



1-MCP and storage conditions on the ripening and production of aromatic compounds in *Conference* and *Alexander Lucas* pears harvested at different maturity stages

Marcos Vinícius Hendges^{a,*}, Daniel Alexandre Neuwald^b, Cristiano André Steffens^a,
Rajko Vidrih^c, Emil Zlatić^c, Cassandro Vidal Talamini do Amarante^a

^a Department of Agronomy, State University of Santa Catarina, Lages, Brazil

^b Competence Center for Fruit Growing – Lake Constance and Physiology of Specialty, Crops University of Hohenheim, Ravensburg, Germany

^c Department of Food Science and Technology, Biotechnical Faculty, Ljubljana, Slovenia

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ABSTRACT

The objective of this investigation was to evaluate the quality parameters and aroma profiles of *Conference* and *Alexander Lucas* pears after 7 months of storage. Fruits of both cultivars were harvested at two different maturity stages and stored under cold (CS) (20.9 kPa O₂ + < 0.04 kPa CO₂), controlled atmosphere (CA) (2 kPa O₂ + < 0.7 kPa CO₂) and ultra-low oxygen condition (ULO) (0.7 kPa O₂ + < 0.7 kPa CO₂). CS and CA storage also included treatment with 1-methylcyclopropene (1-MCP) at 300 nL L⁻¹. After seven months of storage at 0 ± 0.1 °C and 94 ± 2% RH plus seven days at 20 ± 2 °C and 60 ± 5% RH, fruits were evaluated for flesh firmness, peel color ('hue' angle = h°), soluble solids content (SSC), titratable acidity (TA), respiratory rate, ethylene production rate, and production of aromatic compounds. Fruits of both cultivars from the first maturity stage treated with 1-MCP did not develop a buttery texture or yellow color and produced significantly less alcohols and esters. A combination treatment (1-MCP + CA) most severely suppressed aroma development, particularly in the *Conference* cultivar. ULO storage also reduced yellowing, ethylene production and the development of aromatic compounds in both cultivars from the first maturity stage, but this reduction was less pronounced than that observed for 1-MCP treatments. The production of aromatic compounds was lower in fruits from ULO, regardless of their maturity stage. Fruits from the second harvest treated with 1-MCP had significantly more aromatic compounds than did those from the first harvest. In general, the CA condition has no effect on the ripening and production of aromatic compounds in both cultivars compared to CS.

1. Introduction

The development of a buttery texture, yellowing of the peel, synthesis of aromatic compounds, and adequate sugar-acid content are important quality attributes in European pears (Kappel et al., 1995; Plocharski and Konopacka, 1999; Mitcham et al., 2003). *Conference* pears are among the most cultivated in Europe (Chiriboga et al., 2013b) and present juicy, crunchy flesh with sweet taste (Saquet, 2016). This cultivar is harvested from September and stored until February under cold and end of April in controlled atmosphere (Silbereisen et al., 2015). *Alexander Lucas* are cultivated mainly in Germany, Holland, Czech Republic and Poland with not to many evidence about post-harvest behavior. Although not very representative in Europe its production has increased in countries such as Holland and Poland (Groot

et al., 2000; Wawrzyńczak et al., 2006). This cultivar is characterized by large fruits, yellow epidermis when completely mature and, in some cases with reddish spots. The flesh is juicy with sweet and fruity taste (Silbereisen et al., 2015).

Different postharvest technologies, such as cold storage (CS) and controlled atmosphere (CA) storage conditions are used to maintain the fruit quality during and after storage of pears. CA storage delays ripening and maintains fruit quality (Moya-León et al., 2006), but it may cause a significant decrease in the production of aromatic compounds of *Packham's Triumph*, *Doyenne du Comice* (Chervin et al., 2000; Lara et al., 2003), and especially under conditions of ultra-low oxygen (ULO) in *Bartlett* pears (Zlatić et al., 2016). However, this quality reduction may be overcome by harvesting the fruits at later maturity stages (climacteric phase) (Brackmann et al., 1993).

* Corresponding author.

E-mail address: marcos_hendges@hotmail.com (M.V. Hendges).

Alternatively, or in association with CS or CA, treatment with 1-methylcyclopropene (1-MCP) can be used to maintain the quality attributes of fruits. The use of 1-MCP maintains flesh firmness (Calvo and Sozzi, 2004; Chiriboga et al., 2011; Rizzolo et al., 2014), delays yellowing of the fruit peel (Calvo and Sozzi, 2004; Trinchero et al., 2004), and reduces some physiological disorders (Argenta et al., 2003; Rizzolo et al., 2005). However, under certain conditions 1-MCP treatment provokes overly firm flesh and a green peel and may below in aromatic compounds in *d'Anjou*, *Packham's Triumph* and *Conference* pears (Argenta et al., 2003; Rizzolo et al., 2005; Moya-León et al., 2006; Chiriboga et al., 2013a).

Argenta et al. (2003) observed a reduction in the production of aldehydes, alcohols, and esters in *d'Anjou* pears treated with 1-MCP. These compounds are the most commonly found aromatic volatiles in European pears (Suwanagul and Richardson, 1998; Rapparini and Predieri, 2003). Aldehydes present an herbaceous (green) aroma (Plotto et al., 1999) and some alcohols contribute to the fruit's aroma and are precursors of esters (Lara et al., 2003; Echeverria et al., 2004). Esters in particular contribute to the overall aroma of European pears (Suwanagul and Richardson, 1998; Rapparini and Predieri, 2003; Moya-León et al., 2006). Volatile esters such as acetates of butyl, hexyl, pentyl, butyl butanoate and ethyl hexanoate are related to the characteristic pear aroma (Rapparini and Predieri, 2003). Hexyl and butyl acetates may also represent a large amount of volatiles (Chervin et al., 2000) with fruity and sweet aroma (Rapparini and Predieri, 2003) and ethyl (2E,4Z)-deca-2,4-dienoate has a strong impact on European pears (Zlatić et al., 2016). According to Rizzolo et al. (2005) hexanal (herbaceous), ethyl acetate (sweet), hexyl acetate (fruity), ethyl butanoate (fruity, ripe) and butanol (fruity) are identified as important volatiles in the *Conference* pear aroma. However, the olfactory notes of high amount of ethyl acetate are not described as "fruity" (Zlatić et al., 2016).

According to Villalobos-Acuña et al. (2011), the negatively effect of 1-MCP on the ripening of European pears can be affected by several conditions. The different responses of cultivars, harvest time and storage conditions of pears to 1-MCP may be associated with the internal ethylene concentration (Zhang et al., 2009), and the efficacy of 1-MCP treatment depends on the maturity stage of the fruit (Alpalhão et al., 2006). Some scholars have studied the influence of maturity stage at harvest and 1-MCP treatment in *Conference* pears (Rizzolo et al., 2005; Chiriboga et al., 2013b) however, this is not completely clear and there are few studies evaluating that effect in *Alexander Lucas*.

The objective of this work was to evaluate the ripening and production of aromatic compounds in pears of the *Conference* and *Alexander Lucas* cultivars, harvested at two different maturity stages, treated with 1-MCP and stored under CS, CA and ULO condition.

2. Material and methods

Pears of the *Conference* and *Alexander Lucas* cultivars were harvested in 2012 in southwestern Germany's Center for Competence in Fruit Production, Constance Lake, Ravensburg, Baden-Württemberg. The moment of harvest was chosen according to the indication to the best fruit quality for both cultivars by the Streif index (SI) (Neuwald, 2018).

The fruits were harvested at two maturity stages, based on the Streif index (SI): the first maturity stage (1MS) with coefficients of 0.13 and 0.15 (fruits harvested on 09-05-2012) and the second maturity stage (2MS) with coefficients of 0.8 and 0.8 (fruits harvested on 09-18-2012) for the *Conference* and *Alexander Lucas* cultivars, respectively (Table 1). The SI was calculated using the following formula: $SI = \text{firmness of flesh (kgf)} / [\text{soluble solids content (}^\circ\text{Brix)} \times \text{iodine-starch index (1-10)}]$ (Table 1).

The fruits were subjected to treatments under CS (20.9 kPa O_2 + < 0.04 kPa CO_2), CA (2 kPa O_2 + < 0.7 kPa CO_2), 1-MCP (300 nL L^{-1}) plus CS (1-MCP + CS), 1-MCP plus CA (1-MCP + CA), and ULO (0.7 kPa O_2 + < 0.7 kPa CO_2). The treatments were applied with three

replications, and the experimental unit consisted of eight fruits for the analysis of quality attributes (Tables 1 and 2) and ten fruits for the analysis of aromatic compounds (Tables 3 and 4). The temperature and relative humidity (RH) were the same for all treatments ($0 \pm 0.1^\circ\text{C}$ and $94 \pm 2\%$ RH, respectively).

After seven months of storage plus seven days at room conditions ($20 \pm 2^\circ\text{C}$ and $60 \pm 5\%$ RH), the fruits were evaluated for flesh firmness, peel color ('hue' angle = h°), soluble solids content (SSC), titratable acidity (TA), respiratory rate, ethylene production rate, and aromatic compound content: aldehydes (hexanal and 2-hexenal), alcohols (1-butanol and 1-hexanol), esters (ethyl acetate, butyl acetate, pentyl acetate, hexyl acetate and ethyl (2E,4Z)-deca-2,4-dienoate).

Flesh firmness (N) was determined using a penetrometer with a tip of 0.5 cm^2 . Readings were taken after peel removal from an equatorial section of the fruit. Peel color (h°) was determined using an electronic colorimeter (Minolta CR 300C), in which 180° represents full green and 90° full yellow color. The readings were performed at the point of largest diameter of the fruit.

SSC (%) was evaluated with a digital refractometer with automatic temperature correction using a homogeneous sample of filtered juice. TA (mL of NaOH) was determined in a sample of 10 mL of juice diluted in 50 mL of distilled water, titrated with NaOH (0.1 N) to a pH of 8.1. This procedure was performed using an automated titrator (Metrohm) with 52 positions.

The respiratory rate was determined at 20°C in 4.2-L hermetically sealed glass jar in three replicates of four fruits using continuous flow gas analyzers and expressed in $\text{nmol of } CO_2 \text{ kg}^{-1} \text{ s}^{-1}$. The ethylene production rate ($\text{nmol of } C_2H_4 \text{ kg}^{-1} \text{ s}^{-1}$) was measured in 1-mL air samples withdrawn extracted using 10 mL syringes from the hermetic glass jar (4.2-L) that were closed for two hours at 20°C . The samples were injected into a gas chromatograph (Carlo Erba Strumentazione) equipped with a flame ionization detector (FID - Eletrometer - 180) and a stainless Steel, $0.9\text{ m} \times 1/8''$ filled with activated alumina, 60-mesh column. Injector, detector and oven (Fractovap - 2150) temperature were set to 200°C , 240°C and 100°C , respectively.

The aromatic compounds were evaluated in three replications of ten fruits. The fruits were cut longitudinally in a wedge shaped (30 g), remaining flesh and peel, and it was mixed with 30 g of a saturated calcium chloride solution ($CaCl_2$, $700\text{ g } L^{-1}$). The mix (flesh with peel and saturated $CaCl_2$ solution) was homogenized at 13,500 rpm by T18 Ultraturrax® homogenizer (IKA®, Staufen, Germany) mixer and then stored in freezer (-28°C).

The extraction of pear volatiles was carried out using a solid-phase microextraction (SPME) fiber coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) sorbent (1 cm long, 50/30 μm thickness, StableFlex™, Supelco, USA). A 10 mL of sample mix was placed in 20-mL glass vial and 50 μL of ethyl nonanoate (internal standard, 0.175 mg/L solution in ethanol) was added to each vial. All vials were flushed with nitrogen and immediately sealed with a PTFE/silicone septum and closed with crimp cap. The sample vials were conditioned in a temperature-controlled heating module at 40°C for 30 min. After extraction, the fiber was removed from the sample and the analytes were thermally desorbed in the injector port of the GC.

Gas chromatography/mass spectrometry (GC/MS) analysis were conducted on a GC 7890 A gas chromatograph (Agilent Technologies, CA, USA) equipped with a MPS2 Multipurpose autosampler (Gerstel GmbH, Mülheim an der Ruhr, Germany) and 5975C mass spectrometer (Agilent Technologies). Volatile compounds were desorbed into a GC injector port at 250°C in splitless mode for 2 min. The gas chromatograph was fitted with a ZB-WAX capillary column, $60\text{ m} \times 0.32\text{ mm i.d.}$ with $1\text{ }\mu\text{m}$ film thickness. Helium was the carrier gas at a flow rate of 1.2 mL/min at 40°C . Oven temperature was programmed as follows: initial temperature 40°C held for 5 min, then 4°C/min to 230°C . The volatile compounds were identified with a mass selective detector (5975C, Agilent Technologies, CA, USA). The detector operated in the m/z range between 30 and 250, ion source and quadrupole temperature

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