Effect of low oxygen storage conditions on volatile emissions and anaerobic metabolite concentrations in two plum fruit cultivars

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Abstract

Goliáš J., Hic P., Kaňová J., 2010. **Effect of low oxygen storage conditions on volatile emissions and anaerobic metabolite concentrations in two plum fruit cultivars**. Hort. Sci. (Prague), *37*: 145–154.

By harvest time, small amounts of acetaldehyde were accumulated in the flesh of plums, such as 0.31 mg/l for the cv. Stanley and 1.03 mg/l for the cv. Valjevka. This relative difference in concentrations remained constant throughout the whole period of storage in a regular atmosphere. The long-term effects of higher concentrations of CO_2 are the same as for very low oxygen concentrations; and significant amounts of ethanol accumulate in the tissue. Out of a total number of 42 different odour compounds identified in the juice, there were 11 alcohols, 6 aldehydes, 17 esters, 2 terpenes, 3 organic acids, and 1 lactone. Very low oxygen atmospheres slow down the production of esters and aldehydes, but have little effect on the production of lactones and terpenes. It was shown that a very low oxygen concentration, without much CO_2 (Fluctuating anaerobiosis treatment), does not encourage the production of significant amounts of ethanol and acetaldehyde in the fruit flesh, but does significantly slow the biosynthesis of aromatic volatiles.

Keywords: plum fruit; volatiles; ethanol; acetaldehyde; firmness; headspace gas analysis

The quality of plum fruit following harvest is essentially influenced by the temperature and composition of the ambient atmosphere. The storage period is limited by fruit softening, visible signs of wilting and a physiological disease manifested as an internal browning adjacent to the stone (Taylor et al. 1993; Goliáš 2004; Toivonen, Brummell 2008). Knowing the lower oxygen limit for effective aerobic metabolism is critical for managing the composition of the gaseous atmosphere (Gran, Beaudry 1993; Beaudry 1999; Menniti et al. 2006). A gas atmosphere where the ethanol concentration does not increase over time is considered to be optimal for long-term storage (Smagula, Bramlage 1977; Nichols, Patterson 1987; Pesis 2005). If oxygen levels drop below a certain critical point for the aerobic conversion of storage substrates, then pyruvate is converted into acetaldehyde and ethanol with adverse affects on fruit quality.

Fermentation processes which proceed as a consequence of fruit ripening, even without low oxygen levels in the ambient atmosphere, also result in the accumulation of ethanol and consequently the production of ethyl esters in concentrations which may cause off-flavours. Specifically, an unpleasant odour and taste results from the production of ethyl acetate, ethyl butanoate, ethyl 2-methylbutanoate, ethanol, and acetaldehyde (Lara et al. 2007), whereas there

Supported by the Ministry of Agriculture of the Czech Republic, Project No. QH 81235.

is no direct relationship between the production of ethanol in a low oxygen atmosphere and alcohol dehydrogenases (ADH) (Lara et al. 2003). In contrast, the esters connected to the more positive, "fruity" attributes of fruit flavour are the most remarkable contributors to the aroma profile of intact fruits, both quantitatively, and qualitatively (Nursten 1970; López et al. 1998; Nunes et al. 2008).

The effects of four different gas mixtures, FAN (fluctuating anaerobiosis), ULO (ultra-low oxygen), CA (controlled atmosphere), and RA (regular atmosphere), on the concentrations of anaerobic metabolites in stored plum fruits were studied, in order to define the conditions for the production of aromatic volatiles which are released through the skin of the intact fruit.

MATERIALS AND METHODS

Plant material and storage conditions

Plum fruits (*Prunus domestica* L. cv. Stanley, cv. Valjevka) were harvested in the middle of September 2008, at 131 and 141 days from full bloom for Valjevka and Stanley, respectively. At harvest maturity the fruits were completely firm and resistant to mechanical injury, and came from 8-year old trees grown in the orchards of Agro Stošíkovice, Ltd., South Moravia, Czech Republic. The fruits were harvested manually and transported within several hours to the technological laboratory of the Institute of Postharvest Technology of Mendel University in Brno, Lednice, Czech Republic. 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 of +0.5°C to +1.0°C and the various gas mixtures were introduced to individual containers. In each container the fruits of both cultivars were stored together for a period of 36 days and then they were stored in a normal oxygen atmosphere for up to 55 days. Five samples were taken at intervals of about 10 days. The storage temperature was 1.0°C to 1.5°C, the relative humidity of the RA variant was 94–98% and in the hermetically sealed containers it reached 100% water vapour saturation. The controlled atmosphere (CA) was prepared by reducing oxygen levels using activated carbon (Swing-sorb) and by adding CO_2 gas from a pressure bottle to achieve the required value, in other cases the concentration of CO_2 69 remained at the lowest possible achievable levels. The fruits of both cultivars were stored in the following gas mixtures.

FAN (fluctuating anaerobiosis):
0.5–0.6% O_2 + 0.1–0.2% CO_2
ULO (ultra-low oxygen):
1.2–1.4% O_2 + 0.2–0.4% CO_2
CA (controlled atmosphere):
1.9–2.2% O_2 + 8.7–8.9% CO_2
RA (regular atmosphere):
20.8–20.9% O_2 + 0.1–0.2% CO_2

The measurement of the gas mixture was set up on the control panel and the atmosphere was adjusted to achieve the required concentrations of CO_2 and O_2 three times daily with an accuracy of 0.1% of the measured value.

Firmness measurement

The technique of pushing a plunger into the intact fruit under a defined loading rate was used for evaluating the firmness of the skin and flesh and for the determination of the fruit's toughness. Firmness was measured on the Texan 2000 instrument (manufacturer: Mendel University in Brno, Czech Republic), which recorded compression and rate of loading. A steel plunger with 5 mm diameter was pressed into the fruit at a rate of 8 mm/min and the resulting force deformation curve was plotted. For the evaluation of deformation curves and the estimation of the basic physical parameters of fruit quality (skin and flesh firmness, plus toughness of the flesh) the software NextView version 2.5 was used. The break in the curve indicates the puncture point when the plunger breaks the skin (a measure of skin firmness) and the sudden decrease in force observed provides a measure of flesh firmness. The area under the deformation curve measures toughness (the work of compression done by loading to the rupture point). For each fruit two deformation curves were generated. Eighteen fruits were tested for each treatment.

Acetaldehyde and ethanol contents

Both compounds were determined in the juice, which was frozen to a temperature of –25°C. Immediately before the analysis, samples were thawed and 1 µl of non-diluted sample was injected into a packed column (length 1.2 m, the diameter 3 mm)

filled with Porapak P (Waters Ass., Inc., Framingham, Mass., USA). Crushed Teflon was added periodically to the injection space of the chromatograph, to adsorb the balast substance contained in the liquid phase. GC settings were: temperature 92°C, detector temperature 150°C, injection temperature 120°C, carrier gas He 12 ml/min, a flame ionization detector (FID). The quantitative study of acetaldehyde and ethanol was carried out with absolute calibration. The results in the graphs are expressed in mg/l of juice.

Volatile measurement by headspace gas analysis

A concentration method was developed for determining the volatile compounds transpired through the skin of plum fruits. At the end of the storage period in the experimental chambers with the gas mixtures, about 0.120 kg (W) of fruit was transferred immediately into a hermetically sealed spherical jar with a volume of about 0.5 l and at a temperature of 1.0–1.5°C. The fruit was then stored in air, at the same temperature, from day 36 onwards, and the last measurements were taken on day 50 of the storage period. The compounds released were removed by a stream of gas percolating at a rate of 30 ml/min (F) and trapped in a concentrating column with Tenax GC as a sorbent. After one hour, the inflow in the concentration column generated a total volume (V) of 3 l of the percolating gas. The compounds deposited in the Tenax GC trap were recovered by thermal desorption (Scientific Instrument Services, Inc., Ringoes, NJ, USA) and transferred to gas chromatographic (GC) columns using a stream of carrier gas (He). The production (G_i) of the given compounds is expressed as a function of the concentration of the analytes in the percolating gas (c_i, µg/l) the velocity of the percolating gas (F, l/h), and weight of fruit (W, kg) placed in the spherical jar.

$$G_i = c_i F/W \text{ (µg/kg h)}$$

An Agilent Technologies 7890A GC system (Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with FID was used to perform the analysis. The system was coupled to an Agilent G1701EA GC/MS ChemStation software system. A fused silica capillary column 30 m × 0.25 mm i.d.; J&W Scientific coated with a 0.25 µm layer of DB-1 was used. Helium was used as the carrier gas. Thermal desorption of the compounds took place in the GC injection port, equipped with a 0.75 mm i.d. splitless glass liner, at 250°C for 5 min in splitless mode. The split valve was then opened (1:50), and the fiber remained in the injection port for the entire 135 e GC run to ensure complete desorption of the aroma compounds. The detector was operated at 250°C. The oven temperature was programmed to increase from 35°C (maintained for 4 min) to 250°C at a rate of 3°C/min.

The final temperature was maintained for 15 min. The mass spectrometer was operated in the electron impact mode with an electron energy of 70 eV; source temperature 230°C; quadrupole temperature 150°C; mass range m/z 50–500; scan rate 3.62 s/scan; and EM voltage 1,150. Compounds were identified based on NIST mass spectra library search. Most of these compounds were further confirmed by comparing their mass spectra and retention times with those obtained for standards.

Estimated concentrations for all compounds were made by GC/MS peak area comparisons of the external components with the area of a known quantity injected in the sorbent (Tenax GC) sealed in the concentration column using equal quantities of 100 mg. The GC peak area data obtained by the HP-GC/MS analysis were used as a direct estimate of the amount produced of each volatile compound.

Statistical analysis

One of the main variables was the five different types of atmosphere. Observations were made over a total of 55 days and 5 measurements were taken at intervals of about 10 days. For the first 36 days the samples were measured in a gas mixture atmosphere and for the rest of the time they were placed in air in the cool storage chamber at an identical temperature.

Each replicate (n = 3) consisted of 0.12 kg of fruit. Means were compared using ANOVA with LSD tests to examine differences between treatments each time ($P < 0.05$). All analyses were performed using the Unistat software package version 5.45 for Windows.

RESULTS AND DISCUSSION

Levels of anaerobic metabolites in plum fruits

During fruit ripening on the tree and after harvest some essential processes involve the produc-

tion of the anaerobic metabolites acetaldehyde and ethanol. By harvest time, small amounts of acetaldehyde have already accumulated in the flesh of plums, measured as 0.31 mg/l for the cv. Stanley and 1.03 mg/l for the cv. Valjevka. This relative difference in concentrations remains constant throughout the whole period of storage in RA (Fig. 1). Similar differences can be seen in the ethanol concentrations during harvest, being 5.30 mg/l for cv. Stanley and 11.24 mg/l for cv. Valjevka (Fig. 2). The extent of anaerobic conversion of storage substrates is assessed as the change over time during refrigerated storage in the air atmosphere. Only a slight increase of ethanol was seen for the cv. Stanley, which was never more than double during the whole storage time in RA. The ability to degrade accumulated ethanol when there is insufficient oxygen in the fluctuating atmosphere (FAN variant), which is shown by cv. Stanley, suggests an ability to retain aerobic material conversion even in situations when ethanol concentrations exceed the metabolic threshold. The ethanol oxidation to concentrations comparable to the initial ones is shown after 10, 20, and 30 days of storage (Fig. 3). The resulting residual concentrations of ethanol do not differ which means that enzymatic conversion by alcohol dehydrogenase (ADH; EC 1.1.1.1) and by pyruvate decarboxylase (PDC; EC 4.1.1.1) remains efficient even during a permanent lack of oxygen in the ambient atmosphere. According to our current understanding, oxygen concentrations in the ambient atmosphere that are lower than 1.0% oxygen lead to a reduction in metabolism in the fruit and also to the loss of the fruit's ability to degrade accumulated ethanol.

Effects of a higher concentration of CO_2 on the ethanol production in the fruit flesh

Ethanol and acetaldehyde, which are natural aroma components, are accumulated during ripening even under aerobic conditions, but to a much greater extent under partially or totally anaerobic conditions. Increased levels of CO_2 in the presence of apparently adequate levels of O_2 stimulate an increase in ethanol concentrations because partially anaerobic conditions can still occur under the storage conditions in controlled atmospheres. Higher concentrations of CO_2 in the ambient atmosphere, even during the pre-storage treatment, increase the fruit firmness (Larsen, Watkins 1995; Polenta, Murray 2005) and increase the tolerance of fruits to disorders induced by chilling temperatures (Crisosto et al. 1999). The long-term effects of higher concentrations of CO_2 are the same as for very low oxygen levels, namely, the significant production of ethanol which accumulates in the tissue (Figs. 4 and 5). The CO_2 concentration in the ambient atmosphere was 8.7–9%.

If the oxygen supply is sufficient to support aerobic metabolism (1.9–2.2% O_2), the response of the fruits is below the limit for aerobic conversions in the CA treatment. The concentration of 531 mg/l of ethanol, after 36 days of storage in the CA for cv. Stanley, affected its metabolism. Its oxidation in the subsequent aerobic phase, after 36 days, was significantly lower compared to cv. Valjevka (Fig. 4). The ethanol concentrations (Fig. 5) also indicate a high capacity for ethanol production in the cv. Valjevka in all tested atmospheres, as well as ethanol oxidation after the aeration phase, which is noticeably slower.

Softening of fruit in different gas mixture treatments

The deformation after the breaking of the skin was taken as the criterion for plum firmness.

Initial values for skin firmness were 1.12 and 1.20 MPa for the cultivars Stanley and Valjevka, respectively. For both, firmness levels remained unchanged during the storage in gas mixtures while, in the fruit stored in air (RA), firmness levels moderately decreased over 55 days. When Stanley fruits were stored continuously in RA, there was a significant decrease in firmness, reaching 0.70 and 0.63 Pa after 36 and 55 days, respectively (Fig. 6). Skin firmness measured on the intact fruit expresses the fruits' properties better then flesh firmness and toughness. Numerically, toughness would be indicated by a force measured in Pa/mm^2 of plunger contact area leading to the disruption of the skin (Fig. 8). In addition, this parameter of toughness is more useful for fresh plums. Fruit softening, biochemically conditioned by spontaneous pectolysis of the contained pectin compounds, is noticeably slowed down in the atmospheres with lowered oxygen content; a higher content of CO_2 in the atmosphere (CA treatment) does not significantly slow down softening when compared to the low oxygen content. The loss of fruit firmness is a good measure of fruit softening (Crisosto et al. 2004; Valero et al. 2007) and is a factor limiting the shipping, storage and shelf life of

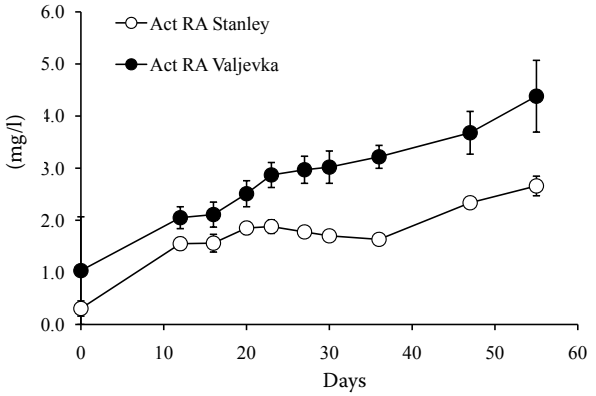

Fig. 1. Changes over time of acetaldehyde (Act) concentration in pulp of Stanley and Valjevka plums stored at RA (regular atmosphere). Each point is the mean of 5 repetitions. Vertical bars indicate standard error

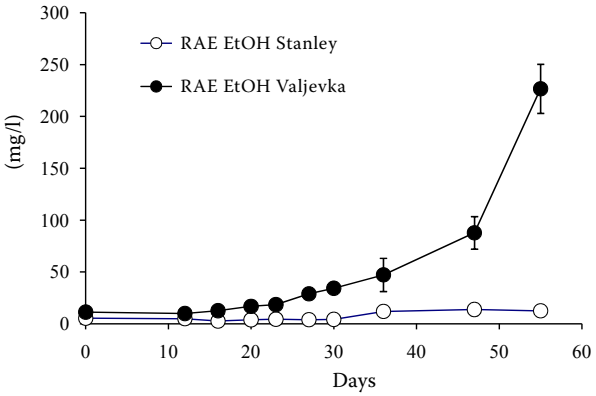

Fig. 2. Changes over time of ethanol (EtOH) concentration in pulp of Stanley and Valjevka plums stored at RA (regular atmosphere). The other descriptors are the same as in Fig. 1

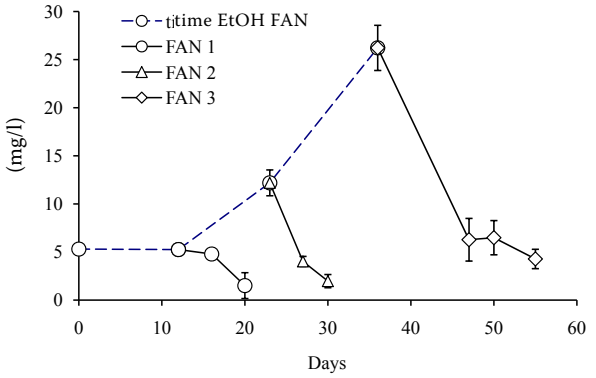

Fig. 3. Ethanol concentration (mg/l) in fruit of cv. Stanley at FAN (fluctuating anaerobiosis) condition (0.5–0.6% O_2 + 0.1–0.2% CO_2). After 10 (FAN1), 20 (FAN2), and 30 (FAN3) days they were transferred to an air atmosphere and further stored at +0.5°C to +1.0°C. The other descriptors are the same as in Fig. 1

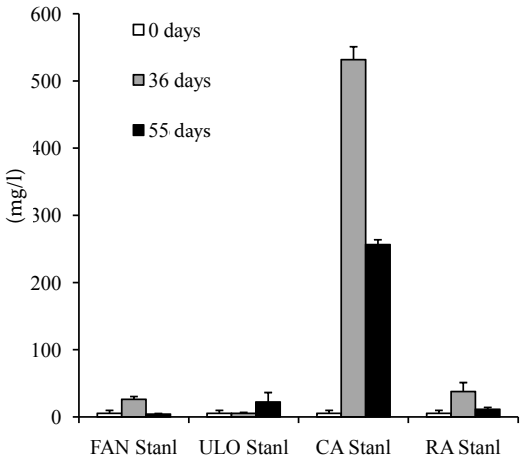

Fig. 4. Effect of CO_2 and O_2 levels on ethanol content (mg/l) in pulp of cv. Stanley (Stanl) fruit in FAN (fluctuating anaerobiosis) 0.5–0.6% O_2 + 0.1–0.2% CO_2, ULO (ultra-low oxygen) 1.2–1.4% O_2 + 0.2–0.4% CO_2, CA (controlled atmosphere) 1.9–2.2% O_2 + 8.7–8.9% CO_2, RA (regular atmosphere) 20.8–20.9% O_2 + 0.1–0.2% CO_2 at +0.5°C to +1.0°C for 0, 36, and 55 days. The other descriptors are the same as in Fig. 1

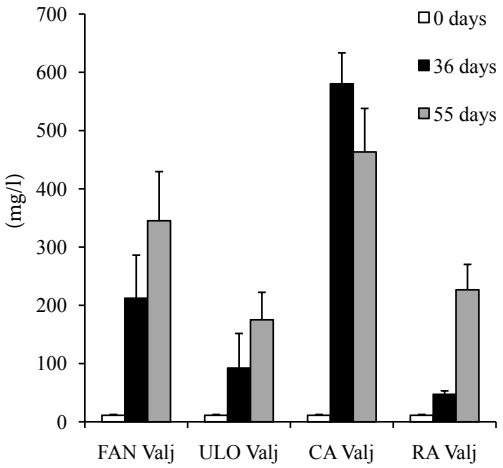

Fig. 5. Effect of CO_2 and O_2 levels on ethanol content (mg/l) in pulp of cv. Valjevka (Valj) fruit in FAN (fluctuating anaerobiosis) 0.5–0.6% O_2 + 0.1–0.2% CO_2, ULO (ultra-low oxygen) 1.2–1.4% O_2 + 0.2–0.4% CO_2, CA (controlled atmosphere) 1.9–2.2% O_2 + 8.7–8.9% CO_2, RA (regular atmosphere) 20.8–20.9% O_2 + 0.1–0.2% CO_2 at +0.5°C to +1.0°C for 0, 36, and 55 days. The other descriptors are the same as in Fig. 1

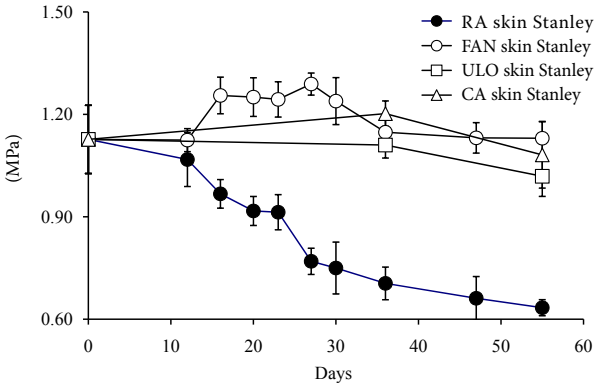

Fig. 6. Changes over time in skin firmness of cv. Stanley plums in FAN (fluctuating anaerobiosis) 0.5–0.6% O_2 + 0.1–0.2% CO_2, ULO (ultra-low oxygen) 1.2–1.4% O_2 + 0.2–0.4% CO_2, CA (controlled atmosphere) 1.9–2.2% O_2 + 8.7–8.9% CO_2, RA (regular atmosphere) 20.8–20.9% O_2 + 0.1–0.2% CO_2 at +0.5°C to +1.0°C. Each point is the mean of 12 repetitions. Vertical bars indicate standard error

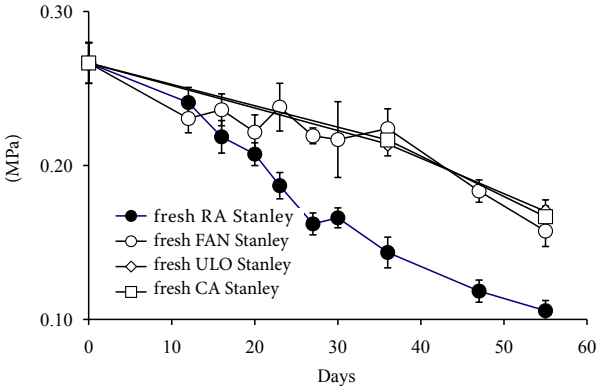

Fig. 7. Changes over time in flesh firmness of cv. Stanley in FAN (fluctuating anaerobiosis) 0.5–0.6% O_2 + 0.1–0.2% CO_2, ULO (ultra-low oxygen) 1.2–1.4% O_2 + 0.2–0.4% CO_2, CA (controlled atmosphere) 1.9–2.2% O_2 + 8.7–8.9% CO_2, RA (regular atmosphere) 20.8–20.9% O_2 + 0.1–0.2% CO_2 at +0.5°C to +1.0°C. The other descriptors are the same as in Fig. 6

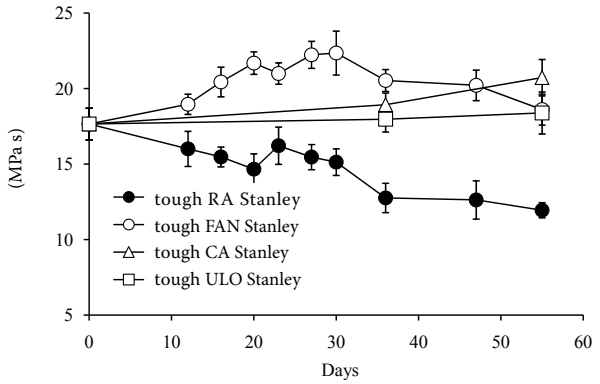

Fig. 8. Changes over time in toughness of cv. Stanley in FAN (fluctuating anaerobiosis) 0.5–0.6% O_2 + 0.1–0.2% CO_2, ULO (ultra-low oxygen) 1.2–1.4% O_2 + 0.2–0.4% CO_2, CA (controlled atmosphere) 1.9–2.2% O_2 + 8.7–8.9% CO_2, RA (regular atmosphere) 20.8–20.9% O_2 + 0.1–0.2% CO_2 at +0.5°C to +1.0°C. The other descriptors are the same as in Fig. 6

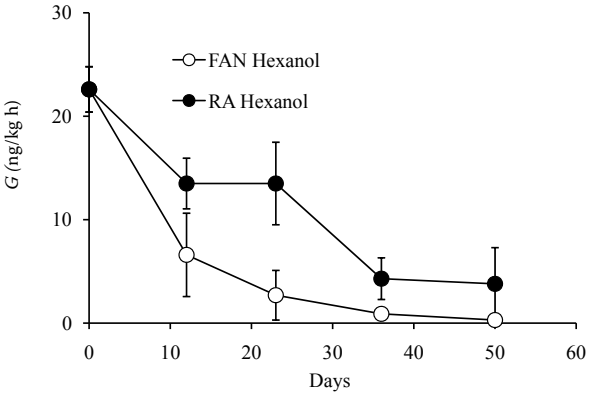

Fig. 9. Hexan-1-ol volatiles expressed as production (ng/kg h) released across skin of intact fruit of cv. Stanley at FAN (fluctuating anaerobiosis) 0.5–0.6% O_2 + 0.1–0.2% CO_2, and RA (regular atmosphere) 20.8–20.9% O_2 + 0.1–0.2% CO_2 conditions at +0.5°C to +1.0°C. Each point is the mean of 3 repetitions. Vertical bars indicate standard error

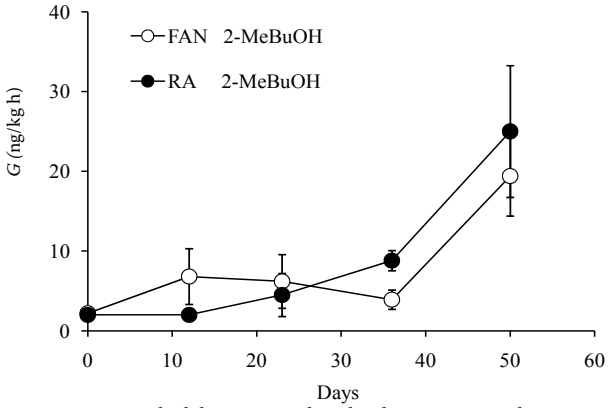

Fig. 10. 2-methyl-butan-1-ol volatiles expressed as production (ng/kg h) released across skin of intact fruit of cv. Stanley. The other descriptors are the same as in Fig. 9

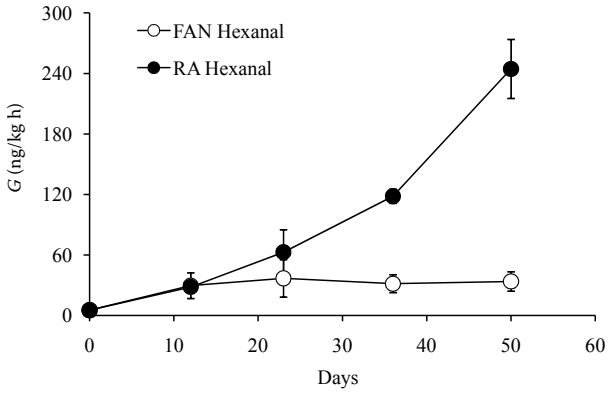

Fig. 11. Hexanal volatiles expressed as production (ng/kg h) released across skin of intact fruit of cv. Stanley. The other descriptors are the same as in Fig. 9

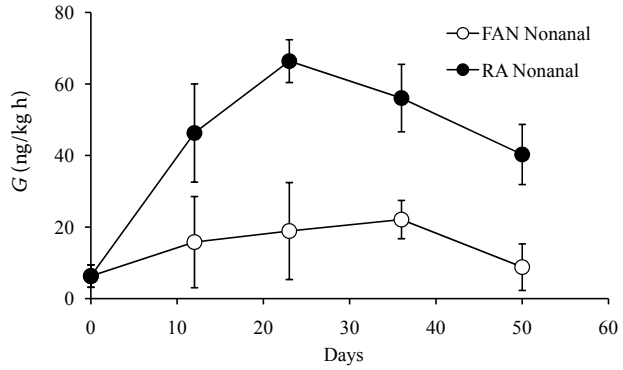

Fig. 12. Nonanal volatiles expressed as production (ng/kg h) released across skin of intact fruit of cv. Stanley. The other descriptors are the same as in Fig. 9

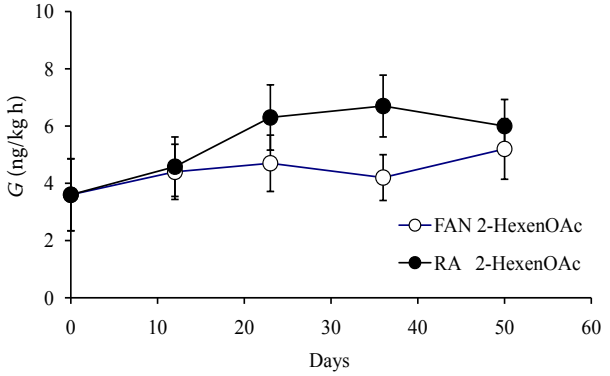

Fig. 13. tr-2-hexenoic acid acetate volatiles expressed as production (ng/kg h) released across skin of intact fruit of cv. Stanley. The other descriptors are the same as in Fig. 9

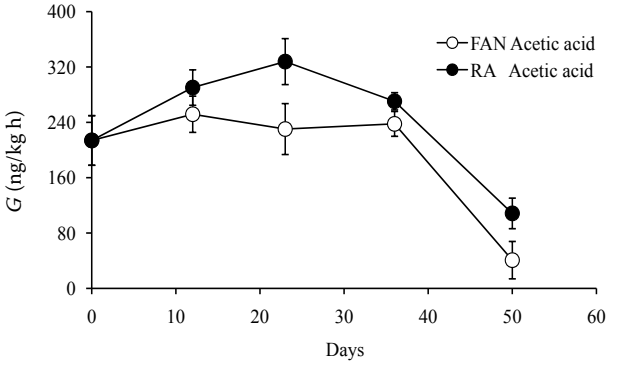

Fig. 14. Acetic acid volatiles expressed as production (ng/kg h) released across skin of intact fruit. The other descriptors are the same as in Fig. 9

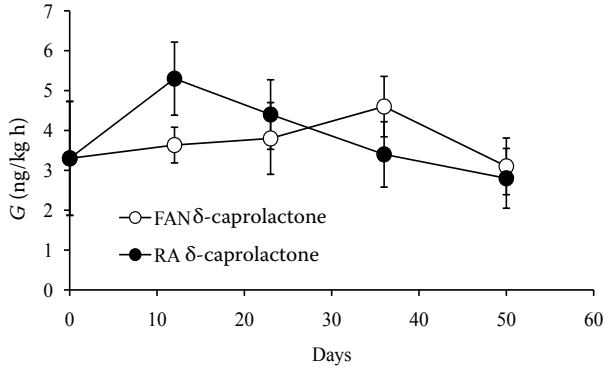

Fig. 15. δ-caprolactone volatiles expressed as production (ng/kg h) released across skin of intact fruit of cv. Stanley. The other descriptors are the same as in Fig. 9

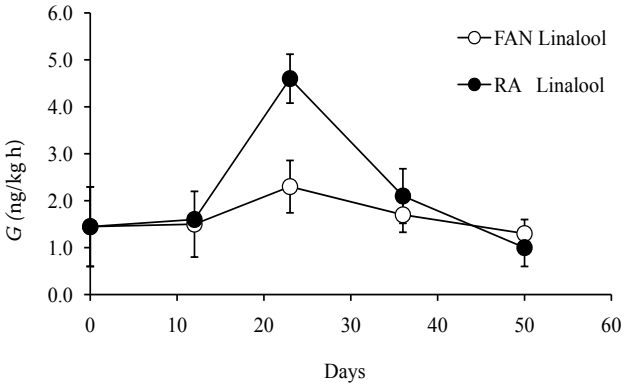

Fig. 16. Linalool volatiles expressed as production (ng/kg h) released across skin of intact fruit of cv. Stanley. The other descriptors are the same as in Fig. 9

plums (Dong et al. 2002; Menniti et al. 2006). The parameter of skin firmness provides more reliable values than flesh firmness (Fig. 7). If fruits are directly influenced by the storage gas mixtures (FAN, CA, ULO), then the loss of mechanical firmness is insignificant; not until after exposure of the fruit to normal oxygen levels does the more noticeable decline in firmness occur.

Emission of volatile compounds across intact skin of whole fruit

Volatiles released from the fruit by diffusion into a percolating gas were concentrated on the sorbent from a large volume. Under these analytical conditions, acetaldehyde, ethanol, and ethyl acetate were not determined because of their low molecular

Table 1. Evaluation of volatiles expressed as production (ng/kg h) released across skin of intact fruit at FAN (fluctuating anaerobiosis) 0.5–0.6% O_2 + 0.1–0.2% CO_2, ULO (ultra-low oxygen) 1.2–1.4% O_2 + 0.2–0.4% CO_2, CA (controlled atmosphere) 1.9–2.2% O_2 + 8.7–8.9% CO_2, RA (regular atmosphere) 20.8–20.9% O_2 + 0.1–0.2% CO_2 at + 0.5°C to + 1.0°C for cv. Stanley LSD test, $P < 0.05$; significant differences are indicated by letters

Treatment	1	2	3	4	5	6	7	8
Initial	6.0^{a}	1.3^{a}	18.0^{a}	27.7^{a}	6.4^{ab}	3.9^{a}	6.0^{a}	6.0^{a}
FAN	103.7^{b}	26.6^{b}	264.6^{ab}	149.4^{b}	7.1^{ab}	7.3^{ab}	3.7^{a}	3.7^{a}
ULO	19.8^{a}	2.6^{a}	377.4^{ab}	45.2^{a}	8.5^{b}	9.8^{abc}	5.1^{a}	5.1^{a}
CA	17.0^{a}	18.3^{ab}	466.1^{ab}	73.0^{ab}	9.2^{b}	13.6^{c}	6.9^{a}	6.9^{a}
RA	63.8^{ab}	15.4^{ab}	671.1^{b}	115.6^{ab}	3.8^{a}	11.6^{bc}	3.5^{a}	3.5^{a}
Treatment	9	10	11	12	13	14	15	16
Initial	3.3^{c}	22.3^{a}	0.3^{a}	0.3^{a}	0.8^{a}	3.6^{a}	78.0^{a}	0.3^{a}
FAN	1.3^{a}	7.6^{a}	17.7^{ab}	54.4^{a}	87.1^{b}	5.2^{a}	251.9^{a}	0.7^{a}
ULO	2.6^{bc}	8.2^{a}	25.3^{b}	24.9^{a}	62.3^{ab}	2.4^{a}	240.7^{a}	1.8^{ab}
CA	2.7^{bc}	15.1^{a}	42.6^{c}	79.7^{ab}	171.0^{c}	1.9^{a}	525.5^{b}	1.6^{ab}
RA	1.7^{ab}	11.2^{a}	9.7^{ab}	200.3^{b}	119.8^{bc}	4.3^{a}	304.6^{ab}	4.7^{b}
Treatment	17	18	19	20	21	22	23	24
Initial	0.8^{a}	0.3^{a}	3.6^{a}	0.7^{a}	0.3^{a}	0.3^{ab}	1.8^{a}	0.3^{a}
FAN	2.6^{ab}	3.5^{a}	3.2^{a}	2.2^{a}	1.5^{ab}	0.4^{ab}	168.2^{b}	0.9^{ab}
ULO	1.2^{a}	2.3^{a}	3.0^{a}	1.0^{a}	1.2^{ab}	0.4^{ab}	75.4^{a}	0.4^{a}
CA	2.1^{ab}	2.8^{a}	2.6^{a}	2.4^{a}	3.1^{c}	0.3^{a}	261.3^{c}	0.6^{a}
RA	3.3^{b}	6.3^{a}	2.8^{a}	8.4^{b}	2.5^{bc}	1.4^{b}	68.0^{a}	1.6^{b}
Treatment	25	26	27	28	29	30	31	32
Initial	1.4^{a}	0.4^{a}	0.3^{a}	0.3^{a}	5.3^{ab}	0.8^{a}	6.3^{a}	2.5^{a}
FAN	2.3^{a}	0.5^{a}	1.9^{a}	5.8^{a}	32.6^{ab}	1.6^{a}	15.4^{a}	2.2^{a}
ULO	2.2^{a}	0.5^{a}	2.9^{a}	5.4^{a}	5.2^{a}	1.6^{a}	13.0^{a}	3.5^{a}
CA	4.1^{a}	0.5^{a}	3.4^{a}	11.3^{b}	22.0^{ab}	3.9^{a}	48.8^{b}	3.3^{a}
RA	2.1^{a}	0.5^{a}	2.7^{a}	12.0^{b}	175.7^{b}	0.6^{a}	34.9^{ab}	1.8^{a}
Treatment	33	34	35	36	37	38	39	40
Initial	19.4^{b}	0.7^{ab}	3.3^{a}	1.8^{a}	213.8^{ab}	56.0^{b}	25.7^{b}	1.9^{a}
FAN	5.2^{a}	0.9^{b}	3.4^{a}	1.9^{a}	173.8^{ab}	3.9^{a}	2.5^{a}	1.0^{a}
ULO	3.0^{a}	0.3^{a}	4.3^{a}	1.0^{a}	157.4^{ab}	3.3^{a}	2.3^{a}	1.3^{a}
CA	6.5^{a}	0.5^{ab}	6.8^{a}	1.8^{a}	277.4^{b}	5.3^{a}	3.9^{a}	2.2^{a}
RA	1.3^{a}	0.2^{a}	3.1^{a}	1.9^{a}	65.9^{a}	0.7^{a}	1.3^{a}	1.3^{a}
Treatment	41	42						
Initial	0.3^{a}	22.6^{b}						
FAN	0.6^{a}	4.5^{a}						
ULO	0.3^{a}	7.2^{a}						
CA	0.5^{a}	8.4^{a}						
RA	0.5^{a}	2.0^{a}						

Table 2. Chemical names of established analytes

1	butan-1-ol	22	ethylhexanoate
2	2-Methyl-butan-1-ol	23	ethyltrans-3-hexenoate
3	2-Methyl-pentan-1-ol	24	*n*-Propylhexanoate
4	3-Methyl-pentan-1-ol	25	*n*-Hexylhexanoate
5	2-Ethyl-hexan-1-ol	26	ethyloctanoate
6	2-Hexen-1-ol	27	trans-methyl-2-octenoate
7	octan-1-ol	28	methylheptanoate
8	octan-2-ol	29	hexanal
9	heptan-1-ol	30	2-Hexen-1-al
10	phenylethylAlcohol	31	*n*-Nonanal
11	2-Methyl-1-propylacetate	32	*n*-Decanal
12	2-Methylbutylacetate	33	2-Furaldehyde
13	hexylacetate	34	benzaldehyde
14	2-Hexen-1-ol,acetate	35	δ-Caprolactone
15	*n*-Butylacetate	36	octan-2-one
16	*n*-Propylacetate	37	aceticacid
17	*n*-Hexylpropionate	38	propanoicacid
18	ethylbutyrate	39	butyricacid
19	*n*-Amylbutyrate	40	linolool
20	*n*-Hexylbutyrate	41	limonene
21	*n*-Butyl-2-methylbutyrate	42	hexan-1-ol

weight. From the total number of 42 determined volatile compounds, 11 alcohols, 6 aldehydes, 17 esters, 2 terpenes, 3 organic acids, and 1 lactone were present (Tables 1 and 2). The most representative compounds were the families of esters and the most abundant were hexan-1-ol, 2-ethylhexan-1-ol, 2-hexen-1-ol, hexanal, and their esters. A very low oxygen atmosphere slows down production of esters and aldehydes (Figs. 9 and 11–14); in contrast, insignificant differences are found in the production of lactones and terpenes. However, in the last stage of fruit storage, in air, there was a marked increase in the level of 2-methyl-butanol-1 (Fig. 10) although, in general, biosynthesis of aromatic volatiles is significantly depressed by the lack of oxygen in the ambient atmosphere. When stored in a gas mixture with subsequent aeration of the ambient atmosphere, the fruit was unable to undertake significant new biosynthesis (Lara et al. 2007). Therefore, low oxygen storage may lead to the decreased commercial value of product because of a reducted production of some aroma volatiles.

CONCLUSION

The storage of fruits that can be easily spoilt, like plums, requires not only an optimal temperature but also a gas mixture which does not induce unfavourable physiological responses in the fruit. It was shown that a very low oxygen concentration with a low content of CO_2 (FAN treatment) do not encourage significant production of ethanol and acetaldehyde in the fruit flesh, but do significantly slow down the biosynthesis of aromatic volatiles. It was further determined that when fruit is stored at low oxygen levels together with high levels of CO_2, softening is minimal. A high proportion of CO_2 in the ambient atmosphere, of 8.7–8.9%, creates an anaerobic environment where the high CO_2 portion limits aerobic metabolism. Forty-two different aromatic volatiles were quantified; according to their chemical structure and biosynthesis, there was a slower rate of production in many of them in atmospheres with very low oxygen content.

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Received for publication December 14, 2009
Accepted after corrections July 9, 2010

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