}$ was produced by ABTS oxidation with potassium persulfate solution. Absorbance at 734 nm and 25°C (A_{734}) was measured after a fixed time of incubation of carotenoid extract (or Trolox) with the $ABTS^{\bullet+}$ solution (diluted in absolute ethanol). The (%) decrease of A_{734} measured after 3 min incubation of extract (or Trolox), with respect to A_{734} of the uninhibited radical cation solution, was calculated; AA was quantified by means of a proper concentration-response curve prepared with Trolox by plotting the (%) decrease of A_{734} as a function of standard antioxidant concentration.

For all three methods, determinations were carried out in triplicate by analyzing at least three different amounts of extract. A linear dependence of the inhibition on the amount of extract was verified by linear regression analysis of data. Then, AA was obtained by comparing the slope derived by linear regression analysis with that of the calibration curve prepared with Trolox.

Statistical analysis

Distribution of data from figure 4 was evaluated using the Shapiro-Wilk and Jarque-Bera tests, and homogeneity of variances was verified by the Bartlett's test. Figure 4 data were submitted to a "one-factor" analysis of variance (ANOVA) and the mean separation was tested by the Duncan's test at 0.01 *P* level of significance. Statistical analysis was performed using Statistica (data analysis software system), version 7 (StatSoft, Tulsa, Oklahoma, USA).

3. Results

Application of the LOX/RNO method to AA assessment of extracts showing high ß-carotene concentration is complicated both by the high absorbance of ß-carotene in the visible light region and by the ability of LOX to cause carotenoid bleaching. Thus, a preliminary investigation regarding this point was carried out.

In figure 2 the absorbance spectra of both RNO and β-carotene aqueous solutions are reported, as recorded in the visible region of the electromagnetic spectrum: a large overlapping of both spectra in the entire investigated visible region is clearly evident, as well as a high absorbance value of ß-carotene at 440 nm, representing the wavelength of the RNO absorption maximum. In light of these absorbance properties of carotenoids, the LOX-1-catalyzed RNO bleaching cannot be easily monitored at 440 nm.

To overcome these problems, in this study a new simple LOX/RNO protocol was developed for AA assessment of carotenoid-enriched extracts. Figure 3 shows evaluation according to the new protocol of the LOX-1-dependent RNO bleaching in the presence of carotenoids extracted from the yellow-fleshed peach fruits of cv. Armking. In particular, in figure 3A a typical experimental trace of the LOX/RNO control (in the absence of extract) reaction is reported: it was spectrophotometrically monitored by continuously measuring the RNO absorbance decrease at 440 nm and it consists of a lag phase (representing the time occurring to consume oxygen in the reaction mixture due to the primary LOX-1 reaction of linoleate hydroperoxidation), and a true RNO bleaching phase (due to the radical species generated by LOX-1 when anaerobiosis is approached in the assay mixture), occurring, in this experiment, at a rate equal to about 0.15 $\Delta A_{440\ nm} \cdot min^{-1}$. In figure 3B the trace relative to the RNO bleaching in the presence of 1.13 µg carotenoids from Armking (RNO *plus* carotenoid bleaching, trace a) is shown. In the same figure, the trace b is reported, representing the experimental curve relative to carotenoid bleaching reaction, obtained

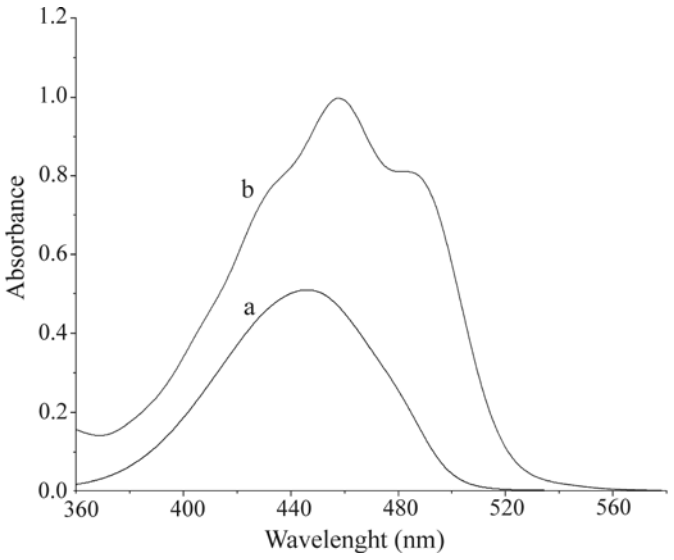

Fig. 2 - RNO (a) and ß-carotene (b) absorbance spectra. Aqueous solution of ß-carotene was prepared as reported in Methods. Absorbance spectra of 15 µM RNO (trace a) and 8 µM ß-carotene (trace b) were recorded in 2 mL of 100 mM sodium borate buffer pH 9.0.

by measuring the absorbance decrease at 440 nm in the absence of RNO: it clearly shows carotenoid absorbance decrease due to oxidation by radicals produced during secondary anaerobic reactions associated to the LOX-1-mediated linoleate hydroperoxidation (Barimalaa and Gordon, 1988; Pastore *et al.*, 2000 a). Moreover, the curve representing the difference between traces a and b, obtained by mathematical processing of these curves using a specific software, is also reported (trace indicated as "a *minus* b"), showing a rate equal to about 0.12 $\Delta A_{440\ nm} \cdot min^{-1}$. This newly built curve only reflects the effect of carotenoids on RNO bleaching, excluding any absorbance decrease due to LOX-1-dependent carotenoid bleaching; thus, its rate may be compared to that of the LOX/RNO control reaction. The comparison of the slopes of the trace "a *minus* b" and the control one shows that the amount of extract from cv. Armking containing 1.13 µg of carotenoids is able to induce an about 25% decrease of the rate of RNO bleaching. In the same experiment, an increasing inhibition of the reaction rate with increasing amount of Armking carotenoid extract, evaluated according to the same protocol, was also observed. In particular, a linear dependence between inhibition and amount of extract was found in the studied 0.75-2.25 µg carotenoid range (Fig. 3D). In figure 3C the Trolox-dependent inhibition of the rate of RNO bleaching as a function of the standard antioxidant concentration is reported, showing a linear dependence of the inhibition in the 20-50% range on Trolox concentration ranging from 2 to 7.5 mM, described by the equation: y(inhibition) = 6.059x(concentration millimolar of

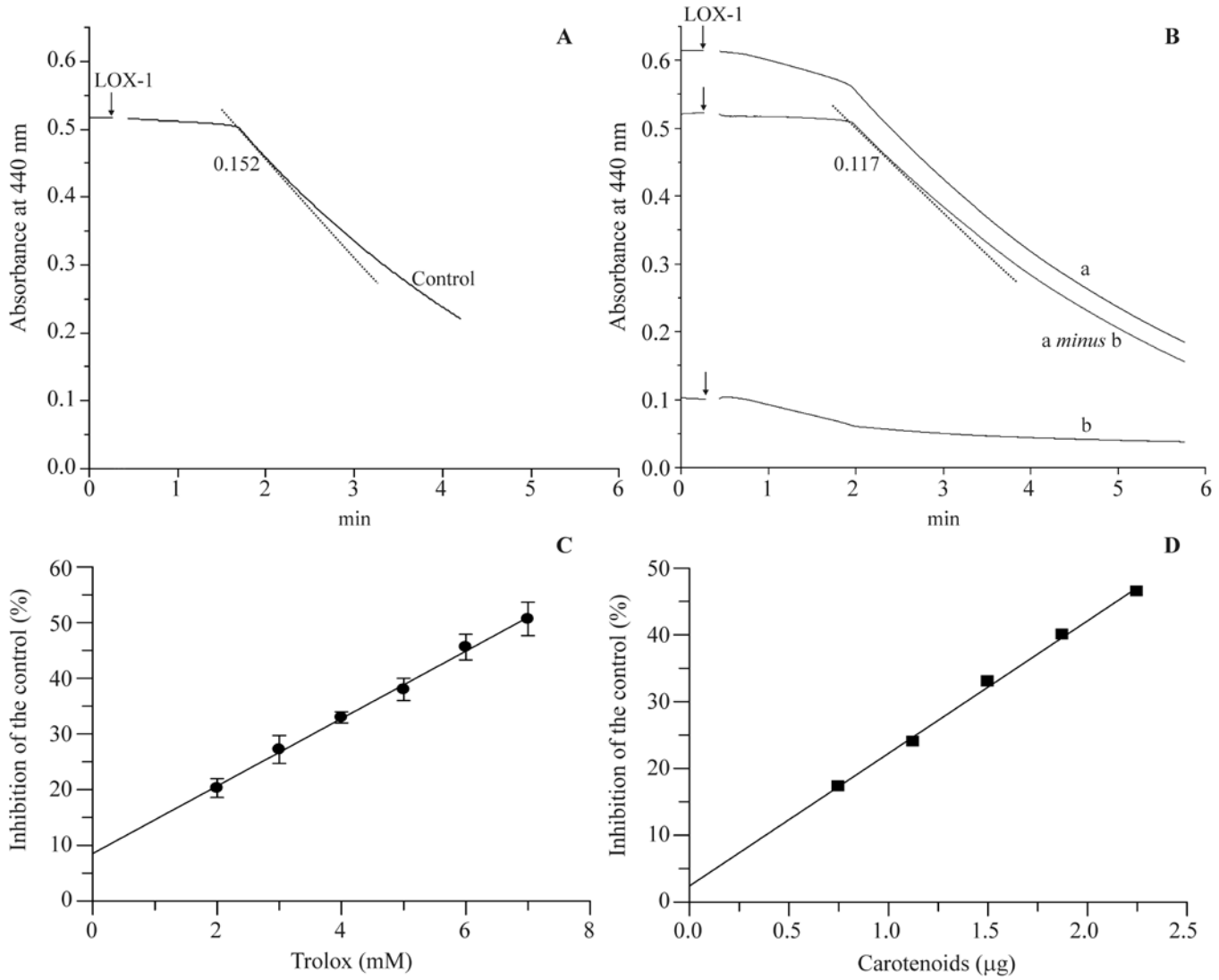

Fig. 3 - The LOX-1-dependent RNO bleaching in the absence (A, control) and presence (B) of carotenoid-enriched extract from peach fruit and linear dependence of the inhibition on Trolox concentration (C) and extract amount (D). The LOX/RNO reaction was monitored as described in Methods in the absence (A, control) and in the presence of 1.13 µg carotenoids extracted from the yellow-fleshed cv. Armking (B, trace a). In (B) the LOX-1-dependent bleaching of the same amount (1.13 µg) of carotenoid extract was also reported (trace b), as well as the curve obtained from the difference between traces a and b (trace "a *minus* b"). In (C) the calibration curve obtained with Trolox is reported; in (D) the (%) inhibition of the rate of the LOX/RNO reaction is reported as a function of extract amount, expressed as carotenoid content. The rates of the RNO bleaching, calculated as the highest slopes (dotted lines) to the experimental curves, are reported, expressed as $\Delta A_{440\ nm} \cdot min^{-1}$. For AA calculation see the text.

Trolox) + 8.505 (r=0.999, $P \leq 0.001$). By means of this Trolox-based calibration curve, the AA value for Armking extract was calculated, resulting, in this experiment, equal to 120±6 µmol eq. Trolox/g fresh weight. The new developed protocol was also applied to measure the AA of carotenoid extract obtained from the white-fleshed cv. Silverking. In this case, a linear dependence between inhibition and amount of extract was obtained in the studied 0.075-0.2 µg carotenoid range; the inhibition corresponded, in that experiment, to an AA value equal to 7.5±2 µmol eq. Trolox/g fresh weight. Interestingly, the ratio between AA values of the yellow-fleshed Armking and the white-fleshed Silverking extracts resulted equal to 16, very similar to the ratio (about 17) between carotenoid content of the extracts (13.5 and 0.8 µg/g fresh weight, respectively, in these experiments).

The study was extended to three other peach varieties, one yellow-fleshed (Redhaven) and two white-fleshed (Caldesi 2000 and IFF331). In Table 1, AA values of carotenoid extracts obtained from all tested genotypes, measured by means of the LOX/RNO, are reported and compared with those obtained by using the ORAC and TEAC assays. The LOX/RNO method provided AA values ranging from 10 (white-fleshed genotypes) to 150 µmol eq. Trolox/g fresh weight (yellow-fleshed genotypes). In Table 1 the carotenoid content of extracts is also reported for all genotypes under study, ranging from 0.25 (white-fleshed genotypes) to 18.5 µg/g fresh weight (yellow-fleshed genotypes). Interestingly, a highly statistically significant positive correlation was obtained between AA values provided by the LOX/RNO method and carotenoid content (r = 0.996, $P \leq 0.001$). AA values obtained by the TEAC assay also resulted significantly correlated to carotenoid content (r = 0.997, $P \leq 0.001$); a lower correlation was obtained for the ORAC protocol (r = 0.824, $P \leq 0.001$); high correlations were also obtained between AA values measured by the LOX/RNO method and that obtained using the other assays (LOX/RNO-TEAC r = 0.992, $P \leq 0.001$; LOX/RNO-ORAC r = 0.837, $P \leq 0.001$; ORAC-TEAC r = 0.839, $P \leq 0.001$).

Figure 4 presents the ratio between the yellow-fleshed cultivars and white-fleshed genotypes under study in terms of carotenoid content, as well as the ratio between AA mean values of yellow- and white-fleshed peach fruits, as evaluated by means of the LOX/RNO, ORAC and TEAC methods. The yellow-fleshed genotypes have a carotenoid content on average 36 times higher than that of white-fleshed varieties. Interestingly, the AA mean value obtained by the LOX/RNO method for the yellow-fleshed genotypes resulted about 14 times higher than those measured for the white-fleshed varieties. The same ratios $AA_{\text{yellow-fleshed fruits}}/AA_{\text{white-fleshed fruits}}$ were only about 2.6 and 3.6 in the case of the ORAC and TEAC assays, respectively.

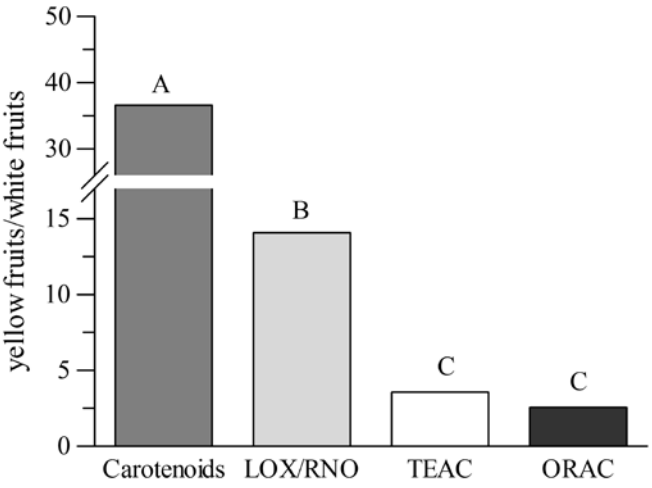

Fig. 4 - Ratio between yellow- and white-fleshed peach fruits in terms of carotenoid content and antioxidant activity, evaluated by means of the LOX/RNO, ORAC and TEAC methods. Data are reported as mean value (n=3 different experiments). Different capital letters indicate significant differences at 0.01 *P* level, according to the Duncan's test.

4. Discussion and Conclusions

In this study, the LOX/RNO method was applied for the first time to assess antioxidant properties of peach fruits, in

Table 1 - Antioxidant Activity (AA), evaluated by means of the LOX/RNO, ORAC and TEAC methods, and carotenoid content of extracts from yellow- and white-fleshed peach genotypes

Genotype	Carotenoid content (µg/g f.w.)	AA (µmol eq. Trolox/g fresh weight)		
		LOX/RNO	ORAC	TEAC
Yellow-fleshed				
Redhaven	18.33±0.75	155.3 ± 10.3	0.325 ± 0.042	0.082 ± 0.002
Armking	14.11±0.71	125.3 ± 9.5	0.444 ± 0.063	0.068 ± 0.001
White-fleshed				
Silverking	0.75 ± 0.08	9.6 ± 3.5	0.211 ± 0.039	0.025 ± 0.001
Caldesi 2000	0.34 ± 0.02	10.5 ± 1.2	0.112 ± 0.007	0.018 ± 0.0001
IFF331	0.24 ± 0.01	9.8 ± 1.5	0.129 ± 0.006	0.020 ± 0.0001

All data refer to fresh weight and are reported as mean value ± standard deviation (n=3 different experiments).

comparison with the widely used ORAC and TEAC assays. In particular, the carotenoid component was studied by comparing some yellow- and white-fleshed genotypes. Unfortunately, application of the LOX/RNO method to AA determination of carotenoid extracts from peach fruits is strongly affected by absorbance properties of carotenoids, whose absorbance spectra largely overlap the one of RNO. Moreover, it should be considered that carotenoids are also subjected to absorbance bleaching due to their co-oxidation associated to the LOX-mediated hydroperoxidation of polyunsaturated fatty acids (Barimalaa and Gordon, 1988; Pastore *et al*., 2000 a). In a previous study, in order to evaluate the effect of ß-carotene on the LOX/RNO reaction, these problems were overcome by measuring the RNO absorbance changes as absorbance difference ($\Delta_{394nm-512nm}$) using a double-wavelength spectrophotometer, an expensive and not widespread laboratory instrumentation (Pastore *et al*., 2009). Interestingly, using that experimental approach, a very high sensitivity to ß-carotene of the LOX/RNO reaction was found, with an IC_{50} value in the micromolar range (Pastore *et al*., 2009), consistent with the inhibition by ß-carotene of LOXes from different sources (Lomnitski *et al*., 1993).

In this paper, to evaluate antioxidant properties of carotenoids by means of the LOX/RNO reaction, a new experimental approach was developed involving the use of a simple spectrophotometer, which is generally found in research laboratories. This is a multi-step protocol involving the following spectrophotometric measurements: *i*) the LOX-1-depedent RNO bleaching in the absence of carotenoid-enriched extract (control reaction); *ii*) the RNO bleaching in the presence of carotenoids (RNO *plus* carotenoid bleaching reaction); *iii*) the carotenoid bleaching (at 440 nm) in the absence of RNO (carotenoid bleaching reaction); *iv*) mathematical processing by specific software of the traces obtained at points *ii*) and *iii*) so as to build a new curve in which the absorbance value at each time is represented by the difference between the respective values of the traces *ii*) and *iii*). Excluding absorbance decrease due to LOX-1-dependent carotenoid bleaching, the rate of the new curve reflects only the effect of carotenoids on RNO bleaching, making it possible to compare the rate of the LOX/RNO control reaction (point *i*), and allowing calculation of the (%) decrease of rate of RNO bleaching and, finally, AA quantification by means of a dose-response curve prepared with Trolox.

To validate the newly developed protocol, measurement of the AA of carotenoid extract obtained from the yellow-fleshed peach fruits of cv. Armking was undertaken, as well as from the white-fleshed cv. Silverking. In both cases, the inhibition of the reaction rate was found to be linearly dependent on the amount of carotenoid extract; the inhibitions measured in these experiments allowed calculation of an AA value of the yellow-fleshed Armking fruits about 15-fold higher than that obtained for the white-fleshed Silverking extract. Interestingly, extract from Armking fruits showed a carotenoid content about 16-fold higher than that measured for Silverking. These data confirm the suitability of the modified LOX/RNO protocol to measure AA of carotenoid compounds.

The study was extended to carotenoid extracts obtained from other yellow- and white-fleshed peach varieties. As already observed in previous studies (Pastore *et al*., 2009; Laus *et al*., 2012 a, b; Laus *et al*., 2013 a, b), the method LOX/RNO provided very high AA values (10-150 µmol eq. Trolox/g fresh weight) which were much higher than those measured by ORAC and TEAC assays (about 85-350-fold and 530-1900-fold higher, respectively) and they showed a statistically significant positive correlation with carotenoid content, higher than that obtained with the other assays in comparison.

Moreover, the ratio between AA values measured by the LOX/RNO method of yellow- and white-fleshed peaches resulted much higher than that obtained using the ORAC and TEAC assays, thus indicating the capability of the newly developed LOX/RNO protocol to highlight much higher differences among the different tested samples with respect to the other compared AA assays.

On the whole, the results of this paper indicate that the LOX/RNO method, applied according to the new protocol developed in this study, is able to measure high AA values of carotenoid-enriched extracts from peach fruits, highly related to carotenoid content, and to easily discriminate among samples. Although TEAC and ORAC values also show correlation with carotenoid content, they measure low AA and few differences among yellow- and white-fleshed varieties. In conclusion, the LOX/RNO method may represent a recommended tool to assess AA of the carotenoid component in peach fruits. Therefore, the use of this assay in studies regarding AA measurements of other fruits and vegetables is worthwhile.

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‘Conference’ and ‘Abbé Fétel’ pears treated with 1-methylcyclopropene: physiological and quality implications of initial low oxygen stress and controlled atmosphere storage

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Key words: α-farnesene, ethylene production, fermentative metabolites, pulp mechanical properties, sensory profiles, storage disorders.

Abstract: Superficial scald is a disorder developed in cold storage by ‘Conference’ and ‘Abbé Fétel’ pears and it has been related to the presence of oxidation products, mainly conjugated trienols (CTols), of which α-farnesene is primary, acting on epidermal cells. Among tested postharvest methods to control scald, there is treatment at harvest with 1-methylcyclopropene (1-MCP) and initial low oxygen stress (ILOS). The investigation presented here studied, in ‘Conference’ and ‘Abbé Fétel’ pears treated with 1-MCP (300 µL L^{-1}), the physiolological and quality implications of storage in controlled atmosphere (CA, 2 kPa O_2 + 0.7 kPa CO_2, -0.5°C) after two 2-weeks ILOS (0.3-0.5 kPa O_2) periods at three-week intervals after 13 and 21 weeks of storage and shelf life at 20°C up to seven days. Results showed that 1-MCP treatment severely reduced α-farnesene, $CTol_{269}$, $CTol_{281}$ and ethanol after ILOS treatment in both cultivars, and ethyl acetate in ‘Abbé Fétel’ pears. Furthermore, it impaired fruit softening, delayed skin yellowing and reduced ethylene production in shelf life. At sensory analyses, 1-MCP treated ‘Conference’ and ‘Abbé Fétel’ pears were described as being firmer and less juicy, sweet and aromatic than untreated fruit. 1-MCP treated pears did not develop superficial scald and soft scald in ‘Abbé Fétel’, nor superficial scald and black speck after 21 weeks of storage in ‘Conference’.

1. Introduction

‘Abbé Fétel’ is the most important pear cultivar in Italy in terms of production (CONERPO, 2010) and it can be stored in normal air (NA) for three to four months and in controlled atmosphere (CA) for up to six months (Bai *et al.*, 2009). However, when stored in NA for more than four months ‘Abbé Fétel’ pears became sensitive to superficial scald (Vanoli *et al.*, 2008). ‘Conference’ pears in Italy are often subjected to superficial scald in cold storage, and it has been reported that under predisposing climatic conditions, up to 70% of fruit developed scald, thus impairing their marketability (Folchi and Bertolini, 2008).

Scald is manifested as brown or black patches on the skin; it can take several forms and, along with superficial scald, it is an expression of damage and/or death within the surface layers of cells (Lurie and Watkins, 2012; Whitaker, 2013). Scald has been related to the presence of oxidation products [conjugated trienols (CTols), primarily α-farnesene], acting on epidermal cells (Whitaker, 2007) and could be prevented or controlled by storage in CA (Bertolini *et al.*, 1997; Lurie and Watkins, 2012). However, the low levels of oxygen used in CA for ‘Abbé Fétel’ pears can induce soft scald (Bertolini *et al.*, 2002; Rizzolo *et al.*, 2010; Vanoli *et al.*, 2010 a).

Up to now, the traditional strategy to prevent superficial scald in pears is a pre-storage treatment with ethoxyquin, which recently has been excluded from the list of active ingredients of chemicals used in food production (EC Council Directive 91/414) (Calvo and Kupferman, 2012). The most effective alternatives to ethoxyquin are treatment at harvest with 1-methylcyclopropene (1-MCP) or storage under controlled atmosphere with low levels of O_2 such as ultra-low oxygen, initial low oxygen stress, and dynamic controlled atmosphere (Calvo and Kupferman, 2012; Lurie and Watkins, 2012).

It was found that 1-MCP inhibited superficial scald and prevented or controlled soft scald and internal breakdown in ‘Bartlett’ pears (Villabolos-Acuña *et al.*, 2011 a, b). In ‘Conference’ doses ranging from 50 to 1000 µL L^{-1} did not prevent the formation of superficial scald, both in CA and in NA, but either controlled it, keeping the incidence of scald within commercially acceptable rates, or reduced symptom severity (Eccher Zerbini *et al.*, 2003; Rizzolo

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et al., 2005; Folchi and Bertolini, 2008). The reduction of symptom severity in 'Conference' fruit was related to lower amounts of α-farnesene and CTols (Rizzolo *et al.*, 2005; Folchi and Bertolini, 2008). In 'Abbé Fétel' pears, after shelf life, CTols and α-farnesene were significantly higher in fruit affected by superficial scald and lower in those affected by senescent scald (Vanoli *et al.*, 2010 b).

Storage under controlled atmosphere with a low level of O_2 was suggested as an alternative to ethoxyquin and to 1-MCP treatment not only in controlling scald development but also in improving overall fruit quality (Prange *et al.*, 2011; Lurie and Watkins, 2012). Widespread adoption of low O_2 regimes has not taken place due to concerns of anaerobic damage when fruit is held below the lower oxygen limit (LOL), that is the environmental O_2 level at which cell metabolism changes from being predominantly aerobic to fermentative (Wright *et al.*, 2012). Zerbini and Grassi (2010) and Rizzolo *et al.* (2008, 2010) found that LOL for 'Conference' pears was 0.4 kPa O_2 and for 'Abbé Fétel' pears 0.6 kPa O_2. Vanoli *et al.* (2008, 2010 a) and Rizzolo *et al.* (2010) found that storage of 'Abbé Fétel' pears at 0.7 kPa O_2 at -0.5°C completely prevented superficial scald development and reduced soft scald incidence compared to 2 kPa O_2, but increased internal browning and internal breakdown. As for the initial low oxygen stress, Wang and Dilley (2000) found that an ILOS with 0.25 kPa O_2 for two weeks, carried out one or two times at two-month intervals, strongly inhibited α-farnesene and its volatile oxidation product (6-methyl-5-hepten-2-one), increased ethanol, and was effective in controlling scald development in several apple cultivars. As for pears, Calvo *et al.* (2002) found that ILOS (0.5 kPa O_2) followed by low oxygen CA (1.5 kPa O_2) significantly inhibited the development of superficial scald after nine months of storage in 'Beurré d'Anjou' cultivar, while Rizzolo *et al.* (2015) reported that in 'Conference' pears after an ILOS (0.2-0.5 kPa O_2) period followed by low oxygen CA (2 kPa O_2) there were lower amounts of α-farnesene, $CTol_{258}$ and acetaldehyde, and higher quantities of ethanol than after CA and NA storage, developing less scald than the other atmospheres.

The objective of the present research was to evaluate in 'Conference' and 'Abbé Fétel' pears the effect of 1-MCP application on fruit stored in a low oxygen CA after two two-week ILOS periods at three-week intervals. Physiological aspects (fermentative metabolites, conjugated trienols) in storage, and ethylene production, quality and sensory characteristic changes with post-storage shelf life and storage disorders are discussed.

2. Materials and Methods

The experiment was carried out in 2012 on 'Conference' and 'Abbé Fétel' pears (*Pyrus communis* L.) (about 1000 fruit/cv) harvested from commercial orchards in the Modena province (Italy) on 20 August and 10 September, respectively, at a commercial degree of maturity [mean ± standard error: 'Abbé Fétel': firmness, 62.5±1.3 N; hue, 104±0.6°; starch hydrolysis, 4.0 ± 0.5 (EUROFRU 1-10 scale); 'Conference': firmness, 71.0±1.6 N; hue, 108.9±0 .5°; starch hydrolysis, 3.0 ± 0.1 (EUROFRU 1-10 scale)] and randomized in 14 boxes. For each cultivar, on the day after harvest, half of the fruits were treated with 300 μL L^{-1} 1-MCP (Smartfresh™, AgroFresh Inc., Rohm and Haas, Spring House, PA, USA) and seven boxes of untreated fruit were used as control. 'Conference' fruits were then put in NA at -0.5°C for four weeks before the beginning of ILOS periods and CA storage, while for 'Abbé Fétel' pears the ILOS experiment began two days after the 1-MCP treatment. For both control and 1-MCP treated 'Conference' and 'Abbé Fétel' pears, two ILOS periods at 0.3-0.5 kPa O_2 for about two weeks were applied with a three-week interval in CA at 2 kPa O_2 + 0.7 kPa CO_2 at -0.5°C. Four containers were used, each one dedicated to one sample (1-MCP dose and cultivar); the gas composition of each container was controlled and checked with centralized analyzers, supervised by a specific Fruit Control Equipment software; fluorescence FIRM™ sensors monitoring (HarvestWatch™, Satlantic, Canada) was carried out in each container from the beginning of the first ILOS period till the first storage time (13 weeks). The first ILOS period was applied from d0 to d17, and the second one from d40 to d53. Then pears were stored in CA at -0.5°C in 2 kPa O_2 + 0.7 kPa CO_2 up to 21 weeks.

Samplings

α-farnesene, CTols and fermentative metabolites were analyzed (6 fruits/1-MCP dose/cultivar) at the beginning and at the end of the second ILOS period, and at the first storage time (13 weeks); hereafter these samplings are referred as d0, d13 and d40.

After 13 and 21 weeks of storage, 3 boxes/1-MCP dose/cultivar were put in shelf life at 20 °C up to 7 days. At 1, 5 and 7 days of shelf life (d1, d5, d7) 20 fruit/1-MCP dose/cultivar were analyzed for background skin color and pulp mechanical properties (firmness, stiffness and energy-to-rupture). Ethylene production was measured at d1, d5 and d7 on ten fruits of sample d7, while sensory analyses were carried out on ten fruits at d5 and d7. After 7 days at 20 °C the incidence of storage disorders was evaluated on 3 boxes/1-MCP dose/cultivar.

α-Farnesene and CTols

α-Farnesene and CTols ($CTol_{258}$, $CTol_{269}$, $CTol_{281}$) were determined in the skin according to Zoffoli *et al.* (1998), by sampling eight skin disks of 0.8 cm^2 area from the equatorial region of two pears (three replications) and extracting overnight at 2°C with 6 mL of HPLC-grade hexane with 1 g of anhydrous Na_2SO_4. The absorbance of the extracts at 232, 258, 269, 281 and 290 nm was measured using a Jasco (model 7800) spectrophotometer. Concentrations of α-farnesene and CTols were calculated according to Huelin and Coggiola (1970) and Du and Bramlage (1993). Data were expressed as nmol cm^{-2}.

Fermentative metabolites

Fermentative metabolites (ethanol, acetaldehyde and ethyl acetate) were determined on the pulp of the same fruit analyzed for α-farnesene and CTols by means of HS-SPME-GC, by pooling the six fruits of each sample. Ten grams of homogenized pulp (three replications) were put into 25 mL vials tightly closed with an aluminum cap with a silicone-Teflon rubber septum; samples were then immediately frozen and kept at -20°C until analysis. After 60 min thawing at room temperature, the SPME headspace volatile sampling was carried out for 30 min at 40°C using a 50/30μm DVB-CAR-PDMS fiber (Supelco), which was desorbed for 5 min in the GC injector port at 250°C. Fermentative metabolites were separated on a Supelcowax-10 column (60 m ×0.25 mm I.D., 0.25 μm film thickness) using the following conditions: carrier gas, helium at a flow of 1.5 mL min^{-1}; temperature program, 40°C×13 min, 15°C min^{-1} to 185°C; FID temperature, 250°C. Fermentative metabolites were quantified by relating the peak area of each one to that of external standards.

Background skin color

Background skin color was measured on the greener side of fruit with a Spectrophotometer CM-2600d (Minolta Co, Japan) using the primary illuminant D65 and 10° observer in the L*, a*, b* color space. From a* and b* values, hue (H°) and chroma (C*) were computed according to $H°=\arctan(b^* a^{*-1})$ and $C^*= (a^{*2} + b^{*2})^{-2}$.

Pulp mechanical properties

The mechanical properties of pear tissue of each fruit were measured on two opposite peeled areas in the equatorial region of the pear using an 8 mm diameter plunger mounted on an Instron Universal Testing Machine (model 4301, Instron Ltd, Great Britain) with crosshead speed at 200 mm min^{-1}. From the force-displacement curve the following pulp mechanical properties were measured (Rizzolo *et al*., 2014): firmness (N), stiffness (N mm^{-1}) and energy-to-rupture (mJ). Firmness, stiffness and energy-to-rupture readings were averaged for each fruit.

Ethylene production rate

The ethylene production rate (EP) was measured by static HS/GC on fruit put in 1.7 L gas-tight glass jars (ten replications, one fruit per jar) for 2 h at 20 °C according to Rizzolo *et al*. (2005). One milliliter of the headspace gas was sampled and analyzed using a deactivated aluminum oxide F1 (80-100 mesh) column (1/8 in 200 cm) at a column temperature of 100°C and FID detection. Quantitative data were obtained by relating the ethylene peak area to that of a 10 μL L^{-1} standard and were expressed as pmol kg^{-1} s^{-1}.

Sensory analysis

Sensory analyses were carried out in a sensory lab using a panel of ten short-term trained judges at d5 and d7 of shelf life at 20°C. For both the cultivars in each session, one peeled slice/1-MCP dose was presented to each panelist. At the beginning of the session, a slice of a fruit not included in the experimental plan was tasted to eliminate the first tasting effect. Drinking water was provided as a palate cleaner between samples. Each sample was evaluated for the intensity of attributes related to fruit structure (firm, juicy, grainy) and taste and flavor (sweet, sour, aromatic, bitter, astringent) using 120 mm unstructured line scales with anchors at 12 mm from the extremes (low, high). In addition, in order to have a rough idea of sample pleasantness, at the end of the tasting session, panelists were also asked to score samples for overall acceptability using a 120 mm unstructured line scale with "low" and "high" anchors near the extremes. Details on panel training and attributes are reported by Rizzolo *et al.* (2014).

Storage disorders

Storage disorders were evaluated on three boxes/1−MCP dose/cultivar. For each box the percentages of healthy fruit and of fruit affected by superficial scald, rots (both cultivars), soft scald ('Abbé Fétel'), early blackening, black speck and black spot ('Conference') were computed.

Statistical analysis

Data were submitted to analysis of variance (Statgraphics ver.7, Manugistic Inc., Rockville, MD, USA). Prior to statistical analysis the rating scores of each sensory attribute were standardized by panelists in order to remove the variability due to their using different parts of the scale (Bianchi *et al*., 2009). Percentage data were submitted to angular transformation before ANOVA.

3. Results

Oxygen levels and chlorophyll fluorescence

Figure 1 shows the oxygen partial pressure and the corresponding response of chlorophyll fluorescence (Fα) for control and 1-MCP treated 'Abbé Fétel' and 'Conference' pears from the beginning of the first ILOS period till the first storage time (13 weeks). By comparing the graphs of O_2 partial pressure of containers of control and 1-MCP treated 'Conference' pears, it is evident that during the first ILOS period the O_2 values ranged from about 0.2 to 0.6 kPa and during the second ILOS from 0.15 to 0.55 kPa. The two ILOS periods induced a remarkable rise in chlorophyll fluorescence (Fα) in untreated fruit and much smaller ones in 1-MCP treated pears, when O_2 concentration decreased below 0.4 kPa. In control 'Abbé Fétel' pears the O_2 concentration decreased below 0.6 kPa only for a short time during the first ILOS period, while during the second ILOS period O_2 values ranged from 0.3 to 0.6 kPa, causing a very slight rise in Fα at the end of the second ILOS period. On the other hand, in the container with 1-MCP treated 'Abbé Fétel' pears O_2 values ranged from 0.1-0.4 kPa during the two ILOS periods, causing a slight rise in Fα in coincidence with the two ILOS periods.

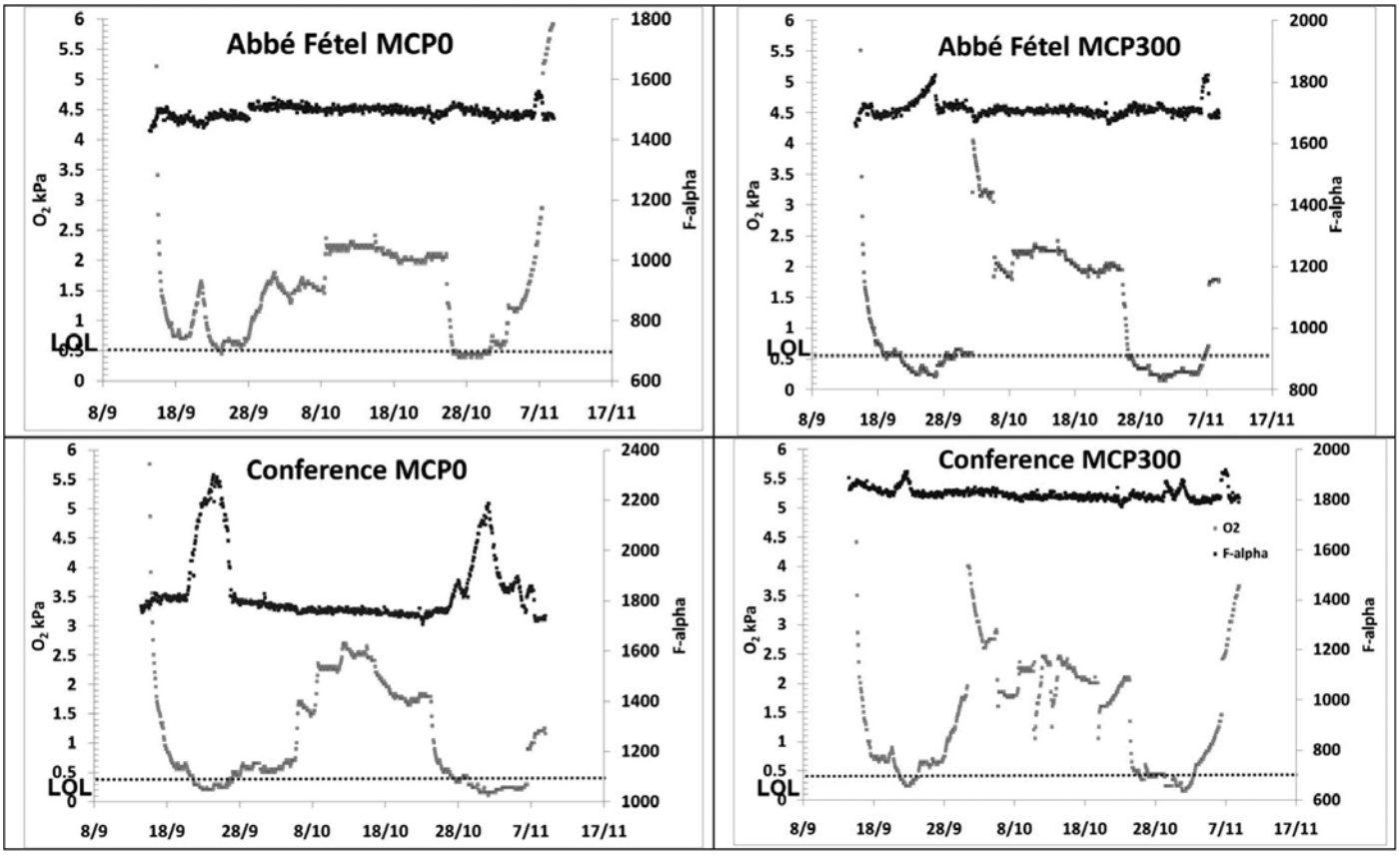

Fig. 1 - Oxygen partial pressure (gray) and corresponding response of chlorophyll fluorescence Fα (black) for control and 1-MCP treated containers of 'Abbé Fétel' and 'Conference' fruit. The dotted lines indicate the O_2 partial pressure inducing stress evidenced by Fα increase ('Abbé Fétel' 0.6 kPa; 'Conference' 0.4 kPa)

α-Farnesene and conjugated trienols

On average 1-MCP treated 'Conference' pears had lower amounts of α-farnesene, $CTol_{269}$ and $CTol_{281}$ than control fruit, and $CTol_{281}$ at the end of the ILOS period (d13) was not detectable in 1-MCP treated fruit (Table 1). In control fruit α-farnesene, $CTol_{269}$ and $CTol_{281}$ significantly increased at d40, in correspondence wth the first storage time. Likewise, 1-MCP treated 'Abbé Fétel' pears

Table 1 - Amounts (mean ± standard error) of α-farnesene (α-FARN), CTols ($CTol_{258}$, $CTol_{269}$, $CTol_{281}$) and fermentative metabolites (ACE, acetaldehyde; EtOH, ethanol; EtAc, ethyl acetate) in control (MCP0) and 1-MCP treated (MCP300) 'Conference' pears at the beginning (d0) and at the end (d13) of the second ILOS period and in correspondence with the first storage time (d40) and ANOVA results

	α-FARN nmol cm^{-2}	$CTol_{258}$ nmol cm^{-2}	$CTol_{269}$ nmol cm^{-2}	$CTol_{281}$ nmol cm^{-2}	ACE µg kg^{-1}	EtOH µg kg^{-1}	Et Ac µg kg^{-1}
	MCP0						
d0	11.32±2.97	0.97±0.24	1.16±0.18	0.21±0.08	27.33±5.38	38.77±13.85	0.10±0.05
d13	9.26±2.81	0.80±0.27	0.90±0.22	0.60±0.33	29.15±3.23	69.45±3.71	0.14±0.09
d40	23.04±8.69	1.80±0.60	2.32±0.58	0.91±0.33	35.34±0.29	24.71±1.13	1.77±1.52
	MCP300						
d0	3.77±0.28	1.01±0.15	1.08±0.12	0.14±0.04	40.26±4.42	55.81±7.39	0.06±0.03
d13	2.96±0.31	0.62±0.15	0.68±0.16	nd	17.31±8.67	21.30±14.83	0.05±0.04
d40	3.90±0.37	0.77±0.15	0.93±0.14	0.04±0.02	33.89±3.82	23.01±2.66	0.87±0.45
	ANOVA [z]						
A: day	NS	NS	*	*	NS	*	NS
B: 1-MCP	**	NS	*	**	NS	NS	NS
A × B	NS	NS	NS	*	NS	**	NS

[z] ***, P<0.001; **, P<0.01; *, P<0.05; NS, not significant.

Table 2 - Amounts (mean ± standard error) of α-farnesene (α-FARN), CTols ($CTol_{258}$, $CTol_{269}$, $CTol_{281}$) and fermentative metabolites (ACE, acetaldehyde; EtOH, ethanol; EtAc, ethyl acetate) in control (MCP0) and 1-MCP treated (MCP300) 'Abbé Fétel' pears at the beginning (d0) and at the end (d13) of the second ILOS period and in correspondence with the first storage time (d40) and ANOVA results

	α-FARN nmol cm^{-2}	$CTol_{258}$ nmol cm^{-2}	$CTol_{269}$ nmol cm^{-2}	$CTol_{281}$ nmol cm^{-2}	ACE μg kg^{-1}	EtOH μg kg^{-1}	Et Ac μg kg^{-1}
	MCP0						
d0	14.80±4.65	0.83±0.14	0.70±0.14	0.42±0.10	10.38±0.31	14.86±5.18	0.92±0.07
d13	15.10±1.41	0.70±0.05	0.63±0.02	0.38±0.01	16.40±3.56	31.10±6.65	0.08±0.06
d40	55.14±9.81	3.83±0.53	6.41±0.91	5.14±0.79	26.78±6.56	39.62±15.98	0.06±0.01
	MCP300						
d0	2.51±0.08	0.43±0.05	0.34±0.06	0.18±0.03	14.03±2.83	17.89±5.11	0.51±0.03
d13	2.69±0.06	0.47±0.01	0.34±0.01	0.18±0.01	16.21±2.10	34.94±5.34	0.09±0.05
d40	10.91±1.94	1.01±0.30	1.01±0.32	0.62±0.17	27.33±3.04	48.99±11.30	0.04±0.02
	ANOVA [z]						
A: day	***	***	***	***	***	*	***
B: 1-MCP	***	***	***	***	NS	NS	**
AxB	**	***	***	NS	NS	NS	***

[z] ***, P<0.001; **, P<0.01; *, P<0.05; NS, not significant.

had lower amounts of α-farnesene, $CTol_{258}$, $CTol_{269}$ and $CTol_{281}$ than control fruit (Table 2). The α-farnesene and CTols concentrations did not significantly change from the beginning (t0) to the end of the second ILOS period (d13) both in control and 1-MCP treated 'Abbé Fétel' fruit, and then they increased at d40. The concentration of α-farnesene in pear skin was three to five times higher in control than in 1-MCP treated fruit, those of $CTol_{258}$ and $CTol_{269}$ two ('Conference') to four-six times ('Abbé Fétel'), and that of $CTol_{281}$ two to eight times in 'Abbé Fétel' and fifteen to twenty-three times in 'Conference'.

In both cultivars the ratios $CTol_{258}/CTol_{281}$ and $CTol_{269}/CTol_{281}$ (Fig. 2) were higher in 1-MCP treated fruit than in control ones, being, on average, three times higher in 'Conference' pears, and about 50% higher in 'Abbé Fétel' treated fruit. In 'Conference' the highest values for both ratios were observed in 1–MCP treated fruit at d 40, while in 'Abbé Fétel' in 1–MCP treated fruit at d0 and d13. Control 'Abbé Fétel' and 'Conference' pears at d40 showed the lowest values for both the ratios.

Fermentative metabolites

In 'Conference' pears the 1-MCP treatment at harvest did not influence the amounts of fermentative metabolites (Table 1). In control 'Conference' pears ethanol increased with the second ILOS period, then it significantly decreased

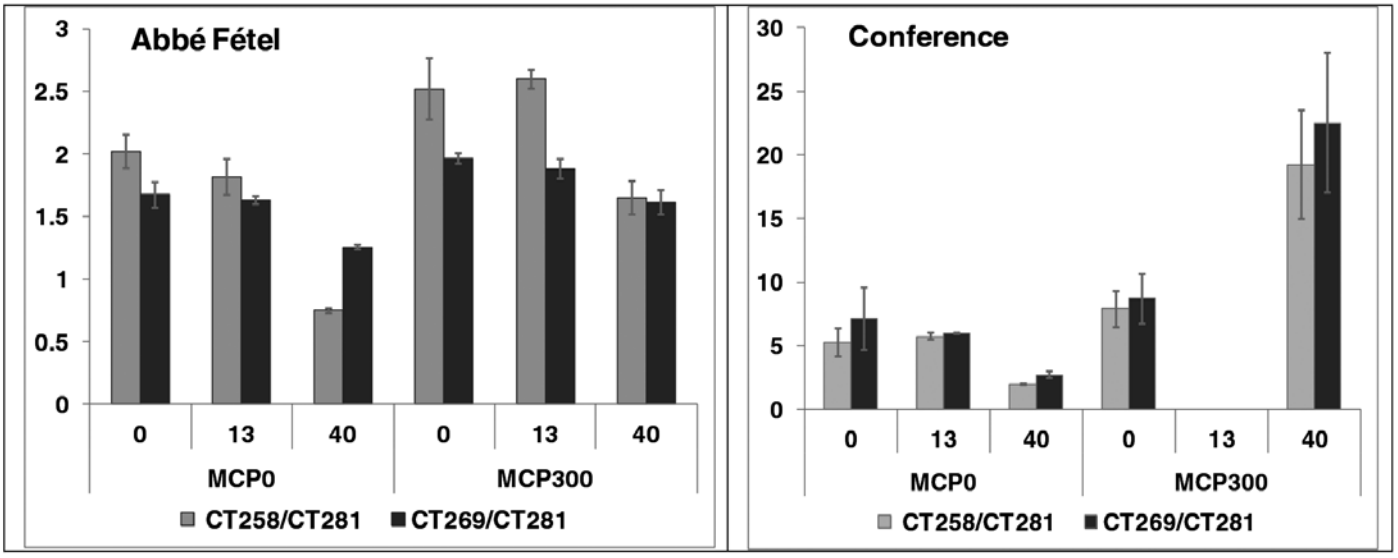

Fig. 2 - $CTol_{258}/CTol_{281}$ and $CTol_{269}/CTol_{281}$ ratios of control (MCP0) and 1–MCP treated (MCP300) 'Abbé Fétel' and 'Conference' pears at the beginning (d0) and the end (d13) of the second ILOS period and in correspondence with the first storage time (d40). Bars refer to standard error of the mean.

at d40 (i.e. 13 weeks storage time), while in 1-MCP treated fruit it decreased with the second ILOS period and did not change further at d40. The sampling time had no significant influence on acetaldehyde and ethyl acetate amounts both in control and 1-MCP treated fruit, probably due to the high standard errors of data, especially for ethyl acetate.

The 1-MCP treated 'Abbé Fétel' pears (Table 2) had, on average, lower ethyl acetate amounts at d0 than control fruit. Ethyl acetate both in control and 1-MCP treated pears decreased steeply with the ILOS period, and afterwards did not change at d 40. Both in control and 1-MCP treated 'Abbé Fétel' fruit acetaldehyde concentration did not change from the beginning (d0) to the end of the second ILOS period (d13), and then increased at d40, while ethanol increased throughout the sampling time.

Ethylene production

Ethylene production in control 'Conference' pears was lower than in 'Abbè Fétel' (Fig. 3). In 'Conference' pears EP was significantly affected only by the 1-MCP treatment (Table 3), whereas in 'Abbé Fétel' fruit both 1-MCP treatment and post storage shelf life significantly influenced EP (Table 3). In both cultivars the 1-MCP treatment reduced EP to values lower than 10 pmol kg^{-1} s^{-1}. In 'Abbé Fétel' control and 1-MCP treated fruit after 13 weeks storage showed a decreasing EP with shelf life, and after 21 weeks a minimum EP at d5.

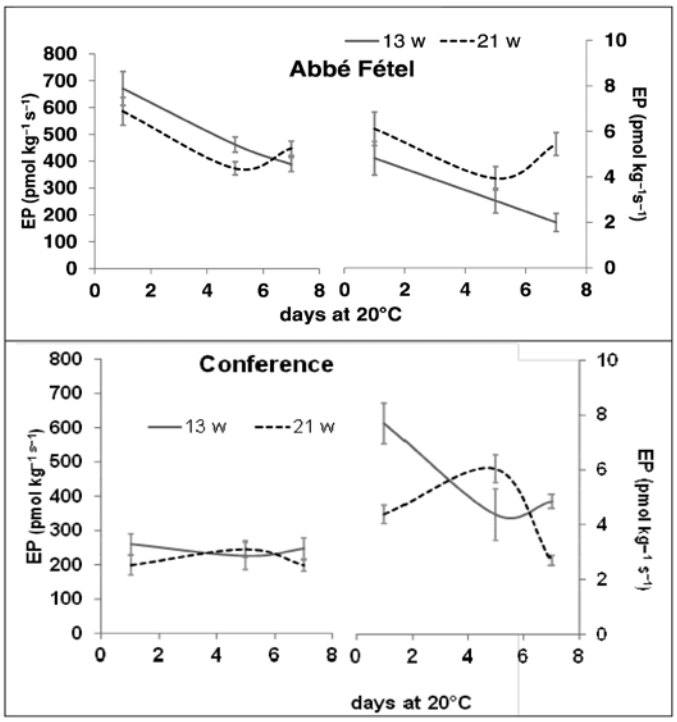

Fig. 3 - Ethylene production rate (EP) of control (left) and 1–MCP treated (right) 'Abbé Fétel' and 'Conference' pears during shelf life at 20°C after 13 and 21 weeks of storage. Bars refer to standard error of the mean. Results of ANOVA analysis are reported in Table 3.

Pulp mechanical characteristics

Upon removal, 1-MCP treated 'Conference' and 'Abbé Fétel' pears maintained firmness similar to that at harvest ('Conference': 71.0±1.6 N; 'Abbé Fétel': 62.5±1.3 N), but lower stiffness and higher energy-to-rupture than at harvest (values at harvest: stiffness: 'Conference', 21.0±0.5 N mm^{-1}; 'Abbé Fétel', 24.5±0.6 N mm^{-1}; energy-to-rupture: 'Conference', 0.106±0.04 J; 'Abbé Fétel', 0.074±0.003 J). The same scenario was found for control 'Conference' pears, whereas control 'Abbé Fétel' fruit had lower firmness and energy-to-rupture than at harvest.

On average, 1-MCP treated 'Conference' pears had higher firmness, stiffness and energy-to-rupture than control fruit, without any difference between storage times; firmness and energy-to-rupture did not change with shelf life, whereas stiffness decreased, but to a lesser extent than control fruit (Fig. 4). In control 'Conference' pears the values of all the mechanical properties decreased with shelf life with the main changes at d5.

As for 'Abbé Fétel' pears (Fig. 4), on average, 1-MCP treated fruit showed higher firmness, stiffness and energy-to-rupture than control fruits, without any difference between the storage times, except for a higher energy-to-rupture of 1-MCP treated fruit after 21 weeks of storage.

Table 3 - Multifactor ANOVA results for pulp mechanical characteristics (firmness, *F*, stiffness, *St* and energy-to-rupture, E_f), color parameters (lightness, L*; chroma, C* and hue) and ethylene production rate (EP) for 'Abbé Fétel' and 'Conference' pears

	Abbé Fétel							Conference						
	EP	*F*	*St*	E_f	L*	C*	Hue	EP	*F*	*St*	E_f	L*	C*	Hue
A: storage time	NS	NS	NS	***	NS	**	***	NS	NS	NS	NS	***	***	***
B: 1-MCP	***	***	***	***	***	***	***	***	***	***	***	***	***	***
C: shelf life	***	***	***	***	***	***	***	NS	***	***	***	***	***	***
A×B	NS	NS	*	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	*
A×C	NS	*	NS	*	NS	NS	NS	NS	*	NS	**	NS	NS	NS
B×C	***	***	***	***	NS	NS	*	NS	***	***	***	**	*	*
A×B×C	NS	**	**	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

***, P<0.001; **, P<0.01; *, P<0.05; NS, not significant)

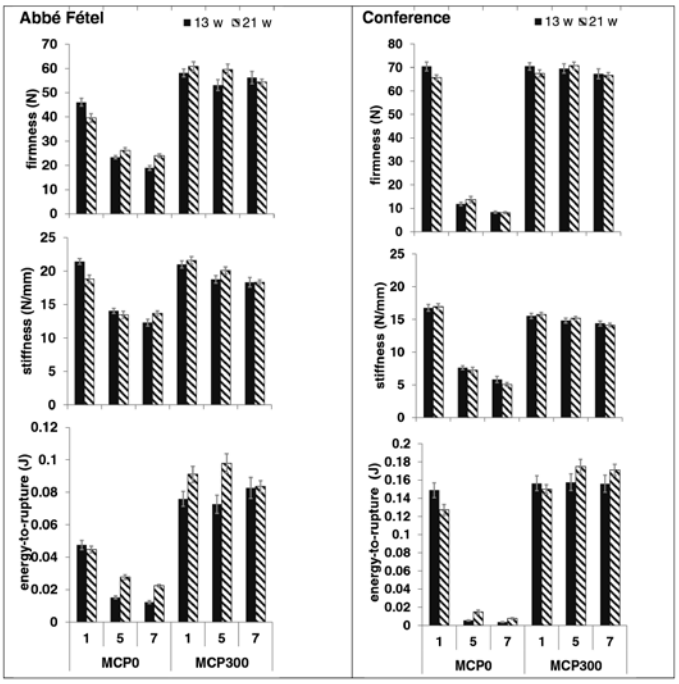

Fig. 4 - Firmness, stiffness and energy-to-rupture of control (MCP0) and 1-MCP treated (MCP300) 'Abbé Fétel' and 'Conference' pears after 1, 5 and 7 days of shelf life at 20°C after 13 and 21 weeks of storage. Bars refer to standard error of the mean. Results of ANOVA analysis are reported in Table 3.

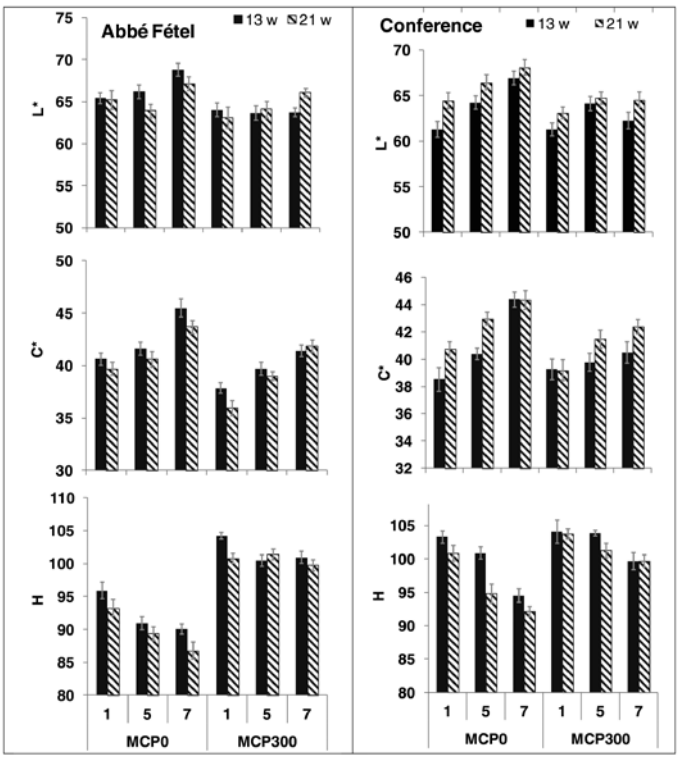

Fig. 5 - Lightness (L*), chroma (C*) and hue angle (degree) of control (MCP0) and 1-MCP treated (MCP300) 'Abbé Fétel' and 'Conference' pears after 1, 5 and 7 days of shelf life at 20°C after 13 and 21 weeks of storage. Bars refer to standard error of the mean. Results of ANOVA analysis are reported in Table 3.

At d1 of shelf life, control 'Abbé Fétel' pears had lower firmness and energy-to-rupture but similar stiffness than d1 1-MCP treated fruit. Firmness, stiffness and energy-to-rupture of control 'Abbé Fétel' pears decreased after 5 days and then they did not change further. In 1-MCP treated 'Abbé Fétel' pears, firmness and energy-to-rupture did not change with shelf life, whereas stiffness decreased after 5 days, but to a lesser extent than in control fruit, without any further change with the increase of shelf life time.

Skin color

On average in 'Conference' pears L* and C* were higher in control fruit after 21 weeks storage (Fig. 5) than in 1-MCP treated fruit at both storage times and in control fruits after 13 weeks storage, while the highest H° was found for 1-MCP treated fruit at both storage times and the lowest for control fruit after 21 weeks. Hue decreased with storage time only in control fruit. At d1 of shelf life control 'Conference' pears had L*, C* and H° values not different from those of 1-MCP treated fruit. With shelf life, L* and C* increased and H° decreased both in control and 1-MCP treated fruit, with control fruit at d7 showing the highest values of L* and C* and the least of H°.

In 'Abbé Fétel' the highest L* value was found in control fruit after 13 weeks of storage and the lowest in 1-MCP treated fruit at both storage times (Fig. 5). Chroma on average was higher in control fruit than in 1-MCP treatment without any influence of storage time, whereas H° was higher in 1-MCP treated fruit than in control pears, without any difference between storage times, with control fruits having the lowest H° value after 21 weeks storage. At d1 of shelf life control 'Abbé Fétel' pears had higher L* and lower H° than 1-MCP treated fruit. L* increased with shelf life in control fruit at both storage times and in 1-MCP treated ones after 21 weeks of storage. H° decreased with shelf life only in control fruit, while C* increased both in control and 1-MCP treated fruit, with control fruit having the highest values at d7.

Sensory analysis

With regard to sensory analysis, 'Conference' 1-MCP treated pears were on average firmer, more grainy, less juicy, sweet, sour and aromatic than control ones (Fig. 6), with the average scores being (1-MCP and control, respectively): 84 and 45 for sensory firmness, 52 and 38 for graininess, 25 and 71 for juiciness, 47 and 62 for sweetness, 19 and 24 for sourness, and 33 and 59 for aromatic. No changes in sensory profile with storage time and shelf life were observed for 1-MCP treated 'Conference' pears. In contrast, in control 'Conference' pears sensory firmness decreased during shelf life, without any differences between the storage times, while sweetness decreased with shelf life after 13 weeks storage and increased after 21 weeks storage, with d5 pears after 13 weeks being sweeter than fruit after 21 weeks. Juiciness in control fruit increased with shelf life only after 13 weeks storage and it

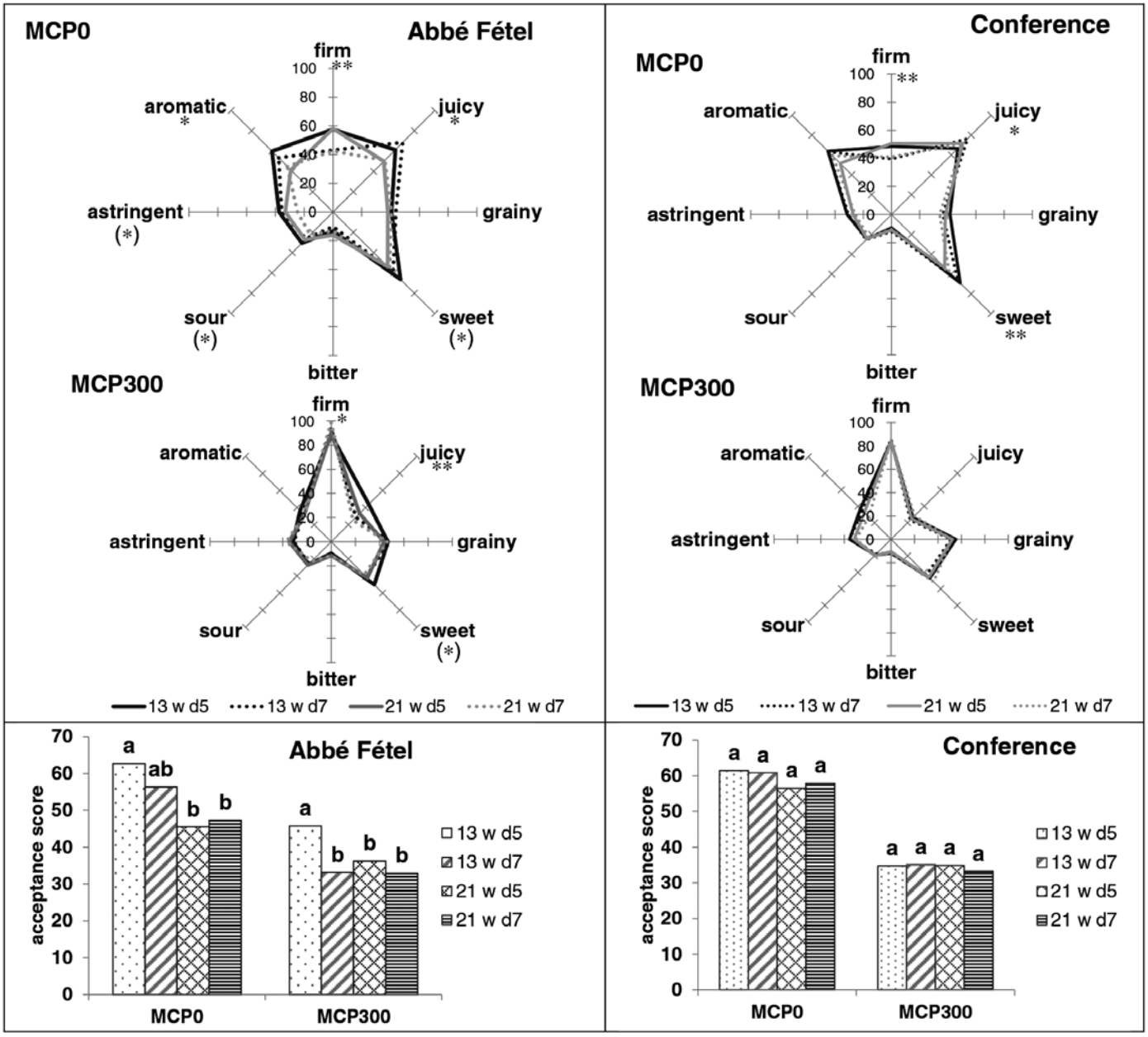

Fig. 6 - Sensory profiles and overall acceptability of control (MCP0) and 1-MCP treated (MCP300) 'Abbé Fétel' and 'Conference' pears after 13 and 21 weeks of storage and 5 (d5) and 7 (d7) days of shelf life at 20°C. Within each 1-MCP dose, for each attribute the significance of F-ratio (**, P<0.01; *, P<0.05; (*), P<0.10; no symbol, not significant) is reported. Within each 1-MCP dose, bars with different letters refer to statistically different means (Tukey's test, P < 0.05%).

was not significantly affected by the storage time. Overall acceptability was higher in control fruit and it was not influenced by storage time and shelf life both in control and 1-MCP treated 'Conference' fruit.

On average control 'Abbé Fétel' pears were less firm and more juicy, sweet and aromatic than 1-MCP treated fruit, the average scores being (1-MCP and control, respectivley): 93 and 50 for sensory firmness, 33 and 58 for juiciness, 43 and 59 for sweetness, and 33 and 50 for aromatic. Similarly to that found for 'Conference' pears, control 'Abbé Fétel' fruit at d5 of shelf life (Fig. 6) were firmer than at d7, without any influence of storage time. Juiciness was higher in d7 fruit after 13 weeks storage than in those after 21 weeks. Sweetness and aromatic scores were lower in d5 fruit after 21 weeks than in d5 ones after 13 weeks, the latter showing also higher sourness and astringency than fruit stored for 21 weeks at the end of shelf life. As for 1-MCP treated 'Abbé Fétel' pears, sensory firmness did not change with shelf life in fruit stored for 13 weeks, whereas it increased in those stored for 21 weeks, with the d7 fruit after 21 weeks being firmer than fruit at d5 of both storage times. 1-MCP treated 'Abbè Fétel' pears at d5 after 13 weeks of storage were less firm and juicier and sweeter than those of the same storage time at d7 and than those stored for 21 weeks. Overall acceptability decreased with storage time and shelf life: control fruit after 13 weeks storage and 5 days of shelf life had the highest overall acceptability, while 1-MCP treated fruit at

Table 4 - Storage disorders in control (MCP0) and 1-MCP treated (MCP300) 'Conference' pears after 13 and 21 weeks of storage and 7 days of shelf life at 20°C and ANOVA results (n=3)

	healthy	Early blackening	Black spot	Superficial scald	black speck	rot
			MCP0			
13 w	33.2 ±0.3	59.4±0.7	0	0	4.6 ±0.4	0.3±0.1
21 w	22.2±0.4	0	71.3±0.3	4.7±0.4	0	0.3±0.3
			MCP300			
13 w	29.0±0.01	64.3±0.01	0	0	5.3±0.01	1.3±0.01
21 w	10.8±0.2	0	88.4±0.2	0	0	0.5±0.1
			ANOVA [z]			
A: storage time	**	***	***	**	***	NS
B: 1-MCP	NS	NS	*	**	NS	NS
A × B	NS	NS	*	**	NS	NS

[z] ***, P<0.001; **, P<0.01; *, P<0.05; NS, not significant.

d7 after 13 weeks and at d5 and d7 after 21 weeks storage had the least overall acceptability. In addition, 1-MCP treated 'Abbé Fétel' pears stored for 13 weeks at 5 days of shelf life had an overall acceptability score that was not different from that of control fruit stored for 21 weeks.

Storage disorders

In 'Conference' pears at the end of storage and after 7 days at 20°C the percentage of healthy fruit was very low and decreased with storage time both in control and 1-MCP treated fruit (Table 4). Three types of peel disorders were detected: blackening, superficial scald and black speck. Two forms of blackening were distinguishable, differing for the color and severity of the disorder: early blackening, characterized by a grey net covering part of the peel, without any specific localization, and black spot, characterized by a very tight black net which, in the most severe forms, covered almost all the fruit surface. The percentages of fruit affected by the two forms of blackening were very high (Table 4). Early blackening was found only after 13 weeks of storage and its incidence was not influenced by 1-MCP treatment; black spot was found only after 21 weeks of storage, with higher incidence in 1-MCP treated fruit than in control ones. Superficial scald incidence was low (less than 5%) and developed only in control fruit after 21 weeks of storage. Black speck incidence was low (about 5%) and was found both in control and 1-MCP treated pears after 13 weeks of storage. Rot incidence was very low and was influenced neither by storage time nor by the 1-MCP treatment.

In 'Abbé Fétel' pears the percentage of healthy fruit on average was 93.3% in 1-MCP treated fruit and 77.4% in control ones and slightly decreased with storage time (Table 5). Two types of peel disorders were detected: soft scald and superficial scald, both of them developed only in control fruit. The incidence of soft scald increased somewhat with the increase of storage time, while superficial scald was detected at percentages lower than 1.5% only after 21 weeks of storage. Rot incidence was low, ranging from 4.2% (1-MCP treated after 21 weeks) to 8.6% (control after 21 weeks), and was not significantly influenced by storage time nor by the 1-MCP treatment.

Table 5 - Storage disorders in control (MCP0) and 1-MCP treated (MCP300) 'Abbé Fétel' pears after 13 and 21 weeks of storage and 7 days of shelf life at 20°C and ANOVA results (n=3)

	Healthy	Soft scald	Superficial scald	rot
		MCP0		
13 w	82.5±0.3	10.6±0.1	0	5.6±0.2
21 w	72.8±0.1	15.1±0.2	1.4±0.4	8.6±0.2
		MCP300		
13 w	95.6±0.03	0	0	4.4±0.03
21 w	91.5±0.2	0	0	4.2±1.2
		ANOVA [z]		
A: storage time	*	NS	NS	NS
B: 1-MCP	***	***	NS	NS
A × B	NS	NS	NS	NS

[z] ***, P<0.001; *, P<0.05; NS, not significant.

4. Discussion

In control and 1-MCP treated 'Conference' pears, the two ILOS periods induced a remarkable rise in chlorophyll fluorescence (Fα) in untreated fruit and much smaller ones in 1-MCP treated pears, when O_2 concentration decreased below 0.4 kPa, which is the lower O_2 limit (LOL) at which metabolism of 'Conference' pears changes from aerobic to fermentative (Zerbini and Grassi, 2010). The lesser Fα increase found in 1-MCP treated 'Conference' pears could be due to respiration reduction induced by 1-MCP, which reduces LOL, as observed in other pear cultivars (Watkins, 2006) and in 'Abbé Fétel' fruit by Rizzolo *et al.* (2008, 2010). In control 'Abbé Fétel' pears the O_2 concentration decreased below 0.6 kPa, which is its LOL, only for a short

period during the first ILOS period, while during the second ILOS period O_2 values ranged from 0.3 to 0.6 kPa. In contrast, in the container with 1-MCP treated 'Abbé Fétel' pears during the two ILOS periods O_2 values ranged from 0.1-0.4 kPa, causing a slight rise in Fα in 1-MCP treated 'Abbé Fétel' pears probably due to the fact that in this container the O_2 values were much lower than the LOL value.

It has been reported that fruit stored in ultralow oxygen pressure, below 2 kPa inducing fermentation, when compared to fruit stored in air develop lower quantities of straight-carbon chain compounds, esters, aldehydes and ketones (Mattheis *et al*., 1991, Fellman *et al.,* 1993) and increased amounts of ethanol, acetaldehyde and ethanol-derived ethyl esters, mainly ethyl acetate (Argenta *et al*., 2004). Furthermore, Lumpkin *et al.* (2014) found for apples that acetaldehyde, ethanol and ethyl esters amounts increased with pO_2, decreasing from 1.5 kPa to 0.3 kPa, mainly during the first weeks of storage, while Mattheis *et al.* (2013) found in 'd'Anjou' pears a significant correlation between pithy brown core incidence and ethanol, suggesting a relationship between disorder development and abnormal oxidative metabolism due to an hypoxic storage environment. According to these findings we can infer that, in control 'Conference' pears and in control and 1-MCP treated 'Abbé Fétel' fruit, the stress due to the low pO_2 applied during the ILOS period, as highlighted by the fluorescence monitoring, impacted on fermentative metabolites. The reduction of respiration induced by 1-MCP significantly influenced fermentative metabolite development, as in 'Conference' no more ethanol was produced during the low pO_2 ILOS period and in 'Abbè Fètel' pears it lowered the production of ethyl acetate.

As for the relationships between ILOS period and α-farnesene and CTols content in the peel, for both cultivars no significant changes in their concentrations following the ILOS period were found, with the exception of $CTol_{281}$ in 1-MCP treated 'Conference' pears, which became not detectable at the end of the ILOS period. Then, after 13 weeks of storage α-farnesene and CTols concentration in the peel increased in control fruit of both cultivars and in 1-MCP treated 'Abbé Fétel' pears. On the other hand, in both cultivars 1-MCP treatment significantly inhibited the production of α-farnesene and CTols not only during the ILOS period but also after the CA storage period till 13 weeks storage time, with 'Abbé Fétel' pears showing a more marked reduction in α-farnesene, $CTol_{258}$ and $CTol_{269}$ concentrations and 'Conference' fruit in $CTol_{281}$ concentration.

The α-farnesene and CTols trends observed are in agreement with previous findings on 'Conference' and 'Abbé Fétel' pears (Lo Scalzo *et al.*, 2002; Folchi and Bertolini, 2008; Eccher Zerbini *et al.*, 2005; Vanoli *et al.*, 2010 b) and could be due to the fact that low temperature storage induces, in scald-susceptible cultivars, a high rate of α-farnesene synthesis, which causes its marked accumulation in the skin during the first two to three months of storage. Then, the concentration of α-farnesene declines as a consequence of its *in vivo* oxidation to the highly reactive conjugated trienols (Gapper *et al*., 2006; Isidoro and Almeida, 2006; Whitaker, 2007), which disrupt cell membranes and lead to polyphenoloxidase-mediated browning of the skin (Bain and Mercer, 1963) and necrosis of the hypodermal cell layers. Moreover, it was found that both in apples and pears inhibition of α-farnesene synthesis by 1-MCP was closely correlated with suppression of the α-farnesene synthase gene Pc*AFS1*, which encodes the last enzyme in the α-farnesene biosynthetic pathway (Lurie *et al.*, 2005; Pechous *et al.*, 2005; Gapper *et al*., 2006). In highly scald-susceptible apple and pear cultivars, inhibition of ethylene production and α-farnesene synthesis by pre-storage 1-MCP treatment is often lost after several months in cold storage, and this coincides with loss of scald control (Gapper *et al.*, 2006; Tsantili *et al.*, 2007). For 'd'Anjou' pears Zoffoli *et al.* (1998) reported that $CTol_{269}$ was the main peak for conjugated trienols and increased during cold storage, as did the other two CT peaks; also for 'Packham's Triumph' pears the three CTols increased during storage, but $CTol_{258}$ was proportionally higher than $CTol_{281}$ and almost the same as $CTol_{269}$, while in 'Bartlett' pears the main CTol was $CTol_{258}$, which increased with storage, with very low amounts for the other two CTols. Whitaker *et al.* (2001) associated the absorbance measured at 258 nm in the skin hexane extracts to a family of p-cumaryl fatty esters which act as antioxidant rather than to an oxidation product of α-farnesene. In view of these findings, our results suggest that the capacity of 'Conference' pears stored in CA after ILOS periods to generate scald-related antioxidants ($CTol_{258}$) is higher than the fruit's ability to produce scald-related α-farnesene oxidation products ($CTol_{281}$), while the opposite scenario was found for control 'Abbè Fétel' fruit. Considering the ratio $CTol_{258}/CTol_{281}$, a potential marker of superficial scald, Du and Bramlage (1993) found that values lower than 1.0 were generally associated to high scald susceptibility while values greater than 2.0 were associated to lower scald susceptibility. In this work, higher values of $CTol_{258}/CTol_{281}$ were found in 1-MCP treated fruit of both cultivars, whereas values below 1.0 and below 2.0 were found in control fruits of 'Abbé Fétel' and 'Conference', respectively, both developing some superficial scald at the end of storage. Du and Bramlage (1993) reported that $CTol_{269}/CTol_{281}$ ratio values generally reflected those of the $CTol_{258}/CTol_{281}$ ratio, but differences were less distinct when comparing lots of apple fruit with different scald potential. On the other hand, Zoffoli (1994), when considering the trends of the $CTol_{258}/CTol_{281}$ and $CTol_{269}/CTol_{281}$ ratios with cold storage time in pears, suggested that $CTol_{258}$ could be the precursor to $CTol_{269}$, and $CTol_{269}$ the precursor to $CTol_{281}$. In the present study, data of the two ratios confirmed Zoffoli's (1994) hypothesis: indeed in 'Abbé Fétel' pears at the beginning and at the end of the second ILOS period $CTol_{258}$ was predominant, both in control and 1-MCP treated fruit, but in correspondence with the first storage time $CTol_{269}$ was predominant in control fruit, while it was almost the same in 1-MCP treated pears. Similarly, in control 'Conference' fruit at the first storage

time, $CTol_{269}$ was predominant. The low incidence of superficial scald found in this work could be due either to seasonal non predisposing conditions, as low superficial scald incidence was observed in 'Abbé Fétel' fruit stored in NA (Rizzolo, Grassi and Vanoli unpublished) or to the beneficial effect of CA in 'Conference' pears with respect to NA stored fruit (Rizzolo *et al.,* 2015).

Both in 'Conference' and 'Abbé Fétel' pears, 1-MCP treatment prevented the development of scald after storage and shelf life. In 'Conference' a high proportion of fruit developed blackening in a less severe form after 13 weeks storage, and with higher incidence and severity after 21 weeks storage. Blackening has been detected in 'Conference' pears for about twenty years, but its incidence and severity has been increasing over the last few years. According to previous observations (Bertolini, personal communication), blackening development is not related to α-farnesene and CTols development and it is neither controlled nor prevented by 1-MCP treatment, rather it induced higher severity than in control fruit. Black speck was developed in 'Conference' only after 13 weeks storage and, similarly to blackening, it was neither controlled nor prevented by 1-MCP treatment. Black speck has been reported for mature-green 'd'Anjou' pears (Lee *et al*., 1990) and it was suggested that it is provoked by fruit stress related to low temperature in conjunction with low oxygen CA storage, as confirmed by Mattheis and Rudell (2011), who found that the low O_2 partial pressure set points established by monitoring fruit chlorophyll fluorescence can prevent 'd'Anjou' scald but may result in black speck development.

The pre-storage 1-MCP treatment drastically reduced ethylene production during post-storage shelf life both in 'Conference' and 'Abbé Fétel' fruit, as found in previous studies on these cultivars (Eccher Zerbini *et al.*, 2003, 2005; Rizzolo *et al.*, 2005, 2008; Vanoli *et al.*, 2008, 2010 a) and on other pear cultivars (Watkins, 2006 and references herein). Control 'Abbé Fétel' pears produced more ethylene than control 'Conference' pears and were less sensitive to 1-MCP treatment, as shown mainly by the sensory analysis results and, secondly, by quality parameters, as found by Eccher Zerbini *et al.* (2003, 2005).

Control fruit from both cultivars with shelf life underwent skin yellowing and pulp softening. However, control 'Abbé Fétel' pears already upon removal after 21 weeks storage in CA soften to firmness values lower than 40 N, the threshold value corresponding to edible-firm texture for this cultivar (Predieri and Gatti, 2009). 'Conference' control fruit softened rapidly with shelf life, reaching the minimal level of acceptable eating quality of 10 N (Chiriboga *et al*., 2013) already after 5 days of shelf life at 20°C. Fruit softening in control pears was similar to that found in previous experiments on CA and DCA 'Abbé Fétel' fruit stored at -0.5°C (Rizzolo *et al.*, 2014; Vanoli *et al.*, 2015) and on 'Conference' fruit stored in NA and CA at -1°C (Folchi and Bertolini, 2008) and in air at -0.5°C (Chiriboga *et al.*, 2013). The treatment with 1-MCP at the concentration of 300 nL L^{-1} prevented ripening during the 7-day shelf life at 20°C, even if slight decreases in firmness, stiffness and energy-to rupture were found for 1-MCP treated 'Abbé Fétel' pears; these slight changes, indeed, influenced the sensory firmness and juiciness. In contrast, in 1-MCP treated 'Conference' pears, only stiffness slightly decreased with shelf life, and no changes in the sensory profile were found with storage time and shelf life. On the contrary, Folchi and Bertolini (2008) observed that in 'Conference' pears harvested at 64.7 N and treated with 300 nL L^{-1} 1-MCP, stored at -1°C up to four months there was a slight softening of 6 N, followed by a further softening to 40 N, prolonging storage up to seven months plus seven days of shelf life at 20°C. This difference could be due to the fact that 'Conference' pears had been harvested at a less advanced stage of maturity as assessed by the firmness value of 71 N and hue value of 108°, which are similar to the values reported by Chiriboga *et al.* (2013) for early and mid harvests. These authors reported that in two years 'Conference' pears harvested before or around the commercial harvest date remained firm after treatment with 1-MCP and lost their ability to soften even after several days at 20°C. In contrast 1-MCP applied at more advanced stages of maturity slowed down the softening process without completely blocking it.

5. Conclusions

The 1-MCP treatment at harvest influenced the physiological and quality changes of 'Conference' and 'Abbé Fétel' pears stored in CA after ILOS periods. 1-MCP dramatically decreased the concentrations of α-farnesene and CTols in the fruit skin at the end of the ILOS period both in 'Conference' and 'Abbé Fétel' pears, and affected the concentrations of fermentative metabolites in the fruit pulp, lowering, after the ILOS period, the ethanol concentration in both cultivars and the ethyl acetate amount in 'Abbé Fétel' pears. The 1-MCP treatment drastically reduced the ethylene production during shelf life, impaired fruit softening to edible texture, and delayed fruit yellowing both during storage and shelf life. Upon sensory tasting, 1-MCP treated fruit was firmer and less juicy, sweet and aromatic than control fruit, without any changes with shelf life in 'Conference' fruit and with a slight decrease in sensory firmness and juiciness in 'Abbé Fétel' pears. It was confirmed that 1-MCP prevents superficial scald in both cultivars, and soft scald in 'Abbé Fétel' pears, whereas in 'Conference' fruit it either has no effect or enhances the incidence and severity of blackening, a disorder which may not be dependent on oxidation products in the skin.

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Decay control of cold stored *Citrus clementina* Hort. ex Tan. fruit by pre- and postharvest application of potassium phosphite

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Key words: aging, chilling injury, citrus fruit, defence stimulators, pre- and postharvest decay.

Abstract: The effectiveness of pre- and postharvest application of potassium phosphite against naturally occuring postharvest decay and storage disorders on clementine Monreal fruit (*Citrus clementina* Hort. ex Tan.) was investigated. Phosphite solutions were applied according to the following experimental protocol: by spraying fruit on the trees (2.5 g/L), at fruit colour breaking and 15 days before harvest; by the combination of preharvest (2.5 g/L) and postharvest dipping application (4 g of a.i./L). Preharvest applications were compared to Phosethyl-Al solution (2.5 g/L) and water control. Decay and disorders were assessed after 30 days of cold storage at 6±1°C and 90-95% RH, followed by 7 days of shelf life at 20±2°C. The combination of pre- and postharvest application of phosphite was more effective in reducing green (*Penicillium digitatum* Sacc.) and blue mould (*P. italicum* Weh.) incidence, as compared to water control, but it was not so effective in reducing the incidence of minor decay. Potassium phosphite treatments, before harvest and in pre-postharvest combination, significantly reduced chilling injury and aging with respect to water control. Based on these results, pre- and postharvest application of potassium phosphite can be considered a useful strategy to be included in an integrated approach for controlling green and blue mould of citrus fruit in storage.

1. Introduction

Clementines (*Citrus clementina* Hort. ex Tan.), due to their high quality, are one of the most important cultivated citrus mandarins in southern Italy. Production in the last decade has increased considerably thanks to remarkable consumer preference. These fruits are very perishable and the occurrence of various fruit diseases and physiological disorders affect their marketing value.

The major postharvest diseases of citrus fruit, including clementines, can be separated into two categories based on their initial infections: preharvest infections including Brown rot (*Phytophthora* spp.), *Alternaria* rot (*Alternaria citri* Ellis et Pierce*, A. alternata* (Fr.) Keissl), Stem-end rot (*Diplodia natalensis* Pole-Evan, *Phomopsis citri* Fawcett), Grey mould (*Botrytis cinerea* Pers.), Anthracnose (*Colletotrichum gloeosporioides* Penz.); and postharvest infections including Green mould (*Penicillium digitatum* Sacc.), Blue mould (*P. italicum* Weh.) and Sour rot (*Geotrichum candidum* Link) (Ohr and Eckert, 1985; Brown and Miller, 1999; Schena *et al.*, 2011).

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The most common and serious diseases, which occur in Italy, during storage and marketing of clementine fruit are green and blue moulds. Infection takes place only through wounds, where nutrients are available to stimulate spore germination and fruit decay begins at these infected injury sites (Eckert and Eaks, 1989; Smilanick *et al.*, 1997; 2006; Ismail and Zhang, 2004). The incidence of other pathogens is generally low, but can be a serious problem in warm, wet years. These diseases, however, can cause significant economic losses during storage, transport and marketing.

Chilling injury (CI) represents the major disorder of citrus fruit occurring during low non-freezing temperature storage (0-10°C), and it depends on species and cultivars; mandarin hybrids are sensitive to CI. The severity of CI is related to the temperature and the duration of exposure (Chalutz *et al.*, 1985; Eckert and Eaks, 1989; Lafuente and Zacarias, 2006). Aging is indicated by the shrivelling and collapse of the stem-end button tissue (Porat *et al*., 2004).

The use of synthetic fungicides in packinghouses, before fruit storage, remains the major means of control for managing citrus postharvest diseases (Eckert and Ogawa, 1988; Ismail and Zhang, 2004; Smilanick *et al.*, 2006). However, the development of resistance in fungal pathogens to fungicides (Schwinn *et al*., 1982; Viñas *et al.*, 1993; Holmes and Eckert, 1999) and the growing public concern regarding the potential impact on human health and environmental haz-

ards, have resulted in a significant interest in the development of alternative methods of disease control.

The development of treatments to enhance plant defences is an attractive area to seek further improvements in postharvest disease control. Among preharvest treatments, phosphite products, which elicit biochemical defences against invading fungi, can offer an alternative means of decay control.

In Italy, phosphite products (potassium, calcium and copper phosphite salts) are registered as fertilizers but not yet authorized as disease control agents; they require oxidation to phosphate prior to use by plants and this process is mediated by microbes (Adams and Conrad, 1953; Landschoot and Cook, 2005). Although foliar phosphite applications increased flower numbers and yields on 'Valencia' orange, their benefits may result from the control of fungal pathogens, as well as mitigating abiotic stresses, among other mechanisms, such as defence stimulators (Albrigo, 1999). Product activity is carried out primarily through two mechanisms: direct inhibition of the pathogen, with modification of the phosphate metabolism, and induction of host defence responses (induced systemic resistance mechanisms), such as the phytoalexins scoparone, scopoletin and umbelliferone (Smillie *et al.*, 1989; Guest and Bompeix, 1990; Guest and Grant, 1991).

Many growers of citrus fruit and other crops often apply phosphites before harvest to protect fruit from postharvest decay from fungal pathogens (Cerioni *et al.*, 2013 a). In fact, they are effective for the control of diseases caused by Oomycetes (*Phytophthora* and related fungi), particularly susceptible to inhibition by phosphite (Gaulliard and Pelossier, 1983; Cohen *et al.*, 1987; Guest and Grant, 1991; Martin *et al.*, 1998; McDonald *et al.*, 2001; Adaskaveg, 2009). On the other hand, few investigations, instead, describe control of *Penicillium* spp. by phosphites and also report the major efficacy of phosphites when applied in heated solution (Amiri and Bompeix, 2011; Bassay Blum *et al.*, 2007; Cerioni *et al.*, 2013 a). In the United States phosphites are exempt from residue tolerances (US EPA, 2006), and two commercial potassium phosphite formulations are registered for postharvest use.

The objective of the present research was to investigate the effectiveness of pre- and postharvest application of potassium phosphite against postharvest decay (in particular green and blue moulds), and physiological disorders (chilling injury and aging) on cold stored clementine fruits. The efficacy of the product was compared to Phosethyl-Al, a phosphate-generating fungicide. In order to simulate actual commercial conditions, experiments were conducted on naturally-infected fruit instead of on artificially inoculated specimens.

2. Materials and Methods

Plant material

Field trials were conducted in the fall 2013, on 20-year-old clementine trees (*Citrus clementina* Hort. ex Tan.) cv. Monreal, located in the "Palazzelli" experimental orchard (Sicily region, southern Italy) belonging to 'Consiglio per la Ricerca in Agricoltura e l'analisi dell'economia agraria - Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee (CRA-ACM)'.

Solution preparation

Commercial formulations of potassium phosphite (DeccoPhosk, Decco Italia s.r.l., Belpasso, Catania, Italy) and Phosethyl-Al (Aliette, Bayer CropScience) were dissolved manually in water to achieve a final concentration of 2.5 g L^{-1}.

Treatments and storage

Scheduled treatments are reported in Table 1. For preharvest treatments, trials were arranged in a completely randomized block design with three replicates of four plants each. Plants were selected for uniformity of fruit development, absence of evident symptoms of diseases and disorders, and sprayed with potassium phosphite, Phosethyl-Al and tap water. Treatments were carried out at fruit colour breaking and 15 days before harvest using a commercial motor-driven back sprayer (approximately 5 L $plant^{-1}$ of solution).

At commercial maturity, fruits were harvested from treated plants and placed into plastic boxes (one box per plant), each containing 50 fruits, with the exception of potassium phosphite treatments (two boxes per plant), in order to use the extra fruit for the postharvest treatment.

For the combination of pre- and postharvest treatments, a group of 600 fruits from plants A, already treated in the field, were immersed in a solution of potassium phosphite (4 g of a.i./L) at 40°C (±0.5°C) for 120 s. The fruits were not rinsed after treatment and were allowed to dry for 2 h at room temperature. All fruits, placed in three plastic boxes per treatment (each containing 200 fruits), were stored for 30 days at 6±1°C and 90-95% RH, followed by 7 days of shelf life at 20±2°C. These storage conditions were used to simulate actual commercial conditions.

At the end of cold storage and after shelf life, decay incidence, chilling injury and aging were assessed. Decay incidence was expressed as the percentage of fruit infected by fungal pathogens. Diseases were visually identified and classified as green mould (*P. digitatum*), blue mould (*P. italicum*), mix of green and blue mould (*P. digitatum* and *italicum* present on the same fruit), and minor decay (*Phytophthora, Alternaria, Rhizopus*, *Botrytis*, *Phomopsis*, *Diplodia*, etc.). Severity of chilling injury (CI) was evaluated

Table 1 - Scheduled treatments on clementine fruits

Treatment	Dose	Period of treatment
Potassium phosphite (A)	2.5 g/L	Two preharvest treatments
Phosethyl-Al (B)	2.5 g/L	Two preharvest treatments
Water Control (W)		Two preharvest treatments
Potassium phosphite (Ap+p)	2.5 g/L 4 g of a.i./L	Two preharvest treatments and a postharvest treatment

using a four-grade scoring system. A subjective rating of 0 (none), 1 (light), 2 (moderate), and 3 (severe) was used to estimate damage of the rind. A light rating indicated damage <10% of peel area, not perceived to be objectionable to the discerning consumer, moderate (10-30%) was injury estimated to be objectionable, and severe (>30%) indicated damage that would cause consumers to reject the product. Aging was expressed as percentage of fruit damaged.

In order to evaluate the effect of treatments on fruit weight loss, 30 fruits per treatment were regularly weighed at the beginning, at the end of cold storage and after one week of shelf life. The percentage of weight reduction was recorded.

Statistical analysis

Data were analysed using one-way analysis of variance (ANOVA) procedures, using Statistica 6.0 software. Percentage data were arcsine transformed to normalize variance. Mean values of treatments were compared by using Tukey's test at P=0.05 level. Data in the figures are actual percentages of decayed fruit.

3. Results

Postharvest rots on clementines at the end of storage were mainly due to *P. italicum* (blue mould) and *P. digitatum* (green mould) alone and present in the same fruit (mix of green and blue mould). Minor decay was caused by *Geotrichum* spp., *Alternaria* spp., *Botrytis* spp., *Phytophthora* spp., etc. In all cases preharvest application of potassium phosphite and the combination of pre- and postharvest applications, showed variable effects in reducing decay incidence, depending on the pathogens involved. Since the trials were conducted on naturally occurring infections, disease incidence in the control treatments was not very high.

After 30 days of storage at 6±1°C followed by a week of shelf life at 20±2°C, preharvest application of potassium phosphite on clementines significantly reduced the percent infection of blue mould, the mix of green-blue mould, and minor decay as compared to the water control (Fig. 1B-1C-1D); no significant reduction was observed on the green mould incidence as compared to the water control (Fig. 1A).

The combination of pre- and postharvest applications of potassium phosphite was, instead, more effective in reducing the incidence of green and blue mould, as compared to water control (Fig. 1A-1B). The improved control of blue mould, known for its greater ability to grow at low temperature, was of particular interest. Conversely, its efficacy in reducing the incidence of minor decay, on preharvest treatments was not improved by postharvest application (Fig. 1D).

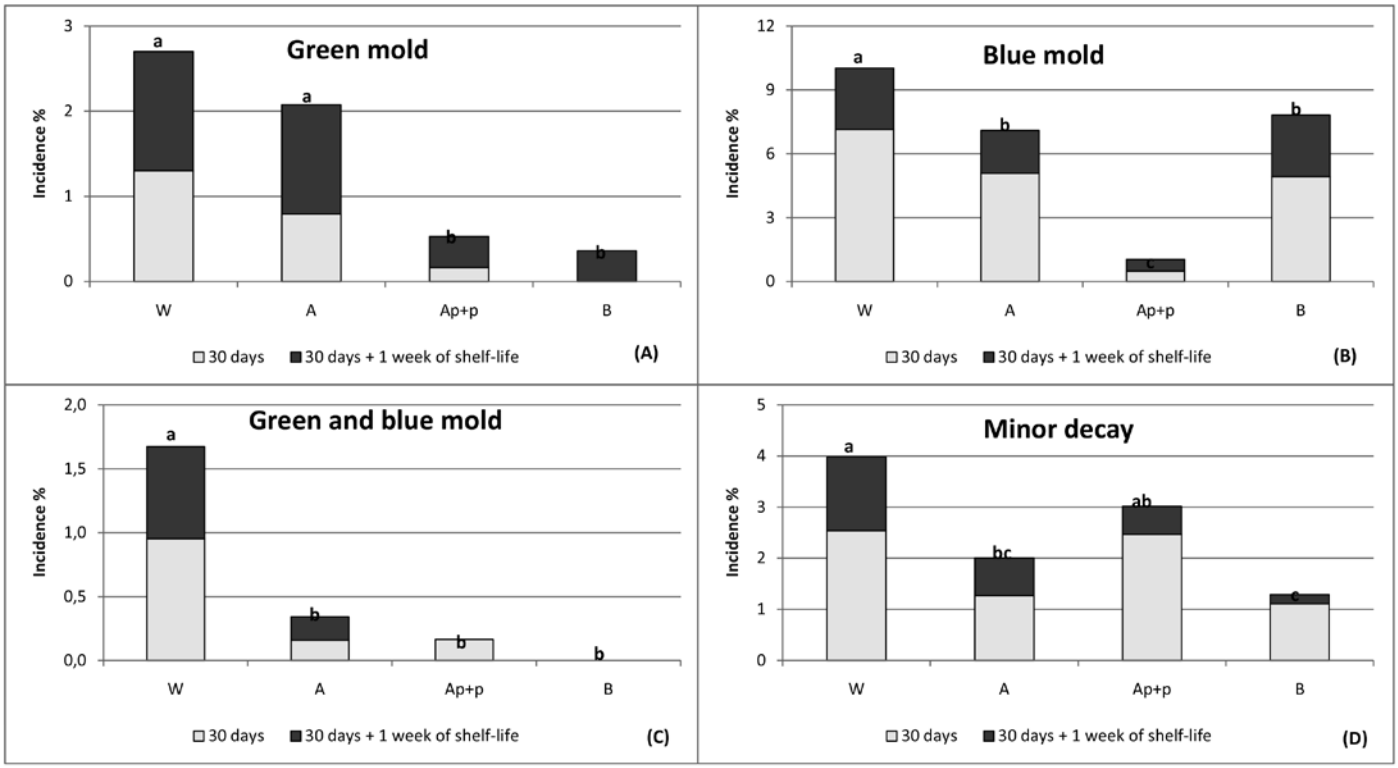

Fig. 1 - Incidence of green mold (*P. digitatum*) (A), blue mold (*P. italicum*) (B), mix of green-blue mold on the same fruit (C) and minor decay (D), on clementine, after 30 days of storage at 6±1°C followed by one week at 20±2°C. Each treatment was applied to three replicates of 200 fruit each. Water treatment was used as control. Columns marked with the same letters are not statistically different according to Tukey's test (P = 0.05). W= Water control, preharvest treatments; A= Potassium phosphite, preharvest treatments (2.5 g/L); Ap+p= Potassium phosphite, pre- (2.5 g/L) and postharvest treatments (4 g a.i./L); B= Phosetyl-Al, preharvest treatments (2.5 g/L).

Concerning CI, potassium phosphite treatments before harvest (A) and in pre-postharvest combination (Ap+p) significantly reduced light and moderate values, as compared to water control and severe values as compared to Phosetyl-Al (Fig. 2). All treatments (A, Ap+p and B) were significantly effective in reducing aging with respect to water control (Fig. 3).

Postharvest treatment with potassium phosphite had no phytotoxic effect on clementines. In addition, after 30 days of storage and one week of shelf life, the general external appearance of fruit was not affected by different treatments.

No statistically significant differences were found for weight loss, among all treatments, both at the end of cold storage and after a week of shelf life (*data not shown*).

4. Discussion and Conclusions

The main objective of the present study was to evaluate the efficacy of pre- and postharvest application of potassium phosphite, in controlling postharvest decay, particularly green and blue moulds of clementine, in order to extend its application for disease control of citrus fruits in Italy.

Reports describing the pre- and postharvest use of phosphite to control diseases caused by true fungi are few. Gutter (1983) reported that the posphite-generating compound Phosetyl-Al, *in vitro* and *in vivo*, had modest activity on the control of *P. digitatum*; Bassay Blum *et al.* (2007) reported that immersion of apple fruit in potassium phosphite solutions controlled blue mould caused by *P. expansum*. Cerioni *et al*. (2013 a) reported that improved control of green and blue mould, in postharvest treatments, was influenced by heating the solution (50°C), and by increasing the phosphite concentration (15 g/L). Regarding post-treatment storage temperature, 10°C were able to control green mould on citrus fruit, but had less effect on blue mould, even when the phosphite solution was heated to 50°C.

Our data showed that treatment with potassium phosphite was more effective against green and blue mould when applied before and after harvest, whereas, when applied only before harvest, it did not influence green mould incidence as compared to the water control. This different result is probably due to defence stimulation that treatment activates on the tree in field trials followed by the defence stimulation activated on fruit, in postharvest treatment (4 g of a.i./L), at the temperature of 40°C. The reduced efficacy of potassium posphite, on minor decay, in pre- and postharvest applications, was unexpected since its field application is effective against different pathogens.

Although not evaluated for the single control of *Phytophthora* brown rot, phosphites have long been known to control this fruit decay (Gaulliard and Pelossier, 1983; Cohen and Coffey, 1986; Graham and Timmer, 2011), which causes significant losses in wet years. Adaskaveg (2009) reported the excellent results obtained for the pre- and postinfection control of *Phythophthora citrophthora* on orange fruit dipped in 0.27 g/L of potassium phosphite. Thus, the phosphite treatments that controlled green and blue mould would be expected to control brown rot.

Phosphite is more costly than other alternatives used in packinghouses (SBC), but are compatible with SBC and with all of the fungicides currently registered for postharvest use such as Imazalil (IMZ) and Thiabendazole (TBZ), improving their performance (Cerioni *et al.*, 2013 a, 2013

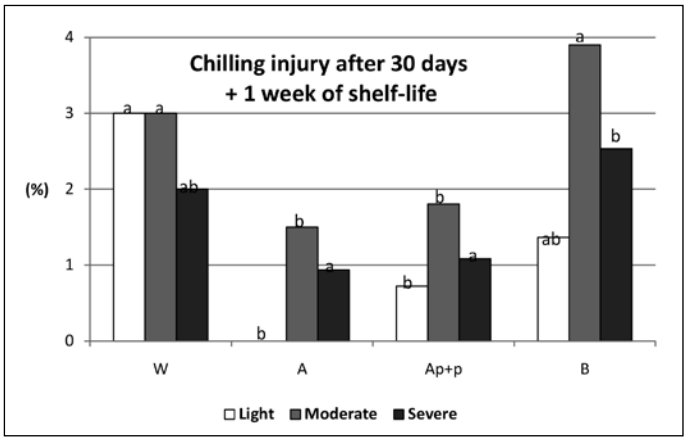

Fig. 2 - Effect of treatments on the severity of chilling injury on clementine, after 30 days of storage at 6±1°C followed by one week at 20±2°C. Each treatment was applied to three replicates of 200 fruits each. Water treatment was used as control. Columns marked with the same letters are not statistically different according to Tukey's test (P = 0.05). W= Water control, preharvest treatments; A= Potassium phosphite, preharvest treatments (2.5 g/L); Ap+p= Potassium phosphite, pre- (2.5 g/L) and postharvest treatments (4 g a.i./L); B= Phosetyl-Al, preharvest treatments (2.5 g/L).

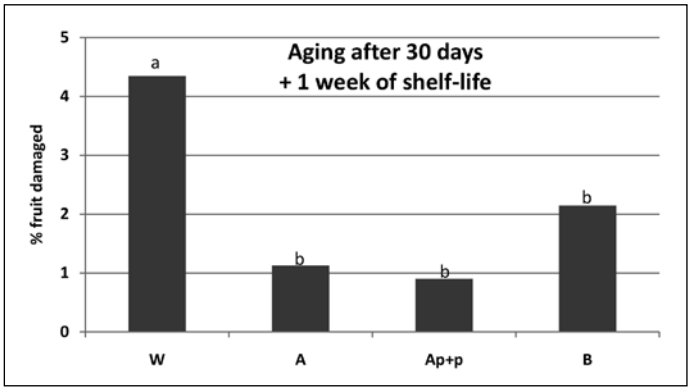

Fig. 3 - Effect of treatments on aging percentage on clementine, after 30 days of storage at 6±1°C followed by one week at 20±2°C. Each treatment was applied to three replicates of 200 fruits each. Water treatment was used as control. Columns marked with the same letters are not statistically different according to Tukey's test (P = 0.05).
W= Water control, preharvest treatments; A= Potassium phosphite, preharvest treatments (2.5 g/L); Ap+p= Potassium phosphite, pre- (2.5 g/L) and postharvest treatments (4 g a.i./L); B= Phosetyl-Al, preharvest treatments (2.5 g/L).

b; Palou *et al.*, 2001; 2002). Thus, the combination of potassium phosphite with SBC could be used to reduce costs, and in combination with IMZ could improve effectiveness for the control of IMZ-resistant isolates of *P. digitatum* (Kinay *et al.*, 2007).

In conclusion, our results have demonstrated that the incidence of green and blue mould on clementine fruit can be reduced by applying potassium phosphite twice before harvest and in postharvest treatments. Pre- and postharvest application of potassium phosphite can be considered a useful strategy to be included in an integrated approach for controlling postharvest diseases of citrus fruit. In any case, less infected fruit on packing lines should also reduce the demand for sanitizers during washing procedures (Lanza and Strano, 2009).

Practical application of potassium phosphite on citrus fruit needs to be further optimized as the obtainable level of protection is affected by various factors, first of all citrus variety, timing and number of applications. Additional research is in progress on different citrus varieties to improve the application strategy.

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Evaluation of postharvest storage and treatments in cut ruscus foliage

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Key words: *Danae*, glycerol, leaf senescence, Thidiazuron.

Abstract: *Danae racemosa* (L.) Moench is an important cut foliage in the ornamental market. During postharvest, leaf senescence symptoms are leaf yellowing, weight loss and/or abscission of leaves. The aim of this work was to evaluate the effect of pre-treatments for 24 h with glycerol and Thidiazuron (TDZ) on *Danae* vase life before and after storage. Treatments were applied in vase water containing glycerol 0.1, 1 or 10 mM, 10 µM TDZ and a combined treatment of 10 µM TDZ plus 10 mM glycerol. The effect of treatments was evaluated through the determination of vase life, chlorophyll content, chlorophyll *a* fluorescence parameters, sucrose and total sugars content. The cut foliage were stored in sealed plastic bags and placed in a dark room at 4°C for two months. The vase life, before and after storage, was determined in a controlled chamber set to 20°C and RH 50-60% with a light intensity of 20 µmol m^{-2} s^{-1}. Results demonstrated that 10 µM TDZ plus 10 mM glycerol was the most effective treatment in maintaining quality during vase life of stored and not stored cut foliage.

1. Introduction

Cut foliage represents an important part of the floricultural industry. They are used as fillers in floral bouquets especially for cut flowers without leaves such as gerberas. The vase life of cut foliage is usually longer than cut flowers, but it may become shorter when they are stored for long periods. Storage methods, wet or dry, affect the vase life of cut foliage (Ferrante *et al*., 2002 a) and play a crucial role for preserving quality: it is important, in particular, to delay the symptoms of leaf senescence considering that the intensity of leaf color is the most important quality parameter (Pacifici *et al*., 2007) and closely associated with the marketability of ornamental cut foliage. During postharvest, quality losses of cut foliage are essentially represented by leaf senescence. The most common symptoms are leaf yellowing, leaf desiccation and/or leaf abscission, and weight losses (Pacifici *et al*., 2007). Leaf yellowing is common in many cut flowers such as *Alstroemeria*, *Chrysantemum*, lilies, tulips, but also in some cut foliage such as cut *Danae racemosa* foliage (van Doorn *et al*., 1992; Ferrante *et al*., 2002 b, 2003, 2005). Postharvest treatments with cytokinins are able to delay leaf yellowing in many cut flowers, but a substituted phenylurea, the Thidiazuron (N-phenyl-N'-1,2,3-thiadiazol-5-yl urea, TDZ), with cytokinin-like activity, has been found to be very effective in many cut flowers sensitive to leaf yellowing (Ferrante *et al*., 2002 b); application of low concentrations of TDZ delayed leaf yellowing (Ferrante *et al.,* 2002 b, 2003; Mutui *et al.*, 2005; Jiang *et al.*, 2008).

During the postharvest chain, another important problem for cut foliage is weight loss. Since these ornamental items are sold by weight, any weight loss is directly translated into economic loss. Cut foliage is essentially composed of branches with leaves, therefore, weight losses are due to water losses by transpiration. During postharvest, the hydraulic conductance of the branches or cut stems progressively declines due to vessel blockages from bacteria growth, embolism, etc. (van Doorn, 1997).

Cut flowers and branches treated with glycerol are able to enhance osmotic potential and absorb great amounts of water, avoiding reduction of the water potential. Glycerol has been used to preserve ornamental plant materials (Dubois and Joyce, 1992) as it causes a greater water removal due to an increment of osmotic potential. In this way, glycerol treatment reduces leaf weight loss, as well as water loss, which reflects on extending vase life (Shanan and Shalaby, 2011).

In this study, the possibility to extend as much as possible the storage of cut foliage in a cold room at 4°C for 60 days was evaluated with the aim of maximizing cut *Danae* availability on the market. This outlook has practical value for some cooperatives and commercialization companies as extention of the storage period could cover periods when production is lacking (Pacifici *et al*., 2013).

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2. Materials and Methods

Plant materials

Cut branches of *Danae racemosa* (L.) Moench. [=*Ruscus racemosus* L.] were provided by a commercial company (Floratoscana, Pescia, PT, Italy) and transported to the postharvest laboratory of the Department of Agricultural and Environmental Sciences of the University of Milan. Cut branches were trimmed to a length of 70 cm to provide homogenous samples for the experiments.

Chemical treatments and storage

Cut foliage was pre-treated for 24 h with a solution containing 0.1, 1 or 10 mM glycerol, 10 µM TDZ and a combined pre-treatment of 10 µM TDZ plus 10 mM glycerol, while control samples were held in distilled water. After pretreatments, the plant material was stored in vases containing distilled water at 20°C and 50-60% relative humidity. Each treatment was composed of eight replicates. A part of the cut branches treated with 10 mM glycerol, 10 µM TDZ and combined treatment 10 µM TDZ plus 10 mM glycerol were stored for 60 days in polyethylene bags at 4°C.

The gas composition in terms of oxygen and carbon dioxide percentages was monitored by means of a "Binder Combigas GA-m³" (from Binder, D) portable gas analyzer equipped with an electrochemical cell for oxygen and CO_2 measurement and an infrared dispersion cell for NH_3 determination.

Vase life evaluation

After storage, cut branches were transferred to a growth chamber with controlled temperature (20°C), relative humidity (60-70%), and light intensity (10-15 µmol m^{-2} s^{-1} PPFD for 12 h per day). Non-stored cut branches (control) were used for vase life assessment immediately after harvest. Ten branches for each storage period were placed in individual bottles for vase life analysis. Vase life was determined as the number of days from when branches were placed in the water to the onset of leaf senescence, yellowing or abscission.

Chlorophyll determination and chlorophyll a fluorescence

Chlorophyll content was measured using a chlorophyll meter (CL-01, Hansatech, UK) that provides an indicator of green color of leaves. This device determines relative chlorophyll content *in vivo* using dual wavelength optical absorbance (620 and 940 nm wavelength).

Chlorophyll *a* fluorescence was measured with a portable Handy Plant Efficiency Analyzer (PEA, Hansatech, UK). Leaves were dark-adapted for 30 min. Using a leaf clip (4 mm diameter), a rapid pulse of high intensity light of 3000 µmol m^{-2} s^{-1} (600 W m^{-2}) was absorbed by the leaf inducing fluorescence, which was measured by the sensor. The fluorescence parameters were calculated automatically. JIP analysis was performed to determine the Performance Index (PI).

Sugars determination

In order to quantify sucrose and total sugars content, about 0.5 g of leaves were ground in 10 ml of distilled water. The homogenate was centrifuged at 10000 rpm for 5 min. For sucrose determination, 0.2 ml of extract were added to 0.2 ml NaOH 2N and incubated at 100°C for 10 min; then 1.5 ml of resorcinol were added and incubated at 80°C for 10 min. A resorcinol solution was prepared by adding 35 mg of resorcinol and 90 mg of thiourea in 250 ml HCl 30%, mixed with 25 ml of acetic acid and 10 ml of distilled water. Samples were cooled at room temperature and spectrophotometer readings were performed at 500 nm. A calibration curve was built with sucrose standards at 0, 0.5, 1, 1.5, 2 mM. Total sugars were calculated by anthrone method: 0.2 g of anthrone were melted in 100 ml of H_2SO_4 and shacked for 30-40 min; 0.2 ml of diluted extract was added to 1 ml of anthrone solution, cooled in ice for 5 min and mixed thoroughly. Samples were incubated at 95°C for 5 min and then cooled on ice. Absorbance readings were measured at 620 nm and a calibration curve was built with glucose standards at 0, 1, 2, 3 and 4 mM. Leaf extracts were diluted 1:10 for total sugars assay and used pure for sucrose assay.

Statistical analysis

Experiments were performed in a completely randomized experimental design with eight replicates for each treatment. The data are reported as means with standard errors. Data presented in Table 2 were subjected to one-way ANOVA.

3. Results

Gas compositions inside plastic bags

The gas composition was determined after 30 and 60 days of storage. Results showed that oxygen was lower in the plastic bags containing cut branches treated with TDZ plus glycerol. At the same time, also higher values of NH_3 were found in the TDZ+Gly treatment (Table 1).

Vase life and chlorophyll content

The vase life was significantly improved by the combined treatment TDZ plus glycerol (33.6 days in average) in comparison with the control (average 21.2 days) and the other treatments (21.8 to 23.6 days) (Table 2). Vase life was significantly reduced in cut foliage stored for 60 days, but treatment with TDZ plus glycerol gave the longest vase life, 22.6 days on average. Vase life of the control was 13.8 days, while in the treatment with glycerol and TDZ alone it was 10.2 and 15.2 days, respectively (Table 2).

The chlorophyll content was higher in all treatments compared to control during the first 15 days of vase life, then declined in 10 mM glycerol (this treatment had the best efficiency among the glycerol treatments) while cut foliage treated with TDZ showed chlorophyll reduction after 20 days. The combined TDZ plus glycerol treatment did not show any chlorophyll reduction until 22 days (Fig. 1). After storage the best results were obtained with TDZ and TDZ plus glycerol treatment. TDZ retained the

Table 1 - Gas compositions inside the plastic bags during storage

Days	Gases	Blank	Treatments			
			Control	10 mM Gly	TDZ	TDZ+Gly
30	CO_2 (%)	0	0.30	0.60	0.10	0.3
	O_2 (%)	20	12.70	19.90	20.90	13
	NH_3 (µL L^{-1})	2	15.00	0.00	12.00	17
60	CO_2 (%)	0.00	0.50	0.40	0.40	0.3
	O_2 (%)	20.90	20.30	19.50	20.00	13
	NH_3 (µL L^{-1})	0.00	8.00	8.00	8.00	17

Table 2 - Vase life before and after storage of cut foliage treated with glycerol (0.1, 1 or 10 mM), 10 µM TDZ or a combination 10 µM TDZ plus 10 mM glycerol

Vase life of fresh harvest cut foliage						
	Control	Glycerol			TDZ	TDZ + glycerol
		0.1 mM	1 mM	10 mM	10 µM	10 µM + 10 mM
Vase life (d)	21.2±1.09 b	21.8±0.45 b	22.2±0.45 b	22±0 b	23.6±1.34 b	33.6±1.14 a

Storage and vase life post-storage				
	Control	Glycerol	TDZ	TDZ + glycerol
		10 mM	10 µM	10 µM + 10 mM
Storage (d)	60	60	60	60
Post-storage vase life (d)	13.8±1.09 b	10.2±1.12 b	15.2±0.45 b	22.6±2.61 a
Storage + vase life (d)	73.8±1.09 b	70.2±1.12 b	75.2±0.45 b	82.6±2.61 b

Values are means with standard errors (n=5).
Data were subjected to one-way ANOVA analysis and different letters indicate significant differences for P<0.05.

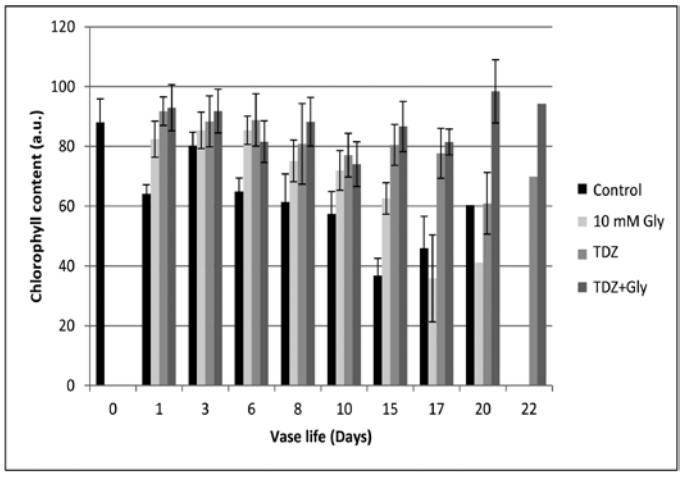

Fig. 1 - Chlorophyll content measured from cut *Danae* branches during vase life with a chlorophyll meter and expressed as relative units. Values are means with standard errors (*n=8*).

chlorophyll content in the treated cut foliage until 10 days, then chlorophyll started to decline. In the combined treatment TDZ plus glycerol, the chlorophyll values remained unchanged and similar to the fresh cut foliage (80 a.u.) until 20 days of vase life (Fig. 2).

Chlorophyll a fluorescence measurements

Chlorophyll *a* fluorescence was measured during storage by way of a non-destructive method to evaluate the leaf health status of *Danae* leaves during vase life, before and after storage. Among the different parameters and indexes calculated, the performance index (PI) is reported in figure

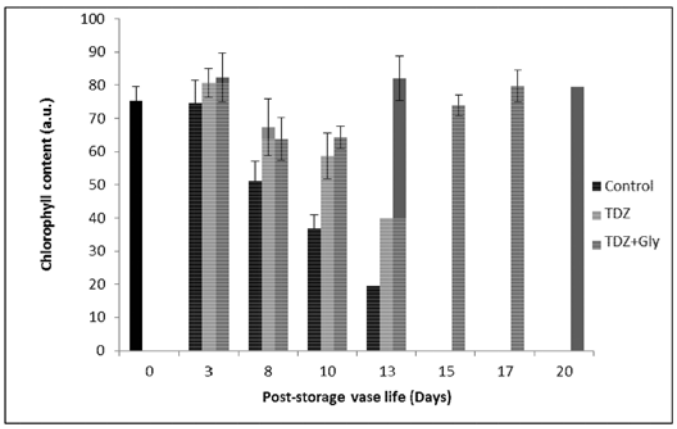

Fig. 2 - Chlorophyll content during vase life of cut *Danae* branches stored for 60 days. Chlorophyll was measured using a chlorophyll meter and expressed as relative units. Values are means with standard errors (*n=8*).

3 and 4. In particular, the PI measured during the vase life of non-stored cut foliage did not change in the combined treatment, while it declined in the control and glycerol treatments. Instead, in the TDZ treated cut branches, the PI declined after eight days of vase life (Fig. 3). After two months of storage, the PI was lower in all treatments and dropped faster in the control and TDZ, while in the TDZ plus glycerol it slightly declined after eight days and remained unchanged until 20 days of vase life (Fig. 4).

Sugars content

The sucrose content of cut foliage at harvest was 15 mg/g FW on average and declined during vase life. In glycerol treatments, and in particular in the 10 mM treatment, the reduction was faster and showed lower values. After 14 days of vase life, higher values were found in TDZ and TDZ plus glycerol. After 19 days only the cut branches treated with TDZ and glycerol were alive and the sucrose content was 4.5 mg/g FW on average (Fig. 5). In stored cut branches the sucrose content was five fold lower compared to the fresh harvested branches. The sucrose content declined in TDZ after eight days of storage and ranged from 1.5 to 2.2 mg/g FW until 15 days of vase life. In TDZ plus glycerol treatment the cut branches did not show reduction of sucrose content until 15 days of vase life and a significant decline was observed after 20 days of vase life (Fig. 6).

Total sugars showed a similar trend of sucrose content during vase life before and after storage. The higher values were found in TDZ plus glycerol treatment, especially at the end of vase life (data not shown).

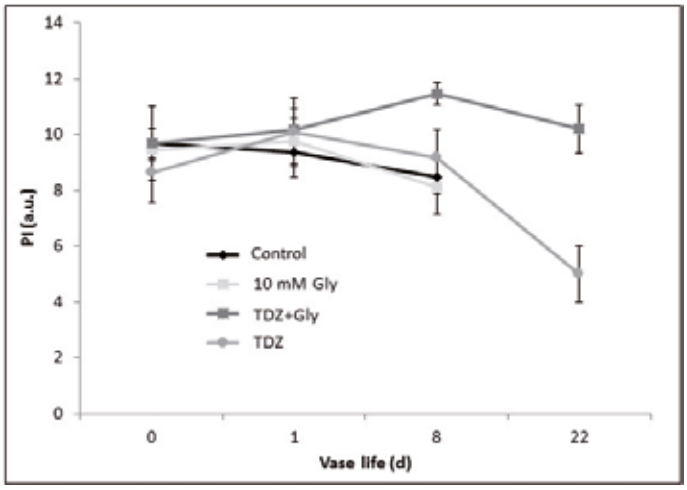

Fig. 3 - Performance index (PI) measured on cut foliage during vase life determination. Values are means with standard errors (n=5).

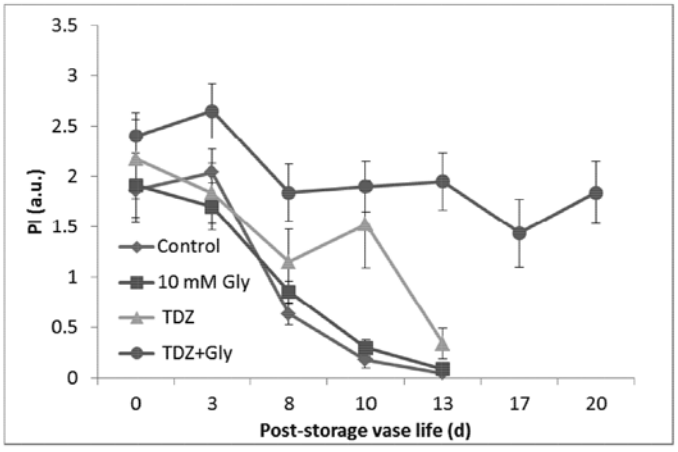

Fig. 4 - Performance index (PI) measured on cut foliage stored for 60 days and then transferred to 20 °C for vase life determination. Values are means with standard errors (n=5).

4. Discussion and Conclusions

The visual appearance of cut foliage is the most important quality parameter, and preservative treatments for

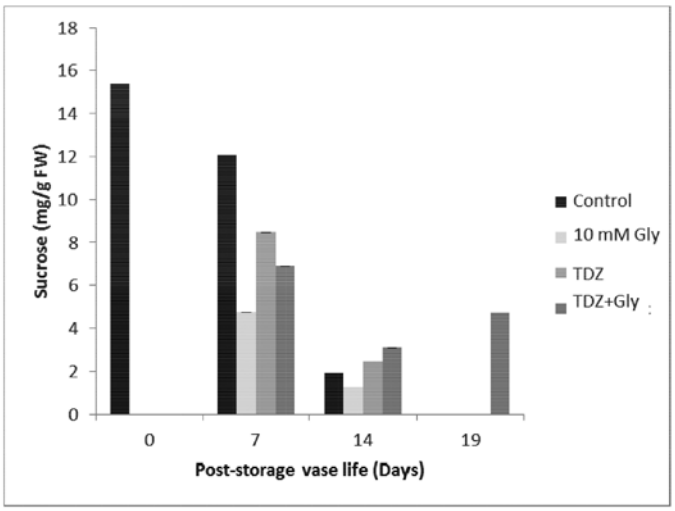

Fig. 5 - Sucrose content measured in leaves of cut *Danae* branches treated with distilled water (control), 10 mM Glycerol, 10 µM TDZ or 10 µM TDZ plus 10 mM Glycerol during vase life. Values are means with standard errors (n=3).

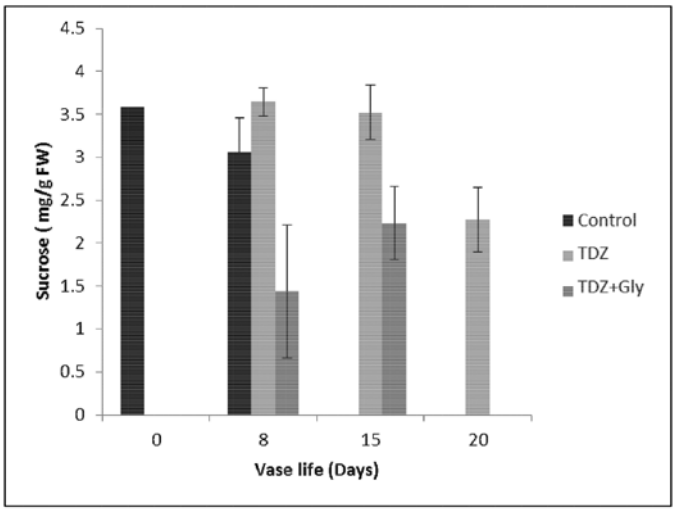

Fig. 6 - Sucrose content measured in leaves of stored cut *Danae* branches treated with distilled water (control), 10 µM TDZ or 10 µM TDZ plus 10 mM Glycerol during vase life. Values are means with standard errors (n=3).

these ornamental items are aimed at delaying leaf senescence or yellowing, which can occur during long-distance transportation or long storage periods. In addition to external quality, another important parameter to control in cut foliage postharvest is weight loss. Since cut foliage is sold on the basis of weight, any weight losses translate into economic losses. *Danae* plants are in active vegetative growth from September to May, thus there are three months without harvest and a lack of cut branches on the market. Flower markets and cooperatives are interested in storing as much cut foliage as possible. Cut *Danae* branches can be stored under mild vacuum packages or in water for two months while maintaining satisfactory vase life (Pacifici *et al*., 2014). However, preservative treatments are needed to delay leaf yellowing during post-storage vase life. The positive effect of cytokinin treatments on chlorophyll retention was observed in cut eucalyptus branches (Ferrante *et al*., 2002 b) and TDZ has been used in cut flowers to inhibit leaf yellowing in sensitive species such as *Alstroemeria*, chrysanthemum and tulips (Ferrante *et al*., 2002 a, 2003, 2005). Our results indicate that cut branches treated with TDZ plus glycerol provide the best post-storage performance. TDZ alone reduced chlorophyll decline but TDZ with glycerol showed a synergistic effect; the positive effect of the combination was observed in all parameters measured.

Chlorophyll *a* fluorescence measurements can be used during postharvest to evaluate the health status of leaves. In the ornamental field, chlorophyll *a* fluorescence and relative derived parameters have been used to evaluate quality losses in cut *Eucalyptus* and *Danae* branches (Pacifici *et al*., 2008, 2013). In potted *Bougainvillea* plants, the use of chlorophyll *a* fluorescence parameters were used to evaluate the efficiency of ethylene inhibitors during the post-production stage (Ferrante *et al.,* 2012). In our experiments, chlorophyll *a* fluorescence showed that TDZ plus glycerol maintained leaf functionality as demonstrated by the Fv/Fm ratio (data not shown) and PI.

Storage of cut *Danae* branches in polyethylene bags at 4°C for 60 days gave good results since the cut foliage was available on the market for more than 80 days from harvest. The reduced gas exchanges avoided excessive water loss by transpiration and probably also conditioned respiration, as demonstrated by the gas compositions at the end of storage, especially in the bags containing cut branches treated with TDZ plus glycerol. Cut *E. parvifolia* branches treated with glycerol showed longer life compared with control (Ferrante *et al.*, 2001) and analogous results were found in *E. cinerea* (Campbell *et al*., 2000). Glycerol uploaded in the cell increases osmotic capacity and induces an initial stress which can be observed by measuring ethylene production (Ferrante *et al*., 2001), but it also helps maintain water balance.

Sugars represent the energy source for stored products and are usually associated with vase life. Among the sugars, sucrose represents one of the most important energy reserves and its degradation provides a direct substrate for respiration (Reid, 1991; Ferrante and Reid, 2006). In the present study, the cut foliage treated with TDZ showed higher sugars content. Further studies are needed to understand sugar metabolism in TDZ treated cut foliage after storage.

These results can be further evaluated in combination with postharvest treatments and the use of passive refrigeration systems, which have been applied to different perishable foods (Costa *et al*., 2013).

In conclusion, the results obtained from this investigation suggest that pulse treatment for 24 h with TDZ and glycerol can be used to extend the vase life of stored and non-stored cut *Danae* branches. However, further studies are required at physiological and biochemical level to elucidate the biological pathways affected by TDZ and glycerol.

Acknowledgements

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Postharvest changes in quality characteristics, antioxidant activity and bioactive compounds of peach and nectarine cultivars [*Prunus persica* (L.) Batsch]

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Key words: acidity, carotenoids, color, DPPH, firmness, Folin-Ciocalteau, hue, phenolics, shelf-life.

Abstract: Three peach ('Honora', 'Dr. Davis' and 'Fairtime') and five nectarine ('Maria Anna', 'Diamond Ray', 'Fairline', 'Nectaross', 'Sweet Red') cultivars were analyzed at harvest and after a postharvest ripening period. Physicochemical characteristics [peel ground color (L*,C*, *h*°), soluble solids content (SSC), flesh firmness and titratable acidity (TA)], the concentration of some bioactive compounds [total phenol content (TPC) and total carotenoids (TC)] and the total antioxidant activity (TAA) were evaluated at harvest and after a shelf-life period of five days at 20 °C. Phenolic compounds and antioxidant activity were assayed on two different extracts of each sample: ethanol/HCl and ethanol/acetone. After shelf-life, all the cultivars showed a decrease in firmness and an increase in the ratio SSC/TA. The *h*° parameter of the peel background color had a good correlation with firmness, SSC, TA and the ratio SSC/TA in some of the cultivars, but no relationships were found in the white-fleshed varieties and in two of the nectarines evaluated. The trend of the carotenoids content after postharvest ripening was found to be cultivar-dependent, while TAA or TPC showed an increase in nectarines and remained unchanged in peaches. The ethanol/acetone mix was able to extract almost the double of antioxidant compounds with respect to the ethanol/HCl extract.

1. Introduction

Peaches and nectarines are widely-consumed summer fruit and, in the last few years, there has been an increasing interest in their nutritional value (Ramina *et al*., 2008; Wolfe *et al*., 2008). Peach fruit contains a wide range of chemical compounds but, from a dietary point of view, the most important fruit constituents are carotenoids, phenolics and fibre (Ramina *et al*., 2008). Yellow-fleshed peaches are considered a good source of β-carotene and β-cryptoxanthin (Gross, 1987) while flavonols, that are glycosilated forms of quercetin and kampferol, are the most abundant phenolics in peaches and other stone fruit (Young *et al*., 1989). All these compounds are reported to have antioxidant activity (Fu *et al*., 2011; Haminiuk *et al*., 2012) and, when added to the human diet, have a protective action against cancer and cardiovascular diseases (Steinmetz and Potter, 1996). After harvest, firmness, acidity and other quality parameters of peaches and nectarines are subjected to important changes (Crisosto, 2006; Ramina *et al*., 2008). Fruit nutritional quality varies greatly among cultivars (Gil *et al*., 2002) and often decreases after refrigerated storage (Di Vaio *et al*., 2001; Tsantili *et al*., 2010). After storage at low temperatures, sensory characteristics, and especially aroma, of peach decreases (Infante *et al*., 2008) while in fruit ripened at 18°C, the level of volatile compounds was found to be similar to tree-ripened fruit (Aubert *et al*., 2003). The aim of this work was to study the evolution of qualitative characteristics and the concurrent change in bioactive compound concentrations in eight peach and nectarine cultivars during postharvest ripening at 20°C.

2. Materials and Methods

A white- ('Honora') and two yellow-fleshed ('Dr. Davis', Fairtime) peach cultivars and a white- ('Maria Anna') and four yellow-fleshed ('Diamond Ray', 'Fairline', 'Nectaross', 'Sweet Red') nectarines were harvested in the experimental field of the CRA-fruit tree culture of Rome. Immediately after harvest, all fruits were sent to the CRA-food technology research unit of Milan where 30 fruits

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per cultivar were selected for size uniformity and absence of damage. Fruits of each cultivar were randomly divided into two sets of 15 fruits each and analyzed immediately [harvest (HR)] or after five days of shelf-life at 20°C (75-80% RH) in a temperature-controlled ripening room (SL).

Quality characteristics

Color (L*, a*, b*, CIE values), soluble solids content (SSC) and titratable acidity (TA) were analyzed on each fruit. Skin background color was measured by a spectrophotometer (CM-2600d, Konica Minolta, Japan) on the two cheeks of each fruit (15 fruit/sampling); flesh color was assessed on two opposite sides of each fruit after removing 2.5 mm of peel and flesh. Hue value ($h°$) was calculated as arctangent of b*/a* and expressed in degrees, while the color saturation index (C*) was calculated as $\sqrt{a^{*2}+b^{*2}}$. SSC was measured by a digital refractometer (RFM 81, Bellingham+Stanley, UK). TA was measured by titrating 10 ml of fruit juice with 0.1 N NaOH to pH 8.1 and calculating TA as g of malic acid/100 g fresh weight. The maturity index (Artés and Salmerón, 1996; Crisosto *et al*., 2001; Crisosto, 2006) was calculated as the ratio SSC/TA.

Bioactive compounds and antioxidant activity

Three replicates (five fruits/replicate), were analyzed at each sampling. Each extract was prepared in duplicate.

Total carotenoids (TC) were assessed by the method of Picchi *et al*., (2012) with some modification. Briefly, 5 g of homogenized flesh were added to 150µL of butylated hydroxytoluene (BHT) (1% in methanol w/v), 0.05 g of ammonium sulphate and 10 mL of extracting solution (hexane: ethyl acetate: ethanol, 2:1:1 v/v). Samples were vortexed for 10 s and then centrifuged (15 min, 4°C, 15000xg) and the supernatant was filtered through cheesecloth and stored at -20°C until analysis. Absorbance was recorded at 450 nm (UV-UVIDEC 320 spectrophotometer, Jasco, Japan) and total carotenoids were estimated by comparison with a standard curve obtained with different amounts of β-carotene. The results were expressed as µg β-carotene equivalent (β-carotene EQ) /100 g F.W.

Total phenolic content (TPC) and total antioxidant activity (TAA) were analyzed preparing two different extracts: 5g of homogenized flesh to 20 mL of Ethanol (96%): HCl 0.04N (1:1 v/v) (E extract) or 20 mL of Ethanol (96%): Acetone (1:1 v/v) (E/A extract). Samples were vortexed for 10 s and centrifuged (15 min, 4°C, 10000xg), and the supernatant was filtered through cheesecloth and stored at -20°C until analysis.

TPC was measured using the Folin-Ciocalteu method (Singleton *et al*., 1999) with some modifications: 150 µL of sample extract, 5 mL of deionized water and 1 mL of Folin-Ciocalteu reagent were put in 10 mL test tubes and, after 5 min, 2 mL of 20% sodium carbonate solution were added. Samples were kept 120 min in the dark and the absorbance at 730 nm was read against a blank (the same reaction mix but without the sample extract). TPC was calculated from a calibration curve, using gallic acid as standard. Results were expressed as mg of gallic acid equivalent (GAE)/100 g F.W.

TAA was measured using the DPPH assay. The effect of peach extracts on the content of 2.2-diphenyl-1.picrylhydrazyl radical (DPPH•) was estimated according to the method of Lo Scalzo *et al*., (2004) with some modifications: 100 µL of sample extract or Trolox standard solution (0.01 to 0.5 mg/mL) were added to 2 mL of ethanol and 500 µL of DPPH• (0.5mM in ethanol) and the decrease in absorbance at 517 nm was recorded after 3 min. Each reading was done against its blank (2.5 mL ethanol, 100 µL of sample extract). The DPPH scavenging capacity of the samples was calculated using a standard curve of Trolox, and expressed as mg Trolox EQ/100 g F.W.

3. Results and Discussion

Quality characteristics

Based on 'Redhaven' peach maturity (July 10 in central Italy) the evaluated varieties were considered (Table 1) as "middle-late" (Honora, Maria Anna, Nectaross), "late" (Dr. Davis, Diamond Ray, Sweet Red) or "very late" (Fairline, Fairtime) maturity cultivars. Firmness at harvest differed considerably from cultivar to cultivar but, as reported by other authors (Gil *et al*., 2002), peach cultivars have, on average, lower flesh firmness than nectarines (40.6 N and 51.1 N respectively, $P<0.01$). After five days of shelf-life, firmness of all the cultivars was comparable, with the exception of 'Dr. Davis' (yellow peach) and 'Fairtime' (yellow nectarine) which showed the highest values (13.7 and 18.8 N, respectively). Flesh firmness alone is not considered a satisfactory maturity index because it can vary among varieties, fruit size or climatic conditions (Crisosto, 1994). In general, mature fruit of early-season peach or nectarine is less firm than late season varieties (Crisosto, 1994).

At harvest all cultivars reached the SSC (10%) proposed by Kader (1997) as the minimum quality standard. All the nectarines and, above all, the "very late" cultivar 'Fairline', had higher SSC than peaches, both at harvest and after shelf-life. After shelf-life the SSC showed a slight increase in 'Fairline' while it remained unchanged in all the other cultivars. TA was higher in nectarines than in peaches (1.3 and 0.7 g/100 g fresh weight respectively, $P<0.01$) and decreased in all the cultivars after shelf-life. In peaches, SSC was shown to correlate well with consumer acceptance (Crisosto and Crisosto, 2005), but SSC and TA can be determined by several factors (Crisosto, 2006) and large differences are reported among peach varieties (Crisosto, 1994).

The ratio SSC/TA (maturity index) was judged by some authors (Lill *et al*., 1989; Artés and Salmerón, 1996) to be a more reliable quality index. In the present work, the maturity index increased in all cultivars after shelf-life and, on average, was higher in peaches than in nectarines (17.7 and 10.8% respectively, $P<0.01$).

Color changes that are associated with ripening strongly influence visual and eating quality of peaches (Ramina *et*

al., 2008). In our experiment, peel background color was affected by postharvest ripening. Almost all the cultivars had a lower (more yellow) h° value after shelf-life (Table 2), except for the white peach 'Honora' and for the nectarine 'Diamond Ray'. In this latter, the red color covered the whole fruit surface and it was very difficult to measure the background yellow color. L* and, above all, C* values of the peel background color markedly differed from cultivar to cultivar but seemed not to be affected by shelf-life.

Hue of the flesh decreased slightly after shelf-life in all the cultivars but not in white-fleshed peaches 'Honora' and 'Maria Anna' and in the nectarine 'Diamond Ray'. *h*° and L* values of the flesh were similar between white- and yellow-fleshed cultivars. The color of the white-fleshed peach ('Honora') and nectarine ('Maria Anna') differed from the yellow cultivars only for a lower C* value, which indicates a lower saturation of the color, rather than a real difference in the hue.

Table 1 - Physical and chemical characteristics (means ±standard error) of different peach and nectarine cultivars at harvest (HR) or after five days of shelf-life (SL) at 20°C

Fruit type	Flesh color	Cultivar	Harvest date	Time	Firmness (N)	SSC	TA (g/100 g F.W.)	SSC/TA
Peach	White	Honora	Aug. 4	HR	41.2±5.2	10.8±0.3	1.1±0.04	9.8±0.2
				SL	4.30±0.1	11.2±0.5	0.9±0.03	12.4±0.2
	Yellow	Dr. Davis	Aug. 18	HR	38.8±1.4	13.1±0.1	0.7±0.00	19.9±0.1
				SL	13.7±0.5	13.1±0.2	0.5±0.01	25.6±0.2
		Fairtime	Sept. 23	HR	41.8±2.8	11.2±0.2	0.7±0.02	16.6±0.2
				SL	7.20±0.3	10.7±0.6	0.5±0.02	20.4±0.3
Nectarine	White	Maria Anna	Aug. 4	HR	25.6±3.1	14.1±0.3	1.7±0.02	8.5±0.2
				SL	2.30±0.2	14.9±0.2	1.0±0.06	15.3±1.1
	Yellow	Diamond Ray	Aug. 10	HR	43.4±2.5	11.8±0.5	1.2±0.05	9.9±0.3
				SL	4.00±0.3	12.4±1.1	1.0±0.02	13.0±0.8
		Fairline	Sept. 16	HR	72.0±2.3	17.3±0.3	1.3±0.02	13.7±0.3
				SL	18.8±2.6	19.8±0.8	1.3±0.03	14.7±0.2
		Nectaross	Aug. 4	HR	56.8±4.8	13.7±0.2	1.6±0.02	8.7±0.0
				SL	4.60±0.4	14.5±0.5	1.5±0.05	9.9±0.6
		Sweet Red	Aug. 18	HR	57.6±3.6	11.1±0.4	1.3±0.05	8.4±0.1
				SL	7.00±0.7	11.4±0.4	1.2±0.04	9.2±0.2

Table 2 - Peel and flesh color parameters (means ±standard error) of peach and nectarine cultivars at harvest (HR) or after five days of shelf-life (SL) at 20°C

Fruit type	Flesh color	Cultivar	Time	Peel background color			Flesh color		
				L*	h°	C*	L*	h°	C*
Peach	White	Honora	HR	62.1±3.0	55.5±5.5	33.3±0.9	77.9±1.6	77.7±6.2	23.7±0.3
			SL	60.2±0.8	52.9±3.5	36.9±1.2	74.8±2.7	76.6±2.0	26.8±0.8
	Yellow	Dr. Davis	HR	75.1±0.2	83.5±0.4	60.3±1.3	82.2±0.4	85.4±0.5	50.3±1.6
			SL	74.7±0.6	79.3±0.7	62.6±0.3	81.6±0.3	83.8±0.3	51.5±0.4
		Fairtime	HR	76.6±0.4	89.7±0.3	51.8±1.4	82.6±0.2	87.4±0.3	47.8±0.9
			SL	75.4±0.7	84.8±0.5	56.3±1.2	80.1±0.4	84.1±0.3	51.3±1.2
Nectarine	White	Maria Anna	HR	70.3±1.7	65.2±3.9	31.3±1.0	78.4±0.7	64.7±3.5	19.5±0.8
			SL	64.8±2.2	54.7±3.6	37.5±1.2	77.2±1.3	73.2±6.1	20.8±0.7
	Yellow	Diamond Ray	HR	41.7±0.7	31.9±0.8	47.6±1.7	69.4±3.3	68.3±4.6	49.8±1.7
			SL	44.5±2.2	35.8±2.3	47.7±1.8	66.8±4.5	68.0±5.8	48.1±2.5
		Fairline	HR	75.3±0.1	87.6±0.3	58.7±0.2	80.5±0.6	86.4±0.3	51.3±0.2
			SL	71.9±0.3	81.2±1.0	61.9±0.6	78.2±0.5	83.0±0.7	55.5±0.6
		Nectaross	HR	71.2±1.2	79.7±1.7	51.8±0.4	79.3±0.7	85.4±0.9	50.4±0.9
			SL	70.4±1.2	75.1±1.9	58.3±1.0	75.0±1.2	79.8±2.1	50.8±0.6
		Sweet Red	HR	72.3±1.5	82.7±2.5	50.6±1.0	79.2±0.5	85.7±0.2	52.3±0.9
			SL	73.2±1.2	77.3±1.0	53.9±0.9	77.8±0.7	81.9±0.7	51.1±0.5

Flesh or peel background color are reported by different authors (Delwiche and Baumgardner, 1985; Byrne *et al*., 1991; Crisosto, 1994; Lewallen and Marini, 2003) to be highly correlated with firmness and other quality parameters of peaches and nectarines, so that background color is often used as maturity index (Kader, 1997). Peel background color or flesh color are not affected by sunlight and, thus, are more dependable indices of maturity than red color (Crisosto, 1994). In this work we found good correlations between peel background color or flesh color ($h°$) and different quality parameters in some of the evaluated cultivars (Table 3). In particular, peel background color of the yellow peaches 'Dr. Davis' and 'Fairtime' showed good correlations with firmness, TA or SSC/TA and, in 'Fairtime', these parameters were also related with flesh color. With regard to the yellow nectarines, peel and flesh color of 'Fairline' and 'Nectaross' were related with different quality parameters but without showing very high *r* values. Good correlations were found only in 'Nectaross' between $h°$ of the flesh and SSC or SSC/TA. 'Sweet Red' nectarine had a high correlation coefficient between flesh color and firmness, without showing any significant correlation coefficient with the other parameters. Similar, but slightly lower, *r* values were found for a* color parameter (*data not shown*). No relationships were found between flesh or peel color and any of the quality characteristics in the white peach ('Honora') and nectarine ('Maria Anna') while 'Diamond Ray' showed only a low correlation coefficient between flesh color and TA and between flesh color and SSC.

Bioactive compounds and antioxidant activity

Peaches and nectarines are rich in bioactive compounds such as carotenoids and phenolics (Gil *et al*., 2002). The major carotenoids in peaches are β-carotene and β-cryptoxanthin (Ramina *et al*., 2008). White and yellow peaches show different levels of carotenoids production, especially in the last phase of maturity (Brandi *et al*., 2011). In our experiment we found a lower level of total carotenoids in the two white-fleshed cultivars (Fig. 1). Carotenoid content increased, after shelf-life, in the two yellow peaches ('Dr. Davis' and 'Fairtime') and in the nectarine 'Diamond Ray', while it remained constant in the other nectarine cultivars ('Fairtime', 'Nectaross' and 'Sweet Red'). Carotenoid content showed a rather good correlation coefficient with the chroma (C*) of the flesh (r=0.64, P<0.01) and of the peel (r=0.62, P<0.01) but not with the $h°$ values (r=0.29 and r=0.33 with the flesh and peel hue values, respectively, P<0.05). The literature is inconsistent regarding the trend of TC after harvest. A decrease is reported by Ramina *et al*. (2008) while other authors (Caprioli *et al*., 2009, Bianchi *et al*., 2015) described an increase in carotenoids after shelf-life at 20°C. As is shown also by our results, the evolution of carotenoids after harvest could be cultivar-dependent.

Phenolic compounds were measured on two different extracts of each sample. In general, the extraction of phenolic compounds in alcoholic solution provides satisfactory results (Perva-Uzunalić *et al*., 2006); on pomegranate, some authors reported that the extraction in a mixture with methanol, ethanol, acetone and water had better results (Li *et al*., 2006). For this reason we decided to perform the classic ethanol/HCl (E) extraction plus an ethanol/acetone (E/A) extraction.

Total phenolic content (Fig. 2), evaluated by the two extraction methods, remained almost unchanged after shelf-life in the peach cultivars while it increased in nectarines, with the only exception being 'Diamond Ray' that

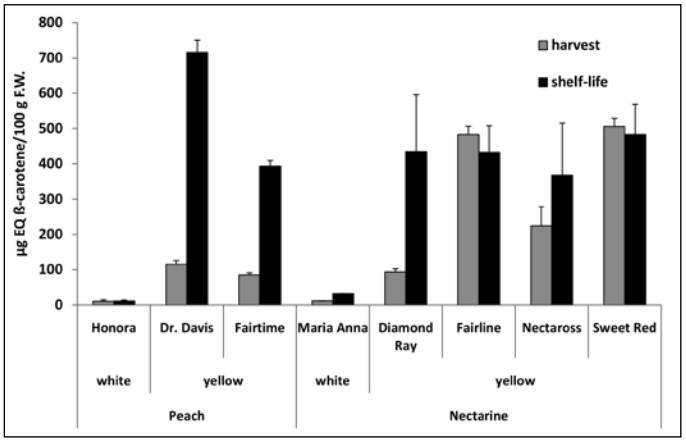

Fig. 1 - Total carotenoids content of the flesh of peach and nectarine cultivars at harvest and after five days of shelf-life at 20°C. Bars refer to standard error.

Table 3 - Correlations among peel background color or flesh color and different quality parameters in yellow or white-fleshed peaches and nectarines

Fruit type	Flesh color	Cultivar	Peel background color				Flesh color			
			Firmness	SSC	AC	SSC/AC	Firmness	SSC	AC	SSC/AC
Peach	White	Honora	NS	NS	NS	NS	NS	NS	NS	NS
	Yellow	Dr. Davis	0.73**	-0.59**	0.76**	-0.82**	NS	NS	0.38*	-0.39*
		Fairtime	0.70**	NS	0.76**	-0.81**	0.77**	NS	0.83**	-0.82**
Nectarine	White	Maria Anna	NS	NS	NS	NS	NS	NS	NS	NS
	Yellow	Diamond Ray	NS	NS	-0.48*	NS	NS	-0.52*	NS	NS
		Fairline	0.58**	-0.68**	NS	-0.56**	0.56**	-0.77**	NS	-0.66**
		Nectaross	0.42*	-0.38*	0.58**	-0.61**	0.52**	-0.45*	0.53**	-0.82**
		Sweet Red	NS	NS	NS	NS	0.82**	NS	NS	NS

Significance of r= P<0.05 (*), P<0.01(**), NS= not significant.

showed a very low TPC content. Between the two methods used, the ethanol/acetone mix (31.4 mg GAE/100g F.W, on average, against 26.4 mg of the ethanol extract, P<0.01) was able to extract a higher quantity of phenolics. Phenolic compounds are a class of compounds that is very broad and complex. In stone fruit the most abundant phenolics are flavonols and cinnamic acids, including chlorogenic and neochlorogenic acids (Ramina *et al*., 2008), but phenolic extracts of plants are always a mixture of different classes of compounds which are selectively soluble in the solvents (Koffi *et al*., 2010). Furthermore, solvent polarity plays a key role in increasing phenolic solubility (Naczk and Shahidi, 2004).

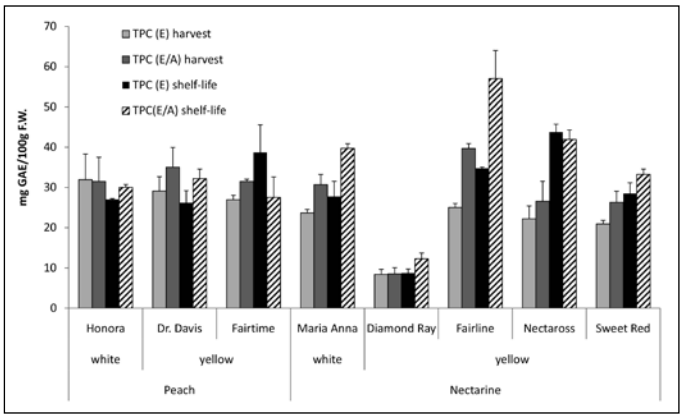

Fig. 2 - Total phenol content (TPC) of ethanol/HCl (E) or ethanol/acetone (E/A) extracts from flesh of different peach and nectarine cultivars at harvest and after five days of shelf-life at 20°C. Bars refer to standard error.

Since there were differences in TPC extraction between ethanol/HCl and ethanol/acetone solution, total antioxidant activity was also assayed on both extracts. As for phenolic compounds, peaches and nectarines showed different behaviors after shelf-life (Fig. 3): in peach fruit, total antioxidant activity, measured on E or E/A extracts, remained unchanged, while in nectarines it showed an increase after five days at

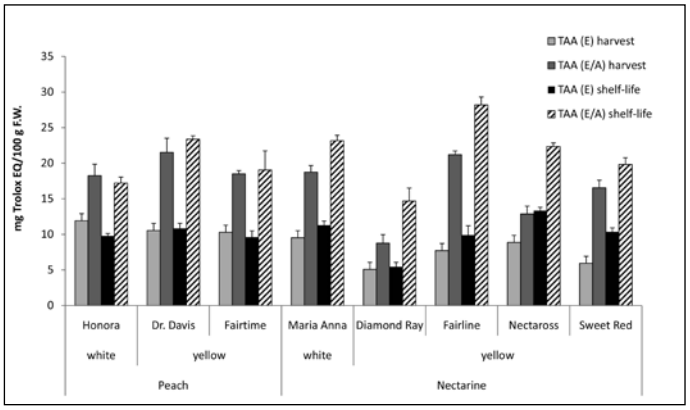

Fig. 3 - Total antioxidant activity (TAA) of ethanol/HCl (E) or ethanol/acetone (E/A) extracts from flesh of different peach and nectarine cultivars at harvest and after five days of shelf-life at 20°C. Bars refer to standard error.

20°C. Other authors also showed a significant increase in TAA in several nectarine cultivars after refrigerated storage (seven days at 2°C) while the increase was not significant or there was a decrease in peaches (Di Vaio *et al*., 2001, 2008).

Antioxidant activity of peaches and nectarines measured on E/A extract was more than double that measured on ethanol/HCl extract (19 mg Trolox EQ/100 g F.W., on average, with respect to 9.4 of the E extract, P<0.01). This fact indicates that probably more antioxidant compounds can be extracted by the combined action of the solvent mix ethanol/acetone. Acetone is a polar aprotic solvent that solvates ions without making bonds. Having also a lipophilic portion [-C-$(CH_2)_2$], it probably allows a better extraction of non-polar compounds like lipophilic phenols or carotenoids with respect to the ethanol/HCl extract. The correlation coefficient between the difference in TAA values measured on the two extracts (TAA ethanol/acetone-TAA ethanol/HCl) showed a slight correlation with total carotenoids (r=0.34, P<0.05), while there was no relationship between TAA measured on E extract and total carotenoids. As shown by Gil *et al*. (2002), total antioxidant activity was highly correlated with TPC (r=0.81 between TAA and TPC measured on E extract and r=0.87 between TAA and TPC measured on E/A extract, P<0.01).

Principal component analysis

To obtain a global picture of the difference in quality and nutritional characteristics of the different cultivars, all the data were subjected to PCA. Four functions were extracted, explaining 84.7% of total variance. Considering the first two principal components (Fig. 4) PC1 (39.2% of total variance) was positively related to all the evaluated

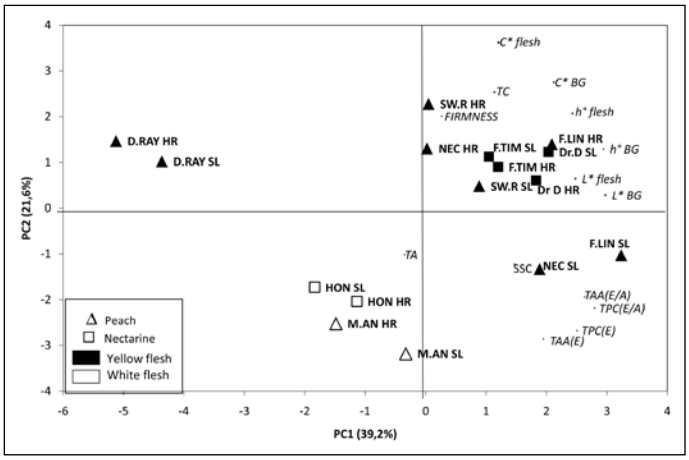

Fig. 4 - Principal component analysis of quality characteristics and bioactive compounds of eight peach and nectarine cultivars at harvest (HR) and after shelf-life (SL). Cultivars: HON= Honora; Dr.D= Dr. Davis; F.TIM=Fairtime; M.AN= Maria Anna; D.RAY= Diamond Ray; F.LIN=Fairline; NEC=Nectaross; SW.R=Sweet Red. Factors: SSC= soluble solids content; *h*°, C*, L*= color parameters; BG= peel background; TC= total carotenoids content; TA= titratable acidity; TAA= total antioxidant activity; TPC= Total phenol content; (E) ethanol/HCl extract; (E/A)=Ethanol/acetone extract.

factors, except for firmness and TA; PC2 (21.6%) grouped C* and *h*° color parameters, firmness and total carotenoids, opposite to SSC and TA. The biplot of PC1 versus PC2 (Fig. 4) revealed four distinct groups. The first group was formed by the two white-fleshed cultivars that showed negative values for both PC1 and PC2 and, hence, were negatively related to C* and total carotenoids. The second group was made up of the two 'Diamond Ray' samples, which showed very negative scores on PC1, probably because of their low values of bioactive compounds. The third group is composed of the two ripe samples (after shelf-life) of 'Nectaross' and 'Fairline' that had positive values on PC1 and negative on PC2 and, hence, linked mainly with a high content of antioxidant compounds. The last group was formed by the remaining yellow peaches and nectarine samples that showed positive values on PC1 and PC2, which are linked with high values in color parameters and high carotenoids content.

The scores of the all the samples on PC1 and PC2 showed important differences from cultivar to cultivar (Fig. 5). After shelf-life PC1 scores increased in all the nectarines but remained unchanged in the peach cultivars, while PC2 scores did not show important changes, except for 'Nectaross' and 'Fairline'.

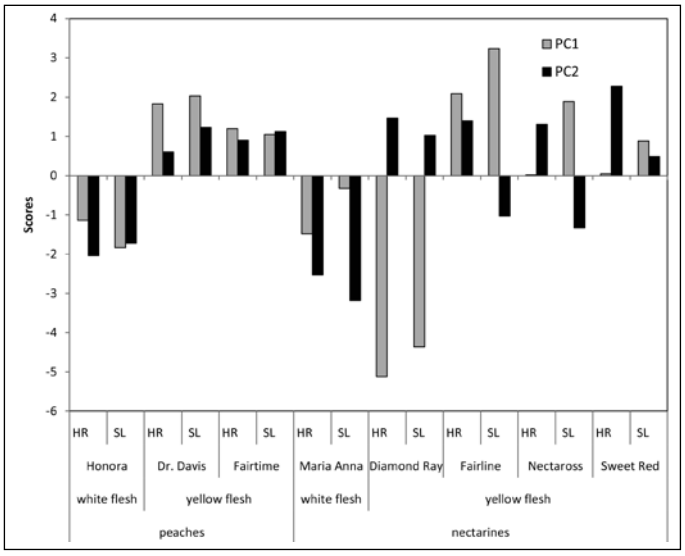

Fig. 5 - PCA scores on PC1 and PC2 of peach and nectarine cultivars evaluated at harvest (HR) and after shelf-life (SL). Bars represent standard error.

4. Conclusions

This study has shown a high variability in peach and nectarine characteristics after postharvest ripening. Peel background color, that is often used as maturity index (Kader, 1997) had, after shelf-life, a good correlation with firmness, SSC, TA and SSC/TA in some of the cultivars, but no relationships were found in the white-fleshed varieties and in two of the nectarines evaluated. For these cultivars it could be desirable to evaluate other nondestructive parameters such as Near infrared spectrometry (NIR) or Time-resolved reflectance spectroscopy (TRS) (Carlomagno *et al*., 2004; Zerbini *et al*., 2006), which might be better related with the ripening stage.

The trend of carotenoids content after postharvest ripening was found to be cultivar-dependent, while TAA and TPC measured on two different extracts (ethanol/HCl and ethanol/acetone) showed an increase in nectarines and remained unchanged in peaches. The E/A mix was able to extract almost double the antioxidant compounds with respect to the simple ethanol/HCl extract, probably because of the higher extraction of non-polar compounds due to the presence of acetone.

With principal component analysis, the nectarine 'Diamond Ray' was grouped differently from the other cultivars, probably because of its low content in bioactive compounds. In general, all the evaluated cultivars did not show, after postharvest ripening, a significant decrease in quality parameters other than firmness. On the contrary, all the cultivars maintained or increased their antioxidant activity and their initial content in bioactive compounds.

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Ethylene and the postharvest performance of cut camellia flowering branches

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Key words: ethylene antagonist, ethylene production, flower longevity, senescence, vase life.

Abstract: Camellias are potentially appreciated as cut branches due to their deep and bright green foliage with a high number of flower buds. The present research investigated the role of ethylene in postharvest flower and leaf abscission of seven cultivars of *Camellia japonica* and evaluated the effect of inhibitors of ethylene action on their vase life. Cut flowering branches were shipped from private companies, mimicking a long and short supply chain. Plant materials were treated with commercial Chrysal, gaseous 1-MCP, and 1-MCP included in β-cyclodextrin-based nanosponges and compared to cut branches exposed to exogenous ethylene (1 ± 0.2 µl l^{-1}) for 24 h and control in tap water. Visual checks for symptoms of variation in senescence, flower opening stage, flower abscission, leaf chlorophyll content (SPAD) and gloss, in addition to ethylene production levels were monitored daily. Cut branches placed in tap water lost ornamental value after *ca* 5.5 days of vase life and reached complete senescence after about 10 days in all the studied cultivars, regardless of supply chain, with the exception of 'Il Tramonto' with 10 and 20 days, respectively. Exogenous ethylene application reduced camellia cut branch longevity in 'Debbie' and 'R.H. Wheeler', while the other cultivars lasted as long as the control. Anti-ethylene treatments prolonged the vase life only in 'Korun Koku' and 'Il Tramonto'.

1. Introduction

Camellia japonica L. is an important ornamental shrub commonly used as potted plant and for landscaping (Accati *et al*., 2006; Scariot *et al*., 2007). Generally, camellia flowers on the plants last for seven to ten days, and flower senescence is mainly characterised by browning regions on the petals and by floral organ abscission. On the contrary, when flowers are removed from the plant, they usually wilt quickly unless they are held at low temperatures and high humidity (Bonner and Honda, 1950). For this reason the use of camellia as cut flower has been restricted to situations where longevity is not especially important, even if the deep and bright green foliage with a high number of flower buds make camellias potentially appreciated also as cut flowering branches. Only a few studies have been performed on this topic. In the 1950's considerable efforts were made to find treatments to extend camellia cut flower vase life by Cothran (1958). This author showed that poor water relations lead to early wilting of cut camellia blooms. Therefore, most of the efforts were directed towards the reduction of vapour pressure deficit or to prevent water loss from the petals. For better conservation, Threlkeld (1962) suggested harvesting flowers with no stem, holding them at high humidity. More recently, Doi and Reid (1996) reported that in the first day of vase life, the camellia flower is unable to acquire sufficient water from the vase solution to supply the needs of petal expansion and transpiration.

The role of ethylene in camellia senescence have been also envisaged. Woolf *et al*. (1992) reported that abscission of camellia flower buds was induced by a foliar spray with 2-4 ml l^{-1} ethephon [(2-chloroethyl)phosphonic acid] and that the vegetative buds and leaves were less sensitive to ethephon than flower buds. Doi and Reid (1996) used silver thiosulphate (STS) to prevent flower abscission.

The negative effects of ethylene can be significantly delayed by treatment with inhibitors of ethylene action, such as 1-methylcyclopropene (1-MCP) (Serek *et al*., 2006). The possible value of such treatment in improving the life of cut camellia flowering branches has not been explored. Recently, a non-volatile formulation of 1-MCP in β-cyclodextrin-based nanosponges (β-CD-NS 1:8) was developed (Seglie *et al*., 2011 a, b). This structure, supplied in the conservation solution, prolonged the vase life of several cut flowers better than gaseous 1-MCP (Seglie *et al*., 2013).

Since there is a lack of knowledge on postharvest characteristics of *C. japonica* cut flowers and foliage, the objectives of this study were to evaluate the role of ethylene in postharvest flower and leaf abscission of seven cultivars

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of *C. japonica* and investigate the effect of inhibitors of ethylene action (commercial Chrysal, commercial 1-MCP and 1-MCP included in β-cyclodextrin-based nanosponges) on camellia flowering branch vase life.

2. Materials and Methods

Flower budded branches (50 cm in length) of seven *C. japonica* cultivars were supplied to the postharvest laboratory of the Department of Agricultural, Forest and Food Sciences (DISAFA) at the University of Torino (Italy) as listed in Table 1. The long supply chain (three days long) was simulated as follows: (1) cut branches were harvested at Verbania (Italy) at two companies (F.li Savioli and Società Cooperativa Val Intrasca) and kept in cold storage overnight; (2) transportation to the flower market of Sanremo (Imperia, Italy) by refrigerated truck; and (3) shipment to DISAFA the next day. In the short supply chain (one day long), cut branches were collected at Verbania and then directly transferred to DISAFA.

At DISAFA, branches were recut to 20 cm length and ten branches per treatment were used. Three treatments were compared with a tap water control: Chrysal Professional 2 (10 ml l^{-1}) for 24 h followed by the application of Chrysal Professional 3 (10 mg l^{-1}) for all the experiment; commercial gaseous 1-MCP (3.3% ai, SmartFresh™, AgroFresh Inc., USA) (0.25 µl l^{-1}) for 6 h in a gas-tight cabinet (112 l); and β-cyclodextrin-based nanosponge - 1-MCP complex (β-CD-NS 1:8) (0.25 µl l^{-1}). Ten branches were also sealed in a box (112 L) and exposed to exogenous ethylene (1±0.2 µl l^{-1}) for 24 h. Subsequently, six branches per cultivar were individually enclosed in sealed tubes (1 L) containing 300 ml tap water, to quantify the daily production of endogenous ethylene.

The study was undertaken during spring 2012 in a controlled room at 20±2°C day/night temperature, 60% relative humidity, and 46 µmol m^{-2} s^{-1} cool white light (meter model HT307; HT, Faenza, Italy).

Every day, the postharvest performance of the branches was evaluated considering both flowers and foliage characteristics, according to the following factors: visual check for symptoms of variation in senescence, flower opening stage, flower abscission, leaf chlorophyll content (SPAD), gloss, and endogenous ethylene production. Variation in senescence was rated on a scale from 0 to 2, in which 0 = no visible senescence, 1 = initial senescence, and 2 = complete senescence. The loss of ornamental value of branches was considered when they reached level 1 on the scale (Seglie *et al*., 2010). Variation in flower opening stage was evaluated on the basis of the following scale: 1 = initial opening, 2 = half opening, and 3 = full opening (Guo *et al*., 2004, modified). Chlorophyll content was indirectly measured in leaves through a Chlorophyll Meter SPAD-502 instrument (Konica Minolta Sensing Inc., Osaka, Japan). Gloss variation was measured in leaves using a Spectrophotometer CM-2600 (Konica Minolta Sensing Inc., Osaka, Japan).

Ethylene concentration was monitored by a digital Agilent Technologies gas chromatograph, 6890N Network GC system (Santa Clara, California). The gas carrier was N_2 at 40 ml min^{-1}, and column temperature was 60°C.

Photo-documentation at different stages of senescence was carried out. Data were registered until all the cut branches appeared completely damaged. Petal, flower, and bud abscission percentages were mathematically transformed using arcsin√P/100. Differences were initially subjected to the homogeneity of the variance test and then the analysis of variance (ANOVA) was established using Ryan-Einot-Gabriel-Welsch-F (REGW-F) post-hoc test. The critical value for statistical significance was $P < 0.05$. All the data were computed by means of the SPSS statistical package (version 21.0; SPSS Inc., Chicago, Illinois).

Table 1 - The studied *Camellia japonica* cultivars and their supply chain length

Cultivar	Supply chain
´Korun Koku´	Long
´Debbie´	Long
´Bonomiana´	Long
´Colonel Firey´	Long
´Charles Cobb´	Short
´R.H. Wheeler´	Short
´Il Tramonto´	Short

Long= 3 days; Short= 1 day.

3. Results

Camellia cut flowering branches placed in tap water lost ornamental value after approximately 5.5 days of vase life and reached complete senescence after about 10 days in all studied cultivars, regardless of supply chain, with the exception of 'Il Tramonto' (Fig. 1) in which senescence symptoms started to appear after 10 days and complete senescence was reached at day 20. Exogenous ethylene application reduced camellia cut branch longevity in 'Debbie' and 'R.H. Wheeler', while the other treated cultivars lasted as long as the control. Only in 'Charles Cobb' did cut branches treated with exogenous ethylene last longer, reaching complete senescence at day 14. The two anti-ethylene treatments (1-MCP and β-CD-NS) similarly prolonged vase life up to 10 days, slightly better than Chrysal in 'Korun Koku' and 'Colonel Firey'. On the contrary, Chrysal outperformed the anti-ethylene treatments in 'Il Tramonto', prolonging its vase life up to 27 days.

Flowers fully opened on branches of 'Korun Koku' treated with β-CD-NS, 'Debbie' treated with both Chrysal and 1-MCP, 'Bonomiana' in tap water, 'Colonel Firey' treated with both exogenous ethylene and 1-MCP, and 'Il Tramonto' in all treatments with the exception of β-CD-

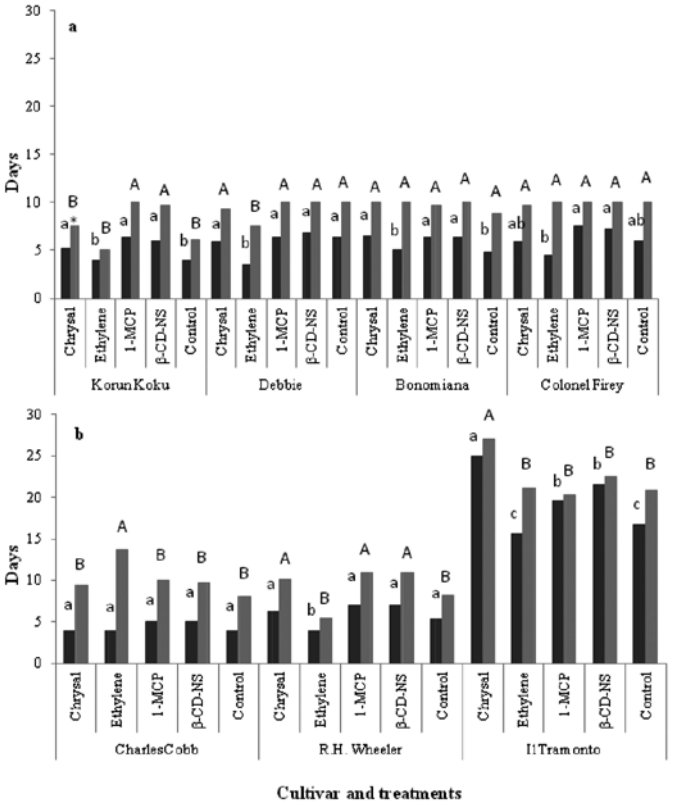

Fig. 1 - Cut flowering branch longevity of the studied *Camellia japonica* cultivar according to five treatments and two supply chains (a. Long, b. Short). The number of days to reach the initial stage of senescence (stage 1, black bar) and complete senescence (stage 2, grey bar) was measured. Mean separation within bars of stage 1 (lower case) and stage 2 (upper case) were computed by the Ryan-Einot-Gabriel-Welsch's multiple stepdown *F* (REGW-F) test, $P \leq 0.05$.

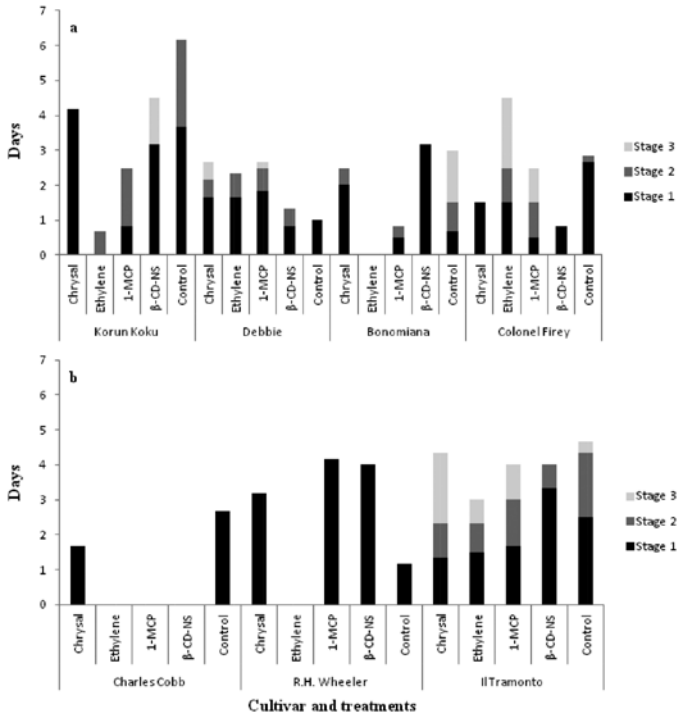

Fig. 2 - Variation in flower opening stages (stage 1 = initial opening, stage 2 = half opening, and stage 3 = full opening) of the studied *Camellia japonica* cultivar according to five treatments and two supply chains (a. Long, b. Short).

NS (Fig. 2). Branches of 'Charles Cobb' and 'R.H. Wheeler' reached, at maximum, stage 1 (initial opening).

Exogenous ethylene increased petal, flower, and bud abscission more than control in 'Korun Koku' (16.67% of flowers), 'Bonomiana' (100% of buds), 'Colonel Firey' (100% of petals and 100% of flowers), and 'Il Tramonto' (50% of buds) (Table 2). Among the other treatments, an increase in abscission was noted in branches of 'Bonomiana' and 'Colonel Firey' treated with β-CD-NS (25 % of flowers and 75 % of petals, respectively), in 'Charles Cobb' and in 'Il Tramonto' treated with Chrysal (25 and 75% of flowers, respectively), and in 'Il Tramonto' also treated with 1-MCP (50% of buds). However, no correlations were found between abscission levels and applied treatments.

Generally, leaf SPAD and gloss values were not affected by treatments. Only in 'Korun Koku', 'R.H. Wheeler', and 'Il Tramonto' were slight differences found. In 'Korun Koku' Chrysal outperformed the anti-ethylene treatments, and conversely in 'R.H. Wheeler' and 'Il Tramonto'.

Figure 3 shows differences in ethylene production among the studied *Camellia* cultivars. A general trend was observed during the experiment with an ethylene increase until day 11. 'Korun Koku', 'Colonel Firey', and 'Bonomiana' produced up to 1.42, 1.10, and 1.04 µl l^{-1}, respectively. On the contrary, in 'Charles Cobb', 'Debbie', and 'R.H. Wheeler', 0.75, 0.67 and 0.66 µl l^{-1} were recorded, respectively. 'Il Tramonto' followed a similar but postponed trend. This latter cultivar started to produce ethylene at day 10 until day 24. Also the ethylene production was much higher (2.10 µl l^{-1} at day 20).

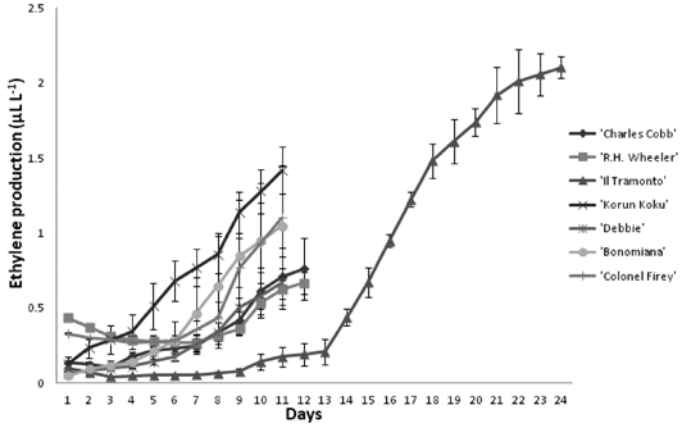

Fig. 3 - Mean values of the endogenous ethylene production in the studied cut flowering branches of *Camellia japonica* cultivars placed in tap water.

Table 2 - Petal, flower, and bud abscission of the studied *Camellia japonica* cultivars subjected to five treatments and measured at the end of the experiment

Cultivar	Treatments	Petal abscission (%)	Flower abscission (%)	Bud abscission (%)
	Chrysal	35.71 b [z]	0.00 b	35.71 b
	Ethylene	100.00 a	16.67 a	83.33 a
´Korun Koku´	1-MCP	60.00 ab	0.00 b	60.00 ab
	β-CD-NS	66.67 ab	0.00 b	66.67 ab
	Control	44.44 ab	0.00 b	44.44 ab
	P	*	**	*
	Chrysal	66.67 a	16.67 ab	50.00 ab
	Ethylene	100.00 a	66.67 a	33.33 ab
´Debbie´	1-MCP	20.00 b	0.00 b	20.00 b
	β-CD-NS	87.50 a	4.17 b	83.33 a
	Control	100.00 a	37.50 ab	62.50 ab
	P	**	*	*
	Chrysal	27.27	27.27 b	0.00 b
	Ethylene	100.00	0.00 b	100.00 a
´Bonomiana´	1-MCP	18.75	0.00 b	18.75 b
	β-CD-NS	25.00	25.00 a	0.00 b
	Control	14.29	0.00 b	14.29 b
	P	NS	**	**
	Chrysal	11.11 b	0.00 b	11.11
	Ethylene	100.00 a	100.00 a	0.00
´Colonel Firey´	1-MCP	25.00 b	0.00 b	25.00
	β-CD-NS	75.00 a	0.00 b	75.00
	Control	12.50 b	0.00 b	12.50
	P	**	*	NS
	Chrysal	75.00	25.00 a	50.00
	Ethylene	80.00	0.00 b	80.00
´Charles Cobb´	1-MCP	100.00	0.00 b	100.00
	β-CD-NS	100.00	0.00 b	100.00
	Control	100.00	0.00 b	100.00
	P	NS	*	NS
	Chrysal	100.00 a	10.00	90.00 a
	Ethylene	37.50 b	0.00	37.50 b
´R.H. Wheeler´	1-MCP	41.67 b	0.00	41.67 b
	β-CD-NS	54.55 b	0.00	54.44 b
	Control	100.00 a	0.00	100.00 a
	P	***	NS	**
	Chrysal	75.00	75.00 a	0.00 b
	Ethylene	50.00	0.00 c	50.00 a
´Il Tramonto´	1-MCP	75.00	25.00 b	50.00 a
	β-CD-NS	33.33	33.33 b	0.00 b
	Control	20.00	20.00 b	0.00 b
	P	NS	*	**

[z] Different letter indicates significant differences at the 0.05 level, Ryan-Einot-Gabriel-Welsch (F) post hoc test.

4. Discussion and Conclusions

Camellias are important ornamental species worldwide. Their morphological (Corneo and Remotti, 2003) and genetic (Ueno *et al*., 1999; Caser *et al*., 2010) diversity as well as multiplication techniques and cultivation practices (Larcher *et al*., 2011; Berruti and Scariot, 2013) were previously evaluated for their breeding and production.

In this study, we investigated camellia attitude to be used as cut flowering branches. In 2001, van Doorn stated that senescence performance and ethylene sensitivity are highly species related. Here, we observed a great variability among cultivars, similarly to what was found in buttercup by Kenza *et al*. (2000), Scariot *et al*. (2009), and Seglie *et al*. (2013), in rose by Chanami *et al*. (2005), in peony by Hoffman *et al*. (2010), and in bluebell by Scariot *et al*. (2008). Exogenous ethylene application in this study anticipated the senescence only in 'Debbie' and 'R.H. Wheeler'. Anti-ethylene treatments prolonged the vase life only in 'Korun Koku' and 'Il Tramonto'. Therefore, precautions against exposure to ethylene cannot be generalised for camellia cultivars. The provided data could be of benefit to growers and those involved in shipping.

In conclusion, 'Il Tramonto' was the most interesting genotype as cut branches. This cultivar kept its ornamental value up to 27 days (when treated with Chrysal), its flowers fully opened, and its foliage maintained colour and brightness during the vase life.

This information could be useful to bring new produce to the flower market and to stimulate further research on postharvest techniques for camellia flowering branches.

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Soilless cultivation of cherry tomato with gutter subirrigation and reused substrate

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Key words: closed system, coconut fiber, horticulture, *Solanum lycopersicum* L.

Abstract: Soilless cultivation systems in horticultural production are modern technologies that involve the supply of water and minerals through the nutrient solution and plant growth on media. The soilless cultivation of vegetables ensures higher yields and better quality than with traditional soil crops. When managed in a closed system with irrigation water of good quality soilless cultivation can significantly reduce the environmental impact of nutrient solutions with respect to crops grown in open systems. Compared with drip irrigation, gutter subirrigation simplifies the management of the closed system because disinfection of recirculated nutrient solution and correction of its chemical composition is not necessary. Gutter subirrigation was developed in previous experiments for the cultivation of tomato on fresh substrate. The objective of this work was to evaluate the use of the same substrate after two consecutive crops of tomato and to study the effect of the presence, if any, of previous radical residues with the same cultivar and rootstock. The experiment was conducted in an unheated iron and polycarbonate greenhouse with forced ventilation. Cherry tomato plants were transplanted into pots containing fresh or reused coconut fiber. The following parameters were considered: biomass, production, product quality (electrical conductivity, pH, titratable acidity, dry residue, dissolved solid content) and chemical analysis of recirculated and radical nutrient solution. The results obtained in this experiment reveal no significant differences in production and fruit quality between plants grown on fresh substrate and those grown on reused substrate (marketable yield was 5.4 kg m^{-2} vs 5.3 kg m^{-2}, respectively).

1. Introduction

Greenhouse soilless horticulture requires a high level of technology, considerable capital investment, and operators with adequate professional skills but it is highly productive, ergonomic, and uses water and space efficiently (Savvas, 2003; Resh, 2012).

Over the last few years research has aimed to achieve the most effective systems able to satisfy restrictions relating to the protection of ground water and soil.

In fact, the conversion of soilless culture to a closed system is associated with environmental policies established to reduce environmental pollution (Voogt *et al*., 2013). To avoid some of the risks (rapid spread of root pathogens, accumulation of Na and mismanagement of electrical conductivity - EC - and pH), disinfection of recirculated nutrient solution (NS) and amendment of its chemical composition are carried out. However, discharge of part of the recirculated solution is still required since NaCl, from the irrigation water, and organic compounds, from long cultivations, accumulate causing an inhibition of growth (Voogt *et al*., 2013). The difficulties associated with managing the recirculated NS hinder the widespread adoption of the closed system, especially in areas where protected agriculture is characterized by a low level of technology.

Subirrigation can simplify the closed loop management of the NS, because unlike the drip-irrigation system, the elements that are not absorbed by the plant do not accumulate in the recirculated solution but rather in the upper part of the substrate, where roots are less present (Venezia *et al*., 2001; Santamaria *et al*., 2003; Venezia *et al*., 2006; Venezia and Piro, 2007; Venezia, 2010). Spatial distribution of salts within the substrate makes it possible to minimize the effects of excessive salinity.

Only a few studies to date about soilless cultivations have assessed the possibility of reusing the substrate (Urrestarazu *et al*., 2008; Venezia *et al*., 2008). The use of inert inorganic substrates involves disposal problems that can be avoided by employing organic substrates. Among them, peat has been widely used in the Mediterranean area. In this context, it is necessary to identify ecologically-friendly alternatives, such as renewable organic substrates that can be reused for several crop cycles, which would provide great benefits to vegetable production.

In the case of reusing the substrate, the presence of roots from the previous crop may result in adverse effects on the development of the crop, especially because in soil-

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less cultivation farmers are specialized in and practice monoculture.

The aim of this work was to evaluate the utilization of ecologically-friendly coconut fiber substrate for repeated crops of cherry tomato cultivated in closed loop gutter sub-irrigation.

2. Materials and Methods

The experiment was conducted at CREA Vegetable Crops Research Centre in Pontecagnano, southern Italy (40°38' N, 14°52' E, 28 m asl).

Cherry tomato plants (*Solanum lycopersicum* L., cv Shiren - Cois '94 - rootstock Beaufort - De_Ruiter) were grown in a closed-loop soilless system with gutter subirrigation in an iron-type greenhouse with polycarbonate covering under natural light conditions. Air temperature thresholds for ventilation and cooling were 25 and 26°C, respectively.

A fresh bulk coconut fiber substrate was compared with the same medium previously utilized for two cropping cycles of cherry tomato Shiren/Beaufort. The latest cultivation on the reused substrate was terminated 40 days before the experiment started. Plots were composed of one row of 20 pots per trough for the fresh substrate and 17 for the reused one. The pots, which contained 10 L of substrate, were positioned on hydraulically independent gutter with 1% slope.

For each gutter the NS was contained in an independent storage tank of 270 L. The ionic compositions of the NS supplied expressed in meq L^{-1} were:

- NS T1, used for irrigation before transplant in the fresh substrate: 0.3 Na^+, 0.4 $NH4^+$, 2.5 K^+, 4.9 Mg^{++}, 8.9 Ca^{++}, 0.4 Cl^-, 11.5 NO_3^-, 2.2 $H_2PO_4^-$, 2.2 SO_4^{--}. EC and pH values were 1.8 dS m^{-1} and 5.5, respectively.
- NS 0,3: 0.3 Na^+, 0.4 NH_4^+, 4.0 K^+, 1.5 Mg^{++}, 4.4 Ca^{++}, 0.2 Cl^-, 6.8 NO_3^-, 0.8 $H_2PO_4^-$, 1.8 SO_4^{--}. EC and pH values were 1.2 dS m^{-1} and 5.7, respectively.
- NS 0,6: 0.3 Na^+, 0.1 NH_4^+, 8.0 K^+, 3.0 Mg^{++}, 6.0 Ca^{++}, 0.3 Cl^-, 13.5 NO_3^-, 1.5 $H_2PO_4^-$, 1.2 SO_4^{--}. EC and pH values were 2 dS m^{-1} and 5.7, respectively.

During the first month of growth, plants were irrigated with NS 0,3; NS 0,6 was used in the second month; subsequently NS 0,6 and NS 0,3 were used alternately in order to maintain a constant EC of 1.5 dS m^{-1} until the end of the cycle.

Plants were transplanted on 17 August 2013, trained to a single stem and grown up to the seventh truss at a density of 2.6 plants m^{-2}. The experiment ended on 17 December 2013. A bumblebee colony was provided to aid pollination.

Consumption, EC and pH of the NS were recorded daily. Ionic composition of the recirculated NS was determined fortnightly, with samples taken after refilling the tanks.

Electrical conductivity, pH and ionic composition of the aqueous extracts (1:1.5 v/v - growth medium:deionized water) from three layers of the substrate (0-5, 5-17 and 17-21 cm from pot bottom) were determined after harvesting. Each layer was mixed well with water for 20 min and then filtered before measurements.

Electrical conductivity and pH values of all samples were determined by Metrohm 856 Conductivity Module; ionic composition by ion chromatography using Dionex ICS-1500/ICS-1600 RFIC.

The leaf chlorophyll concentration was determined weekly on the fifth true leaf of four plants per plot by using a hand-held SPAD-502 meter device that estimates chlorophyll *in vivo* by transmittance of red (650 nm) and infrared (940 nm) radiation through the leaf, and calculating a relative SPAD meter value that should "correspond to the amount of chlorophyll present in the same leaf" (Minolta, 1989).

Dry above-ground biomass was determined on two plants per plot. Plant organs were separated into fruits, stems, leaves, and roots and dried in a forced-air oven at 80°C for 72 h.

Fruit quality was assessed by measuring the EC, pH, titrable acidity, dissolved solid content and dry residue of blended fruit. To determine titrable acidity and dissolved solid content (°Brix) the 905 Titrando-Metrohm and the Refracto 30 PX-Mettler Toledo were used, respectively. To determine the dry residue, the samples of blended tomato berries were dried on ceramic plates at 65°C for 72 h.

3. Results and Discussion

The daily consumption of NS was highest at mid-cycle (1 L per plant per day) and there were no differences between the two treatments. With the recirculated NS, there were no significant effects of substrate reuse on EC. The average EC value was 1.3 dS m^{-1} in the first month after transplantation, it then increased to 1.7 dS m^{-1} in the second month and gradually decreased until the end of the cycle up to a value of 1.5 dS m^{-1} according to the management and composition of the NS supplied. In aqueous extracts from the three layers sampled at the end of the cycle, the EC of fresh substrate tended to increase from the base (0.7 dS m^{-1}) towards the upper part of the vessel (2.5 dS m^{-1}). In pots with reused substrate, EC values were higher for both the intermediate (2.6) and top (9.6 dS m^{-1}) layer. This confirms that excess salts tend to accumulate upwards due to capillary rise. In aqueous extracts of the three layers, the concentration of all elements increased towards the upper part of the vessel and more intensely in the reused vessels.

The initial average pH value of the recirculated NS was 5.7 and increased up to 6.3 for both treatments during the first 30 days after transplantation (DAT). With reuse in the advanced stage of the cycle (80 DAT), the pH of the recirculated solution decreased (5.7); the pH value was 6.2 for fresh and 5.7 for reused substrate at the end of the cycle. Regarding aqueous extracts, pH values were lower in the upper layer: in pots with fresh substrate pH was 6.9 for the bottom and 5.9 for the top layer; in reused substrate, the average value was 6.5 for the bottom and 6.3 for the top.

For both treatments the initial average SPAD value was 39.1; at 75 DAT for plants cultivated on fresh substrate it was on average 41.7, and 37.9 on reused substrate. At the end of the cycle, SPAD decreased to a value of 32.3 for both.

Reuse of substrate resulted in a slight decrease in production (3.4%): the average marketable yield was 5.4 kg m^{-2} on fresh substrate and 5.3 kg m^{-2} on reused substrate. The effect of reuse was minimal (3.7%) also on fruit weight with an average of 22.6 g on fresh and 21.7 g on reused substrate noted. With an average value of 106 g/m^2 plants cultivated on reused coconut fiber showed a negligible increase of stem biomass (0.2%); also fruit biomass suffered a small change from 498 g/m^2 to 519 g/m^2 while leaf biomass was reduced by 12.2% with an average value of 266 g/m^2 and 233 g/m^2 on fresh and reused substrate, respectively.

Titratable acidity, pH and °Brix decreased linearly with the order of the cluster: pH average value was reduced from 4.17 to 4.10; titrable acidity from 0.61 to 0.51% and °Brix between 5.5 and 4.3. Electical conductivity and dry residue had a more constant trend ranging between 4.54 - 4.21 dS $m^{-1}$and 6.9-6.6%, respectively.

Urrestarazu *et al*. (2008) grew a tomato crop on almond shell residue reused for 530 days (after four crops) in an open, drip irrigated system without significant yield and fruit quality parameter differences compared to the fresh substrate. They did not encounter phytotoxicity problems because the run off from the open system eliminated root exudates and excess residual salts from previous tomato crops as observed in a tomato crop grown on a substrate added with a compost which was very rich in salts (Stipic *et al*., 2012).

In our work the system was closed with zero run off and for the subirrigation, salts and root exudates of the reused substrate accumulated in upper layers due to capillary rise, but still remained in the system. This spatial distribution and the presence of a suitable microflora allowed a normal yield compared to the fresh substrate despite the intense monoculture after two consecutive crops of tomato with the same cultivar and rootstock in pots containing 10 L of substrate with all previous radical residues.

The experiment is still underway with a tomato crop growing on a coconut fiber substrate reused after four tomato crops to confirm these results and to characterize the microflora.

4. Conclusions

This work has demonstrated that it is possible to carry out a soilless monoculture of tomato with gutter subirrigation, a technique still not widespread for the cultivation of fruit vegetables. Reuse of the coconut fiber did not induce phytotoxicity and there were no significant effects on the quantity and quality of the product obtained. Subirrigation allows all the excess ions to accumulate in the upper part of the substrate; in this way there is no increase of the EC in the lower part of the vessel where roots are massively present nor is there alteration of the re-circulated nutrient solution composition.

This confirms that, compared with traditional drip irrigation, gutter subirrigation with coconut fiber simplifies management of the re-circulated nutrient solution in soilless closed loop systems of a fast-growing species such as cherry tomatoes. The comparison between fresh and reused substrate showed that it is possible to reuse coconut fiber at least three times, thus reducing costs in terms of production and the environment.

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Effect of short heat treatments with a sodium bicarbonate solution on storability of the yellow germoplasm plum 'Meloni'

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Key words: decay control, GRAS compounds, *Penicillium expansum*, quality.

Abstract: The behavior of a Sardinian plum, cv. Meloni, was investigated with regard to short-heat treatments at 20, 50, 55 or 60°C in water with 0 or 2% $NaHCO_3$ (SBC) for 0, 15, 30, 45 or 60 seconds. Fruits were stored for one month at 5°C and 95% RH followed by a simulated marketing period (SMP) at 20°C and 80% RH for six days. Quality and decay percentage were monitored. In addition, fruits were artificially inoculated with *P. expansum* and stored for 10 days at 25°C and 95% RH. Compared to the control, all short-heat dip treatments lowered the degree of decay, and the efficacy was positively correlated with temperature and treatment duration. The use of SBC increased the efficacy of decay control and the best results were attained at 55 and 60°C. Heat treatments increased levels of total flavonoids and antioxidant activity after SMP. None of the heat treatments induced rind damage (browning or discoloration), but the overall appearance decreased significantly when fruit was treated at 55 or 60°C for 60 s after SMP. Scanning electron microscopy (SEM) observations showed that treatments at 55 and 60°C with SBC cause damage and loss of cuticular wax on fruit surface.

1. Introduction

In the past century, the use of synthetic agro-chemicals has contributed to the exponential increase of food production, particularly in industrialized countries. However, it is more and more evident that safeguarding human health and the environment depends upon an appropriate use of synthetic chemicals in food and ecological chains (Frangenberg, 2000). As a result, new and more restrictive regulations have become mandatory, significantly limiting the use of agro-chemicals. Among fungicides, only a few are authorized in postharvest and the levels of residues are strongly restricted. In addition, European Union (EU) regulation establishes for some crops (*e.g.* stone fruits) that postharvest treatments with conventional agro-chemicals is not allowed (European Commission, 2008). Furthermore, the control of postharvest diseases may be jeopardised by the outbreak of resistant biotypes of postharvest fungal pathogens to fungicides (Holmes and Eckert, 1999; Ma *et al*., 2003). This is a real challenge for highly perishable crops such as stone fruits (peach, nectarine, plum and prune) where postharvest physio-pathological disorders can inflict severe economic losses. In recent years, alternative control of postharvest diseases of fresh commodities has become a chief field for research (González-León and Valenzuela, 2007; Palou *et al*., 2008). Among the investigated alternatives, having hardly any restriction, are some food additives, preservatives and generally recognized as safe (GRAS) compounds that provide an acceptable control of decay, although lower than synthetic fungicides (Palou *et al*., 2009; Molinu *et al*., 2010). The efficacy of these agents was shown to depend upon the commodity and the pathogen. Indeed, carbonic acid salts, especially sodium carbonate and bicarbonate, proved to be fungistatic and to control *in vivo Penicillium digitatum* Sacc., the agent of citrus green mould, whereas *P. italicum*, the agent of citrus blue mould, was much lesser affected, especially with mandarin fruit (Smilanick *et al*., 1999; Palou *et al*., 2002). Palou *et al*. (2009) evaluated, in a primary *in vivo* screening, the efficacy of several safe compounds against the major postharvest pathogens of stone fruits and found that sodium carbonate exerted an inhibitory activity on most pathogens. However, when applied at 20°C for 1 m in a small-scale *in vivo* trial, the efficacy and persistence resulted unacceptable, whereas when solutions were heated to 55 or 60°C an increased efficacy was attained, but it was not superior to that of the heated water alone. Brief hot water treatment was employed by Karabulut *et al*. (2010) to control *Monilinia fructicola* on artificially inoculated Californian plums and they observed that hot water applied at 60°C for 60 s completely inhibited the disease after five days of incubation at 20°C ad 90% RH.

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Conducting a comparative study between local prune and international plum varieties, Molinu *et al*. (2010) found that decay was significantly reduced on prunes but not plums when adding 2% SBC to water heated at 55°C. The results attained with plums, nectarines and peaches are in contrast with those attained with *Citrus* fruits where a synergic interaction increased the efficacy of heated salt solutions. In *Citrus* fruits, in addition to the direct fungistatic activity of carbonic acids, a clear induction of natural resistance contributed to increase decay control efficacy (Venditti *et al*., 2005). This aspect has received slight attention in pome and stone fruits treated with GRAS compounds. In addition, limited data are available on the effect of postharvest treatments with GRAS compounds on the quality of prune fruit and no literature is available concerning changes in bioactive components during storage. In order to improve our understanding on the effect of postharvest treatments with heated GRAS solutions, a factorial design experiment was carried out employing 2% SBC with immersion duration and water temperature as quantitative variables.

2. Materials and Methods

Plant material and processing

Fruit. European plum or prune fruit (*Prunus domestica* L. cv. Meloni) was harvested at commercial maturity in an *ex situ* germplasm conservation orchard belonging to the ISPA-CNR located in Oristano (Sardinia - Italy). After harvest, fruits were selected and randomized in order to obtain homogeneous sets. The fruit surface was disinfected by 2 min. immersion in a 1% (v/v) commercial sodium hypochlorite solution, followed by a deionised water rinse and finally air drying.

Treatment and storage. The sets of fruit were arranged in a 3-factorial randomized complete block design (RCBD) (2x5x4) with two treatments (with 0 or 2% SBC), five immersion times (0, 15, 30, 45 or 60 s) and four solution temperatures (20, 50, 55 or 60°C). Each set was made up of three replicates of 30 fruits. After treatment, fruit was left to dry and then stored at 5°C and 95% RH for one month, followed by a six-day simulated marketing period (SMP) at 20°C and 80% RH.

Inoculation treatments. Artificial inoculation was performed with a wild isolate of *P. expansum* Link, obtained from a decayed plum ('Stanley') subjected to a two-month storage at 2°C. The inoculum was obtained from a 12-day-old sporulating culture by adding 5 mL of sterile water with 0.05% (v/v) Tween 80 and gently scrubbing the agar. After filtering, the spore concentration was determined using a haemacytometer and concentration adjusted to $1x10^4$ conidia/mL with sterile water.

The artificial inoculation experiment was performed by wounding, once, 1200 fruits at the equatorial area with a sterile stainless rod (2 mm wide by 2 mm deep) and by introducing, 1 h later, 15 µL (~ 150 conidia) of the inoculum into half of the wounds (20 sets of 30 fruits), and into the remaining fruits (20 sets of 30 fruits) 15 µL of distilled water (controls). One hour later, fruit was treated according to the experimental plan. When dry, each set was placed into a plastic container, covered and stored at 25°C and 95% RH in the dark. The percentage of decay was monitored after 10 days and treatment efficacy was expressed as inhibition percentage compared to control.

Weight loss and appearance. Mass change of prunes, expressed as % of weight loss compared to harvest weight, was determined on 30 fruits after storage and SMP.

Visual quality was evaluated by employing a rating scale composed of five categories (9, excellent; 7, very good; 5, good, limit of marketability; 3 fair, limit of usability; 1, very poor, unusable) (Chena and Zhub, 2011). The extent of quality loss was described as an index, which was determined by summing the products of prunes in each category by the value of each category, and then dividing this sum by the total number of prunes assessed.

Chemical analysis

pH, Titratable acidity and Total soluble solid content. All chemical analyses were performed on centrifuged and filtered juice obtained by homogenizing de-stoned prunes using a blender. pH, titrable acidity (TA) and total soluble solid content (TSS) were carried out in three replicates of ten fruits each at harvest, after cold storage, and after SMP. A 5 mL sample of juice was titrated with 0.1 N sodium hydroxide to an end point of pH 8.2 using a digital pH meter (ORION, model 420A) and the acidity was calculated as % of malic acid content (g/100 mL of juice). Soluble solids concentration was measured using an ATAGO 0-32°Brix temperature compensating refractometer (Atago Co., Japan). After storage and SMP fruit was rinsed in deionised water before analysis.

Antioxidant activity, Total flavonoids and Phenolics. The total antioxidant activity, total flavonoids and phenolics were measured at harvest, after storage, and SMP in fruit immersed for 60 s in water at 20 and 55°C, with or without SBC.

Antioxidant activity (AA) was measured using two different spectrophotometer methods: ABTS and DPPH assay according to Surveswaran *et al.* (2007). For each assay, 0.1 mL of diluted juice (1:10 in water) was used, a calibrated standard curve with Trolox (3-15 µM; R_2=0.992 for DPPH assay and R_2=0.998 for ABTS assay) was made and results expressed as TEAC units (mmol Trolox equivalents per 100 g of fruit). For both assays, absorbance was recorded with an Agilent spectrophotometer (8453 UV-Visible Spectrophotometer, Agilent Technologies, Palo Alto, CA, USA). Samples were analysed in triplicate.

Total flavonoids (TF) were determined according to the colorimetric assay described by Kim *et al.* (2003). An aliquot of diluted juice was used for the assay and the TF in samples were quantified by catechin calibration curve (2.5-20 µg/ml, R_2=0.999). The absorbance was measured at 510 nm with an Agilent spectrophotometer (8453 UV-

Visible Spectrophotometer, Agilent Technologies, Palo Alto, CA, USA). The results were expressed as mg of catechin equivalent (CE) per 100 g of fruit. Analyses were performed in triplicate on each sample.

Total phenolics (TP) levels were measured in juice using the Folin-Ciocalteu assay (Singleton and Rossi, 1965). Aliquots of diluited samples were mixed with Folin-Ciocalteu reagent (1:1) and 10 mL of 7.5% sodium carbonate in a 25-mL volumetric flask. Reaction mixture was incubated for 120 min at room temperature and the absorbance measured at 750 nm with an Agilent spectrophotometer (8453 UV-Visible Spectrophotometer, Agilent Technologies, Palo Alto, CA, USA). Results were expressed as mg of gallic acid equivalents (GAE) per 100 g of fruit by means of a calibration curve of gallic acid (10-100 mg/L, R_2= 0.989). Samples were analysed in triplicate.

Scanning Electron Microscopy (SEM)

Prunes subjected to the different treatments were used to study the effect of the treatment on the cuticle and epicuticular waxes by means of SEM. Observations were performed, on replica of the fruit rind, adapting the methodology of Dore *et al.* (2010). Replicas of the same marked area were made on dry fruit before and after the treatment (1 and 30 d post-treatment). Samples were observed with a DSM 962 SEM (ZEISS, Oberkochen, Germany) at 20 kV.

Statistical analysis

A one-way or three-way analysis of variance was applied to data using OpenStat (2007). Data from disease incidence were transformed to the arcsine of the square root of the proportion of decayed fruit. When appropriate, means were separated by Fisher's protected least significant difference test with a significance level of $P = 0.05$. Synergy testing of the combined treatments was performed according to Plascencia-Jatomea *et al.* (2003), calculating the expected and registered efficacy by Limpel's equation: $E_e = X + Y - (XY/100)$, where E_e was the expected additive response to hot water treatment and BCS immersion, and X and Y were the percentages of inhibition relative to each factor (heat or SBC) used alone.

3. Results

Decays

Concerning the development of molds during the storage trial (Table 1), over 90% of natural infections were caused mainly by *P. expansum*, while the remaining 10% consisted of *M. fruticola*, *Botrytis cinerea,* and *Rhizopus stolonifer*. It is possible to observe that salt, temperatures, and immersion times significantly influenced decay control. As a general rule, decay control was improved by increasing the immersion duration and by heating the SBC solution. After storage, the lowest degree of decayed fruit was attained in the sets dipped in the SBC solution at 55 or 60°C for 45 s and at 60°C for 60 s. As a result, compared to untreated fruit (28.5% decay) those treatments significantly reduced the natural infection to 4.1, 3.8 and 7.1%, respectively. After the SMP decay augmented in all sets of fruit, however, the best control remained that attained by dipping the fruit for 45 s in the heated salt solution at 55 or 60°C (7 and 6.7% of rots) with about 80% reduction of decay compared to control (35.4% of rots).

Table 1 - 'Meloni' prunes with decay (%) after 1 month of storage at 5°C and 95% RH and 6 days at 20°C and 80% RH (SMP) when treated with (+) or without (-) a 2% $NaHCO_3$ solution at 20, 50, 55 or 60°C for 15, 30, 45 or 60 s or left untreated (Control) [z]

Temperature (°C)		Immersion duration (s)							
	Storage	15	15	30	30	45	45	60	60
	Control	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)
	28.5 aA								
20		21.0 bB	20.8 bB	22.1 bB	19.1 bB	22.1 bB	18.1 bB	21.1 bB	23.4 bB
50		20.4 bB	19.1 bB	22.4 bB	19.4 bB	18.8 bB	11.4 cC	15.1 cB	13.4 cC
55		22.4 bB	18.1 bB	19.4 bB	17.1 bB	10.4 cC	4.1 dD	15.8 cB	12.4 cC
60		19.4 bB	16.1 bcC	15.4 cC	10.4 cD	7.4 cD	3.8 dE	8.8 dC	7.1 dD
	SMP	15	15	30	30	45	45	60	60
	Control	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)
	35.4 aA								
20		26.7 bB	26.7 bB	26.0 bB	22.0 bC	26.0 bB	21.3 bC	25.0 bB	20.3 bC
50		26.3 bB	23.0 bB	25.3 bB	24.3 bB	22.7 bB	15.3 cC	20.0 dB	16.3 cC
55		26.3 bB	22.0 bB	23.3 bB	23.0 bB	15.3 cC	7.0d D	19.7 dB	15.3 cC
60		26.3 bB	19.0 bB	19.3 bB	15.3 cC	11.3 cC	6.7 dD	11.7 cC	10.0 cC

[z] Values are means with N=90 each time. Capital letters relate to comparisons within rows, lower case letters to comparisons within columns, different letters indicate differences at $P \leq 0.05$ according to Newman-Keuls test.

Inoculation treatments

The *in vivo* experiment with artificially inoculated fruit (Fig. 1) evidenced that the immersion for 15, 30 or 45 s in a 2% SBC solution at 20 or 50°C did not improve decay control compared to water dips. The same occurred when fruit was treated for 15 s at 55 or 60°C, indicating that with immersions up to 45 s no additive effects took place by heating the solution up to 50°C; we observed the same behavior at higher temperatures with 15 sec dips. It is interesting to note that synergistic interactions took place at 55°C for 30, 45, 60 s while no such interaction occurred at 60°C, except for the 30-s dip. The best result was obtained when fruits were immersed in SBC solutions at 55°C for 45 and 60 s with an increase of decay inhibition by 49 and 53% respectively compared to the heat treatment only (20 and 25% respectively).

Weight loss and appearance

Weight loss during storage and SMP ranged between 2 and 4% for control fruit. The immersion of fruit at 20°C, with or without SBC, had no effect on weight loss, while it increased when fruit was immersed in heated water, reaching a maximum of 9.5% after SMP with 60 °C for 60 s. A significant increase of weight loss took place when fruit was immersed in the SBC solution at 55 and 60°C for 45 or 60 s (8.7, 9.7, 11.3 and 13%, respectively).

The visual quality of fruit in the storage experiment evidenced a harmful effect on fruit when immersed in water at 55 and 60°C for 60 s (visual quality score between 2 and 3). The reason for this result was related to fruit shrivelling, which was particularly evident after SMP. The SBC solution was not the cause of shrivelling but contributed to increasing the impairment when employed at 55 or 60°C. Indeed, at 50°C no shrivelling was observed, while at 55°C it appeared when fruit was immersed for more than 30 s, and it resulted slightly more visible with the SBC solution.

pH, Titratable acidity and Total soluble solid content

At harvest the pH, TA and TSS (°Brix) resulted 3.69, 0.69 and 19.2, respectively. During storage the values changed slightly in untreated fruit, while after SMP the pH and TSS increased (3.84 and 21.6 °Brix, respectively) and TA decreased (0.56). With respect to untreated fruit (control), the different treatments did not affect the pH value, both during storage and SMP. Total acidity decreased during the SMP except for a notable increase observed in fruit of the set treated at 55 and 60°C for 60 s. Consistent increases of TSS occurred during SMP in fruit from sets immersed at 55°C for 45 or 60 s without SBC (24.2 and 23.1, respectively) or with SBC (22.3 and 23.7, respectively). On the other hand, fruit treated at 60°C, with or without SBC (20.2 and 20.1, respectively), had lower TSS compared to untreated fruit (control) or that treated at 55°C.

Antioxidant activity, Total flavonoids and Phenolics

Figure 2 shows the data regarding total antioxidant activity measured by the DPPH assay after storage and SMP in fruit immersed for 60 s in water at 20 and 55°C, with or without SBC.

With respect to harvest, the antioxidant activity decreased during storage and treatment temperature did not affect this trend nor did the SBC solution. Compared to the end of storage, an increase of the AA occurred in all sets of fruit after the SMP. The increase in heat treated fruit with or without SBC reached values higher than the AA at harvest. Data relating to Abts assay were well correlated with DPPH (r^2=0.70).

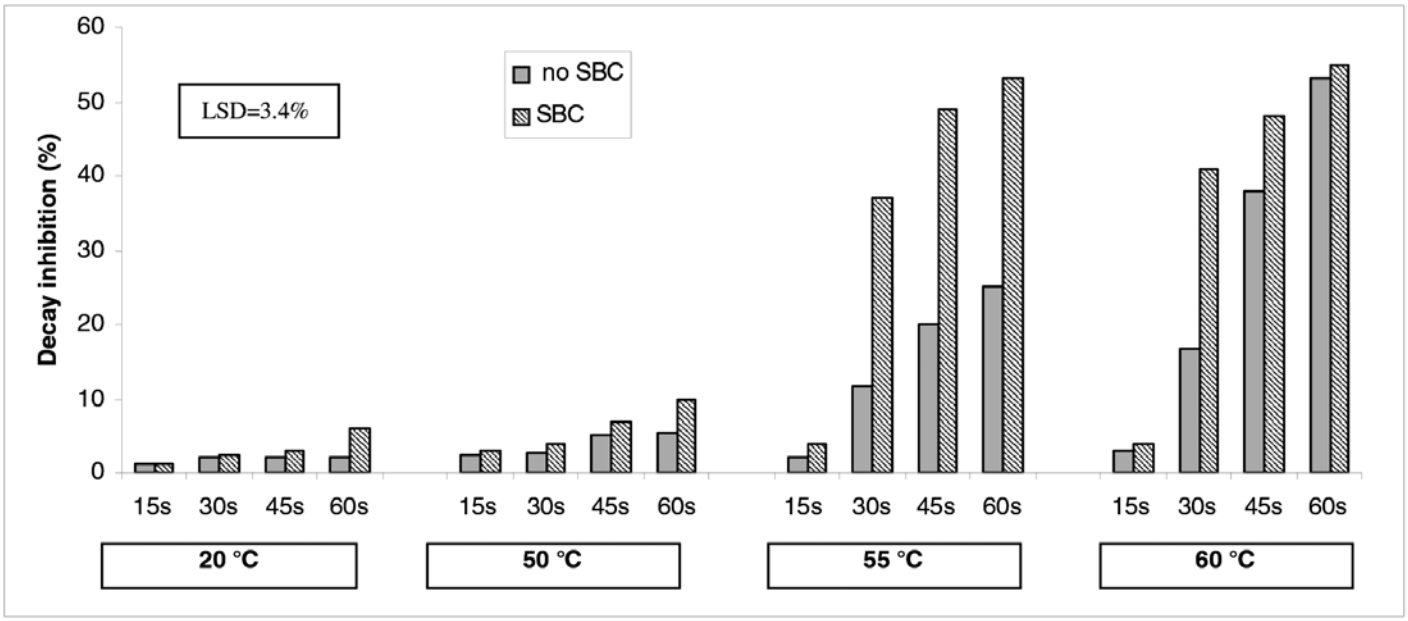

Fig. 1 - *P. expansum* inhibition (%) in 'Meloni' plum artificially inoculated 24 h before immersion at 15, 30, 45 or 60 s in water at 20, 50, 55, or 60°C with (SBC) or without (no SBC) 2% $NaHCO_3$ after 10 days at 25°C and 95% RH. Mean decay control= 90.5%.

The correlation between total polyphenols and total flavonoids resulted good (r^2= 0.70) and showed a similar trend with the AA values, thus increasing during the SMP and reporting higher values for heat treated fruit (data not shown). Following SMP the TF of fruit dipped at 55°C for 60 s, with or without SBC, resulted significantly higher (81.9 and 76.2 CE/100 g, respectively) compared to cold-water treated fruit (59.4 CE/100 g). Heat treatment increased both the TF and the AA values, measured by DPPH assay, attaining a high correlation (r^2= 0.80) between them, while the TP and DPPH values resulted less correlated (r^2= 0.63).

Scanning Electron Microscopy (SEM)

SEM observations of the fruit surface at harvest evidenced a constant layer of epicuticular wax with several wax plates (Fig. 3, micrograph A). After immersion for 45 or 60 s in water, at 50 and 55°C, the epicuticular wax layer was melted (B), while when immersed under the same conditions in the 2% SBC solution, new wax with a different crystalline structure appeared on the surface (micrographs C-D). When the immersion was performed for 45 or 60 s at 60 °C the cuticle was damaged by evident cracks near the stomata (E). When those conditions were applied with the 2% SBC solution, an erosion of the epicuticular wax was observed in most samples (micrograph F).

4. Discussion and Conclusions

The hot water treatments at 55 and 60°C with 2% of SBC were effective in controlling *P. expansum* decay during one month of storage at 5°C and the subsequent SMP in both experiments.

The most effective treatments remained those of dipping the fruit for 45 s at 55 or 60°C in SBC solution; this result confirms the findings reported by Molinu *et al.* (2010) for other *cvs* of sardinian germplasm.

Karabulut *et al.* (2010) indicated 55 and 60°C as effective temperatures for decay control of "Casselman" plums inoculated with *M. fructicola* 12-16 h before treatments. The authors observed a total rot control at 60°C for 1 min, while with 'Meloni' prunes, inoculated 24 h pre-treatment, we obtained under the same conditions an inhibition percentage of about 53%, which rose to 55% with the SBC solution.

Also Palou *et al.* (2009) reported a significant increase of treatment efficacy by heating the solutions to 55 or 60°C with several GRAS compounds in stone fruits, inoculated *in vivo* with seven major postharvest pathogens.

The trends at 55 °C with SBC are similar to those attained with *Citrus* fruit, but with a much lower efficacy (Smilanick *et al.*, 1999). This difference was also observed by Palou *et al.* (2009) in stone fruits. The observed diversities between species is probably related to notable differences in fruit tissue texture, rind structure, cuticle thickness and chemical composition within the potential infection courts. Indeed, in wounded *Citrus* fruits, alkaline hydrolysis of pectins is promoted by carbonic acid salts along with an increased pH of the albedo and a remarkable induction of phytoalexin biosynthesis (Dore *et al.*, 2010). These additional factors affecting pathogenicity have not yet been investigated in stone fruits and may contribute to explaining the higher or lower efficacy according to species.

Concerning overall appearance, a significantly lower score was attributed to fruit immersed in water at 55 and

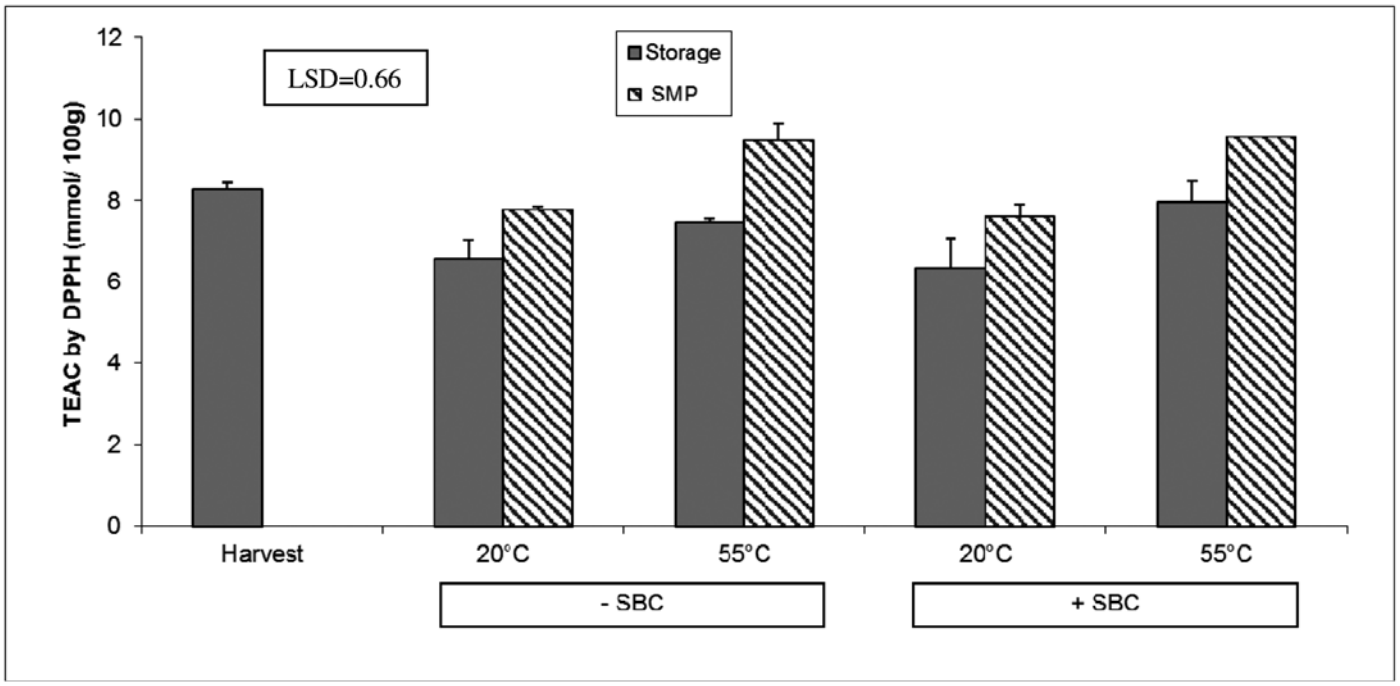

Fig. 2 - Total antioxidant activity in 'Meloni' plum immersed for 60 s in water at 20 and 55°C, with (+) or without (-) BCS, expressed as TEAC units (mmol Trolox equivalents per 100 g of fruit) by DPPH assay, after 1 month of storage at 5°C and 95% RH and 6 days at 20°C and 80% RH (SMP).

60°C for 60 s. This as the result of fruit shriveling, particularly evident after SMP in heat treatments with the SBC solution. Also fruit weight loss was affected by the heat-SBC treatments and the same trend was reported for other crops even though to a lesser degree.

In *Citrus* fruit, the greater weight loss was attributed to an increase of fruit transpiration following alterations on the cuticular permeability (Dore *et al.,* 2010). Peel-impairments, especially at the cuticle level near stomata, were evidenced by SEM on 'Meloni' fruit immersed in the SBC solution at 60°C for 45 and 60 s. As reported for

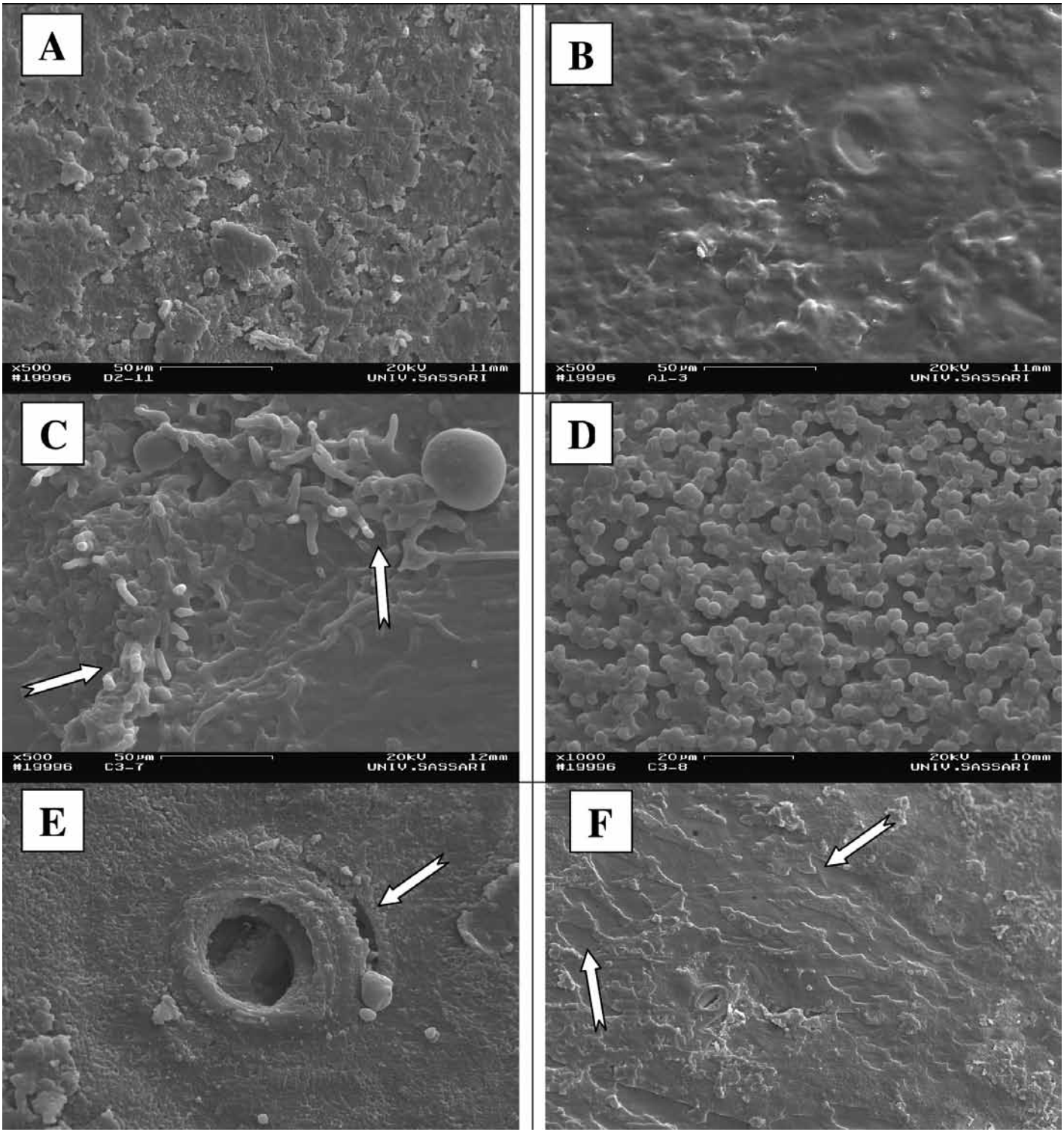

Fig. 3 - *Micrographs A-F*. Scanning electron microscopy micrographs of 'Meloni' prune epicuticular wax at harvest (A), following immersion at 55°C for 60 s in water (B) and in a 2% $NaHCO_3$ solution (C) with arrows indicating crystalline wax (C), and a magnification of the new wax layer (D). In micrograph 'E' damage to the cuticle and wax erosion (F) following immersion at 60°C for 60 s in the 2% SBC solution are evidenced (arrows).

Citrus fruits, it is likely that also for stone fruits harming of the cuticle produces the observed greater fruit weight loss and wilting.

With regard to chemical analysis, the variations occuring during storage and SMP in untreated and in 20°C treated fruit are consistent with literature. In fact, Díaz-Mula *et al.* (2009) reported a 40-45% decrease of total acidity detected in eight plum cultivars after 35 days of storage at 2°C and a significant increase of TSS in almost all the cultivars studied. Compared to treatments at 20°C, fruit dipped at 55°C had higher TSS and TA contents. According to Serrano *et al.* (2004) this trend could be related to a reduction of the respiration rate caused by the heat treatment. Somewhat less clear is the significant drop of TSS in fruit dipped at 60°C. A similar phenomenon was reported for *Citrus* fruit when the treatment temperature reached the fruit-damage threshold, and this drop was attributed to fruit physiology connected to stress-induced metabolic responses (Schirra and d'Hallewin, 1997). With respect to harvest, AA, TP and TF decreased during cold storage, while an increase was observed after SMP and, in heat treated fruit, values were higher compared to harvest. Díaz-Mula *et al*. (2009) monitored the bioactive compounds in several plum cultivars during cold storage and reported rising and dropping trends according to cv. and ripening stage at harvest. The increase of AA, TP and TF following heat treatments has been reported also for pomegranate and strawberry (Mirdehghan *et al.*, 2006; Vicente *et al.*, 2006), supporting our findings that heat affects nutraceutical properties in a positive way. The results attained with the heated SBC solution suggest that only heat and not SBC plays a role inducing AC, TP and TF.

As far as we are aware, no literature is available on the changes of bioactive compounds and antioxidant activity during cold storage and subsequent SMP of stone fruits according to treatment with SBC solution.

From the results attained with the European plum 'Meloni' it appears clearly that decay is furthermore reduced, compared to water at 55°C, when 2% of SBC is added and immersion is performed for 45 or 60 s. In addition, as for C*itrus* fruits, we observed that SBC induces the production of crystalline wax and that treatments at 60°C induce cuticular damage and loss of epicuticular wax from the fruit surface. These effects on the cuticle and epicuticular waxes can increase fruit transpiration, as demonstrated by the greater weight loss and shrivelling. After SMP, postharvest immersion of 'Meloni' prunes in hot water resulted in an increase of bioactive compounds and SBC did not contribute to this increase. Thus, heating a SBC solution seems not to affect nutraceutical properties but from our results it significantly improves the efficacy of this GRAS compound in containing *P. expansum* infection during storage. It is also important to evidence that a synergic anti-penicillium effect took place by employing the heated SBC solution.

Epicuticular damage highlighted by SEM undermines or hide the effects on fruit quality and probably further research is needed in order to establish the most appropriate concentration of the salt aimed at reducing the epicuticular damage and at clarifing the salt influence on the nutraceutical properties of fruit.

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Effect of film packaging and storage temperature on physical and chemical changes in fresh-cut green asparagus

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Key words: antioxidants, modified atmosphere, quality attributes, toughness.

Abstract: The effect of two packaging materials, Film 1 (polyvinylchloride film, manually extensible, 12 µm thickness, O_2 permeability of 22,000 $cm^3/m^2/24$ h/atm) and film 2 (polyethylene film, 13 µm thickness, heat shrinkable, O_2 permeability of 8,500 $cm^3/m^2/24$ h/atm) on changes in oxygen, carbon dioxide and ethylene concentrations within film packaging, weight losses, chemical parameters and textural properties of fresh-cut green asparagus (*Asparagus officinalis* L.) were evaluated during three weeks of storage at 2 or 10°C. During the first two days of storage, in-package carbon dioxide and ethylene concentration increased progressively, while oxygen level decreased. An overall decrease in pH, sucrose and fructose content was observed while an increase in titratable acidity was observed in non-packaged asparagus. A significant increase in total phenols and total soluble solids was recorded, while in Film 2 at 10°C significant decreases were detected in total soluble solids. Antioxidant activity did not change in asparagus packaged at 2°C while in unpackaged and in Film 1 and 2 at 10°C there were significant decreases. Ascorbic acid contents declined rapidly after storage in all samples. Weight loss increased markedly in non-packaged asparagus; in asparagus packaged with Film 1 at 10°C significant differences were detected with respect to the other packaged treatments. Both packaging materials preserved rheological properties of spears whereas un-packaged asparagus lost crispness rapidly. The overall results showed that the best storage conditions to extend the shelf-life of fresh-cut green asparagus were achieved by combining packaging and storage at 2°C.

1. Introduction

Asparagus (*Asparagus officinalis* L.) is a highly perishable product with a very short shelf-life due to its high respiration rate, which leads to a rapid degradation of chemical compounds and toughening of the spears (Kader, 1992). During storage, physiological and compositional changes include loss of sugar, vitamins and water, toughening, degradation of pigments and bract opening that reduce spears quality (Villanueva *et al*., 2005). Among these, changes in texture, colour and brightness are the main factors which affect asparagus acceptance. Environmental humidity is also an important factor in determining asparagus freshness; weight loss of 3-6% makes the product unacceptable (Albanese *et al.,* 2007). Rapid cooling after harvest and low storage temperature notably reduce postharvest changes in quality characteristics. Modified atmosphere packaging (MAP), combined with low temperatures, has been used to extend the shelf-life of minimally processed vegetables by reducing respiration rate, retarding the maturation and senescence, reducing microbial proliferation and quality deterioration (King *et al.,* 1986; Zagory and Kader, 1988; Gontard *et al.,* 1996; Fonseca *et al.,* 2002).

These effects are related to the surrounding atmosphere of products: the depletion of oxygen and accumulation of carbon dioxide inside the packaging. All these factors are affected by metabolic activity, storage temperature and film gas diffusion characteristics.

The purpose of the present study was to evaluate the effect of two film-packaging materials on maintaining the quality of fresh cut green asparagus stored at 2°C (recommended storage and transport temperature) or 10°C (to simulate final display) (King *et al.,* 1993).

2. Materials and Methods

Plant material

Asparagus (*Asparagus officinalis* L. cv. Grande) spears were harvested from a commercial greenhouse located in Alghero (Sardinia). Commercially mature spears (25 cm long) were randomly harvested with some standing stalk in April and were immediately transported to the laboratory and refrigerated at 2°C and 10°C.

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Preparation of samples and storage conditions

The asparagus samples were selected according to diameter and cut at a length of about 20 cm. Samples of about 300 g each were then un-packaged or packaged with Omni Film (a polyvinylchloride film manually extensible, 12 µm thickness, O_2 permeability of 22,000 $cm^3/m^2/24$ h/atm and water vapour transmission rate of 515 $g/m^2/24$ h/1 atm.) (Film 1) or with Bolphane BX polyethylene film (Bolloré Plastic Films Division, Dayville, France, 13 µm thickness, heat shrinkable, O_2 permeability of 8,500 $cm^3/m^2/24$ h/atm and water vapour transmission rate of 16 $g/m^2/24$ h/1 atm) (Film 2). Both films were applied tightly to each asparagus bunch and sealed by a hand-wrapping machine to stretch the film (SW-500 E, Sambo Tech Corporation Lovero, Gyeonggi-do, Korea) in the case of Film 1 and heat-shrunk by a Bellpack packaging machine (Tecnopack Packaging Equipment, Livorno, Italy) in the case of Film 2. In both packaged treatments, in-package gas atmosphere was achieved passively. All samples were stored for 21 days at 2°C (recommended storage and transport temperature) or 10°C to simulate retail outlet display.

Quality characteristics were determined at harvest and after 7, 15 or 21 days. These included weight loss, toughness, pH, titratable acidity, total soluble solid (TSS), sucrose, glucose, fructose, total phenols, antioxidant activity and ascorbic acid. Chemical analyses were determined on asparagus juice obtained using a domestic juicer and results were referred to 100 g of fresh weight (FW). Crude juice was centrifuged in an Eppendorf tube using a K3 System centrifuge (Centurion Scientific Ltd, West Sussex, England) at 12,000 rpm for 20 min; the clear supernatant was filtered through a 0.45 µm acetate cellulose filter (Sun *et al.,* 2005). Analyses were performed in triplicate. All reagents were of analytical or better grade.

Modified atmosphere packaging

The atmosphere composition (O_2, CO_2 and C_2H_4) inside the packages was determined after 1, 3, 7, 14 and 21 days of storage. Gas samples (1 mL) were withdrawn from each package with a gas-tight syringe. Ethylene was determined using a Varian 3300 GC (Varian Analytical Instruments, Walnut Creek, CA, USA) equipped with a flame ionisation detector (FID), Carbowax 20 M 80/120 mesh Carbograph 1 AW 30 column (Alltech, Milan, Italy), column temperature of 60°C, injector 110°C, and detector 180°C. Oxygen and CO_2 concentrations were determined with a Agilent 6890 GC system equipped with a thermal conductivity detector (TCD), CTR I column 6'X1/4' outer & 6'X1/8' inner (Alltech, Milan, Italy); column temperature was 60°C, injector 120°C, and detector 160°C. Helium was used as carrier gas.

Assessment of physical parameters

The weight loss of each lot was determined by a precision scale (Sartorius CP 22025-OCE, Gottingen, Germany). Textural properties were measured using two different methods based on a cutting test at 7.5 and 15 cm from the tip using a 1.2-mm thickness blade (speed 3 mm s^{-1}) and a puncture test at 15 cm from the tip using a 2-mm needle (speed 1 mm s^{-1}, depth 3 mm) (Rodrıguez *et al*., 2002 a). Both test were accomplished using a texturometer interfaced with a computerized system with specific software (DO-FB0.5TS, Zwick Roell, Ulm, Germany).

Chemical analysis

Titratable acidity was determined by titrating 5 mL of juice using a potentiometric titrator (Titrino 720 SM, Metrohm, Herisau, Switzerland) with 0.1 N NaOH till pH 8.1. The pH was measured by a pH meter (Orion 720A). Total soluble solids (TSS) were determined using a digital refractometer (PR-101, Atago, Tokyo, Japan) and expressed as °Brix; ascorbic acid by extraction with metaphosphoric acid and determined volumetrically by titration with 2,6-dichlorophenolindophenol (AOAC Method 967.21). Total phenolic content was analyzed according to the Folin-Ciocalteau colorimetric method (Singleton and Rossi, 1965). Total phenols were expressed as gallic acid equivalent. Antioxidant activity was assessed using the free radical 2,2'-diphenyl-1-picrylhydrazyl (DPPH) (Bonded *et al.,* 1997). The mixture, containing 3 mL of a methanol solution of 0.16 mM DPPH and 0.025 mL of asparagus extract, was allowed to react in a cuvette and the absorbance of the DPPH solution was determined at 515 nm after 15 min of reaction. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a reference to compare the antioxidant activity. The activity was expressed as mM Trolox equivalent antioxidant activity (TEAC) related to 100 g of fresh weight. Analysis of carbohydrate was performed by coupling a liquid chromatograph system consisting of a D-7000 manager, L-7100 pump, L7200 auto-sampler (LaChrom, Merck-Hitachi Ltd., Tokyo, Japan) with an evaporative light scattering detector (ELSD Sedex 60Lt, Alfortville, France). A Bio-Rad aminex fast carbohydrate column (100 mm x 7.8 mm, 9 µm, lead form, Bio-rad, Milan, Italy) with a guard column (Bio-rad micro-guard Carbo-P aminex cation exchange resin, lead form) thermostated at 80°C, was employed. The isocratic mobile phase was H_2O ultra-pure and a flow rate of 0.8 mL/min was employed. The ELSD detector was set as follows: drift tube temperature 45°C; nebulizer gas (air) pressure, 2.5 bar; and photomultiplier 8. Stock standard solutions of each carbohydrate were prepared in ultra-pure water and their quantification in asparagus juice was calculated according to the linear calibration curves of standard compounds. The analyses were conducted in triplicate for all parameters.

Visual assessment

The external appearance of spears (turgidity, formation of longitudinal striation, colour changes and presence of off-flavour) was assessed by a panel of six untrained technicians. Spear quality was evaluated using a 1 to 5 subjective scale (1= unacceptable; 2= acceptable; 3= good; 4= very good; 5= excellent, freshly harvested appearance).

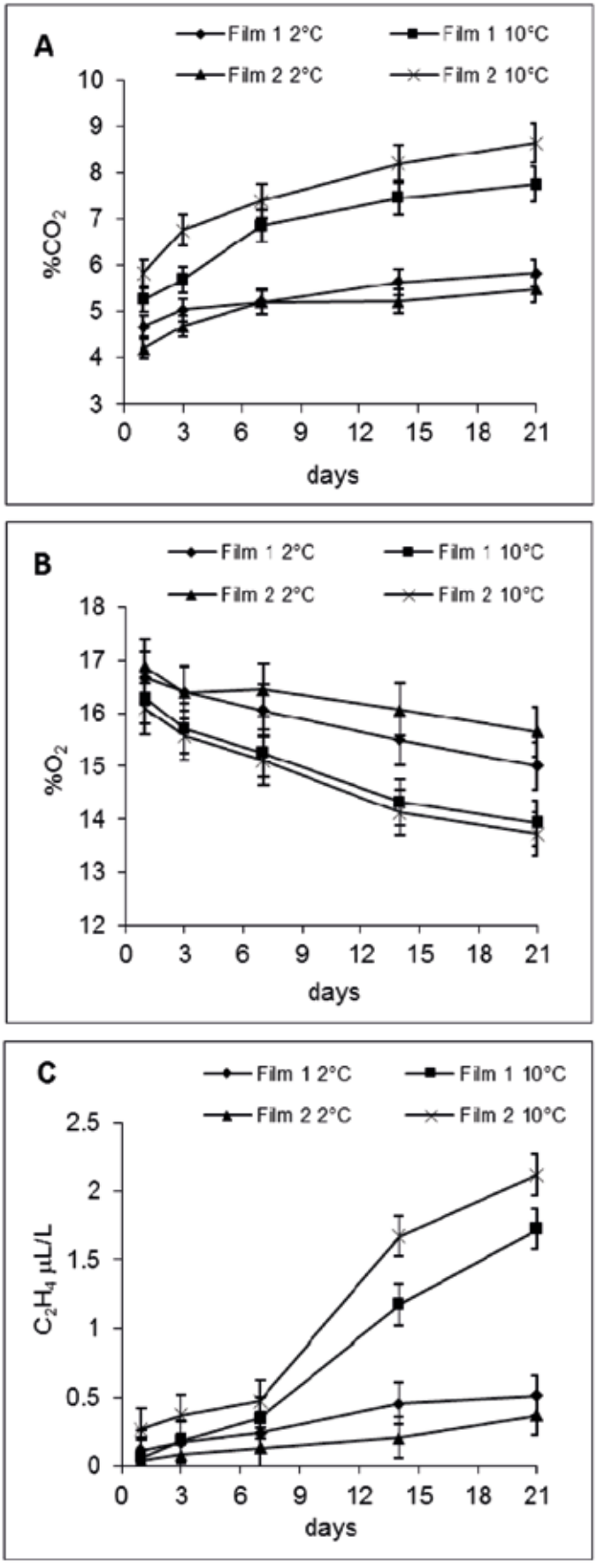

Fig. 1 - Influence of film packaging (Film 1, Omnifilm PVC film; Film 2, Bolphane BX polyolefinic film) and storage conditions on in-package CO_2 (A), O_2 (B) and ethylene levels (C). Vertical bars represent the standard deviation. LSD are given at the 5% level.

Statistical analysis

Statistical analysis was carried out using Statgraphics software (Timberlake, version 5 professional, 2000). Separation of means was performed by the LSD test, $P \leq 0.05$.

3. Results

Modified atmosphere packaging

The levels of in-package CO_2 and O_2 were significantly affected by storage temperature (Fig. 1). At 2°C, CO_2 partial pressure was always lower than at 10°C (Fig. 1A), while O_2 levels were higher (Fig. 1B). In-package CO_2 increased steadily in all treatments during the first 3-7 days, thereafter it increased at a lower rate in packages stored at 10°C, while it was quite stable or increased slightly in those stored at 2°C. In contrast, in-package O_2 partial pressure decreased gradually in all packages with final values in the range of 13.8-16.5 kPa.

Irrespective of the type of film, when asparagus spears were stored at 10°C, ethylene rates were slightly higher than samples stored at 2°C during the first 7 days, thereafter a dramatic increase occurred in both films, especially in samples sealed with Film 2 which always displayed levels significantly higher than those sealed with Film 1 (Fig. 1C).

Weight losses

Weight losses increased markedly in non-packaged asparagus, slightly in samples sealed with Film 1 and stored at 10°C but with values always significantly higher than in the other packages, and were negligible in samples sealed with Film 2 at both storage temperature and in those sealed with Film 1 stored at 2°C. In particular, at the end of storage weight losses averaged 1% and 4% in asparagus spears packaged with Film 1, 0.3% and 0.8% in those packaged with Film 2 and 12 and 30 % in un-packaged spears stored at 2°C or 10°C, respectively (Fig. 2).

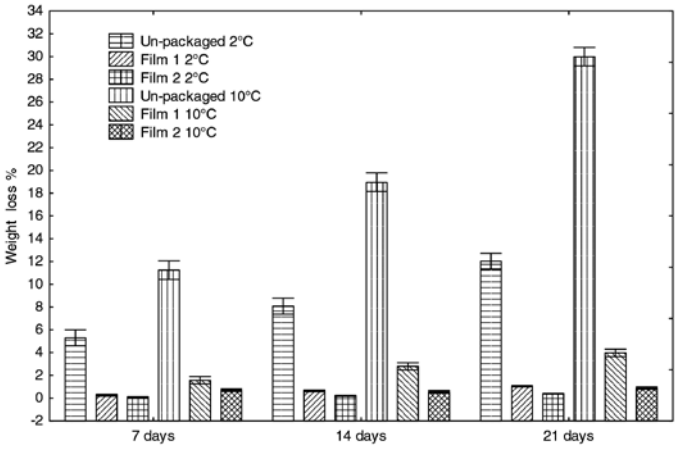

Fig. 2 - Influence of film packaging and storage conditions on weight loss in green asparagus. Vertical bars represent LSD, $P \leq 0.05$.

Texture

Figure 3 illustrates results with regard to changes in maximum shear stress to cut and displacement of F max of asparagus spears. Over the storage period, the profile of the curves and F max following cutting at 7.5 (Fig. 3A) and 15 cm (Fig. 3B) from the tip were very similar. The maximum shear force required for cutting (Figs. 3A, 3B) and force displacement (deformation L (mm) at F max) (Figs. 3C, 3D) was affected by packaging, storage time and temperature. Changes at harvest time (time 0) were notably higher in un-packaged asparagus, especially in samples cut at 15 cm of both storage temperatures and in those cut at 7.5 cm and stored at 10°C. The smallest changes occurred in packages stored at 2°C, where the breaking force and displacement at F max were significantly lower than all other treatments, perhaps due to the lower weight loss. For both parameters, differences between the two films were negligible. Overall results of puncture test revealed minor changes both in F max (Fig. 4A) and deformation (Fig. 4B) in packaged samples, especially in those stored at 2°C; in contrast, both parameters increased in unpackaged asparagus, especially in those stored at 10°C.

Chemical analysis

In Table 1 the influence of film packaging and storage condition on pH, TSS, TA, sucrose and fructose is reported. With respect to harvest time, pH decreased at both storage temperatures. Titratable acidity increased significantly in unpackaged asparagus stored at 10°C; changes were obvious after 14 days of storage when the asparagus spears were in advanced decay, while in the other treatments values were fairly stable. A slight increase in TSS

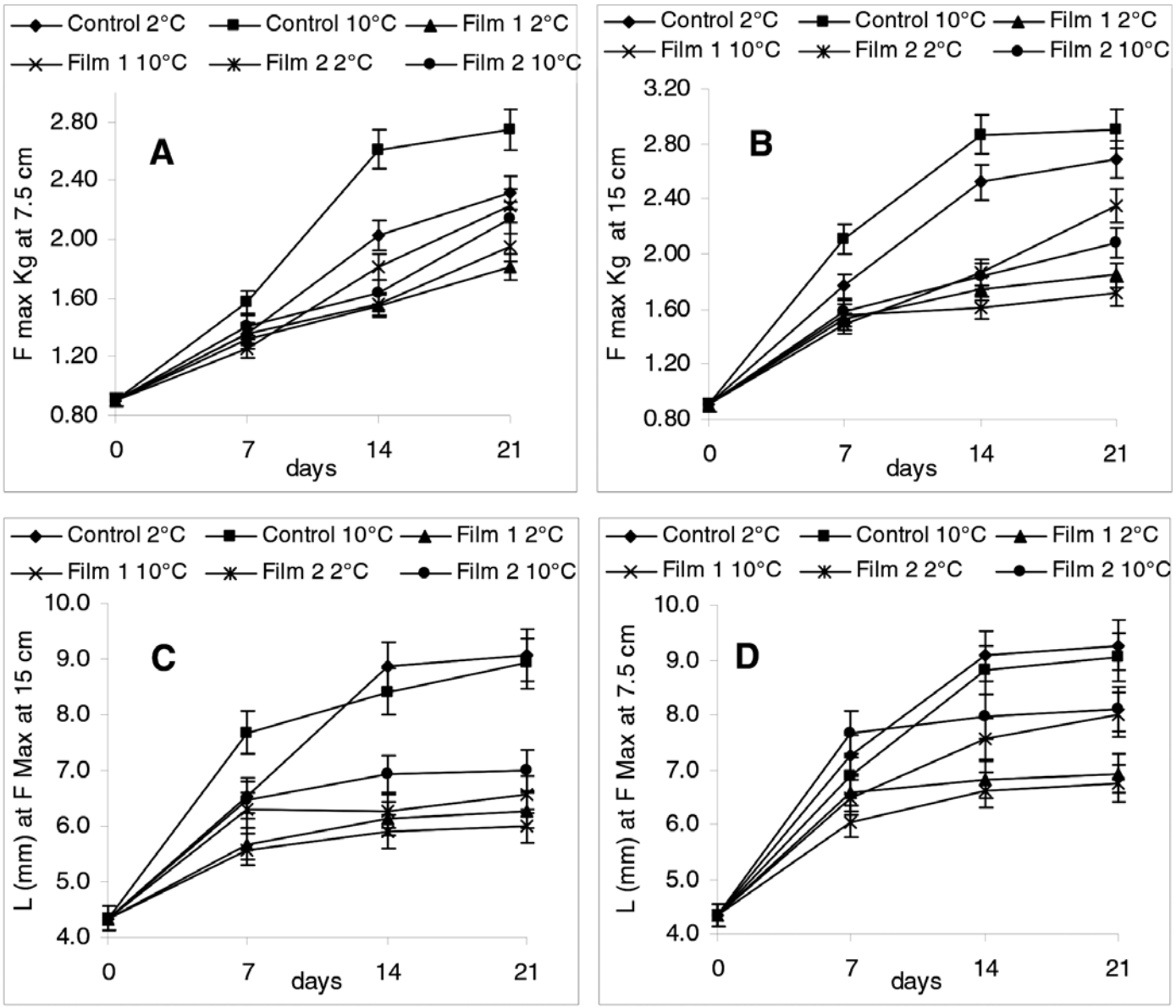

Fig. 3 - Effect of film packaging (Control, un-packaged; Film 1, Omnifilm PVC film; Film 2, Bolphane BX polyolefinic film) and storage conditions (2°C and 10°C) on cutting force (F max) and force displacement (L at F max) in green asparagus during 21 days of storage at 7.5 cm (A and C) and 15 cm (B and D) of the tip. Vertical bars represent the standard deviation. LSD are given at the 5% level.

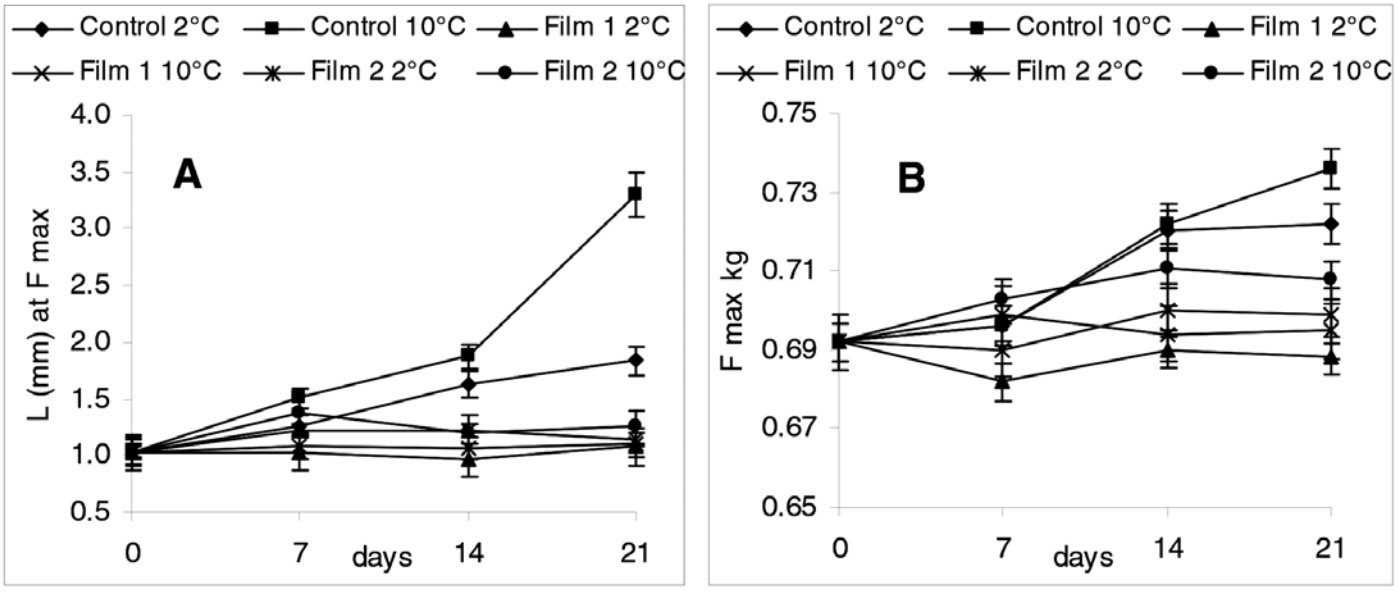

Fig. 4 - Influence of film packaging (Control, un-packaged; Film 1, Omnifilm PVC film; Film 2, Bolphane BX polyolefinic film) and storage conditions (2°C and 10°C) on force puncture test (F max) (A) and displacement of force (L at F max) (B) in green asparagus during storage. Vertical bars represent the standard deviation. LSD are given at the 5% level.

Table 1 - Influence of film packaging (Film 1, Omnifilm PVC film; Film 2, Bolphane BX polyolefinic film) and storage conditions on pH, total soluble solids (TSS), titratable acidity (TA), sucrose and fructose in asparagus spears

Storage period	pH	SST (°Brix)	TA (g/100g as citric acid)	Glucose (g/100g)	Fructose (g/100g)
7 days storage					
Harvest	6.15 a	5.20 bc	0.11 abc	1.29 a	1.51 a
Un-packaged 2°C	5.93 c	5.23 bc	0.12 ab	1.19 b	1.40 abc
Film 1 2°C	6.04 b	5.86 a	0.09 c	1.19 b	1.42 ab
Film 2 2°C	6.18 a	5.16 c	0.11 abc	1.03 c	1.30 cd
Un-packaged 10°C	5.84 d	5.33 bc	0.13 a	1.04 c	1.31 bcd
Film 1 10°C	6.04 b	5.40 b	0.10 bc	0.93 d	1.35 bcd
Film 2 10°C	6.07 b	5.30 bc	0.11 abc	0.96 cd	1.27 d
14 days storage					
Harvest	6.15 a	5.20 bc	0.11 b	1.29 a	1.51 a
Un-packaged 2°C	5.88 d	5.53 ab	0.12 b	1.19 b	1.31 b
Film 1 2°C	6.07 b	5.80 a	0.11 b	1.21 b	1.24 b
Film 2 2°C	5.95 c	5.30 bc	0.09 c	1.04 c	1.28 b
Un-packaged 10°C	5.90 cd	5.10 c	0.15 a	1.02 c	0.98 c
Film 1 10°C	6.06 b	5.23 bc	0.11 b	0.84 d	1.04 c
Film 2 10°C	6.07 b	4.80 d	0.11 b	0.75 e	1.02 c
21 days storage					
Harvest	6.15 a	5.20 b	0.11 b	1.29 a	1.51 a
Un-packaged 2°C	5.70 e	5.73 a	0.12 b	1.01 c	1.28 b
Film 1 2°C	5.93 c	5.73 a	0.11 b	1.18 b	1.07 c
Film 2 2°C	5.86 d	5.13 b	0.10 b	1.00 c	1.05 c
Un-packaged 10°C	5.88 cd	5.67 a	0.23 a	0.88 d	0.85 d
Film 1 10°C	6.03 b	5.03 bc	0.12 b	0.84 d	0.98 d
Film 2 10°C	6.07 b	4.70 c	0.12 b	0.81 d	0.75 e

Values in columns followed by unlike letters of each storage period and harvesting time are significantly different according to the Duncan's test of the least significant difference at $P \leq 0.05$.

after storage occurred in unpackaged asparagus or in samples packaged with Film 1 at 2°C, while in Film 1 at 10°C a significant decrease was detected; few changes occurred in the other samples. Glucose and fructose were the predominant sugars, with slightly higher levels of fructose with respect to glucose (Table 1), while sucrose was detected in traces with concentrations lower than 0.3 g/100 g FW (data not shown). At harvest the concentrations of glucose and fructose were 1.29 and 1.51 g/100 g, respectively. During storage their content gradually declined, especially in samples stored at 10°C, which always showed higher losses. The effect of film wrapping was not so evident. Significant differences detected at some sampling dates were not confirmed in others, denoting an inconsistent and overall not significant effect of the films. With regard to ascorbic acid, differences were observed among all samples (Table 2). The obtained data show that ascorbic acid content was higher at harvest and then decreased quickly during storage. The kinetics of degradation of ascorbic acid content during storage fit an exponential function of the following kind: $y = a\, e^{-bx}$, where the coefficient "a" represents the initial concentration and coefficient "b" refers to the rate of ascorbic acid degradation of stored asparagus (Fig. 5). The percentages of ascorbic acid retention after 14 days of storage at 2°C were 63.5, 57.7 and 45.8% while at 10°C they were 34.9, 29.7 and 20.5% in normal atmosphere, packaged with Film 1 and Film 2 respectively. Total phenolic compounds at harvest were determined to be 69.16 mg/100 g FW (Table 2). Over the storage period, significant increases were detected in packaged and unpackage asparagus spears stored at 2°C and a similar trend was observed in asparagus packaged and stored at 10°C, although the increase was less than at 2°C, while in unpackaged spears stored at 10°C total phenols remained similar to those at harvest. Antioxidant activity was fairly stable in asparagus stored at the lower temperature, while in asparagus stored at 10 °C it decreased more than samples stored at 2°C; most of the decline occurred during the first week of storage and with the highest losses detected in unwrapped spears stored at 10°C. (Table 2).

Table 2 - Influence of film packaging (Film 1, Omnifilm PVC film; Film 2, Bolphane BX polyolefinic film) and storage conditions on ascorbic acid, total phenols and antioxidant activity in asparagus spears

Storage period	Ascorbic acid (mg/100g)	Total phenols (mg/100g)	Antioxidant activity (mM TEAC)
7 days storage			
Harvest	22.70 a	69.16 cd	1.71 ab
Un-packaged 2°C	18.23 b	69.98 bcd	1.61 b
Film 1 2°C	17.20 b	74.19 a	1.77 a
Film 2 2°C	13.57 c	72.93 abc	1.79 a
Un-packaged 10°C	11.20 d	68.44 d	1.41 c
Film 1 10°C	11.00 d	74.48 a	1.47 c
Film 2 10°C	8.30 e	73.72 ab	1.46 c
14 days storage			
Harvest	22.70 a	69.16 c	1.71 ab
Un-packaged 2°C	14.43 b	80.35 a	1.63 bc
Film 1 2°C	13.10 b	78.79 a	1.73 ab
Film 2 2°C	10.47 c	82.21 a	1.79 a
Un-packaged 10°C	7.93 d	70.60 bc	1.35 d
Film 1 10°C	6.77 d	74.05 b	1.44 c
Film 2 10°C	4.67 e	73.84 b	1.45 c
21 days storage			
Harvest	22.70 a	69.16 c	1.71 a
Un-packaged 2°C	11.30 b	84.14 a	1.63 b
Film 1 2°C	9.68 c	83.27 a	1.73 a
Film 2 2°C	9.43 c	85.97 a	1.76 a
Un-packaged 10°C	7.63 d	69.52 c	1.35 d
Film 1 10°C	5.17 e	76.91 b	1.49 c
Film 2 10°C	4.63 e	75.10 b	1.48 c

Values in columns followed by unlike letters of each storage period and harvesting time are significantly different according to the Duncan's test of the least significant difference at $P \leq 0.05$.

Visual assessment

After 7 days of storage at the test temperatures, packaged spears were judged as excellent, unpackaged ones scored 3 at 2°C and 2 at 10°C (data not shown). The main alterations detected in unpackaged samples were longitudinal striations due to excessive water loss, especially toward the base. These phenomena increased during storage and were more evident in spears stored at 10°C. As a result, after 14 days unpackaged asparagus stored at 10°C were unmarketable (score 1) (Villanueva *et al.,* 2005). After 21 days, spears stored at 2°C and packaged with Film 2 and Film 1 were scored 3 and 2, respectively, with the main defect being a loss of green color, which also occurred in unpackaged samples. After 21 days of storage even unpackaged samples stored at 2°C were unmarketable, while packaged spears, with only slight discoloration, were still marketable.

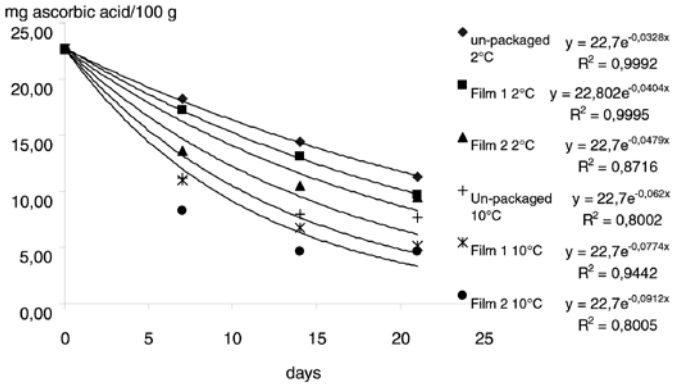

Fig. 5 - Kinetic model of ascorbic acid degradation during storage.

4. Discussion and Conclusions

This study confirms a delay of the processes of physical and chemical deterioration in asparagus spears in response to storage temperature and modified atmosphere resulting from two different packaging solutions. The response was evident in visual appearance, weight loss, and some chemical parameters.

Modified atmospheres are known to reduce the metabolic activity of harvested products. In the present experiment the modified atmosphere was passively created by the accumulation of CO_2 released by respiration and its composition was affected by storage temperature and film permeability to gases. The final partial pressures of CO_2 and O_2 were within the tolerance limits for this commodity, which are above 10k Pa for O_2 and below 15 kPa for CO_2 (Berrang *et al.,* 1990). Ethylene concentration was generally higher at 10°C than at 2°C, especially, after 7 days. Moreover, when asparagus were stored at 10°C, differences in ethylene concentrations were also affected by the type of film. Nevertheless, the in-package gas composition was remarkably effective in all conditions at delaying chemical and physical compositional changes, regardless of storage temperature. It is likely that the potential negative effect of ethylene, especially in packages stored at 10°C, was counteracted by CO_2, which notoriously can mitigate the negative effect of ethylene by interacting with its receptors (Burg, 2004).

The rate of weight loss was dependent on storage temperature, packaging, and type of film used. Prevention of weight loss due to the maintenance of high relative humidity is a major advantage of packaging, and the beneficial effect of this practice was particularly evident in this study. However, the different permeability to water vapor of the two films led to significant differences in weight loss between Film 1 and Film 2, especially at 10°C. As a result, overall appearance of asparagus stored at 2°C and sealed with Film 2 (the less permeable) declined at a slower rate and allowed better maintenance of market quality.

Asparagus toughening has generally been associated with the lignification process (Siomos *et al*., 2000). Postharvest texture changes are influenced by numerous biochemical modifications of plant tissues, such as compositional modifications of cell walls, lignification of the pericyclic fibers, increases in phenolic compounds such as ferulic acid and *p*-coumaric acid, deposition of polysaccharides, mainly xylan, and storage conditions (Rodriguez *et al.,* 2002 b; 2004; 2005). The results of the present study are in agreement with previous findings (Waldron and Selvendran, 1990; Rodriguez *et al.,* 1999 a, b). Moreover, textural properties were influenced by water loss; toughness increases as a consequence of turgor reduction.

The increased titrable acidity detected in unpackaged asparagus stored at 10°C might be induced by the advanced decaying process, which did not occur in packaged samples. A slight increase in TSS after storage occurred in unpackaged or packaged asparagus with Film 1 at 2°C, while in Film 1 at 10°C a significant decrease was recorded; few changes occurred in the other samples. Packaged and unpackaged spears showed significant reductions in glucose and fructose content during storage, particularly in those stored at 10°C. Changes in TSS, glucose and fructose generally denoted marked differences between unpackaged and packaged samples and, above all, between the two storage temperatures. The few differences in chemical composition between packaged and non-packaged asparagus, in contrast with the marked differences detected in weight loss and textural properties, may indicate that the overall beneficial effect of packaging is a consequence of the reduced transpiration occurring in packaged asparagus rather than the physiological effect due to the increased levels of CO_2 and reduced availability of O_2. As for ascorbic acid content, the results reflect a significant decrease with storage which is more accentuated in unpackaged samples and in those stored at 10°C (Esteve *et al.*, 1995). The kinetics of degradation confirmed this trend, in fact the highest rate of degradation was approximately 0.4 mg ascorbic acid/100 g/day and 0.8 mg ascorbic acid/100 g/day for packaged asparagus and 0.3 mg ascorbic acid/100 g/day and 0.6 mg ascorbic acid/100 g/day for unpackaged samples, refrigerated at 2°C and 10°C, respectively. Total phenolic content increased during storage: changes were clear after 14 days in packaged spears and stored at 2°C. This trend can be ascribed to an increase of phenols in asparagus cell walls (Rodriguez *et al*., 2002 b). However, in unpackaged spears stored at 10°C no change occurred in phenols content when they were in advanced decay, most likely because decaying cells are no longer able to synthesize phenolic compounds. Antioxidant capacity was fairly stable during storage in asparagus spears packaged with Film 1 and Film 2 stored at 2°C. In contrast, antioxidant capacity of packaged and unpackaged spears stored at 10°C declined during both periods with values significantly lower than at harvest. The antioxidant activity in foods depends on the content of components such as phenolic compounds, carotenoids and ascorbic acid and their fate during processing or storage. In this study the antioxidant capacity might have been balanced by the decreasing trend of ascorbic acid and the increasing tendency of total phenols. As a result, higher levels of antioxidant activity was detected in packaged asparagus stored at 2°C, which contained more total polyphenols and lost vitamin C at a slower rate than all other treatments.

However, considerations about changes in chemical parameters should take account the effect of transpiration, as a loss of water inevitably leads to an increase of solute concentration, and this effect might be particularly marked in unpackaged samples where weight loss over the storage period ranged between about 6 and 32 %.

The overall results of this study show the positive effect on quality retention of cold-stored asparagus, both from a market point of view and from the chemical and nutraceutical perspective. In particular, the best results were achieved with the less permeable film (Film 2) and storage at 2°C.

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Influence of azoxystrobin dip treatments on postharvest decay of second-crop fig (*Ficus carica*) fruits from Sardinian germoplasm

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Key words: azoxystrobin, cold storage, fig fruit, fig decay, postharvest treatment.

Abstract: Fig (*Ficus carica* L.) fruits from the second-crop, cultivars Verde, San Pietro, Perdingiana, and Carcanzi Trota, were harvested on 25 August and 8 September 2005, subjected to 50 or 100 mg/L azoxystrobin (AZO) dip treatments for 30 s and stored for seven days at 18°C (simulated marketing conditions, SMC) or for seven days at 5°C (CS) plus seven days at SMC. After seven days at 5°C, the external decay incidence in control fruit was 3-10%. Treatment with AZO completely suppressed external decay in 'San Pietro', and 'Carcanzi Trota' and resulted in 2 and 8% decay in 'Verde' and 'Perdingiana', respectively. After CS plus 3 days at 18°C, decay in control fruit was measured as 23-41% and 71-85% of August and September harvests, respectively, while in those treated with AZO, average losses were 4-11%. At the end of SMC, all fruit in all treatments decayed, although the rotten area was smaller in AZO treated fruit. Similarly, in fruit stored directly in SMC, AZO significantly reduced decay during the first three days; after seven days all fruit decayed. Internal decay originating from the syconium cavity was higher in fruit harvested in September and was not affected by AZO treatments.

1. Introduction

Decay represents the major cause of postharvest loss of fig (*Ficus carica*), especially in fruit of the second crop when high humidity levels and precipitation cause skin cracking, ostiole splitting, and the growth of pathogens. Postharvest life of figs can vary from a few days to one to two weeks (Ferguson *et al*., 1990; Crisosto and Kader, 2004).

More than in other fruits, the ripening process in figs is very rapid; under favourable environmental conditions, flesh tissue changes from a spongy dry state to a juicy, sweet condition in one-two days. These sudden changes have long been the object of controversy, and whether figs should be considered climacteric or non-climacteric fruit. Indeed, if on one hand the rapid changes of rheological and compositional features are typical of climacteric fruit, on the other hand figs do not share the ability to continue the ripening process once harvested. Normally, unripe harvested figs never rich an optimum eating stage as happens with other climacteric fruits, such as peaches, pears, kaki or apples. Surely, the maturing and ripening processes are so close and rapid to overlap, not allowing a clear sequential separation between these two physiological stages. Nevertheless, the classification of figs as a climacteric species is generally accepted (Marei and Crane, 1971; Ferguson *et al*., 1990).

Susceptibility of figs to decay and physical damage dramatically increases with ripening: as fruit ripens, the defence mechanism of unripe fruit is rapidly lost and various pathogenic microorganisms can develop. Infection sites may involve the outer tissue of the fruit, with pathogens starting to develop on the peel or the underneath tissue through wounds or cracks, or from inside, through infections originating in the syconium cavity, in most cases transported by wasps or other insects (Crisosto *et al.*, 2011).

In parthenocarpic cultivars with closed or partially closed ostiole, which do not need caprification to produce, visits by fig wasps and other insects inside the syconium cannot take place or are markedly reduced. Consequently, infections starting from inner tissues like endosepsis (*Fusarium monilifome)*, souring or fermentation incited by different types of yeast and bacteria carried by different insects, are easier to control than in cultivars with open ostioles (Ferguson *et al.*, 1990; Michailides *et al.*, 1996). In all cases, an efficient control of decay can be achieved by field treatments with insecticides and fungicides.

Azoxystrobin is a strobilurin-like partial-systemic fungicide with broad-spectrum activity against several important pathogens (Gullino *et al.*, 2000). It is considered a

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reduced-risk-fungicide by the United States Environmental Protection Agency and has been registered for field application or postharvest treatments on several crops.

The present study evaluates the efficacy of postharvest treatments with azoxystrobin to control decay on four cultivars of second-crop fig fruits of Sardinian germplasm. Forniti fruits of these cultivars have an open ostiole, so they are very prone to internal decay.

2. Materials and Methods

The investigation was carried out on four fig cultivars from Sardinia germoplasm (Chessa and Nieddu, 1994): Verde, San Pietro, Perdingiana, and Carcanzi Trota. Fruits of the second crop were picked on 25 August or 8 September from the collection field of the "*AGRIS Sardegna*" in Sassari. Trees received standard agricultural practices, but no chemical treatment to control pests or diseases had been applied in the previous three years. Fruits were harvested early in the morning and immediately transported to the laboratory, which was located about 10 km from the orchard.

Fruit without defects from each cultivar were selected and divided into three groups. Each group was dipped for 30 s, at room temperature, in: water (control) or water with 50 or 100 mg/L of azoxystrobin (AZO) (Ortiva , Syngenta Crop protection Milan, Italy). After dipping and before storage, all figs were dried at room temperature and each treatment group was divided into two subgroups including eight replications of 25 fruits. The first subgroup was stored for three or seven days at 18°C and 90% relative humidity (RH) (simulated marketing conditions (SMC), while the remaining subgroup was stored for seven days in cold storage (CS) at 5°C and subsequent three or seven days of SMC. Afterwards, CS and SMC fruit were inspected for external and internal decay (endosepsis and souring).

Data were subjected to analysis of variance after transformation of average decay-percentage values in $\sqrt{x}$ or $\arcsin\sqrt{x}$ depending on the range of variation of decay. Separation of the means was accomplished according to Fisher's test of the least significant difference (LSD); actual values are reported.

3. Results

The development of external and internal decay was greatly influenced by picking date and storage conditions. Fruits harvest in August were significantly less prone to decay than those harvested in September, regardless of the cultivars (Figs. 1, 2; Tables 1-4). 'Perdingiana' figs (Table 3) of both harvest dates were the most susceptible with high percentages of external and internal decay, whereas no relevant differences were detected among other cultivars. After seven days of storage at 5°C, the percentage of fruit showing external decay was low in all treatments and harvest dates. When fruits were transferred to SMC,

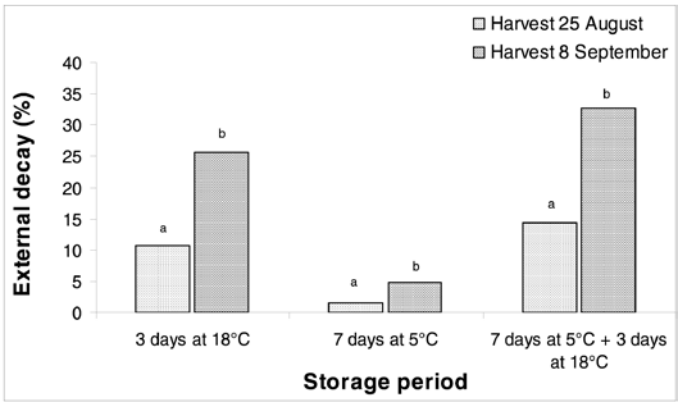

Fig. 1 - Influence of picking date and storage conditions on external decay incidence in second crop production of Verde, San Pietro, Perdingiana and Carcanzi Trota figs. For each storage period histograms with different letters are significantly different, $P \leq 0.05$.

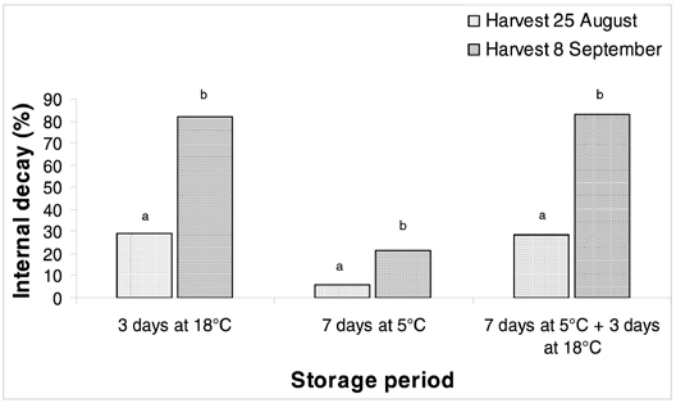

Fig. 2 - Influence of picking date and storage conditions on internal decay incidence in second crop production of Verde, San Pietro, Perdingiana and Carcanzi Trota figs. For each storage period histograms with different letters are significantly different, $P \leq 0.05$.

Table 1 - Influence of 30-s-dip treatments with azoxystrobin (AZO) at 20°C on external and internal decay incidence in second crop 'Verde' figs after three days at 18°C and 65% RH, or for seven days at 5°C plus three days at 18°C

Treatments	3 days at 18°C		7 days at 5°C		7 days at 5°C plus 3 days at 18°C	
	External decay %	Internal decay %	External decay %	Internal decay %	External decay %	Internal decay %
	Harvested in August					
Control	21 b [z]	23 a	6 b	0 a	31 b	18 a
50 mg/L AZO	5 a	35 a	0 a	0 a	7 a	31 b
100 mg/L AZO	4 a	27 a	0 a	0 a	8 a	24 ab
	Harvested in September					
Control	70 b	88 a	9 b	10 a	82 b	86 a
50 mg/L AZO	9 a	75 a	2 a	18 a	11 a	75 a
100 mg/L AZO	9 a	83 a	1 a	16 a	9 a	81 a

[z] For each storage period and harvesting time values in columns followed by different letters are significantly different at P≤0.05 according to Fisher's test of the least significant difference.

Table 2 - Influence of 30 s-dip treatments with azoxystrobin (AZO) at 20°C on external and internal decay incidence in second crop 'San Pietro' figs after three days at 18°C and 65% RH, or for seven days at 5°C plus three days at 18°C

Treatments	3 days at 18°C		7 days at 5°C		7 days at 5°C plus 3 days at 18°C	
	External decay %	Internal decay %	External decay %	Internal decay %	External decay %	Internal decay %
	Harvested in August					
Control	19 b [z]	32 a	3 a	4 a	23 b	28 a
50 mg/L AZO	3 a	25 a	0 a	7 a	10 ab	34 a
100 mg/L AZO	0 a	27 a	0 a	9 a	2 a	29 a
	Harvested in September					
Control	35 b	73 a	5 a	41 b	71 b	76 a
50 mg/L AZO	8 a	65 a	0 a	37 ab	7 a	71 a
100 mg/L AZO	6 a	72 a	0 a	28 a	9 a	67 a

[z] For each storage period and harvesting time values in columns followed by different letters are significantly different at P≤0.05 according to Fisher's test of the least significant difference.

Table 3 - Influence of 30-s-dip-treatments with azoxystrobin (AZO) at 20°C on external and internal decay incidence in second crop 'Perdingiana' figs after three days at 18°C and 65% RH, or for seven days at 5°C plus three days at 18°C

Treatments	3 days at 18°C		7 days at 5°C		7 days at 5°C plus 3 days at 18°C	
	External decay %	Internal decay %	External decay %	Internal decay %	External decay %	Internal decay %
	Harvested in August					
Control	35 b [z]	33 a	9 b	11 a	41 b	44 b
50 mg/L AZO	4 a	31 a	0 a	12 a	6 a	41 ab
100 mg/L AZO	3 a	35 a	0 a	14 a	4 a	33 a
	Harvested in September					
Control	76 b	91 a	13 b	24 ab	85 b	100 a
50 mg/L AZO	10 a	98 a	8 ab	27 b	19 a	95 a
100 mg/L AZO	13 a	87 a	5 a	19 a	16 a	100 a

[z] For each storage period and harvesting time values in columns followed by different letters are significantly different at P≤0.05 according to Fisher's test of the least significant difference.

sharp increases in decay development were recorded in all fruit samples especially those of the second harvest date. In particular, after three days at 18°C external decay percentage in control samples ranged between 71 (San Pietro) and 85% (Perdingiana), whereas after seven days at 18°C all fruit decayed, regardless of the treatments (data not shown). AZO treatments significantly reduced external decay in all cultivars. However, the protective activity of AZO lasted few days in fruit held at 18°C. After seven days at 18°C all AZO-treated fruit showed external decay, although the extent of the diseased area was considerably lower than in untreated fruit (data not shown). No statistical differences were detected between the two concentrations of AZO (Tables 1-4). Various pathogens developed on the same fruit. In fruit harvested in August, alternaria rot (*Alternaria alternata*) and to a lesser extent, cladosporium rot (*Cladosporium herbarum*) accounted for more than 90% of decay, whereas in fruit harvested in September the number of pathogens increased. However, alternaria rot was always the main cause of decay, followed by cladosporium rot, grey mold (*Botrytis cinerea*), and Penicillium mold (*Penicillium spp*.). Moreover, moulds of these pathogens, which first initiated the infections, in a nested fashion, were often overwhelmed by Rhizopus rot (*Rhizopus stolonifer*). Internal decay severely developed in all cultivars, especially in 'Perdingiana'. Fruit affected by internal decay were significantly more in samples harvested in September. Storage at 5°C reduced the development of internal decay; when fruits were moved to SMC it dramatically increased (Tables 1-4). The influence of AZO against internal decay was negligible.

4. Discussion and Conclusions

Results of this experiment confirmed the high postharvest perishability of the studied fig cultivars, especially 'Perdingiana'. The susceptibility to microbiological deterioration was highly affected by the harvesting period. Fruits harvested in August experienced significantly less decay than those harvested in September. This is because the higher environmental humidity in September is more favourable to field infection than in August, when weath-

Table 4 - Influence of 30-s-dip treatments with azoxystrobin (AZO) at 20°C on external and internal decay incidence in second crop 'Carcanzi Trota' figs after three days at 18°C and 65% RH, or for seven days at 5 °C plus three days at 18°C

Treatments	3 days at 18°C		7 days at 5°C		7 days at 5°C plus 3 days at 18°C	
	External decay %	Internal decay %	External decay %	Internal decay %	External decay %	Internal decay %
	Harvested in August					
Control	26 b[1]	24 a	0 a	3 a	29 b	18 a
50 mg/L AZO	6 a	29 a	0 a	8 a	8 a	21 a
100 mg/L AZO	4 a	27 a	0 a	5 a	3 a	24 a
	Harvested in September					
Control	58 b	85 ab	10 b	16 a	77 b	82 a
50 mg/L AZO	10 a	77 a	0 a	14 a	6 a	81 a
100 mg/L AZO	4 a	91 b	0 a	9 a	2 a	79 a

[z] For each storage period and harvesting time values in columns followed by different letters are significantly different at P≤0.05 according to Fisher's test of the least significant difference.

er conditions are usually dry. AZO was highly effective against all the main pathogens causing external diseases, confirming its broad spectrum of activity (Gullino *et al*., 2000). However, its effectiveness lasted only three days in fruit held in SMC. After seven days at 18°C all fruit treated with AZO exhibited visible infections, although the extension of the lesions were notably less than in control fruit.

AZO was ineffective against internal decay. All studied cultivars had open ostiole. Figs with open ostiole may be more susceptible to endosepsis caused by *Fusarium moniliforme* and souring, incited by different kinds of yeasts and bacteria (Crisosto *et al*., 2011). Infections of both diseases are caused by entrance into the syconia of fig wasps and vinegar flies (*Drosophyla spp.),* dried-fruit beetles (*Carpopilus spp*.), and thrips (*Thrips spp*. and *Frankliniella spp*.) (Michailides *et al*., 1996; Crisosto *et al*., 2011). In figs with close or narrow ostiole, in which only wasps and bees can enter, internal decay is generally lower.

Proper postharvest technologies, such as precooling, film-wrapping, and conditioning in modified atmosphere environment are shown to extend the keeping quality of fig fruit (Turk, 1989; Piga *et al.*, 1995, 1998; D'Aquino *et al.*, 1998, 2003). However, under shelf-life conditions, the market life of figs decreases dramatically, especially in second crop fruit.

The present results reveal that postharvest application of AZO, even at very low rates which could leave on fruit reside levels lower than field treatments with higher rates, resulted only in a slight delay of decay development, as most of the infections generally occur in the orchard and remain latent until fruit ripen. Thus, postharvest AZO treatments associated with low temperatures can give better results if pest management includes a preventive disinfestation program aimed at reducing the insect populations which act as carrier of pathogens' conidia.

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Importance of food labeling as a means of information and traceability according to consumers

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Key words: consumer behavior, consumer profiles, food quality, multidimensional scaling, QR code.

Abstract: Consumption patterns have considerably changed over recent years. We are witnessing more and more frequently to a lack of information (i.e. information asymmetry) between food producers and consumers, this generate in the consumer the latter need to access information related to processes of production of food and food distribution. Decision-making, in absence of further information, leads consumers to pay attention to food labeling. Food labels become the only tool for consumers to acquire additional information about products in order to make the purchase decision. In today's modern and globalized market, labels limitations can be partially overcome by using *Mobile Marketing* tools, such as the QR Code (Quick Response Code). Therefore the objective of this study is: (1) categorize profiles of consumers according to the importance given to various information patterns shown on food labeling; (2) discover consumer behaviors when making a purchasing decision based on food information they require in the label; (3) discover consumer profiles with regards to food quality and the use QR Code to acquire further information about food products. Two Italian regional capitals were chosen, as representative of Northern and Southern Italy, basing on the geographical division of the Country made by the ISTAT (Istituto Nazionale di Statistica). The interviews were carried out by telephone, using a questionnaire. Data collected were processed by Multidimensional Scaling We discovered eight profiles of consumers with different purchasing behaviors. Some consumer profiles, with lifestyles typical of contemporary life, use the QR Code to obtain additional information about food products. The innovative purchasing behavior identifies a consumer who is particularly interested in food quality and safety. Agri-food businesses that focus on quality productions could target the innovative profile and communicate further information trough the use of modern communication technologies.

1. Introduction

Consumption patterns have considerably changed over recent years. Consumers are progressively more and more aware of the issues related to food and impacts on the economy and the environment. They understand the close relationship between food quality , the environment and the wellbeing of society in general, so nowadays they are turning gradually toward those food products which are an expression of this interaction. Consumers perceive, evaluate, and choose each product on the basis of the range of food/environmental/social characteristics that it demonstrates (Lancaster, 1971), sometimes not all explicitly recognizable or conveyed by the producer. However, the process of consumption is not homogeneous: not all consumers have the same values and want the same features, however, despite agreeing with certain issues (for e.g. sustainability, social justice goals, etc.), they do not necessarily change their consumption behavior (Weinstein, 1988). A lack of information (i.e. information asymmetry) between producers and consumers, might prohibit consumers from making informed purchase decisions and not allow them to have insights into the implications of their purchase decisions on the food supply chain (Aprile *et al*., 2012). Indeed, food safety issues often arise from problems of asymmetric information between consumers and food producers with regard to product-specific attributes or characteristics (Ortega *et al*., 2011). Food labels become the only tool for consumers to acquire additional information about products for their purchase decisions; in fact, studies have shown that there is a relationship between the objective characteristics of the label and the reactions of consumers (Cavicchi, 2008; Bialkova and Van Trijp, 2010; Di Pasquale, 2011; Grunert, 2011; Veneziani *et al*., 2012; Vianelli and Marzano, 2012; Siriex *et al*., 2013). Each label conveys a set of characteristics (such as text, color, shape, etc.) that provides information about the product; however, the space available is always limited by the size of the package as well as by the regulations set out by law. In today's modern, globalized market, these limitations can be partially overcome by using *Mobile Marketing*, such as the QR Code (Quick Response Code), that combines the possibility to provide information with that of promoting and enhancing the value of the product and/

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or of the brand, thanks to new communication systems. *QR Mobile Marketing*, used for traceability of food products, transforms the physical identifiers (adhesives and labels on products, packaging, price tags, etc.) into something new and interactive, which can provide much information about the product's production process and general information about the Company (Shiang-Yen *et al*., 2010; Kwak, 2013). This system, applied to labels of food products, whether fresh or processed, can become a tool for the consumer to acquire more information about traceability, provenance, producer's background, production, breeding and cultivation techniques used, etc.

The purpose of this study was to determine what information, of that possibly provided on the labels of food products, consumers are particularly interested in knowing and what information consumers are looking for as added value as a guarantee of food quality and safety, e.g. traceability, absence of GMO, organic production, etc. More particularly, our aim was to (1) categorize profiles of consumers according to the importance given to various information patterns shown on food labeling; (2) discover consumer behaviors when making a purchasing decision based on food information they require in the label; (3) discover consumer profiles with regards to food quality and the use of QR Code to acquire further information about food products. Each consumer profile corresponds to a different, homogeneous market segment for similar purchasing behavior and socio-demographic characteristics.

2. Materials and Methods

Sampling design

For this research, two Italian regional capitals were chosen, as representative of northern and southern Italy, based on the geographical division of the country made by the ISTAT (Istituto Nazionale di Statistica): Milan for the north (N_1) and Palermo for the south (N_2). A sample of consumers equal to n = 267 (with p = 95% and ε = 7%) was extracted using the *Stratified-proportional* sampling schema (with random extraction from each stratum) from the population of residents in the two cities. Population size was equal to $N_1 + N_2 = N$ = 1,917,088 (ISTAT, 2012), where N_1 = 1,262,101 and N_2 = 654,987 (proportional sub-sample sizes are n_1 = 176 and n_2 = 91). The sample units (i.e. respondents) were extracted randomly from the telephone directories of the two cities. Respondents had to be aged between 20 and 80 years, and those who did not fit with this requirement were asked not to continue the interview (Table 1). Education and income levels of respondents had to be proportionally equal among consumers in the sample.

Measurements

The interviews were carried out by telephone, using a proper questionnaire divided into two sections. The first section contained questions on consumer socio-demographic characteristics; the second listed 20 food attributes usually displayed in food labels (i.e. information about foods) and respondents were asked to score, using a scale of 1 (not at all) to 10 (a lot), the attributes in relation to their personal preferences (i.e. subjective importance) based on what they wanted to find in food labels. To evaluate preferences, consumers had to subsequently order the 20 scored attributes with the aim of ranking of them. The attributes were selected based on Caswell's classification of food attributes (Caswell, 2000) (Table 2). These attributes were categorized basing on consumer purchasing behavior variables (i.e. Purchasing Style, Perceived Quality); categorization is reported in Table 3.

Data analysis

Multidimensional scaling (MDS) is a set of data analysis techniques that display the structure of distance-like data as a geometrical picture (Young, F., 1985). MDS has its origins in psychometrics but now has become a general data analysis technique used in a wide variety of fields (Schiffman *et al*., 1981) including marketing studies. MDS portrays the structure of a set of objects from data that approximate the distance between pairs of the objects. The data, which are called similarities, dissimilarities, distances, or proximities reflect the amount of similarity (or dissimilarity) between pairs of objects or events (i.e. in this study the agreement in judgments between pairs of consumers). The graphical output is a representation which consists of a geometric configuration of points on a map (Cox and Cox, 2005). Each point in the configuration corresponds to one of the objects. Two points near each other indicate that there are similarities in the attributes for which the similarity (or dissimilarity) has been calculated (Rebollar *et al*., 2012). This configuration reflects the "hidden structure" in the data, and often makes data much easier to comprehend (Kruskal and Wish, 1984). In addition, the map of stimuli can be interpreted based on just a few dimensions corresponding to the attribute measured (Cox and Cox, 2005; Rebollar *et al*., 2012). The space is usually a two (or three) dimensional Euclidean space (but may be not Euclidean). Classic MDS may be

Table 1 - Sample stratification and sub-samples by range of age and cities (based on Population Strata size)

Range of age	Cities		%
	Milan (P_1 = 1.262.101; n_1 = 176)	Palermo (P_2 = 654.987; n_2 = 91)	
20-30	15	8	8.79
30-40	33	17	18.68
40-50	45	24	26.37
50-60	41	21	23.08
60-70	30	15	16.48
70-80	12	6	6.59
Total	176	91	100

Table 2 - Attributes selected

Caswell's classification	Our classification
Intrinsic quality attributes	
1. Food Safety Attributes	
• Foodborne	
• Pathogens	
• Heavy Metals and Toxins	
• Pesticide or Drug Residues	
• Soil and Water Contaminants	
• Food Additives, Preservatives	
• Physical Hazards	
• Spoilage and Botulism	
• Irradiation and Fumigation Other	
2. Nutrition Attributes	
• Calories	• Calories/kilojoules content
• Fat and Cholesterol Content	• Nutritional information
• Sodium and Minerals	• Ingredients
• Carbohydrates and Fiber Content	• Probiotic product
• Protein	• Nutritional properties
• Vitamins	
• Other	
3. Sensory/Organoleptic Attributes	
• Taste and Tenderness	
• Color	
• Appearance/Blemishes	
• Freshness	
• Softness	
• Smell/Aroma	
• Other	
4. Value/Function Attributes	
• Compositional Integrity	• Shelf life and best before dates
• Size	
• Style	
• Preparation/Convenience	
• Package Materials	
• Keepability	
• Other	
5. Process Attributes	
• Animal Welfare	• Organic products
• Authenticity of Process/Place of Origin	• Production zone
• Traceability	• Traceability
• Biotechnology/Biochemistry	• Genetically modified organism (GMO)
• Organic/Environmental Impact	
• Worker Safety	
• Other	
Extrinsic Quality Indicators and Cues	
1. Test/Measurement Indicators	
• Quality Management	• Organic certification
• Systems	• Quality certification (POD, PGI, DOC, etc.)
• Certification	
• Records	
• Labeling	
• Minimum Quality Standards	
• Occupational Licensing	
• Other	
2. Cues	
• Price	• Price
• Brand Name	• Brand
• Manufacturer Name	• Territory of origin of the product
• Store Name	• Green economy product
• Packaging	• Traditional product
• Advertising	• Easy to cook product
• Country of Origin	• Information on promotions and discounts
• Distribution Outlet	• Food preservation methods
• Warranty	
• Reputation	
• Past Purchase Experience	
• Other Information Provided	

Table 3 - Variable description and Variable code

N.	Variable description	Variable code
1	Shelf-life and Best before dates	Shelf_life_Bestbefore_dates
2	Ingredients	Ingredients
3	Nutritional information	Nutritional_information
4	Production zone	Production_zone
5	Genetically modified organism (GMO)	Genetically_modified_organism_GMO
6	Organic products	Organic_product
7	Green economy product	Green_economy_product
8	Probiotic product	Probiotic_products
9	Food preservation methods	Food_preservation_methods
10	Kalories/kilojoules content	Kalories_kilojoules_content
11	Price	Price
12	Brand	Brand
13	Nutritional properties	Nutritional_properties
14	Territory of origin of the product	Product_territory_of_origin
15	Traceability	Traceability
16	Organic certification	Organic_certification
17	Quality certification (POD, PGI, DOC, ecc.)	Quality_certification
18	Traditional product	Traditional_product
19	Easy to cook product	Easy_to_cook
20	Information on promotions and discounts	Promotions_discounts

Metric or *Nonmetric*. In Non Metric MDS data can be at the ordinal level of measurement (ordinal data), moreover data may be complete or incomplete, symmetric or asymmetric, and it is possible to measure similarities or dissimilarities. For these reasons in this study nonmetric MDS was the most appropriate. The nonmetric MDS uses quantitative data models to describe qualitative data. Nonmetric MDS tries to find a configuration of points that minimizes the squared differences between the optimally scaled proximities and the distances between the points. In other words, coordinates have to be found that minimize the so-called stress, which. MDS programs automatically do to obtain the MDS solution. Among the different versions of stress in literature, Kruskal (1964) provided some guidelines for the interpretation of the stress value with respect to the goodness of fit of the solution. According to Kruskal's findings, stress decreases as the number of dimensions increases (thus a two-dimensional solution always has more stress than a three-dimensional one). There are two additional techniques commonly used for judging the adequacy of an MDS solution: the screen plot and the Shepard diagram.

Among the different MDS techniques, the Alternating Least squares SCALing (ALSCAL) method (Takane *et al*., 1977) was used in this study to analyze the data obtained. This model appeared the most suitable in this case because it can analyze data that are nominal, ordinal, complete, or with missing observations, conditional or unconditional, symmetric or asymmetric, replicated or un-replicated, continuous or discrete (Takane *et al*., 1977). The ALSCAL method uses the Euclidean model as a basis to compute optimal distances between objects in a n-dimensional stimulus space (Kruskal and Wish, 1978). To determine the badness-of-fit between the hypothesized structure and the original data, ALSCAL method minimizes the loss function called S-STRESS (SS), which is minimized using an alternating least squares algorithm (Cox and Cox, 2005). A value of zero indicates a perfect fit (Kruskal and Wish, 1978), but Kruskal and Wish (1984) consider the solution to be acceptable when the S-STRESS values are less than 0.1 (Rebollar *et al*.,, 2012). R-square is a squared correlation index that indicates the proportion of variance of the optimally scaled data that can be accounted for by the MDS procedure; according to literature, values of 0.60 or better are considered acceptable. While R-square is a measure of goodness-of-fit, stress measures badness-of-fit, or the proportion of variance of the optimally scaled data that is not accounted for by the MDS model (Cil, 2012). Stress values of less than 10% are considered acceptable.

The vector model (Davison, 1983) was used to interpret the dimensions of preference in function of observable attributes. This model helps to interpret the dimension of the space of similarities using the attributes which make up the similarities between the stimuli. Following the explanation of the vector model made by Rebollar *et al*. (2012), the attribute-vector is displayed as a line in the space representing consumer preferences based on personal purchasing behavior which the projection of each stimulus corresponds to with the degree of importance assigned by respondents. If two stimuli (i.e. points or objects) are strongly related to each other, then the stimuli projections will coincide very closely and the correlation between them will be quite high. When two objects lie in the same direction, this also indicates a high correlation between the two. When the points that represent the vector are close to a dimension and far from the centre, these are important for explaining that dimension. If a point is midway between two dimensions, this indicates that this attribute explains both dimensions. If an object is close to the center of the stimulus space, this means that it is not important in the explanation of the dimension in this space (i.e. it does not depend on either of the two dimensions). This configuration reflects the "hidden structure" in the data, and often makes data much easier to comprehend (Kruskal and Wish, 1984). This model allowed consumer's preferences (i.e. information contents required in food labels) to be ordered based on the respondents' attribute evaluations. It also made it possible to determine which attributes present a high correlation in the evaluation of the stimuli. Furthermore, it was possible to group the objects on the basis of the distances on the map, highlighting characteristics that were statistically correlated, directly or inversely (Kruskal, 1964; Young *et al*., 1978). In this study these groups represent different consumer's profiles correlated by the information required in food labels (preferences).

The data was processed using the statistical software SPSS (version 21).

3. Results

The preference classification of food attributes based on respondents' ranking is shown in Figure 1. The S-stress of the configuration in the space of the first two dimensions was 0.09102, indicating a good fit in those dimensions. The R-square value was 96%, confirming the good fit in the two dimensions. The figure shows how food attributes required by consumers in food labels were classified according to the dimensions. Dimension 1 differentiates the attributes regarding quality information required by respondents; Dimension 2 is particularly important in this space because it differentiates attributes according to the respondents' purchasing styles. If we look at correlation values in the figure it is possible to graphically analyze similarities (and dissimilarities) of different points and groups of them representing preferences. Different groups of preferences represent similarities in respondents' rankings, and therefore these segments of preferences, similar (homogeneous) inside, are the discovered profiles of consumers. These profiles are characterized by quality information required (namely "Quality") and purchasing styles (namely "Behavior").

Dimension 1 shows two major groups of variables, one in the left part of Figure 1, with respect to the vertical axis (located at zero point), and another in the right part of the axis. Also Dimension 2 has two major groups, one in the lower region and the other in the upper region with respect to the horizontal axis passing through the zero point. The results highlight four main aggregations of points, derived from the combination of variations of the two dimensions. These aggregations have been named as: 1. Extra; 2. Regular; 3. Classic; 4. Innovative (see Fig. 1 and Table 4). Furthermore, it's possible to note four other sub-groups of variables identified in Figure 1 and Table 4: 5. Traditional; 6. Basic; 7. Niche; 8. Prime. Two of these sub-groups, namely Niche and Prime, seem particularly interesting because variables are independent from Dimension 2, and correlation is almost equal to zero.

4. Discussion and Conclusions

Analysis of the results revealed eight profiles of consumers homogeneous for similar preferences of food product attributes required in food labels. Profiles with characteristics and purchasing behaviors are listed in Table 4. According to this classification, the first profile, "1. Extra," includes consumers interested in nutritional/health aspects (e.g. traceability, organic cultivation, GMO-free, etc.) and other attributes like area of production and origin of the product. Profile "2. Regular" represents consumers that base their purchases on regular and general information about food; information about nutrition and product origin are also important. The "3. Classic" profile refers to a consumer who purchases according to brand, price, ease of food preparation, food preservation methods, and health features such as calorie content. The "4. Innovative" profile is interested in personal health and issues like environmental protection and animal welfare; this consumer looks for food quality, food certifications, food safety, and traceability. Profile "5. Traditional" requires information on energy content of food, food preservation methods, and promotional information. Profile "6. Basic" is a consumer who chooses exclusively on the basis of brand and product price. Profile "7. Niche" chooses mainly traditional products that are well-known and familiar; probiotics and healthy/functional foods are of interest. The profile "8. Prime" is a consumer who is interested in primary elements of food products, like best-before date and ingredients, but these primary elements also include other attributes (variables) of food which are closely related to the "made-in" concept, i.e. "territory of origin" and "traditional product" which may provide benefits to health.

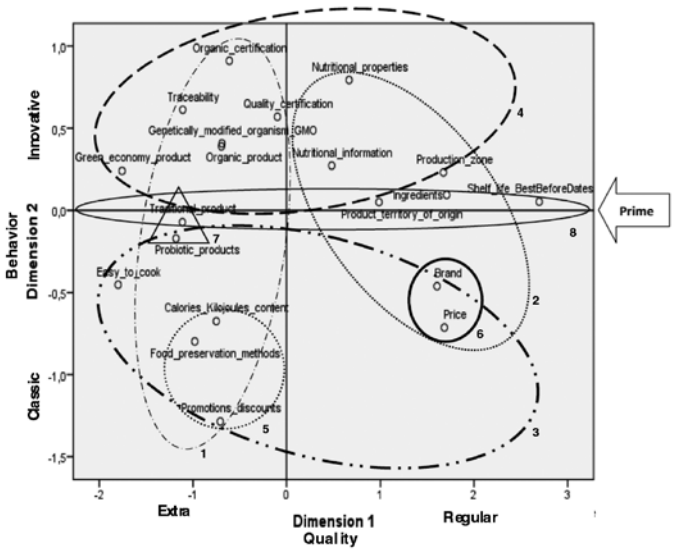

Fig. 1 - Preference classification of food attributes based on respondents' ranking.

The Classic purchasing behavior (profile 3) identifies a consumer who is less interested in innovation. In contrast, the Innovative purchasing behavior (profile 4) identifies a consumer who is particularly interested in food quality and safety. It is interesting to see that Innovative behavior represents consumers who have developed a new concept of product quality: not only intrinsic attributes but also ethics and health attributes, and information regarding quality certification and traceability.

Agri-food businesses that focus on quality productions could therefore target the Innovative profile and communicate further information through the use of modern communication technologies that facilitate the transfer of information via QR Code. This system can be used by producers, distributors, and retailers and it represents a new way to interact with the consumer. Thanks to a website link or to

Table 4 - Characteristics of discovered profiles

Profile	Variables	Quality information required (Quality)	Purchase Styles (Behavior)	Behavior
1. Extra	- Probiotic product - Traditional product - Organic product - Organic certification - GMO - Quality certification - Traceability - Calories/kilojoules content - Food preservation methods - Information on promotion and discount	Extra	From Classic to Innovative	Purchase mainly certified food products
2. Regular	- Price - Brand - Ingredients - Nutritional information - Nutritional properties - Territory of origin of the product - Production zone	Regular	From Classic to Innovative	Purchase basing on regular and general information
3. Classic	- Price - Brand - Calories/kilojoules content - Food preservation methods - Information on promotion and discount - Easy to cook	From regular to Extra	Classic	Little time available for preparing meals but health-conscious
4. Innovative	- Probiotic product - Nutritional information - Nutritional properties - Organic certification - Organic product - GMO - Quality certification - Traceability - Green economy product - Production zone	From regular to Extra	Innovative	Living well in harmony with the environment
5. Traditional	- Calories/kilojoules content - Food preservation methods - Information on promotion and discount	Extra	Classic	Combine classic information with saving
6. Basic	- Price - Brand	Regular	Classic	Very simple purchase decision based solely on price and brand
7. Niche	- Traditional product - Probiotic product	Extra	Classic or Innovative	Healthy tradition-bound
8. Prime	- Shelf life and best before dates - Ingredients - Territory of origin of the product - Traditional product	From extra to regular but very primary attributes	Classic or Innovative	Purchase based on primary elements of food products

videos with information about the food production process, food production area, traceability or alternative ways to use and store the product, this system allows producers to survey consumers' interest toward their products.

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Effects of modified atmosphere packaging on quality parameters of minimally processed table grapes during cold storage

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Key words: fresh-cut, MAP, postharvest performance, *V. vinifera.*

Abstract: 'Vittoria' and 'Red Globe' table grapes were packed in microperforated polypropylene films (passive MAP) and non-perforated polyethylene (active MAP: 1) 20% CO_2 + air; 2) 5% O_2 + 15% CO_2 + N) and stored at 5 °C for 28 days. Microperforated polypropylene packages had the highest postharvest performance in both cultivars until 14 days of cold storage, with reduced weight losses, rachis and berry decay. Total soluble solids content slightly increased in non-perforated polyethylene boxes as a consequence of the higher weight losses, particularly in active MAP with 5% O_2 + 15% CO_2 + N, while no significant differences were found for titratable acidity. Non-perforated polyethylene packages showed excessively high CO_2 concentrations (30-60%) in headspace gas composition, causing berry and rachis decay. The highest post harvest performances in terms of weight losses, Total soluble solids, titratable acidity, crunchiness, juiciness and berry decay were found with micro-perforated polypropylene packages after 14 d of 5 °C cold storage, and 6 days of shelf-life (15-18°C).

1. Introduction

In recent years, consumer demand for ready-to-eat fruits and vegetable products, has increased considerably due to product added value and easy consumption. A major challenge facing this industry is maintaining and preserving the quality of minimally processed produce because the physical damage caused by preparation accelerates the metabolism with associated increases in certain biochemical reactions responsible for quality loss (Sabir *et al*., 2011).

Table grape is a non-climacteric fruit with a low rate of physiological activity that is very sensitive to water loss and fungal infection (*Botrytis cinerea*) during postharvest handling and cold storage. Gray mold is the most aggressive post harvest disease because of its ability to develop at low temperatures. For this reason, it is standard practice to fumigate table grapes with sulfur dioxide (SO_2) immediately after packing (Crisosto and Mitchell, 2002). However, sulphite residue is an important consumer problem. To avoid it, different technologies have been developed such as controlled atmosphere (CA), storage with high CO_2 (15-25%), and hot water and ethanol (Crisosto *et al*., 2002; Karabulut *et al*., 2004), although injuries occur, like rachis browning and off-flavors.

One of the most effective technologies in terms of quality commodity preservation is the combination of modified atmosphere and packaging (MAP) that protects from water loss and browning, and reduces chilling injury symptoms (Kader *et al*., 1989). Martinez-Romero *et al*. (2003) showed that the use of MAP maintained table grape berry quality.

The aim of the present work was to study the effects of MAP on quality parameters of minimally processed white 'Vittoria' and red 'Red Globe' table grape cultivars during 28 days of cold storage.

2. Materials and Methods

Plant material

White and red table grapes (cv. Vittoria and cv. Red Globe) were harvested from a commercial vineyard, located in Mazzarrone Italy (37° 05' N, 14° 34' E, 128 m asl, CT). The vineyard has a row spacing of 2.8 m × 2.8 m (with 1275 plants/ha) with "tendone" training system, covered with net.

White 'Vittoria' (July) and red 'Red Globe' (September) table grapes were hand-picked at the commercially ripe stage (≥12.5°Brix), suitable for the fresh fruit market. Immediately after harvest, table grape quality parameters were analyzed on three clusters per cultivar.

Sample preparation and packaging

After harvest, 'Vittoria' and 'Red Globe' clusters were immediately transported to the laboratory and selected on

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the basis of uniform size, color, firmness, lack of diseases, health and greenish rachises. Table grape clusters were then cut with sanitized scissors to obtain a 200 g cluster. The samples were sterilized with sodium hypochlorite solution (150 ppm active chlorine) and were irradiated with ultraviolet-C (UV-C) to control microbial spoilage.

After washing, each 200 g cluster sample was packaged in a 15×8 cm rigid polypropylene retail box and sealed with two different films: microperforated polypropylene and non-microperforated polyethylene. The boxes sealed with microperforated polypropylene film were packaged in air (passive MAP), while, the boxes sealed with non-microperforated polyethylene film were packaged under two different initial headspace gas compositions (active MAP: 1) 20% CO_2 + air; 2) 5% O_2 + 15% CO_2 + N). The sealed boxes were then stored at 5°C with a relative humidity of 90% for 28 days, with quality evaluation performed on days 0, 7, 14, 21, and 28, after the shelf-life (5 days at 15-18°C).

Quality parameters: firmness, soluble solids, titratable acidity and decay

For each treatment, 12 randomly chosen boxes, were taken for shelf life at each sampling date (7, 14, 21, 28 days) and analyzed for weight loss, soluble solid content (SSC), pH, titratable acidity (TA) and decay. Berry weight loss was calculated as a percentage of its fresh weight (at the beginning of each shelf life). Total soluble solids (TSS) were determined by digital refractometer (Palette PR-32, Atago Co., Ltd) and titratable acidity (TA) by titration of 10 ml homogenized berry flesh juice with 0.1 N NaOH to an endpoint of pH 8.1 and expressed as the percentage of tartaric acid. (mod. S compact titrator, Crison Instruments.

Headspace gas composition

O_2 and CO_2 contents of the packages were measured using an O_2 and CO_2 portable analyzer (Checkpoint, Dansensor Italia, Segrate, Milano, Italy) during each shelf-life, after 7, 14, 21, 28 days at 5°C.

Statistical analysis

Data were submitted to one-way analysis of variance (ANOVA) and means were separated with Tukey's test at P≤0.05. The statistical analysis was carried out using Systat 10 (Systat, USA).

3. Results and Discussion

Weight loss (Fig. 1A) of 'Red Globe' packaged with non-microperforated polyethylene sealed boxes (active MAP: 20% CO_2+air (PET1); 5% O_2+15% CO_2+N (PET2) increased sharply during storage, reaching values lower (8% and 14 % respectively) than those measured at the beginning of the shelf-life. Weight losses of 'Red Globe' grape packaged with microperforated polypropylene boxes (passive MAP (PP) were significantly lower than PET1 and PET2, with weight losses of 4% during shelf-life.

TSS content did not significantly change (P<0.05) in 'Red Globe' table grapes wrapped with PP (passive MAP) at the end of the shelf-life (Fig. 1B), while a significant increase (P<0.05) in TSS was found in table grapes packaged in both active PET1 and PET2 (active MAP), with an increase of 4% and 6% at the end of the shelf-life (Fig. 1B).

Non-perforated polyethylene packages (PET1 and PET2) showed excessively high CO_2 concentrations (from 30 to 60%) in headspace gas composition of 'Red Globe' and 'Vittoria' table grapes, causing berry and rachis decay (Fig. 1C, 2C).

Non-microperforated polyethylene sealed boxes of both cultivars (PET and PET2), showed a similar trend in

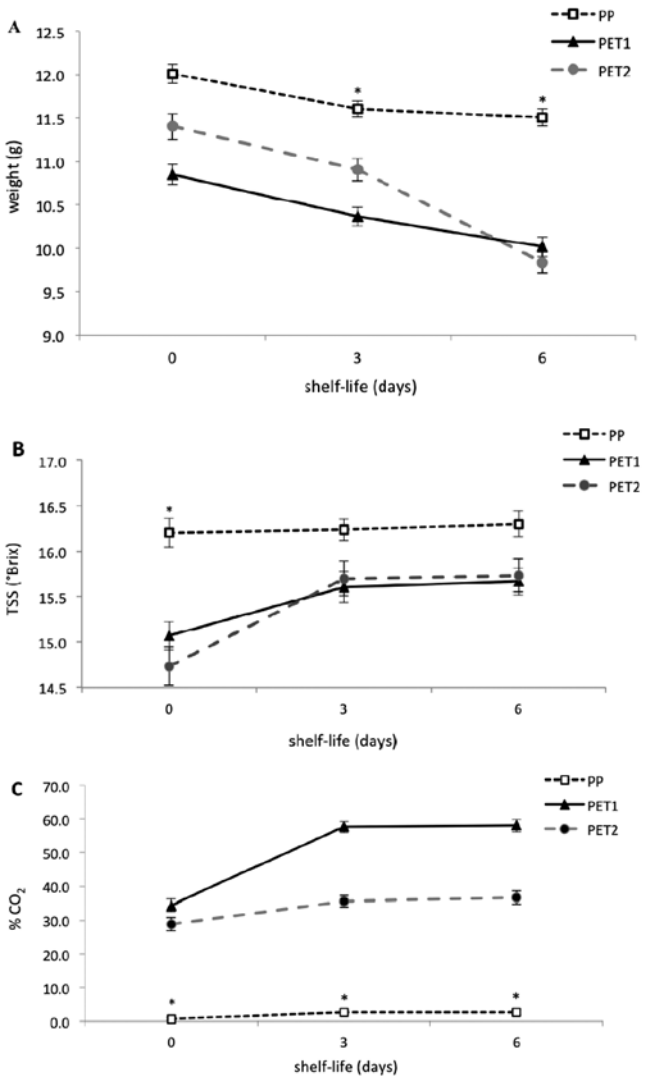

Fig. 1 - Changes in weight loss (A), soluble solid content (B) and headspace CO_2 concentration (C) of minimally processed 'Red Globe' grape berries during shelf life after 14 days of cold storage (5°C): microperforated polypropylene packaging in air (PP); non-microperforated polyethylene packaging in 20% CO_2+air (PET1); non-microperforated polyethylene packaging in 5% O_2+15% CO_2+N (PET2). Data are mean±SE. * indicate significant differences for values (P≤0.05).

terms of weight loss. Indeed, berries had 9% and 14% decrease of weight loss during the shelf-life while PP table grape boxes showed a significantly lower decrease (4%) compared to PET1 and PET2. (Fig. 2A).

TSS of PET1 and PET2 'Vittoria' berries were significantly higher (P<0.05) than those of PP treatments, with an increase of 5% and 8% respectively at the end of the shelf-life (Fig. 2B). A good visual aspect and no off-flavor were detected until 21 days of cold storage in 'Vittoria' PP table grapes (data not shown). Packaging did not affect TA in either of the cultivars.

Microperforated polypropylene packages (PP) had the highest postharvest performance in both cultivars until 14 days of cold storage, also in terms of rachis and berry decay.

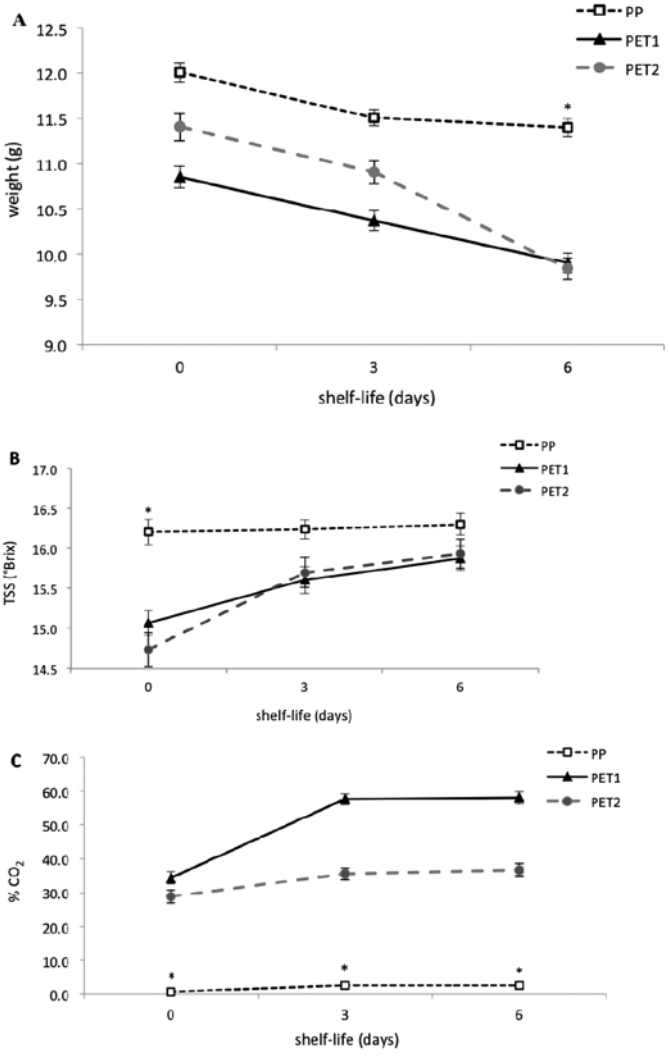

Fig. 2 - Changes in weight loss (A), soluble solid content (B) and headspace CO_2 concentration (C) of minimally processed 'Vittoria' grape berries during shelf life after 14 days of cold storage (5°C): microperforated polypropylene packaging in air (PP); non-microperforated polyethylene packaging in 20% CO_2 + air (PET1); non-microperforated polyethylene packaging in 5% O_2+15% CO_2+N (PET2). Data are mean±SE. * indicate significant differences for values (P≤0.05).

4. Conclusions

'Vittoria' and Red Globe' grapes showed the best postharvest performances in terms of weight losses, TSS, TA, crunchiness, juiciness and berry decay when packed with micro-perforated polypropylene packages after 14 days of 5°C cold storage and 6 days of shelf-life (15-18°C). This study has demonstrated that active MAP (PET1 and PET2) did not affect the postharvest performances of minimally processed table grapes. As Costa *et al*. (2011) reported, the conditions created in the package to modify the initial headspace composition probably compromised the mass loss and the sensory acceptability of the fresh produce.

'Vittoria' berries and rachises from PP boxes remained healthy and green, even after 21 days of 5°C storage, which could be useful for consumers interested in ready-to-eat products.

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