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Relationship between volatiles and antioxidant compounds in scald on 'Alexander Lucas' pear in different postharvest conditions

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ABSTRACT: This study aimed to evaluate the effects of storage conditions on the development of superficial and senescent scald and its relationship with volatiles and antioxidant compounds in 'Alexander Lucas' pears (*Pyrus communis*) harvested at two maturity stages in three orchards. A two-factorial design combined two maturity stages with five storage conditions: standard oxygen plus cold storage (control; 20.9 kPa O_2, 0.03 kPa CO_2), controlled atmosphere (low O_2) (2.0 kPa O_2, 0.7 kPa CO_2), each without and with 1-MCP (300 nl·L^{-1}), and ultra-low O_2 atmosphere (0.7 kPa O_2, < 0.7 kPa CO_2). The fruit were grown in three regions in Germany and stored for seven months (0 °C; 94% relative humidity) plus seven days simulated shelf-life. Scald development was positively correlated to the 6-MHO and α-farnesene (after three months of storage) and negatively correlated to an ascorbate level. The application of 1-MCP led to a reduction in the production of volatile compounds and antioxidants, except by ascorbate, which appeared to be associated with delayed ripening and the less severe development of scald. Ultra-low O_2 reduced the scald, but the effects depended on the production region.

Key words: ultra-low oxygen, 6-methyl-5-hepten-2-one, 1-methylcyclopropone, α-farnesene, phenolics content, controlled atmosphere.

INTRODUCTION

Superficial scald is a physiological disorder that occurs in the absence of preventive treatments during the storage and shelf-life of pears, thus causing economic and quality losses (Isidoro and Almeida 2006, Guerra et al. 2012). The symptoms result in brown or dark colored spots on the fruit epidermis, which often enlarge after removal of the fruit from storage (Whitaker et al. 2009). Superficial scald is associated with oxidation of α-farnesene into conjugated trienols (Gapper et al. 2006, Vanoli et al. 2010) and 6-methyl-5-heptene-2-one (6-MHO), which cause cell damage that leads to the onset of scald symptoms (Pesis et al. 2010).

Senescent scald is the browning of the peel surface that can extend into the underlying flesh and affect European pear fruit during prolonged storage (Lum et al. 2017), and the incidence of senescent scald following storage can be affected by the maturity at harvest (Zhao et al. 2020).

Mitigating actions against ethylene production such storage under low-O_2 or ultra-low-O_2, controlled atmospheres and application of 1-methylcyclopropene (1-MCP), which delay fruit senescence and/or decrease synthesis and oxidation of α-farnesene, can reduce the scald development (Shang Ma and Chen 2003, Gapper et al. 2006, Isidoro and Almeida 2006, Xie et al. 2014). According to Larrigaudière et al. (2016), the prevention of α-farnesene oxidation can determine the fruit sensitivity to scald. They considered that the oxidation prevention capacity of α-farnesene is determined by the antioxidant potential or the specific antioxidants in the fruit. Thus, the development of this physiological disturbance might depend on the balance between the oxidative processes that are triggered by the products generated from oxidation of α-farnesene (conjugated trienols, 6-MHO) and the presence of antioxidant compounds (phenolic compounds, ascorbate) in the tissue (Guerra et al. 2012).

Furthermore, Lindo-García et al. (2021) found that the regulatory processes triggered by cold stress that leads to the development of scald in pears are complex and cultivar dependent. While the 'Blanquilla' pear is related to the fruit's ability to produce ethylene mediated by cold, the 'Flor d'Hivern' pear seems to be mainly associated with a better process of acclimatization to the cold. In 'Blanquilla' pears, disorder incidence depended on the initial fruit maturity at harvest and was expressed only after removal from cold storage. In contrast, disorder incidence in 'Flor d'Hivern' cultivar developed during cold storage and regardless of initial harvest date (Lindo-García et al. 2020).

According to Whitaker et al. (2009), in addition to post-harvest storage conditions, some factors such as pre-harvest temperatures, fruit maturity levels, and production regions might be important for the development of superficial scald. The maturity stage of fruit affects the incidence of scald (Zhao et al. 2020), but the relationship between maturity stages and superficial scald is still not clear (Whitaker et al. 2009, Hui et al. 2016).

This study aimed to evaluate the effects of 1-MCP and storage conditions on development of scald in 'Alexander Lucas' pears harvested at two maturity stages in three orchards, and to define their relationships with the synthesis of α-farnesene, 6-MHO, antioxidant phenolic compounds, and ascorbate.

MATERIALS AND METHODS

Fruit, storage conditions, and tissue sampling

'Alexander Lucas' pear (*Pyrus communis* L.) fruit at two maturity stages MS1 (September 5, 2012) and MS2 (September 18, 2012) were harvested in Baden-Württemberg state, southwest Germany. The orchards are located in the municipalities of Ravensburg (RV; 47°46'N latitude, 9°36'E longitude, average altitude 447 m), Langenargen (LA; 47°35'N latitude, 9°32'E longitude, 402 m average altitude), and Öhringen (OHR; 49°11'N latitude, 9°30'E longitude, 228 m average altitude). The harvest dates were chosen according to indications for the best fruit quality for 'Alexander Lucas' pears as evaluated by the Streif index (SI) (Neuwald 2018).

The fruit from both maturity stages were stored for seven months under the following conditions:

- Control (CS) = 20.9 kPa O_2/ 0.03 kPa CO_2;
- Controlled atmosphere (CA) = 2 kPa O_2/ 0.7 kPa CO_2;
- Control with 1-MCP = 300 nl·L^{-1} (CS+MCP);
- Controlled atmosphere with 1-MCP = 300 nl·L^{-1} (CA+MCP);
- Ultra-low O_2 atmosphere (ULO) = 0.7 kPa O_2/ < 0.7 kPa CO_2.

All these treatments were maintained at 0 ± 0.1 °C and 94% ± 2% relative humidity. Following these seven months of storage, the pears were all kept for seven days of shelf-life under ambient conditions (20 ± 2 °C/60% ± 5% relative humidity). The fruit were evaluated for occurrence of superficial and senescence scald (SI), and also for respiration rate, ethylene release, α-farnesene, 6-MHO, phenolic compounds, and ascorbate. α-farnesene and 6-MHO were also evaluated after three months of storage plus seven days of shelf-life under the same conditions.

The experimental design was completely randomized using a factorial scheme (two maturity stages and five storage conditions) as three replicates of 30 fruit to evaluate the severity of scald, four fruit to analyze respiration rate and ethylene release, 10 fruit to analyze α-farnesene and 6-MHO production, and five fruit to analyze antioxidant compounds (phenolic compounds, ascorbate).

Measurements of maturity parameters and ripening index by Streif

Measurements of maturity parameters were made as reported previously by Hendges et al. (2020), and the maturity parameters are given in Table 1.

Table 1. Maturity attributes of the 'Alexander Lucas' pears harvested at the two maturity stages from the three production regions.

Orchard	Maturity stage	Circumference (mm)	Weight (kg)	Flesh firmness (N)	Starchiodine (1–10)*	Soluble solids (%)	Peel color (°hue)	Titratable acidity (mL NaOH)	Streif index
Ravensburg	MS1	72.73	0.23	54.97	2.90	12.70	116.7	6.31	0.15
	MS2	79.90	0.31	46.74	4.53	12.27	115.6	5.03	0.08
Langenargen	MS1	78.10	0.28	53.70	3.94	11.10	116.9	4.22	0.12
	MS2	78.69	0.30	45.47	5.87	11.80	116.7	3.98	0.06
Öhringen	MS1	71.83	0.23	58.80	4.57	13.80	117.2	6.72	0.09
	MS2	79.00	0.25	52.33	4.71	13.20	115.9	5.17	0.08

MS1: harvested on September 5, 2012; MS2: harvested on September 18, 2012; *Starch iodine (1- immature to 10- mature); Streif index: firmness/SSC × starch.

The ripening index (RI) was calculated according to Eq. 1:

$$RI = \frac{\text{flesh firmness (kg·cm}^{-2})}{\text{soluble solids (\%)} \times \text{iodine-starch index (1-10)}} \quad (1).$$

Evaluation of scald and measurements of respiration rate and ethylene release

Scald index was determinate by Eq. 2 using scald incidence (number of fruit affected by scald) and the percentage of the fruit epidermis affected by scald (scald severity). The scald severity was evaluated according to the following scale:

- 0 = no scald;
- 1 = 1 to 25% surface affected;
- 2 = 26 to 50% surface affected;
- 3 = > 50% surface affected.

The scald index was determined according to Eq. 2:

$$\text{Scald index} = \frac{(\text{number of fruit x 0}) + (\text{number of fruit x 1}) + (\text{number of fruit x 2}) + (\text{number of fruit x 3})}{([(\text{total number of fruit x 3})]\ \text{x 100}} \quad (2).$$

The respiratory rate was determined at 20 °C in a 4.2-L hermetically sealed glass jar in three replicates of four fruit using a continuous-flow gas analyzer. To measure the ethylene release rate, four fruit were placed in a hermetically sealed container for 2 h, and a 10-mL air sample was collected with a syringe. One mL of the container atmosphere was injected into a gas chromatograph (Carlo Erba Strumentazione), with temperatures of 200, 240, and 100 °C for the injector, detector, and column, respectively.

Extraction and quantification of α-farnesene and 6-MHO

To determine the α-farnesene and 6-MHO levels, the method described by Hendges et al. (2018) was applied. Briefly, 30 fruit separated into three replicates were cut longitudinally into wedges. Thirty grams of fruit wedges, including the skin and pulp, were homogenized at 22,413 × g (IKA®, Staufen, Germany) with 30 g of saturated solution of calcium chloride (700 $g \cdot L^{-1}$ $CaCl_2$) and stored at -28 °C for further studies.

For the analysis, 10 g of sample mix was transferred into 20-mL glass vial, and 50 µL of ethyl nonanoate (internal standard, 0.175 $mg \cdot L^{-1}$ in ethanol) was added to each sample vial. The vials were flushed with nitrogen and immediately closed with PTFE/silicone septum and sealed with screw caps. A solid phase microextraction fiber (divinylbenzene/ carboxen/ polydimethylsiloxane sorbent length, 1 cm; thickness, 50/30 µm; StableFlex, Supelco, United States of America) was used for volatile extraction. Identification of α-farnesene and 6-MHO was performed by comparisons of their mass spectra with those of available commercial standards and with spectra published in the National Institute of Standards and Technology mass spectral library. Quantification of α-farnesene and 6-MHO was carried out by preparation of calibration curves for each volatile compound. The concentrations of the sample compounds were calculated from the peak areas and the corresponding standards of known concentrations.

Extraction and quantification of ascorbate

For ascorbate determination, the fruit epidermis was removed from the region with the largest fruit diameter. After removal, the epidermis was fragmented, frozen in liquid nitrogen, and stored at -28 °C. Prior to the analysis, the epidermal samples were ground in a coffee mill that was pre-cooled with liquid nitrogen. A total of 15 mL 3% metaphosphoric acid solution (HPO_3, 65% purity, 30 $g \cdot L^{-1}$) was added to 6-g fruit peel powder. The mixture was homogenized (Turrax; 60 s, 22,413 × g), protected from light in a water bath (4 °C, for at least 20 min). After that, the samples were centrifuged (14,000 × g, 20 min), and the supernatant was filtered through a nylon filter (45 µm) coupled to a 13-mm syringe. Forty microliters were injected into the HPLC (Bischof, Germany) manually, of which 20 µL were dispensed into a chamber and then transferred to the column of the HPLC, with 0.8 $mL \cdot min^{-1}$ eluent flow rate, as 2.5 g tetra-n-butylammonium hydrogen sulfate. The column was incubated at 30 °C. The content of ascorbate was calculated using standards containing 5 mg ascorbate in 100 mL 3% metaphosphoric acid.

Extraction and quantification of phenolic compounds by HPLC-DAD

Fruit epidermis was removed from the region with the largest fruit diameter and immediately frozen in liquid N_2. Subsequently, the samples were stored at -80 °C, freeze dried and ground in a ball mill for extraction of phenolic compounds. This was performed by adding 1 mL of methanol and 200-µL methanolic solution of 3-methoxyflavone (c = 0.1 $mg \cdot mL^{-1}$) as internal standard, to 400 mg dry powder for 30 min in a cooled ultrasound water bath (7 °C). After centrifugation (11,180 × g, 10 min, and 4 °C), the supernatant was evaporated, and the residue was redissolved in 200-µL methanol. A 10 µL sample of these extract was injected into a Kontron HPLC system (Kontron Instruments, Germany) equipped with two pumps, autosampler (model 231, Gilson Abimed Systems) and a diode array detector (DAD). The phenolic compounds were separated on a Nucleosil C18 column (240 × 4 mm i.d., Macherey-Nagel) and eluted with a mixture of water containing 5% formic acid (solvent A) and methanol (solvent B) at a flow rate of 0.5 $mL \cdot min^{-1}$. The gradient profile was as follows:

- 0–5 min = 5% B;
- 5–10 min = 5–10% B;
- 10–15 min = 10% B;
- 15–35 min = 10–15% B;
- 35–55 min = 15% B;
- 55–70 min = 15–20% B;
- 70–80 min = 20% B;
- 80–95 min = 20–25% B;
- 95–125 min = 25–30% B;
- 125–145 min = 30–40% BA;
- 145–160 min = 40–50% B;
- 160–175 min = 50–90% B;
- 175–195 min = 90% B.

For selective detection of flavan-3-ols using post-column derivatization with p-dimethyl-aminocinnamic aldehyde, a further Gynkotek HPLC pump (model 300 C, Germering, Germany) and a VIS-detector at 640 nm (Kontron Detector 432, Kontron Instruments) were used. Chlorogenic acid was identified in comparison to a standard solution, and as for the other hydroxycinnamic acids it was measured at 320 nm. The flavonols were detected at 350 nm.

Statistical analysis

The experimental design was completely randomized using a factorial scheme (two maturity stages and five storage conditions). The treatments were composed of three replications and unit experimental of 10 and 30 fruit for the analysis of volatile compounds and scald index, respectively. The scald index (percentage values) was transformed in to "arc sin $[(x + 0.5)/100]^{½}$" before being subjected to analysis of variance. Tukey's test was used to compare the means ($p < 0.05$).

The statistical interactions between treatments were tested, and, when significant, their values (two maturation stages *versus* five storage conditions) were compared. When the interactions between the treatments were not significant, only the average of the treatments was compared.

The data were analyzed individually for each production region. Principal component analysis was used to summarize the information in the data set of studied parameters. It enables determination of parameters that contribute the most to the classification of the pears and visualization of the classification in a two-dimensional space.

RESULTS AND DISCUSSION

Scald index

The application of 1-MCP diminished the scald index in pears from all three of the orchards. 1-MCP treatment can extend the shelf-life of fruit, maintaining active compounds in peels in addition to reducing superficial scald (Tedeschi et al. 2023). When applied to fruit stored under the standard control CS conditions, 1-MCP reduced the scald index in fruit from LA and OHR, as also for the CA condition for RV (MS2 only) and LA (Table 2). Vanoli et al. (2016) also reported the actions of 1-MCP for the reduction of superficial scald in 'Abate Fetel' pears stored under their CS and CA conditions, and Isidoro and Almeida (2006) showed that 1-MCP was effective in further reducing scald in pears stored in controlled atmosphere. Argenta et al. (2002) reported that 1-MCP treatments prevent or reduce superficial and senescent scald in 'd'Anjou' pears stored at 1 °C. 1-MCP treatment under CS condition maintains higher level of antioxidant compounds compared to CS, preserving high antioxidant activity even with an early harvest (Tedeschi et al. 2023).

In our study, for the fruit harvested under MS1 in RV and under both maturity stages in LA, the development of scald was completely inhibited by CA+MCP. These results showed that the effects of 1-MCP on scald of 'Alexander Lucas' pears that was primarily seen for CS might be dependent on preharvest factors (e.g., pedoclimatic conditions, maturity stage). The reason for the greater control of superficial scald in the fruit under CA+MCP might be related to the joint actions of the two conditions (i.e., low O_2, 1-MCP) on the delay of fruit senescence and the reduction in the ethylene release and the synthesis and oxidation of α-farnesene, along with the degradation of the cell membranes.

No effects were seen for the CA condition on the scald index according to maturity stages and production regions (Table 2). In general, CA inhibits the development of superficial scald; however, its effectiveness is influenced as a function of the cultivar and storage period (Lurie and Watkins 2012). The inability of the CA storage on the senescent scald development control found in the present work could be the result of some changes in the metabolism of fruit in function of internal disorders observed. These disorders can lead to changes in ripening and consequently an imbalance on the fruit. In pears, the symptoms of scald-like disorders can result from multiple and different metabolic changes (Lindo-García et al. 2020). The CA stored pears from the LA and OHR orchards showed higher scald indices, and OHR also showed significantly higher values as compared to CS and the other storage conditions.

Table 2. Scald index, α-farnesene, and 6-methyl-5-heptene-2-one levels by 'Alexander Lucas' pears harvested at the two maturity stages from the three production regions according to the different storage conditions (0 ± 0.1 °C; 94% ± 2% relative humidity) followed by seven days under ambient conditions (20 ± 2 °C; 60% ± 5% relative humidity)*.

Treatment	Data according to orchard and fruit maturity stage								
	Ravensburg			Langenargen			Öhringen		
	MS1	MS2	Mean	MS1	MS2	Mean	MS1	MS2	Mean
Scald index (%)									
CS	4.8Bab	23.1Aa	13.9	33.8	37.5	35.6a	7.3	10.3	8.8bc
CS+MCP	3.9Aab	9.9Aab	6.9	0.7	7.1	3.9b	0.0	4.1	2.1d
CA	6.6Aab	12.6Aab	9.6	39.5	47.3	43.3a	27.6	37.8	32.7a
CA+MCP	0.0Ab	1.3Ac	0.7	0.0	0.0	0.0b	2.5	3.0	2.8cd
ULO	16.4Aa	7.2Bbc	11.8	7.4	0.7	4.1b	23.8	12.7	18.3ab
Mean	6.3	10.8		16.3A	18.5A		12.3A	13.6A	
CV%	41.1	22.2		39.0			36.6		
α-Farnesene (μg·L^{-1}) (three months of storage followed by seven days under ambient conditions)									
CS	1,988.4	1,734.7	1,861.6a	1,016.9	1,194.8	1,105.9a	1560.3	1968.9	1764.6a
CS+MCP	304.5	492.9	398.5b	210.4	700.9	455.7b	473.5	985.5	729.5bc
CA	2,310.7	1,267.4	1,789.0a	949.3	1,309.1	1,129.2a	1,093.3	2,156.3	1,624.8a
CA+MCP	141.5	365.9	253.7b	53.4	117.3	85.3b	228.5	252.2	240.4c
ULO	761.2	1,122.7	942ab	451.3	834.8	643.1ab	578.3	1,799.9	1,189.2ab
Mean	1,101.2A	996.7A		536.3B	831.4A		786.8B	1,436.6A	
CV%	54.14			53.62			40.29		
α-Farnesene (μg·L^{-1}) (seven months of storage followed by seven days under ambient conditions)									
CS	645.4	386.4	515.9a	245.5	275.41	260.5a	148.0Bb	339.3Aab	243.7
CS+MCP	77.2	179.5	128.4c	168.2	185.68	177.0a	192.8Aab	124.0Ac	158.7
CA	462.1	279.4	370.7ab	333.3	309.09	321.2a	158.1Bb	503.9Aa	331.0
CA+MCP	322.6	230.7	276.7bc	306.9	197.74	252.3a	216.9Aab	361.0Aab	289.0
ULO	270.2	322.9	296.5bc	317.9	204.63	261.3a	385.9Aa	281.1Bbc	333.5
Mean	355.5A	279.8A		274.4A	234.4A		220.3	321.8	
CV%	38.5			42.1			34.2	20.1	
6-Methyl-5-hepten-2-one (μg·L^{-1}) (three months of storage followed by seven days under ambient conditions)									
CS	253.3	260.0	256.67a	276.7	436.7	356.67a	263.3Aa	276.7Aab	270.0
CS+MCP	60.0	86.7	73.30b	46.7	140.0	93.33dc	43.3Bb	126.7Abc	85.0
CA	316.7	263.3	290.00a	203.3	283.3	243.30b	210.0Aa	420.0Aa	315.0
CA+MCP	33.3	40.0	36.67b	13.3	43.3	28.33d	36.7Bb	76.7Ac	56.7
ULO	266.7	193.3	230.00a	103.3	176.7	140.00c	220.0Aa	243.3Abc	231.6
Mean	186.00A	168.67A		128.6B	216.00A		154.7	228.7	
CV%	28.82			31.51			20.85	28.09	
6-Methyl-5-hepten-2-one (μg·L^{-1}) (seven months of storage followed by seven days under ambient conditions)									
CS	136.0Aab	113.0Aab	120.0	286.0Aa	220.0Aa	250.0	186.0Aa	90.0Bab	130.0
CS+MCP	93.0Abc	106.0Ab	100.0	143.0Ab	163.0Aab	150.0	103.0Ab	66.0Ab	80.0
CA	170.0Aa	170.0Aa	170.0	160.0Ab	170.0Aa	160.0	183.0Aa	153.0Aa	170.0
CA+MCP	56.0Ac	100.0Ab	80.0	63.0Bc	110.0Ab	70.0	80.0Ab	103.0Aab	90.0
ULO	146.0Aab	113.0Aab	130.0	116.0Abc	123.0Ab	120.0	110.0Ab	143.0Aa	130.0
Mean	120.2	120.4		153.6	157.2		132.4	111.0	
CV%	16.7	18.5		18.6	14.4		19.06	25.51	

CS: standard oxygen control cold storage; CA: controlled (low-oxygen) atmosphere; ULO: ultra-low oxygen atmosphere; MCP: 1-methylcyclopropene; MS1: harvested September 5, 2012; MS2: harvested September 18, 2012; *values within a column followed by the same lower-case letter are not significantly different, and values within a line followed by the same capital letter are not significantly different (Tukey's test; $P < 0.05$); CV: coefficient of variance.

The storage under ULO reduced the scald in the fruit harvested from RV for MS2 and from LA for both maturity stages, compared to fruit kept under CS. The action of ULO is probably associated with the reduced oxidative processes within the cell membranes that involve α-farnesene. ULO storage atmosphere can inhibit the oxidase enzymes activities, and especially ACC oxidase, that most likely is a key factor determining the action of this storage condition on scald inhibition (Larrigaudière et al. 2019).

As observed in the studies conducted by Giné-Bordonaba et al. (2013) and Larrigaudière et al. (2019), the ULO conditions are more efficient for the reduction of superficial scald as compared to the CA conditions in apples and pears. Despite this, the effect of ULO can be dependent on the fruit production region (Giné-Bordonaba et al. 2013). Differently to 1-MCP, that inhibits ethylene metabolism and fruit ripening, ULO condition only delays this process, as a result of the action of both cold temperature and low O_2 partial pressure (Larrigaudière et al. 2019). Larrigaudière et al. (2019) found that inhibition of scald, in part, may be mediated by increased ethanol accumulation, which may act as a weak antioxidant or protect against the effects of cold.

Farnesene production

After three months of storage plus seven of the ambient conditions, fruit treated with 1-MCP (CS or AC) showed lower α-farnesene production, regardless of the orchard and maturity at harvest. After seven months of storage, the treatment with 1-MCP reduced α-farnesene production in the RV pears stored under CS regardless of maturity stage, and in the OHR pears for MS2 only. According to Isidoro and Almeida (2006) and Xie et al. (2014), 1-MCP treatment effectively reduces the accumulation of α-farnesene and conjugated trienes in 'Rocha' and 'd'Anjou' pear epidermis. Gapper et al. (2006) reported that the application of 1-MCP to 'd'Anjou' pears inhibited the expression of the PcAFS1 gene, which encodes α-farnesene synthase, and is positively regulated by ethylene. Thus, 1-MCP would act indirectly through the reduction of the ethylene actions, leading to lower synthesis of α-farnesene, and primarily reducing α-farnesene oxidation and diminishing the α-farnesene metabolites from this process. However, after seven months of storage plus seven days simulating shelf-life, 1-MCP treatment had no effect on fruit harvested from LA for both maturity stages and from OHR (MS1 fruit) (Table 2).

CA (without 1-MCP) treatment did not differ to produce α-farnesene within the fruit, regardless of storage period and production location. There was higher α-farnesene production with the harvest delay in the OHR fruit stored under CS and CA (Table 2).

In this work, ULO had a variable effect, which depended on the production location. According to Larrigaudière et al. (2019), ULO is less effective compared to 1-MCP treatment to prevent α-farnesene accumulation, a result that may be related to the increase in physiological maturity. For fruit harvested from RV, ULO reduced α-farnesene production; however, for the OHR fruit from MS1, an increase in α-farnesene production was seen, as compared to that seen for CS. Larrigaudière et al. (2019) did not find a clear relationship between α-farnesene and conjugated trienes in the development of scald in fruit stored with ULO, as observed in 1-MCP.

Methyl-5-hepten-2-one production

Except for fruit from the OHR (MS2) orchard, the 1-MCP condition (CS or AC) reduced 6-MHO production after three months of storage plus seven days of the ambient conditions, regardless of orchard and maturity at harvest. There was reduction in 6-MHO production in the fruit treated with 1-MCP as a function of the storage conditions and production regions after seven months of storage plus seven days of shelf-life (Table 2).

For the CS+MCP condition, 6-MHO synthesis was reduced in MS1 fruit from LA and OHR. The CA+MCP condition reduced 6-MHO production in the MS1 fruit from RV and OHR, and for both maturity stages from LA. In 'Dangshansuli' pears, 1-MCP treatment was reported to inhibit 6-MHO synthesis and prevent the appearance of superficial scald after six months of refrigerated storage (Hui et al. 2016). The oxidation of α-farnesene and the formation of conjugated trienols and 6-MHO are important factors in the development of superficial scald (Pesis et al. 2010). 6-MHO production coincides with the appearance of superficial scald symptoms; in addition, this volatile is more stable than the conjugated trienols, and for this reason, it has been associated with superficial scald (Farneti et al. 2015, Hui et al. 2016). According to Hui et al. (2016), there

is an endogenous 6-MHO concentration threshold above which superficial scald occurs. In addition, they reported a positive correlation between the production of conjugated trienols and 6-MHO, and between these compounds and scald incidence.

After seven months of storage, plus seven days at shelf-life, the highest 6-MHO production in the present study was observed in fruit stored under CS and CA, which did not differ significantly from the ULO fruit (from RV) for both maturity stages, and from OHR harvested at MS2 (Table 2).

Compared to CS or CA, ULO reduced 6-MHO production in the LA orchard and when compared to CAin the OHR orchard (MS2 fruit) after three months of storage. ULO also restricted 6-MHO production in the LA fruit harvested at both maturity stages, and in the OHR fruit harvested at MS1 after seven months. These effects on 6-MHO reduction might be the result of increased alcohol dehydrogenase activity under ULO, and the consequent use of 6-MHO as a substrate to detoxify the cells by converting it into 6-methyl-5-hepten-2-ol (Pesis et al. 2010).

Ascorbate production

Ascorbic acid and ascorbate are antioxidants that regulate fruit ripening by eliminating reactive oxygen species and are associated with the control of some physiological disorders such as superficial scald (Arabia et al. 2024). The CA+MCP condition (for both maturity stages) showed positive effects in the maintenance of the ascorbate content in the fruit from OHR, as compared to the control CS treatment. According to to Larrigaudière et al. (2016), the total antioxidant potential did not explain scald sensitivity. However, ascorbate might inhibit the enzyme polyphenol oxidase (PPO) activity and thus have an important role in superficial scald control (Larrigaudière et al. 2016; Arabia et al. 2024). Giné-Bordonaba et al. (2020) mention that 1-MCP can prevent the development of scald through repression of the PPO gene, and also through chloroplast-induced protection.

With the harvest delay, there were reduction in the ascorbate content (as means of all treatments in the RV fruit), and increase in the ascorbate content in the fruit from LA (CS, ULO) and OHR (CS, CA).

Production of phenolic compounds

Treatment with 1-MCP (CS) reduced the contents of catechin, procyanidin B2, epicatechin, and chlorogenic acid in fruit from the RV and LA orchards, and CA+MCP decreased catechin, procyanidin B2, and chlorogenic acid in RV fruit (Table 3). It is possible that 1-MCP acts indirectly in the maintenance of the antioxidant compounds due to its action on ethylene, and consequently on the ripening of the fruit. It is reasonable to suggest that inhibition of ethylene production by 1-MCP may lead to reduce concentrations or interrupt the normal metabolism of phenolic compounds in 1-MCP treated fruit (Hoang et al. 2011). The delay in harvest showed high variability regard to the phenolic compounds according to the storage conditions and production region.

Phenolic compounds are important antioxidant substances and can contribute to the reduction of scald development. However, their accumulation might also influence the appearance of this disorder due to their oxidation by the PPO (Lurie and Watkins 2012). The disintegration of the cell membrane and the process of phenol oxidation in the pear fruit are linked to the onset and development of superficial scald (He et al. 2022). 1-MCP treatment affects several phenolic metabolism related genes (Zhou et al. 2020). Low-temperature storage can compromise the integrity of the internal membranes of cells, promoting the reaction between PPO and chlorogenic acid leading to browning of the peel of the fruit. During the development of scald, the skin of fruit treated by 1-MCP is characterized by the lower polyphenol accumulation than untreated fruit, and the expression of PPO is also strongly inhibited by 1-MCP (Giné-Bordonaba et al. 2020).

Fruit storage under CA condition (without 1-MCP) reduced the contents of phenolics in RV (MS1 and MS2) and LA fruit (MS1) (Table 3). In fruit from OHR, no effects on the content of phenolic compounds were seen for the different storage conditions. Silva et al. (2010) reported little or no effects on the free radical scavenging activity of 'Rocha' pears as a function of post-harvest storage conditions. In 'Cripps Pink' apples, the storage atmosphere (CA compared to NA) did not significantly affect the concentrations of the total phenolic compounds or individual phenolics (Hoang et al. 2011). According to Hoang et al. (2011), physiological diseases such as superficial scald are not directly related to the levels of antioxidants in whole fruit. The superficial scald development seems to depend on the balance between the oxidative processes initiated by the conjugated trienols and the scavenging capacity of the tissue (Guerra et al. 2012).

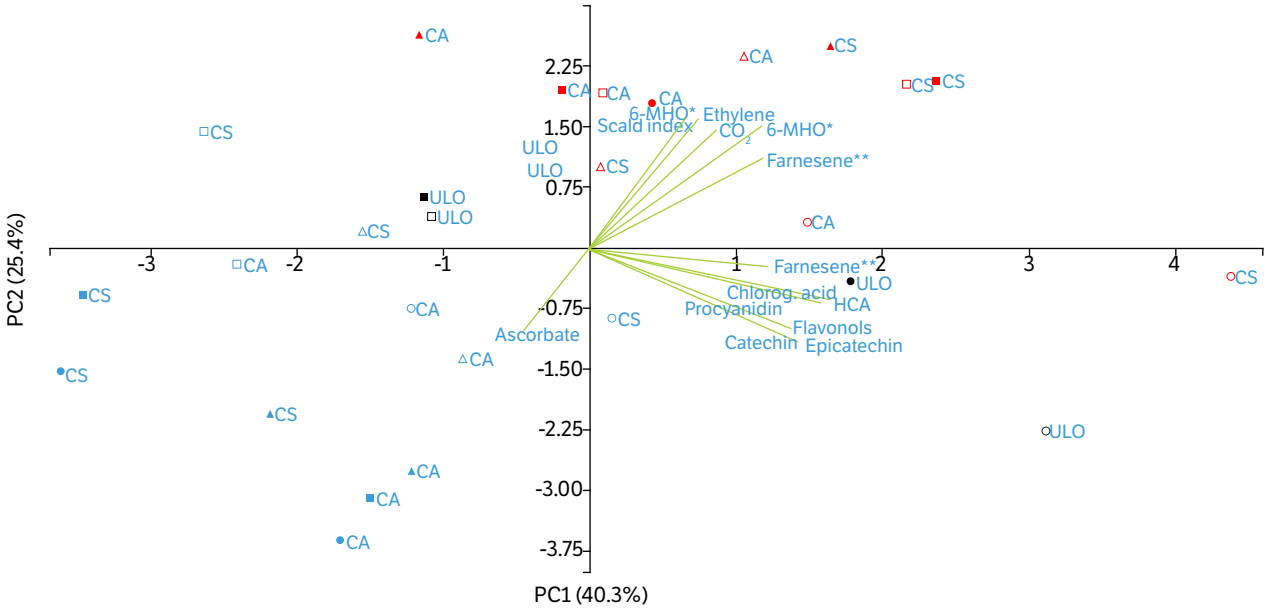
Table 3. Ascorbate and phenolic compounds by 'Alexander Lucas' pears harvested at the two maturity stages from the three production regions according to the different storage conditions for seven months under refrigeration (0 ± 0.1 °C; 94% ± 2% relative humidity) followed by seven days under ambient conditions (20 ± 2 °C; 60% ± 5% relative humidity)*.

Treatment	Data according to orchard and fruit maturity stage								
	Ravensburg			Langenargen			Öhringen		
	MS1	MS2	Mean	MS1	MS2	Mean	MS1	MS2	Mean
Ascorbate (mg·kg^{-1} fresh weight)									
CS	44.0	36.0	40.0a	32.0Bab	52.0Aa	42.0	27.0Bc	48.0Ab	37.0
CS+MCP	56.0	43.0	49.0a	39.0Aab	50.0Aa	45.0	54.0Aab	45.0Ab	50.0
CA	46.0	35.0	41.0a	42.0Aab	52.0Aa	47.0	28.0Bbc	49.0Ab	38.0
CA+MCP	55.0	47.0	51.0a	46.0Aa	41.0Aa	44.0	58.0Aa	70.0Aa	64.0
ULO	55.0	42.0	44.0a	29.0Bb	40.0Aa	34.0	33.0Aabc	44.0Ab	39.0
Mean	49.0A	40.0B		37.0	47.0		40.0	51.0	
CV%	19.7			16.8	14.3		25.0	13.5	
Catechin (mg of 3-methoxyflavone equivalents per kg^{-1} dry mass of epidermis)									
CS	2,721.5	2,114.1	2,417.9a	2,263.3Aa	1,391.6Aa	1,914.6	1,554.0	1,233.1	1,425.8a
CS+MCP	1,151.4	1,752.7	1,452.1b	926.2Ac	688.2Bb	783.4	1,342.2	1,214.3	1,278.3a
CA	1,330.0	1,757.0	1,543.5b	1,027.4Ac	1,060.3Aab	1,043.9	1,040.2	1,242.1	1,141.2a
CA+MCP	1,803.0	1,334.2	1,568.6b	1,750.4Aab	952.8Bab	1,431.4	1,758.0	1,545.1	1,651.6a
ULO	1,793.8	2,036.7	1,915.3ab	1,096.9Abc	1,207.2Aab	1,152.1	1,150.0	1,274.3	1,199.7a
Mean	1,760.0A	1,799.0A		1,447.6	1,042.7		1,369.0A	1,309.2A	
CV%	22.89			16.25	17.12		22.76		
Epicatechin (mg of 3-methoxyflavone equivalents per kg^{-1} dry mass of epidermis)									
CS	140.2.5	99.7.0	119.9.8a	99.4.7Aa	76.3.6Aa	90.2.2	75.2.6	42.2.6	62.0.6a
CS+MCP	50.8.5	79.9.7	65.4.2b	41.9.4Ac	28.7.5Ab	35.3.5	65.0.4	49.5.0	57.2.7a
CA	66.6.4	88.4.2	77.5.3ab	47.8.2Abc	51.7.5Aab	49.7.8	49.8.8	59.4.9	54.6.8a
CA+MCP	87.4.7	67.8.9	77.6.9ab	83.9.6Aab	43.7.5Bab	67.8.7	88.6.3	72.3.2	80.4.5a
ULO	96.0.6	106.3.3	101.2.0ab	52.8.4Abc	57.5.3Aab	55.1.8	57.0.8	63.3.4	59.5.8a
Mean	88.2.5A	88.4.6A		66.8.6	52.1.3		67.1.8A	58.0.8A	
CV%	29.52			19.94	20.09		25.34		
Procyanidin B2 (mg of 3-methoxyflavone equivalents per kg^{-1} dry mass of epidermis)									
CS	8,533.6	6,683.4	7,608.5a	5,594.0Aa	9,444.0Aa	7,904.1	4,503.2	2,507.1	3,704.8a
CS+MCP	2,895.0	4,522.5	3,708.7c	3,574.6Ac	3,266.6Ab	3,389.8	4,306.2	2,712.0	3,509.1a
CA	3,162.6	4,233.8	3,698.2c	4,610.5Abc	4,803.2Aab	4,706.9	3,658.8	3,382.4	3,520.6a
CA+MCP	4,447.3	4,172.6	4,310.0bc	6,874.9Aab	4,145.9Aab	5,783.3	5,735.5	3,989.1	4,862.4a
ULO	5,944.4	6,767.8	6,356.1ab	4,320.6Abc	4,592.2Aab	4,456.4	3,824.0	3,652.4	3,755.4a
Mean	4,996.6A	5,276.0A		5,921.4	4,420.4		4,405.6A	3,274.6B	
CV%	23.69			16.07	15.71		20.73		
Chlorogenic acid (mg of 3-methoxyflavone equivalents per kg^{-1} dry mass of epidermis)									
CS	4,576.4Aa	3,366.4Aa	3,971.5	1,833.2Aab	2,401.7Aa	2,117.4	2,560.7	263.3.1	2,589.7a
CS+MCP	1,662.5Bb	2,345.8Ab	2,004.2	1,711.0Ab	1,762.0Ab	1,736.7	1,894.6	244.8.6	2,227.0a
CA	1,834.7Bb	2,379.1Ab	2,106.9	2,068.4Aa	2,041.8Aab	2,055.1	1,961.4	208.3.4	2,022.4a
CA+MCP	1,912.5Ab	1,993.1Ab	1,952.7	1,848.4Aab	1,829.8Ab	1,834.1	2,275.0	226.2.0	2,267.6a
ULO	2,872.9Ab	3,187.8Aa	3,030.4	2,087.6Aa	1,834.7Bb	1,961.2	2,338.0	210.9.2	2,246.5a
Mean	2,571.8	2,654.5		1,910.0	1,973.0		2,227.8A	2,297.4A	
CV%	20.08	9.01		5.40	10.32		12.90		

CS: standard oxygen control cold storage; CA: controlled (low oxygen) atmosphere; ULO: ultra-low oxygen atmosphere; MCP: 1-methylcyclopropene; MS1: harvested September 5, 2012; MS2: harvested 18 September 18, 2012; *values within a column followed by the same lower-case letter are not significantly different, and values within a line followed by the same capital letter are not significantly different (Tukey's test; $P < 0.05$); CV: coefficient of variance.

Multivariate analysis

Principle component analysis was performed to select a subset of variables contributing the most to classify pears regarding the 1-MCP treatment, storage conditions, and maturity stage in the three orchards. It was therefore possible to explain 65.7% of the variability in the studied parameters by the first two principal components (PC). Positive PC1 values were associated with the production of phenolic and volatile compounds, as well as the scald index. Negative PC1 was related to ascorbate production. Positive PC2 values were related to scald index and volatiles compounds, while negative PC2 with production of antioxidants compounds (Fig. 1). A positive correlation was observed between the scald index, α-farnesene (only after three months of storage), 6-MHO, CO_2, and ethylene production.



CS: standard oxygen control cold storage; CA: controlled (low oxygen) atmosphere; ULO: ultra-low oxygen atmosphere; RV Orchard: Dot; LA Orchard: Triangle; OHR Orchard: Square; MS1: Empty dot, tringle and square; MS2: Filled dot, tringle and square; Without 1-MCP: Red color; With 1-MCP: Blue color; ULO: Black color; PC: principal component.

Figure 1. Principal component analysis for the two maturity stages, three production locations and different storage conditions of the 'Alexander Lucas' pears, according to scald, volatiles and antioxidante compounds. The extracted principal components accounted for 65.7% of the variability in the data.

The samples are well differentiated into two groups. Principal component analysis effectively classified the samples treated with 1-MCP (CS or CA) and the other storage conditions (CS, CA, and ULO). Except for the RV orchard, the ULO results are positioned in the most central region of Fig. 1 in relation to the other storage conditions, demonstrating a reduction in the production of phenolic and volatile compounds, as well as scalding (PC1 negative). Except for the RV orchard, in which the results from CS storage are positioned in a region with greater production of phenolic compounds, CS and CA, without 1-MCP, presents positive PC1 and PC2, which show a greater production of volatile compounds, as well as scald development. When harvested late, fruit stored in CS from RV orchard showed extreme positive PC1 values (data not shown). The fruit of the 1-MCP treatment showed negative PC1 and PC2, characterizing a lower production of phenolic and volatile compounds, as well as the development of scald.

Some other studies have related superficial scald to α-farnesene content (Gapper et al. 2006, Vanoli et al. 2010); however, the oxidation of α-farnesene, rather than its production, is important for the development of this disorder (Larrigaudière et al. 2016). Farneti et al. (2015) and Hui et al. (2016) reported positive correlations between 6-MHO content and superficial scald in apples and pears. The ascorbate content showed an inverse correlation with the occurrence of scald's disorder. The result found by Larrigaudière et al. (2016) and Giné-Bordonaba et al. (2020) suggest the protective role that ascorbate may have in controlling the development of this physiological disorder. Ascorbic acid/ascorbate

possibly acts as a PPO inhibitor and a scavenger of generated reactive oxygen species inside the cell during superficial scald of pear (Arabia et al. 2024). Zhou et al. (2020) showed that arbutin, chlorogenic acid, catechin, and epicatechin were the main phenolic compounds in the peel, and, except by catechin, 1-MCP treatment significantly inhibited the accumulation of these phenolic compounds.

As verified in this work, 1-MCP in combination with low O_2 limits senescent disorders during prolonged low temperature storage and inhibition on scald is evident with a combination of CA and 1-MCP (Lum et al. 2017). However, it is important to keep in mind that this condition can be responsible to lead to an increase in internal browning disorders in 'Alexander Lucas' pears (Hendges et al. 2015, Balkees et al. 2022). In addition, CA+1MCP can cause reduction in the production of volatile compounds and ripening delayed (Hendges et al. 2018, Hendges et al. 2020).

As already reported by others (Whitaker et al. 2009, Larrigaudière et al. 2016) and reinforced in the present study, scald is associated with genetic characteristics, pre-harvest and post-harvest factors, and the interactions between them. These factors had significant influences on the scald appearance of 'Alexander Lucas' pears here, and therefore new studies should be carried out to relate pre-harvest conditions (maturity stage, production region, and others) to the biochemical processes involved in the development of scald during and after storage.

CONCLUSION

Fruit treatments that combined controlled atmosphere or cold storage plus 1-MCP reduced scald, but the effects varied between orchards.

1-MCP treatment decreased the production of volatiles and antioxidant compounds, excepted by ascorbate, which appeared to be associated with delayed ripening and the less severe development of scald.

Use of ultra-low O_2 reduced the scald, but the effects depended on the production region. Storage under controlled atmosphere without 1-MCP application did not reduce the appearance of scald in these pears stored for seven consecutive months plus seven days of exposure to ambient conditions.

There were positive correlations between the development of scald and the production of 6-MHO and α-farnesene (after three months of storage). Ascorbate was negatively correlated with scald.

CONFLICT OF INTEREST

Nothing to declare.

AUTHORS' CONTRIBUTION

Conceptualization: Hendges, M. V., Steffens, C. A. and Neuwald, D. A.; **Supervision:** Steffens, C. A., Amarante, C. V. T., Kittemann, D. and Neuwald, D. A.; **Formal analysis:** Vidrih, R., Zlatic, E. and Neuwald, D. A.; **Methodology:** Hendges, M. V., Steffens, C. A., Neuwald, D. A. and Balkees, B.; **Investigation:** Hendges, M. V., Vidrih, R., Zlatic, E. and Neuwald, D. A.; **Writing – Original Draft:** Hendges, M. V.; **Writing – Review and Editing:** Steffens, C. A., Neuwald, D. A., Amarante, C. V. T., Vidrih, R., Zlatic, E., Balkees, B. and Kittemann, D.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are available from the corresponding author.

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