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Effect of controlled atmosphere storage on production of volatiles and ethylene from cv. Zaosuli pears

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Abstract

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Harvest-mature fruits of the pear cv. Zaosuli (*Pyrus bretschneideri* Rehd.) were stored at 1.0–1.5°C under two different experimental atmospheres. The controlled atmosphere (CA) had low oxygen (2.0%) and high CO_2 (7%), while the regular atmosphere (RA) had 20.9% O_2 and 0.1% CO_2. Sixty-four volatile compounds were subsequently detected and quantified by solid phase micro-extraction (SPME) including 1 hydrocarbon, 16 alcohols, 15 aldehydes, 4 ketones, 15 esters, 8 terpenes, 2 lactones and 2 fatty acids. The principal component analysis of data was carried out to assess the effects of these post-harvest storage conditions in comparison to fruit stored in air at room temperature for 5 days. Thirteen compounds were found to be sufficient to differentiate the two different pear treatments, which were followed by subsequent warming to 20°C. The observed differences in the production of volatiles between the start of storage and 40 days later (RA), or 40 days later (CA), are principally due to just four compounds, namely n-hexanol, 2-furaldehyde, cis-geraniol and α-damascenone. Ethylene production is also lower in the CA treatment. The higher concentration of CO_2 in the CA treatment causes a rise in respiration rates due to anaerobic respiration.

Keywords: asian pears; volatiles; ethylene production; respiration rate; PCA

The summer variety of the Chinese pear *Pyrus bretschneideri* Rehd., known as cv. Zaosuli (synonym cv. Zaosu; Liu et al. 2014), has recently started to be grown in South Moravia. The skin is yellow-green with small lenticels, and the snow-white flesh is tender, juicy, crisp, and sweet, with a pleasantly fragrant flavour. The important question for determining the optimum commercial fruit storage conditions is whether the fruits are climacteric fruits, and produce large amounts of ethylene during the ripening process, which is important for achieving the desired changes in sugar to acid ratios, flavour and aroma, as well as fruit texture (Li et al. 2014a; Chen et al. 2006). Chinese pears *Pyrus bretschneideri* Rehd. are known to suffer from a slight loss in flesh firmness during storage, with an accompanying very small decrease in soluble solids. YAmane et al. 2007 also examined the varietal differences among pears in this group regarding their production of ripening-associated ethylene. Ma and Chen (2003) demonstrated that there was an increase in ethylene production with storage time when initially transferred to a temperature of 20°C. As in European pears, it is observed

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that during ripening, when the fruits become soft and edible and produce an intense aroma, there is a parallel increase in ethylene production (Puing et al. 1996; Makkumrai et al. 2014; Zhou et al. 2015). Most commercial fruit, however, is still kept under normal air storage conditions, and so far the use of controlled atmospheres to extend the storage period and reduce the loss of firmness, acidity, sugars and volatiles (El-Sharkawy et al. 2003; Zhou et al. 2015) has not been widely adopted. Among the important compounds of pear cultivars with Bartlett-like aromas are ethyl (E)-2-, (Z)-2-decadienoate and hexyl acetate (Jenning 1961; Suwanagul, Richardson 1998). In contrast, Chinese pear cultivars produce aromas composed mainly of esters and oxygenated compounds such as aldehydes and ketones, including the most prominent volatile compounds such as ethyl esters with short- to medium-length carbon chains (Kou et al. 2012; Li et al. 2014b).

The objective of this study was to determine whether cold treatment induces a response in Zaosuli pears similar to those in other climacteric fruits, including the revival of the typical ripening process after time spent in storage. The impact of controlled atmospheres during a cold period followed by storage at 20°C was evaluated by the effect on the profile of aroma volatiles, ethylene and CO_2 production, and on non-volatile parameters such as firmness, soluble solids and titratable acidity.

MATERIAL AND METHODS

Pear fruits (*P. bretschneideri* Rehd., cv. Zaosuli) were harvested in the middle of September 2012, 131 days after full bloom, and at harvest maturity the fruits were completely firm and resistant to mechanical injury. They were produced by 4-year old trees grown in the orchards of Mendel University at Lednice, in the Czech Republic. The fruits were harvested manually and transported within just a few hours to the technological laboratory. Immediately before storage in the gas mixtures they were sorted again in order to remove mechanically damaged fruits and fruits without stems. Subsequently they were cooled to a temperature ranging from +0.5°C to +1.0°C, and the two gas mixtures were introduced to the individual containers. The controlled atmosphere (CA) was prepared by reducing oxygen levels to 2.0% using activated carbon (Swing-sorb as method for oxygen reduction), and levels of 7.0% CO_2 were achieved by flushing the containers with CO_2 from a reservoir bottle. A representative sample of fruit was taken at regular intervals, specifically 10, 20, 30, and 40 days of storage at 1°C in the two atmpospheres (CA and RA), and then transferred to normal air conditions at 18°C for 5 days to observe the immediate response to warming. The effects on certain physiological and physico-chemical parameters are shown in Figs 1–5.

Ethylene production and respiration rates were measured for intact fruit using a static system. At each sampling date, five fruits were weighed and placed in 1 l jars. The jars were sealed and kept at 20°C for 1 h prior to measurement. C_2H_4 and CO_2 were monitored by injecting 1 ml head gas samples into an Agilent 4890D gas chromatograph (Agilent Technolgies, Inc., Wilmington, USA). Both gases were determined simultaneously using dual columns. A HP-Plot/Q column 30 m, I.D. 0.53 mm, film 40 mm was used for ethylene (C_2H_4) and detected on FID, and a HP-AL/KCL column 30 m, I.C. 0.53 mm, film 15 mm was used for CO_2 and detected on TCD. Helium at 1.2 ml/min was used as a carrier gas. The oven was programmed to rise from 80 to 120°C at a rate of 10°C/minute. The rate of production of ethylene is expressed as micro-litres per kilogram per hour, that for CO_2 is expressed as mg per kilogram per hour.

A manual solid-phase micro-extraction (SPME) fibre-holder coated with a 100 µm layer of poly-dimethylsiloxane (PDMS) (Supelco, Bellefonte, USA), was chosen to absorb the volatile compounds obtained from the fruit samples. Firstly, the extraction head of the SPME was conditioned in the sample valve of the GC-MS, at a temperature of 250°C for 5 min, and then the needle of the SPME device pierced the septum of the vial and the fiber was exposed for 30 min to the headspace of the vial at 50°C. Subsequently, the needle was removed from the sample vial. Finally, the needle was manually inserted into the gas chromatography injector, where the analytes were thermally desorbed and analyzed. The desorption time was 5 min at 250°C. Gas chromatography with mass spectrometry (GC-MS) measurements were made using a gas chromatograph (Agilent Technologies 7890A, Inc., Santa Clara, USA) interfaced to a quadrupole mass spectrometer (Agilent GC MSD 5975 C), using the NIST 98 library of mass spectra. Analytes were separated using a DW WAX fused silica capillary column of 30 m × 0.25 mm with a phase thickness of 0.25 µm from J & W Scien-

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tific (Agilent Technologies Inc., Santa Clara, USA), which was inserted directly into the ion source of the MS. Compounds were provisionally identified using the NIST mass spectra library search, and the identity of most of these compounds was confirmed by comparing their mass spectra and retention times with those obtained using a standard.

Fruit firmness was measured as being the load in kilograms needed to break the peeled surface of fruit in the equatorial zone, using a 8-mm diameter plunger and expressed as MPa (Fruit Firmness Tester, Turoni, Forli, Italy).

Titratable acidity (TA) was determined by titrating with 0.1 N NaOH to pH 8.1 and calculated as malic acid equivalents.

Soluble solids were measured with a digital refractometer (model PR-1; Atago, Tokyo, Japan) and expressed as °Brix.

All statistical analyses were performed using the SAS statistical software package version 9.2. (SAS Institute Inc., Cary, USA). For each treatment, descriptive statistics (mean ± standard error) at each stage of the post-harvest period were calculated for each parameter under investigation. A stepwise log regression model was used to explore the effect of all volatile parameters as explanatory variables of the observed variation in a binary response (RA/CA). Principal component analysis (PCA) components were expressed as a linear combination of selected variables to differentiate the different treatments of fruits and were used to discriminate between the treatments at each stage of warming. Before proceeding with the statistical analysis the data matrix was standardized by setting mean values at zero.

RESULTS AND DISCUSSION

Physiological characterization of ripening stages

The fruit of cv. Zaosuli pear is clearly climacteric, and the ripening process is accompanied by a burst of ethylene production during shelf life at 20°C (Fig. 1), but the initial rates of production, as well as those during storage at 1°C, are at a minimum of 0.1 ml/kg·h. Ethylene production increased roughly 50-fold over the course of the 5 day experiment, signalling the climacteric stage of fruit development. From the onset of storage response on the higher temperature are lower, but in the controlled atmosphere ethylene production was permanently discontinued. According to Bower et al. 2003, temperature control is a more important factor in maintaining pear quality than scrubbing with ethylene. The higher metabolic activity of pears stored in CA, with a high level of CO_2 in the ambient atmosphere, was reflected by higher CO_2 production, which reached almost 100 mg/kg·h. In contrast, when the fruit was subsequently exposed to the air phase, with the temperature elevated to 20 °C, then respiration values approached those of fruit stored in air only (Fig. 2).

Fruit firmness

Flesh firmness is a very important physical property of ripe fruits, as it directly affects eating quality and texture. Fruit firmness is the parameter that has been most commonly used to follow the progress of ripening, as reported in previous studies of

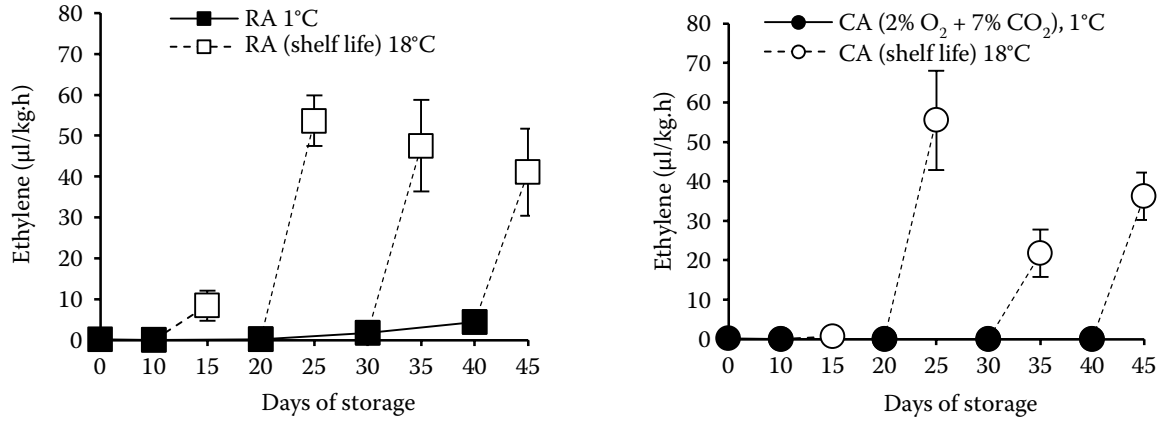

Fig. 1. Ethylene production (ml/kg·h) in fruit stored at 1°C in (regular atmosphere (RA) and controlled atmosphere (CA) for 10, 20, 30 and 40 days, followed by 5 days at 20°C

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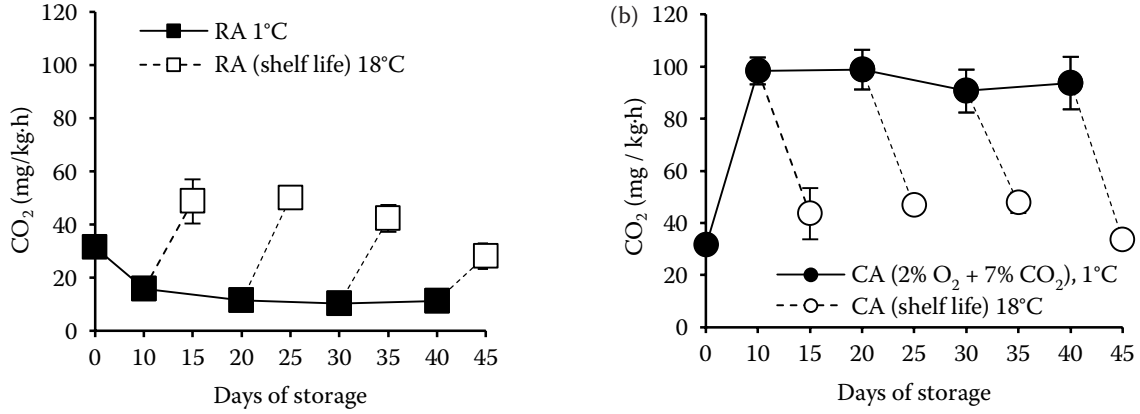

Fig. 2. Respiration rate (mg CO_2/kg·h) of fruit stored at 1°C in regular atmosphere (RA) and (b) controlled atmosphere (CA) for 10, 20, 30 and 40 days, followed by 5 days at 20°C

European and Asian pear cultivars (Murayama et al. 2002; Gómez et al. 2005; Predieri, Gatti 2009; Pasquariello et al. 2013). A change in flesh firmness may be due to a modification of the chemical structure of the cell wall in response to storage conditions. Pear firmness in this study decreased very slowly during storage, and the temporal changes are not statistically significant (Fig. 3). The effects of low oxygen combined with considerably higher than normal CO_2 levels, accompanied by subsequent exposure to temperatures of 18°C, do not lead to a significant softening of the flesh. In contrast, the ripening of European pears is usually characterized by softening (Suwanagul, Richardson 1998; Argenta et al. 2003; Hiwasa et al. 2003) and changes in aroma (Rizzolo et al. 2005; Chiriboga et al. 2011; Makkumrai et al. 2014), with corresponding changes in levels of sugars, organic acids and the production of volatiles.

Changes in soluble solids and titratable acidity

Levels of soluble solids (SS) remained almost stable in the ambient atmosphere as well as in the controlled atmosphere, and accounted for an average between 12–13°Brix of the refractometer measurements. Only small fluctuations in the levels of SS during the warming phase, statistically insignificant, means that the metabolism of the fruits has been stabilized and that there is no tendency to depletion of SS during storage (Fig. 4). Pasquariello et al. 2013 also observed no significant changes in SS at harvest and after cold storage, and neither have others studying different pear cultivars (Ma, Chen 2003; Predieri, Gatti 2009). Even though measurements of total soluble solids are commonly used as an index of ripening in fresh fruit, the differences observed after a period of post-harvest

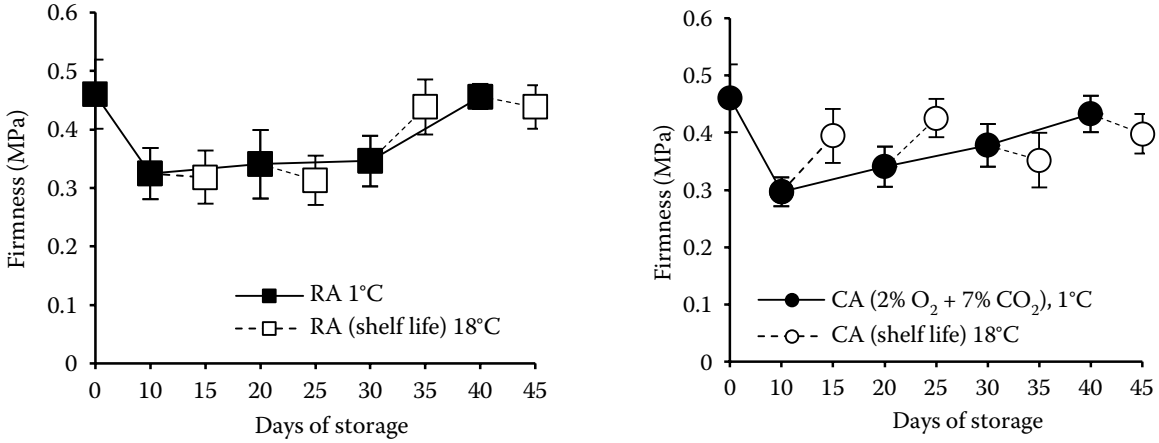

Fig. 3. Firmness (all values mean ± standard error) of pears stored at 1°C in regular atmosphere (RA) and controlled atmospheres (CA) for 10, 20, 30 and 40 days, followed by 5 days at 20°C in RA atmosphere

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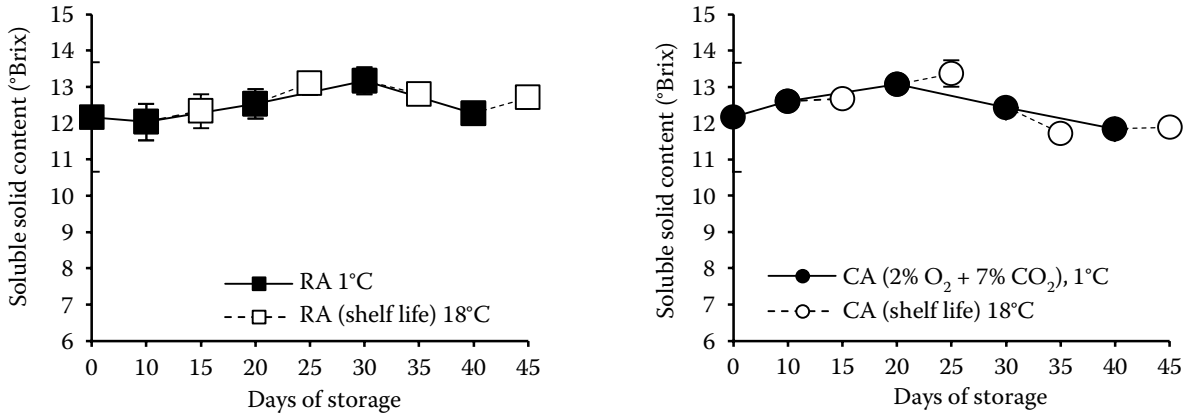

Fig. 4. Soluble solids content with mean ± standard error for fruit stored at 1°C in two atmospheres (regular (RA) and controlled (CA)) for 10, 20, 30 and 40 days, followed by 5 days at 20°C in a regular atmosphere

storage were found to be insignificant. The acidity values of the pears also showed a declining trend, during storage under both conditions, of only about one-tenth of a percent (Fig. 5). The reduction in acid values is largely due to the utilization of organic acids as respiratory substrates, ending as finished products of aerobic oxidation.

The composition of volatiles

A total of 64 different compounds were identified in the headspace of intact fruit of the cv. Zaosuli (*P. bretschneideri* Rehd.), comprising hydrocarbons, alcohols, aldehydes, ketones, esters, terpenes and lactones. The mean concentrations of all the volatile compounds in each functional group at the beginning of storage and then after storage are summarized in Table 1. Out of a total of 8 major chemical groups, the dominant compounds are the alcohols, with straight and branched chain structures of lengths C4 and C5. The second most significant class of compounds identified were the esters, and fifteen (Table 1) were found in all the samples. Esters are formed by the combination of free fatty acids and alcohols. The most abundant of the aldehydes in the pear fruits was benzaldehyde, with 929.4 ± 53.6 mg/kg at the beginning of storage, dropping to 715.0 ± 98.5 mg/kg after 45 days of storage in RA. Benzaldehyde was always present, having a characteristically fruity, sweet, nutty, and caramel-like odour when in sufficient concentration. The other aldehydes were at low levels (2-methylbutanal, (E)-2-octen-1-al, 2-(E)-6(Z)-nonadien-1-al, (E)-2-nonenal). In contrast, n-hexanal and (E) -2-hexenal are present at much greater concentrations than hexanol and other acetates. C6 volatile compounds in similar proportions have been found in *P. serotina* (Takeoka et al. 1992), *P. ussuriensis*

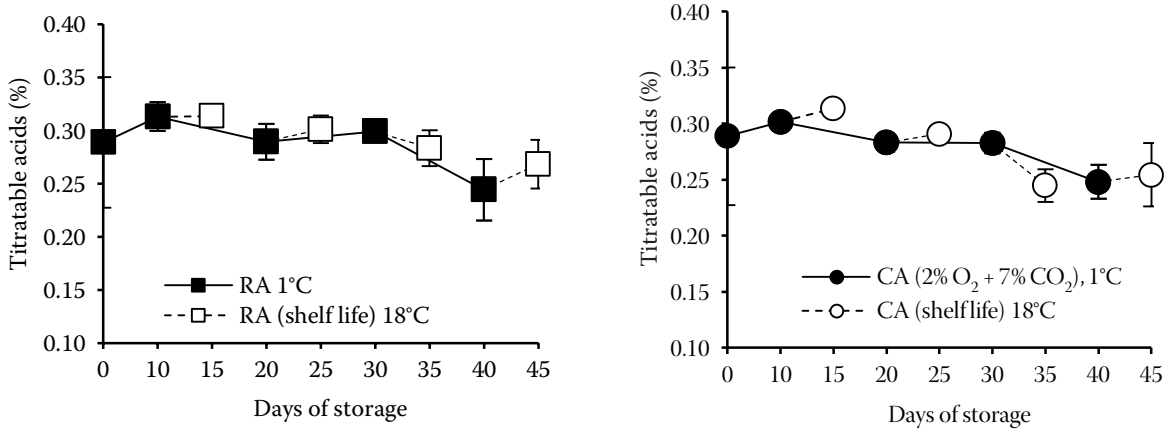

Fig. 5. Titratable acids (g/100 g) with means ± standard error for fruit stored at 1°C in two atmospheres (regular RA and controlled CA) for 10, 20, 30 and 40 days, followed by 5 days at 20°C in a regular atmosphere

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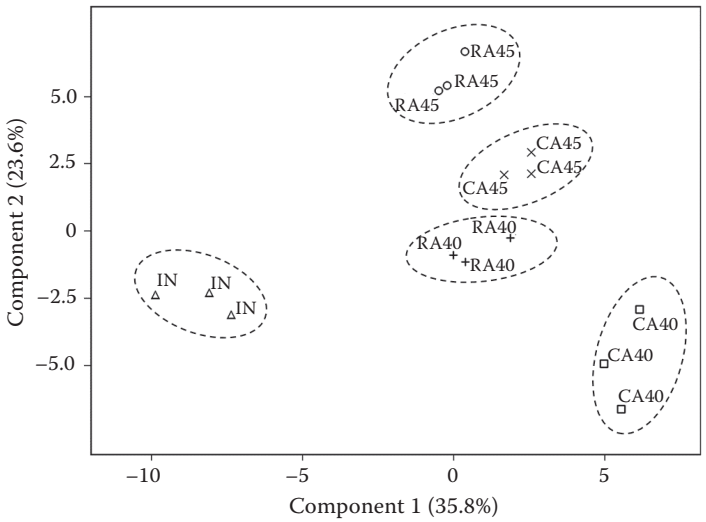

Fig. 6. Patterns of first two principal component analysis (PCA) scores for stored fruit initially (IN), and then after exposure to the two experimental treatment atmospheres (regular (RA) and controlled (CA)) and followed by 5 days at 20°C in a regular atmosphere

(Quin et al. 2012) and other Chinese pear cultivars (Kou et al. 2011). Terpenes are represented in these pear fruits by eight compounds, each at different concentrations. Limonene, regarded as an unstable terpene (Steingasse et al. 2014), is one of a group of terpenes found at high concentrations, and actually increased during storage from 248.0 ± 2.26 mg/kg to 1184 ± 56 mg/kg, a concentration identical in both treatments. γ-caprolactone and γ-octalactone are found in other fleshy fruits such as apricots, but in concentrations of 6–8 mg/kg and 240–320 mg/kg respectively. Both lactones are thought to be responsible for the fruity, sweet, peach-like aroma of fully-ripe pears.

Influence of gas mixtures and warming periods on volatile composition

After 40 days concentrations of volatiles were higher in CA then RA conditions, primarily due to the production of butyl acetate and 2-methyl-1-ol. The formation of both compounds should be intensive just from the effects of raising temperature to 20°C. A comparison of total concentrations after warming the fruit to 20°C revealed an almost one third higher final concentration, showing that an increase in temperature has a more significant effect than the gas mixtures. 2-methylbutan-1-ol, 4-methyl-1-pentanol, 2-heptanol, n-hexanal, (E)-2-hexenal, benzaldehyde, butyl 2-methylbutyrate and limonene were the most abundant compounds, with concentrations of about 500 to 900 mg/kg, whereas butyl acetate represented more than 90%

of the total ester production. The major esters contributing to the aroma of asian pears (*P. serotina*) are hexyl acetate and butyl acetate, while other common esters are also observed (Takeoka et al. 1992; Suwanagul, Richardson 1998; Makkumrai et al. 2014). Our results were similar. The sensory qualities of the above-mentioned compounds

Table 1. Concentrations (mg/kg f.w.) of volatile compounds in different chemical groups initially (IN) and again after 40 days and 60 days at 1°C in regular (RA) and controlled (CA) atmospheres followed by 5 days at 20°C in a regular atmosphere

Storage conditions	Alcohols	Aldehyds	Esters	Terpens	Ketons	Lactons	Fatty acids	Hydro-carbons	Total
IN	6,135^{a}	2,626^{a}	7,038^{a}	312^{a}	301^{a}	37^{a}	330^{c}	8^{a}	16,787
RA 40 days	11,533^{b}	4,071^{b}	7,012^{a}	891^{b}	479^{a}	49^{a}	197^{a}	4^{a}	24,236
RA 45 dyas	16,851^{d}	4,754^{c}	13,310^{d}	1,105^{bc}	1,081^{b}	46^{a}	338^{c}	6^{a}	37,491
CA 40 days	16,162^{c}	4,903^{c}d	10,177^{b}	1,030^{bc}	4,151^{c}	47^{a}	188^{a}	4^{a}	36,662
CA 45 days	16,017^{c}	5,332^{d}	12,127^{c}	1,202^{c}	1,359^{b}	51^{a}	252^{b}	4^{a}	48,144
Quantity	16	15	15	8	4	2	3	1	64

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Table 2. Eigen vectors of PCA components calculated for aroma compounds after cool storage (1°C) and followed by 5 days at 20°C

Variable	Principal compounds	
	Comp1	Comp2
(Z)-2-Penten-1-ol	–0.0996	0.1966
n-Butan-1-ol	0.0495	–0.1934
(Z)-3-Octen-1-ol	–0.0272	0.1917
n-Hexanol	–0.1823	–0.0637
2-Furaldehyde	–0.1821	–0.0868
Benzaldehyde	0.0167	–0.2074
3-Methylbutanal	0.1930	–0.0276
n-Hexyl hexanoate	0.0087	0.2064
(E)-2-Hexenyl acetate	0.0305	0.2344
Butyl acetate	0.0196	0.2034
Z-Geraniol	–0.1953	–0.0364
2-Heptanone	0.0131	–0.2072
a-Damascenone	–0.1938	–0.0375

PCA – principal component analysis

have been evaluated by Makkunrai et al. (2014), being compounds which positively correlate with fruity aroma, pear flavour, butteriness, juiciness and the perception of sweetness.

PCA analysis

Principal component analysis (PCA) is expressed as a linear combination of selected variables in order to differentiate the different experimental treatments of the pear fruits. Variables making a considerable contribution to the PCA components (overall variability) are listed in Table 2 (contributions with absolute values > 0.18 are highlighted). Thirteen compounds are sufficient for distinguishing the two different pear treatments with gas mixtures and the subsequent warming of fruit to 20°C. For stored fruit, the first principal component (PC1) explained 35.8 % of the data variation and showed a high correlation with 3-methylbutanal (0.193). Moreover, four parameters showed a negative correlation with PC1, namely cis-geraniol (–0.195), α-damascenone (–0.193), n-hexanol (–0.182) and 2-furaldehyde (–0.182). The second principal component (PC2), for the same treatment, explained 23.6 % of the data variation and correlated with the volatile compounds *n*-hexyl hexanoate (0.2064), (E)-2-hexenyl acetate (0.234) and butyl acetate (0.203), and negatively with 2-heptanone (–0.207) and benzaldehyde (–0.207). Figure 6 shows the initial concentrations and the results of the different treatments and the warming phase as defined by PC1 and PC2, which together explain 59.4% of the data variation. The differences between the post-harvest storage treatments are spatially quite distinct. Above all, it is apparent that the effect of 40 days storage at 1°C on differences in the production of volatile compounds is comparatively low. As can be seen, PC1 shows a high negative value for the onset of storage and high positive values for the other two treatments (RA and CA). Therefore PC1 mainly shows a separation between the time of storage. On the differences observed between the start of storage and 40 days later for RA conditions, and 40 days for the controlled atmosphere, is explained by the compounds n-hexanol (–0.182), 2-furaldehyde (–0.182), cis-geraniol (–0.195) and α-damascenone (–0.193). Fruit transferred to the warming phase from RA40 and CA40 compared to RA45 and CA45 are clearly differenciated in COMP2 (Table 1) by 5 compounds, namely (Z)-2-penten-1-ol (0.196), (Z)-3-octen-1-ol (0.192), hexyl hexanoate (0.206), (E)-2-hexenyl acetate (0.234) and butyl acetate (0.203). Interestingly, fruit stored at 20°C showed a clear separation not just among themselves, but also in the cooled variants.

CONCLUSION

Measures of simple physical and chemical parameters of fruit ripeness (firmness, soluble solids and titratable acidity, as well as the production of volatiles) were performed after 10, 20, 30 and 40 days at 1°C, and again after a seven-day period at 20°C (representing normal shelf-life conditions). Thirteen volatiles found in the different post-harvest storage conditions were identified which could be used to distinguish the effects of the two gas mixtures and the subsequent increase in temperature from 1°C to 20°C. On the other hand, however, Headspace solid phase microextraction (HP SPME) methods were successful in satisfactorily analyzing the aroma profiles of the cv. Zaosuli (*Pyrus bretschneideri* Rehd). The temperature during storage has a much greater effect on the production of ethylene released from intact fruit than the gas mixtures. In cold storage, as well as in the phase of rising tem-

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peratures, firmness changes are insignificant, and are not associated with any sensory perception of softening. A controlled atmosphere with low oxygen content combined with higher than normal CO2 has a significant effect on increasing the respiration rate, which were decelerated on the production some level, corresponding to the non-treated fruits at a temperature of 20°C. The results demonstrate that the volatile flavour compounds identified by SPME were responsible for the aroma of cv. Zaosuli pear fruits. The identification of a wide profile of aroma compounds was achieved using PCA analysis, and suggests that various groupings of volatile compounds might be useful as potential chemical markers for identifying the storage conditions in which commercial fruit has been held.

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