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Short communication

The paradox of oleuropein increase in harvested olives (*Olea europea* L.)

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ABSTRACT

Olives are non-climacteric fruit. In a previous article, oleuropein (OE) increased substantially in fresh green olives exposed to 20 °C for 7 d, but the increases were lower in preharvest treated fruit with an ethylene synthesis inhibitor. The present aim was to investigate whether phenolic compounds, including OE, were affected by ethylene treatment in green harvested olives. Postharvest treatments with the ethylene perception inhibitor, 1 -methylcyclopropene (1-MCP) at 1.5 μL L^{-1} for 12 h, and/or ethylene at 1000 μL L^{-1} at 20 °C for up to 10 d were applied to fruits of 'Konservolia' cultivar. The results showed that ethylene and/or 1-MCP had similar effects on total phenolics (TP), total antioxidant capacity (TAC) and OE and these results are revealed for the first time in olives. Ethylene had no effect on green loss, but 1-MCP prevented it slightly. In all treated fruit, but not in controls, TP and TAC were increased soon after harvest and remained almost stable throughout exposure, whereas OE increased in controls and all treated at later stages (as confirmed by HPLC-DAD-ESI-MS) independently of degreening. The present experiments could be applied to studies of ethylene perception and transcription related responses in these non- climacteric fruit. In practice, harvested olives do not lose their antioxidant capacity, but the OE elevation in short-stored olives at ambient temperature might have an impact on olive products quality.

1. Introduction

Olive fruit, destined either for oil or for table use, are highly appreciated due to their high nutritional value attributed to their large proportion of unsaturated fatty acids, phenolic compounds, tocopherols, phytosterols, squalene and other nutrients and micronutrients. Olive phenolics are known for health promoting effects due to their capacity to scavenge free radicals (Visioli et al., 2002). The secoiridoid oleuropein (OE), the major phenolic compound in olives, was shown to be particularly beneficial to human health, but also the most responsible compound for pungency in olive products. Decreases in oleuropein occur during fruit development (Morelló et al., 2004) and render the raw material less pungent, shaping the flavour of products.

Olives exhibit some peculiarities in ripening physiology that deserve scientific attention, such as plums (Lippert and Blanke, 2004). Olives are considered as non-climacteric fruit, do not synthesize high ethylene levels and do not respond to exogenous ethylene (Kader, 2002). Nevertheless, the application of the ethylene perception inhibitor 1-methylcyclopropene (1–MCP) to harvested green 'Konservolia' olives prevented skin colouring (reddening) and flesh softening (Ramin, 2007), indicating a role of ethylene for olive ripening. Preharvest treatment of olives with the ethylene synthesis inhibitor 1–aminoethoxyvinyl glycine prevented reddening on the tree, as well as green loss and softening in harvested fruit at 20 °C (Tsantili et al., 2012). In contrast, Shulman et al. (1974) found that ethylene prevented the degreening in olives harvested at the green maturity stage. Therefore, there was a further need to investigate the ethylene effect on OE and other phenolics. In general, the use of the ethylene perception inhibitor, 1-methylcyclopropene (1-MCP), was considered essential to clarify the ethylene effect on ripening processes in fruit (Watkins, 2006).

2. Materials and methods

2.1. Plant material and treatments

Dark green stage fresh olives (*Olea europaea* L. cv, Konservolia) were harvested on 30 October from eight trees grown in Fthiotida, Greece. 'Konservolia' is the main Greek cultivar used for Spanish-style table olives. Olives were mixed, divided into groups of 12 and placed in 5 L sealed treatment jars at 20 °C. Olives were treated with 1-MCP or

Abbreviations: DPPH, 2,2-diphenyl-1-picrylhydrazyl; GAE, gallic acid equivalents; HPLC, high performance liquid chromatography; HT, hydroxytyrosol; LC/MS, liquid chromatograph–mass spectrometer; Lut, luteolin; Lut 7, luteolin 7-*O*- glucoside; 1-MCP, 1-methyl cyclopropene; OE, oleuropein; TAC, total antioxidant capacity; TE, trolox equivalents; TP, total phenolics; Tyr, tyrosol

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ethylene or first 1-MCP and then ethylene, with the treatments being 1-MCP, ETH and 1-MCP + ETH, respectively. Untreated fruit corresponded to controls (CON). In particular, 1-MCP was generated from tablets of Smartfresh™ after their placement in a 15.5 L gas tight sealed jar for 2 h with one activator tablet, according to the applications recommended by the manufacture (AgroFresh, Spring House, PA). Then, a calculated volume of 1-MCP was withdrawn from the stock jar and added to treatment jars for 12 h. The estimated 1-MCP concentration in olives was 1.5 μL L^{-1}. Then, olives in all jars were adequately aerated before re-sealing. Afterwards, ethylene was added at a concentration of 1000 μL L^{-1} to magnify the effects and prepared according to Tsantili and Pontikis (2004). Controls included a sachet with ethylene absorbent (Bi-On Ethyl stopper, super absorbent sachets, water resistant, Bioconservacion S.A., Gava, Spain). All jars contained ~25 g $Ca(OH)_2$ to absorb CO_2 emitted from fruit respiration. Jars were opened once/twice a day for up to 10 d and ethylene was re-established in jars. Each sample consisted of three replicates of 12 olives each on each sampling day. All determinations were carried out after 1.5, 5 and 10 d.

2.2. Concentrations of ethylene, CO_2 and O_2 during treatment

Ethylene concentration in treatment jars was analyzed by injection of 1 mL gas sample in a gas chromatograph (Sigma 300; Perkin-Elmer, Norwalk, CT, USA) equipped with a flame ionization detector (Tsantili, 2014). The detection limit was about 10 nL L^{-1}. Concentrations of CO_2 and O_2 were analysed by 0.5 mL injection of gas into a chromatograph (Hewlett Packard 5390 Series II) with a thermal conductivity detector, according to Tsantili et al. (2002).

2.3. Peel color

Peel colour was estimated by a chromatometer (CR-300; Minolta, Ahrensburg, Germany) and expressed in lightness (L^*), hue angle (h^o) and chroma (C^*) (Tsantili et al., 2012).

2.4. Extraction procedures and determinations of total phenolic concentration (TP), total antioxidant capacity (TAC) and phenolic compounds

Slices, freeze-dried and stored at −80 °C, were powdered with mortar in liquid nitrogen. Powdered tissue of 500 mg was extracted with 80% v/v acetone, thrice. After being centrifuged at 4000*g* the combined supernatants were divided into 2 parts, A for TP and TAC evaluations and B for phenolics extraction, according to Tsantili (2014).

The TP concentration was determined by the use of Folin-Ciocalteu reagent (Singleton et al., 1999) and TAC following the radical scavenging capacity (DPPH) assay (Brand-Williams et al., 1995), according to Tsantili (2014). The TP results were expressed as gallic acid (mg GAE) equivalents and TAC as trolox acid equivalents (μmol TE) on a freeze-dried weight basis.

The identification and quantification of phenolic compounds was performed by high performance liquid chromatography (HPLC) system, according to Tsantili (2014). Results were expressed in mg per g of freeze dried weight.

The identification of OE was further confirmed by a Shimadzu liquid chromatograph – mass spectrometer (LC/MS – 2010A, Kyoto, Japan), including a HPLC system (Shimadzu LC – 10AD VP), diode-array detector (DAD), electron spray ionisation (ESI), mass selective detector (MSD) and computing integrator LC/MS Solution Version 3 01.162), according to Tsantili et al. (2012) (Fig.S1).

2.5. Data analysis

All attributes were analyzed by two – way ANOVA, with treatments and time of exposure being the two factors. One-way ANOVA was carried out to test the effect of exposure time on controls from day 0.

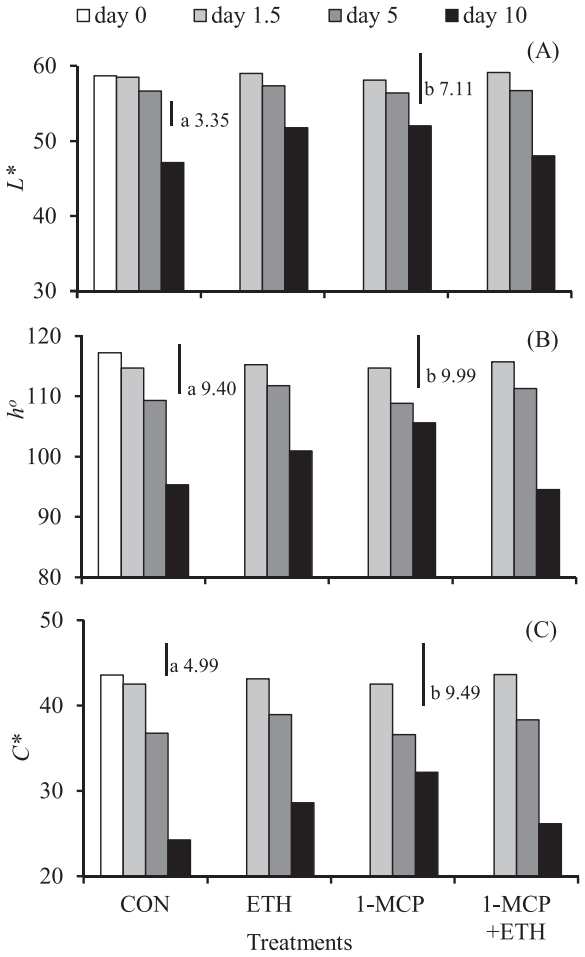

Fig. 1. Effect of ethylene and/or 1-MCP on colour parameters L^*, h^o and C^*, depicted in (A), (B) and (C), respectively, in green harvested 'Konservolia' olives exposed to 20 °C for up to 10 d. CON, controls; ETH, 1-MCP and 1-MCP + ETH, olives treated with ethylene, 1-MCP and 1-MCP + ETH, respectively. HSD, Tukey's honest significant difference values at $P = 0.05$. Bars a, from one-way ANOVA including all controls; Bars b, from two-way ANOVA including all samples apart from initials. $Pd^a < 0.001$ in (A), (B) and (C). Ptr^b is NS, but $Pd^b < 0.001$ in all three (A), (B) and (C), whereas $Ptr\ x\ d^b < 0.05$ in (B) and NS in (A) and (C). NS, non-significant. *P*, Probabilities of the effect: treatment (*Ptr*), days (*Pd*) and their interaction (*Ptr* × *d*).

The significant differences were estimated at $P < 0.05$. Data analyses were made using JMP 7.0.1 (SAS Institute, Cary, NC, USA).

3. Results and discussion

To ensure the treatment effects, the tested levels of ethylene treatment were continuously close to 1000 μL L^{-1}. The ethylene concentrations in jars with controls were not detectable. The levels of O_2 and CO_2 concentrations were constantly higher than 19% and between 0.03 and 0.04%, respectively, in all jars.

Initially, values of L^*, h^o and C^* were about 58, 118 and 42, respectively (Fig. 1A, B and C). Olives, CON, ETH and 1-MCP + ETH treated, underwent the degreening process after 1.5 d, the higher green reduction occurred after 5 d exposure, although fruit still retained some green colour on day 10, averaging about 49, 98 and 28 in L^*, h^o and C^*, respectively. Neither ETH nor 1-MCP + ETH treated fruit exhibited an effect on peel colour different from CON in all three colour parameters, but 1-MCP prevented h^o decreases (at significance limits). These results agree with the preventing effect of 1-MCP on colouring, according to Ramin (2007). The discrepancy of results between these works and that of Schulman and Lavee (1974) could be attributed to different

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