



Comparative analysis of aroma compounds in 'Bartlett' pear in relation to harvest date, storage conditions, and shelf-life



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ABSTRACT

In this study 'Bartlett' pear fruit from two harvest dates were stored under normal atmosphere and ultra-low oxygen (ULO: 0.8 kPa O₂, <0.5 kPa CO₂) at +1 °C and –1 °C. Storage under ULO at –1 °C greatly delayed ripening, as demonstrated by maintained firmness and suppressed synthesis of aroma volatiles after storage. ULO storage also suppressed synthesis of esters, including two character-impacting compounds: methyl and ethyl (2E,4Z)-deca-2,4-dienoate. Synthesis of hexyl acetate was suppressed under ULO storage regardless of temperature, while ethyl acetate synthesis was suppressed only by ULO at –1 °C. The levels of most aroma volatiles were recovered after the following 10 d of shelf-life, although with significantly lower recovery for methyl and ethyl (2E,4Z)-deca-2,4-dienoate in fruit under ULO storage. Although synthesis of aroma volatiles was most suppressed under ULO at –1 °C, butyl and hexyl acetate levels recovered better in fruit under ULO storage at –1 °C than at +1 °C. Acetaldehyde and nonanal were the principal aldehydes present, with levels that were higher in early harvested fruit and in fruit stored at +1 °C. Using multivariate analysis, we found two clusters one for after storage and the other for after shelf life samples, with the exception of pears after storage and after shelf life at +1 °C and normal atmosphere positioned on the lower side of graph. Overall, this analysis discriminated between mentioned storage conditions that are specifically defined by hexylacetate and decanal on the one side and ethyl butanoate, pentyl acetate and α-farnesene on the other side.

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1. Introduction

Pears (*Pyrus communis* L.) are one of the most important fruit grown around the world, with the 'Bartlett' pear cultivar as the most commonly grown pear in most countries outside Asia. USA pear production mainly includes the four cultivars of 'Bartlett',

'd'Anjou', 'Bosc', and 'Doyenné du Comice' (Suwanagul and Richardson, 1998a,b), where 'Bartlett' represented 47% of the total of 716 × 10⁶ kg in 2014 (Perez and Plattner, 2014). Due to their flavor and sweetness, 'Bartlett' pears are well suited for many forms of processing, like canned half pears, pear juice, and colorless pear brandy. However, approximately one third of production is destined for fresh consumption.

As reported by Li et al. (2012a,b), 'Bartlett' pears are classified as a fruit with a very intense aroma. They are considered as summer pears that are usually harvested in the first half of August. As 'Bartlett' pears are susceptible to even slightly elevated temperatures (Trincherio et al., 2004), they can be stored for no more than three months. Due to this relatively short storage period compared to other pear varieties, controlled atmospheres, storage temperature of –1 °C, and 1-methylcyclopropene (1-MCP) treatment (Trincherio et al., 2004) have been used. The ethylene inhibitor 1-MCP is widely used to delay ripening in a range of fruit (Watkins,

Abbreviations: NA, normal atmosphere; ULO, ultra-low oxygen atmosphere; PCA, principal component analysis; LDA, linear discriminant analysis; S₊₁_NA, after storage, +1 °C, normal atmosphere; SL₊₁_NA, after shelf life, +1 °C, normal atmosphere; S_{–1}_NA, after storage, –1 °C, normal atmosphere; SL_{–1}_NA, after shelf life, –1 °C, normal atmosphere; S₊₁_ULO, after storage, +1 °C, ultra low oxygen; SL₊₁_ULO, after shelf life, +1 °C, ultra low oxygen; S_{–1}_ULO, after storage, –1 °C, ultra low oxygen; SL_{–1}_ULO, after shelf life, –1 °C, ultra low oxygen.

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2006), including pear (Mwaniki et al., 2005). For pears, postharvest application of 1-MCP has been reported to provide valuable benefits, such as decreased softening, good color development, and delay in respiration and ethylene synthesis (Trincherio et al., 2004).

However, although 1-MCP has been shown to have these benefits, it also has deleterious effects on the aroma-volatile synthesis. Decreased volatile production is a well-known effect of controlled atmosphere storage, as reported by Lara et al. (2003) for 'Doyenne du Comice' pears, and as also reported for 'Packham's Triumph' pears (Chervin et al., 2000). Among other storage parameters used with fruit, a temperature of -1°C was proposed to prolong the storage life of 'Doyenne du Comice' (Shang Ma and Chen, 2003), 'Anjou' (Bai et al., 2009; Xie et al., 2014), 'Abate Fetel' (Predieri and Gatti, 2009), and 'Bartlett' pears (Villalobos-Acuña et al., 2011; Whitaker et al., 2009). On the other hand, most European pears, including 'Bartlett', show resistance to ripening after harvest and require low temperatures and/or ethylene treatments to counteract this phenomenon (Makkumrai et al., 2014; Villalobos-Acuña and Mitcham, 2008). Low-temperature conditioning can result in higher production of esters and fruity flavor attributes, while ethylene treatment is associated with low levels of esters and high levels of aldehydes (Makkumrai et al., 2014).

Kondo et al. (2006) quantified 38 aroma volatiles in 'Bartlett' pears, which included alcohols, esters, ketones, aldehydes, terpenes and enols. High levels of aldehydes accentuate an apple-like aroma, while esters are responsible for fruity flavor attributes. These short-chain to medium-chain esters of alcohols include hexyl acetate as a contributory flavor compound with a sweet fruity note (Gan et al., 2014), and methyl (2*E*,4*Z*)-deca-2,4-dienoate and ethyl (2*E*,4*Z*)-deca-2,4-dienoate as character-impact compounds.

The objective of the present study was to determine the effects of harvest date, storage temperature ($+1^{\circ}\text{C}$, -1°C), and atmosphere (normal atmosphere [NA], ultra-low oxygen [ULO]) on the evolution of aroma volatiles immediately after storage, and after the following 10 d of shelf-life. Additionally, we carried out multivariate analysis for the experimental conditions in terms of the storing of the pear samples, to determine those that differentiated significantly according to the positive parameters of the aroma volatiles.

2. Materials and methods

2.1. Plant material, treatments and storage

The fruit of 'Bartlett' pear were harvested on two different harvest dates: on the commercial harvest date, and 7 d later. On the day of harvest, the fruit were randomly divided in four treatment groups. The fruit were kept in an experimental storage house under either NA or ULO (0.8 kPa O_2 , <0.5 kPa CO_2) conditions and at $+1^{\circ}\text{C}$ and -1°C , for 120 d. Low CO_2 was used to prevent internal browning (Chen, 2004). After storage, the fruit were held at 20°C for 24 h, and then analyzed for fruit firmness and aroma volatiles. The same analyses were repeated after the following 10 d of shelf-life at 20°C .

2.2. Flesh firmness

Fruit firmness was measured at harvest, 24 h after removal from cold storage, and after 10 d of shelf life at 20°C . The same 15 fruit were analyzed first for aroma compounds and afterwards for firmness. For the firmness analyses, the skin was removed from two areas of 15 mm in diameter on opposite sides of the equatorial region of each fruit. The firmness was measured using a Chatillon DFG-50 digital force gauge (Ametek Test & Calibration

Instruments; Largo, Florida, USA), equipped with an 8-mm plunger.

2.3. Determination of volatile compounds

Isolation of aroma compounds was performed by the stir-bar sorptive extraction (SBSE) technique of Raffo et al. (2009), with some modifications. A single fruit was put in a hermetically closed glass jar (volume, 2.5 L) together with a stir bar (PDMS-coated, 1 mm thickness, 10 mm length) for a period of 2 h at 20°C . Fifteen replications were carried out for each treatment combination.

Repeatability tests were carried out by extractions of samples of 15 fruit with 15 different stir bars. All of the analyses were performed on an Agilent GC 7890 with a 5975 mass selective detector (Agilent Technology, Santa Clara, CA, USA). The instrument was equipped with a thermal desorption unit (TDU, Gerstel) and a PTV inlet (CIS 4, Gerstel). Introduction of the stir bar into the TDU unit was done by an MPS 2 autosampler (Gerstel).

The volatile compounds were desorbed from the stir bar under the following conditions: during the TDU desorption step, the PTV inlet was in solvent vent mode (split ratio, 1:50; vent pressure, 62 kPa [9.0 psi]) and the TDU was in splitless mode. The TDU initial temperature was set at 50°C for 0.5 min, then increased to 250°C at a rate of $60^{\circ}\text{C min}^{-1}$, and held for 5 min. The desorbed compounds were trapped in the PTV inlet at -40°C . After the cryofocusing step, the inlet was heated at $12^{\circ}\text{C min}^{-1}$ to 280°C (held for 5 min) in splitless mode (splitless time, 1.2 min). The separation was carried out on a fused silica ZBwax 60-m capillary column with 0.32 mm internal diameter and 1.0 μm film thickness (Agilent Technologies). The gas chromatograph oven temperature was initially set at 40°C (5 min isothermal), then ramped at $4^{\circ}\text{C min}^{-1}$ to 200°C (5 min isothermal). Helium was used as the carrier gas, at a linear flow velocity of 29 cm s^{-1} . The mass selective detector was operated at 70 eV with electron impact ionization. The transfer line was set to a temperature of 230°C . Electron impact mass spectra were acquired in full-scan ($30\text{--}300\text{ m/z}$).

The concentrations of the sample compounds were calculated from the peak areas of the selected ions and the corresponding standards of known concentration. α -Farnesene was quantified in equivalents of limonene. The external standard solutions (1 μL) prepared in methanol were introduced into a 2.5-L glass 'headspace' jar, which was then closed with a PTFE-coated silicon septum. The jar was left for at least 2 h at room temperature, to completely evaporate the sample inside the jar, and then the analysis was carried out. A stir bar was then inserted into the headspace jar, which was then closed again. An open glass insert was used to suspend the stir bar inside the jar (available from Gerstel). After this absorption, the stir bar was removed from the jar insert and placed in an empty TDU tube for analysis. Acetaldehyde, methyl acetate, ethylacetate, ethanol, propyl acetate, methyl butanoate, ethyl butanoate, butyl acetate, 2-methylpropan-1-ol, pentyl acetate, limonene, *E*-2-hexenal, nonanal, hexyl butanoate, ethyl octanoate, 1-heptanol, α -farnesene, methyl (2*E*,4*Z*)-deca-2,4-dienoate, and ethyl (2*E*,4*Z*)-deca-2,4-dienoate were from Sigma. Butanal, hexanal, 1-butanol, 1-pentanol, hexylacetate, 6-methyl-5-heptene-2-one, 1-hexanol, decanal, and benzaldehyde were from Fluka. Identification of the compounds was performed by comparison of their mass spectra with those of the National Institute of Standards and Technology (USA) mass spectral database, for the corresponding standards. Kovats retention indices (Table 1) were calculated using a mixture of *n*-alkanes ($\text{C}_5\text{--}\text{C}_{20}$), according to the method of Kovats (1958). The data for the volatile compounds are expressed in $\mu\text{g L}^{-1}$ of each compound standardized to a one hour collection with a 100 g fruit sample.

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