Effect of low oxygen and high carbon dioxide atmospheres on the formation of volatiles during storage of two sweet cherry cultivars

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Abstract

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The aroma profiles of two sweet cherry cultivars Kordia and Vanda were investigated during storage at different oxygen and carbon dioxide levels and at a low temperature using solid phase microextraction gas chromatography combined with mass spectrometry (SPME-GC-MS). The most abundant aroma volatiles observed in both sweet cherry cultivars were alcohols, esters, terpenoids and aldehydes. Fifteen alcohols (but principally ethan-1-ol, (*E*)-2-hexen-1-ol and phenethyl alcohol) provided approximately 39% of the total volatile production and eight esters (principally (*E*)-2-hexenyl acetate and pentyl butyrate) were responsible for another 39% of the volatile production. Four terpenoids (principally limonene and α-linalool) were responsible for a further 15% of volatile production, and 10 aldehydes (principally (*E*)-2-hexenal and (*E*)-2-octen-1-al) were responsible for the remaining 7% of total volatile production. However, out of all the volatile compounds detected, a total of just 6 compounds (phenethyl alcohol, (*E*)-2-hexenal, (*E*)-2-octen-1-al, pentyl butyrate, (*E*)-2-hexenyl acetate and limonene) made up 80% of the total volatile production. Fruit stems remained green during all 54 days of the storage period, although one tenth of the stems slowly dessicated in each of the three controlled atmospheres. This is in marked contrast to the stems of fruit held in a regular atmosphere, which turned completely brown.

Keywords: modified atmosphere; monoterpenic hydrocarbons; alcohols; esters

The optimisation of post-harvest storage conditions for sweet cherry fruits, at temperatures near 0°C and with low oxygen and high CO_2 levels, significantly prolongs the storability of the fruit. In addition to choosing the optimal time for harvest, and the rapid cooling of harvested fruit, it has been shown that maintaining the level of oxygen just above the limit for aerobic respiration, and raising CO_2 levels to around 10%, result in better quality of stored fruit and extend post-harvest life (Girard, Kopp 1998; Hevia et al. 1998; Esti et al. 2002; Crisosto et al. 2003; Sharma et al. 2010; Goliáš et al. 2010). Sweet cherries have a higher tolerance to elevated CO_2 concentrations than most stone fruit crops (Tian et al. 2001, 2004; Petracek et al. 2002). The impact of controlled atmosphere storage (CA) on the production of volatile compounds by sweet cherry fruits was studied by analysing the compounds released from fruit into the ambient atmosphere (Mattheis et al. 1991; Meheriuk et al. 1995; Bernalte et al. 1999; Goliáš et al. 2010). Dynamic headspace gas chromatogramy (GC) methods show that the pre-

dominant flavour volatiles are (*E*)-2-hexenol, benzaldehyde, hexanal and (*E*)-2-hexenal, but some compounds observed after 4 weeks of storage appeared to be unrelated to the storage conditions and may reflect metabolic changes which occur during fruit senescence. In cherries, the main problems during storage are desiccation and subsequent browning of the stems, and two factors which were implicated in this phenomenon are temperature and humidity. A relative humidity between 90 and 95% is particularly important in maintaining a green stem colour, because cherry stems lose water about 14 times faster than the flesh of the fruit itself (Sekse 1996).

The aim of this work was to study the production of volatiles emanating from intact fruit into the ambient atmosphere and the concentration of volatiles in juice extracts in two sweet cherry cultivars, stored in four different atmospheres. Two distinct gas chromatography methods (HP/GC/MS and SPME/GC/MS) were used.

MATERIAL AND METHODS

Plant material and storage conditions. Fruits from the sweet cherry (*Prunus avium* L.) cultivars Kordia and Vanda were harvested, with the appropriate size 26–28 and 24–26 mm for cvs Kordia and Vanda, respectively, and dark color for Kordia, red color for Vanda, just one day before commercial maturity in orchards Agro Stošíkovice in South Moravia, Czech Republic. The fruits were harvested by hand and exposed to the ambient atmosphere and transported witout cooling in three hours to the technological laboratory of the Department of Postharvest Technology of Horticultural Products at Mendel University 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. The fruits with diametre from 24 to 28 mm uniform according to cultivar were cooled to a temperature of +0.5 to +1.0°C and various gas mixtures were introduced to individual containers. The storage temperature was +1.0–1.5°C, the relative humidity (RH) of the regular atmosphere variant (RA) was 94–98%, and in the hermetically sealed containers it was 100%. The controlled atmosphere (CA) was prepared by reducing oxygen levels using activated carbon (Swing-sorb; Besseling, Zwaag, The Netherlands) and by adding CO_2 gas from a pressure bottle to achieve the required value. In other cases the

concentrations of CO_2 remained at the lowest possible achievable levels of 0.5%. In each container 20 kg of fruit from both cultivars was stored in each of the following gas mixtures:
Fluctuating anaerobiosis (FAN):
    O_2 0.5–0.6%, CO_2 0.2–0.3%
Controlled atmosphere 1 (CA1):
    O_2 0.9–1.0%, CO_2 9.0–9.2%
Controlled atmosphere 2 (CA2):
    O_2 1.5–2.0%, CO_2 14.6–15.0%
Regular atmosphere (RA):
    O_2 20.9%, CO_2 0.1%
The gas mixtures were monitored automatically and the atmospheres were adjusted to achieve the desired concentrations of CO_2 and O_2 three times a day, with an accuracy of 0.1%, for a period of 40 days.

Ethanol measurement. Juice samples, made from 12 fruits of each cultivar and for each treatment (*n* = 3), were stored at –27°C. Immediately before analysis the samples were thawn and 1 µl of non-diluted juice was injected into a packed column (length 1.2 m, diameter 3 mm) filled with Porapak P (Waters Associates, Inc., Framingham, USA). GC settings: temperature 92°C, detector temperature 150°C, injection temperature 120°C, carrier gas He 12 ml/min, flame ionisation detector (FID). A quantitative determination of ethanol levels was performed using an absolute calibration. The results are expressed in mg/l of juice.

Volatile measurement by solid phase microextraction (SPME). The analyses were performed at the start of the experiment, then 40 days later when the storage period in gas mixtures ended, and again at the end of the experiment after a total of 56 days. The sealed vials were immersed in a water bath and held at 50°C to equilibrate, and after 30 min a 2 cm fused silica fiber, coated with divinylbenzene(DBV)/carboxen(CAR)/polydimethylsiloxane(PDMS) 50/30 µm was introduced and exposed to the headspace environment for 30 minutes. Concentrations are measured in µg/l.

Determination of volatiles from intact fruit by headspace analysis with thermal desorption. Observations were made on days 10, 24 and 40 (when the storage in gas mixtures ended) and then on days 45, 50 and 56 when the fruit was stored in air at +0.5 to +1.0°C. A fresh sample of 0.150 kg fruit was transferred to a hermetically sealed spherical jar, with a volume about 0.5 l at a temperature of 0°C. The volatile compounds released from the fruit were flushed out by a stream of gas percolating at a flow rate of 50 ml/min and then trapped in an en-

richement column with Tenax TA (Scientific Instrument Services, Inc., Ringoes, USA) as the sorbent.

Statistical analysis. The experiment had a factorial design with two storage periods (40 and 54 days) four storage atmospheres (RA, FAN, CA1 and CA2), and two cultivars. Statistical analysis was carried out using the statistical package SAS v. 9.2 (SAS Institute, Inc., Cary, USA). At each time point, three samples of the two cherry varieties in each of the four treatments were analyzed and summarised by means and their standard errors. Analysis of variance (ANOVA) and multiple analysis of variance (MANOVA) were used to analyse the chemical changes in the fruits of the two cherry cultivars held in each of the four atmospheres.

Evaluation of visual quality and stem browning. At the end of the storage period all the fruit in each treatment was individually evaluated for overall visual quality and the incidence and severity of stem browning. Browning was evaluated according to the following five-point scale: 1 = no browning, 2 = 1–25%, 3 = 26–50%, 4 = 51–75% and 5 = 76–100% of the surface of the stem affected. The scale for evaluating visual quality, or the visible presence of fruit rot, was identical to that used for stem browning.

RESULTS AND DISCUSSION

The volatiles produced in sweet cherry fruits while in storage

The most common aroma volatiles in both cultivars were 15 alcohols (mainly ethan-1-ol, (*E*)-2-hexen-1-ol and phenethyl alcohol), which were responsible for 39–40% of total volatile production, 8 esters (mainly (*E*)-2-hexenyl acetate and pentyl butyrate), which were also responsible for 39–40%, 4 terpenoids (mainly limonene and α-linalool) which were responsible for another 14–15% and 10 aldehydes (mainly (*E*)-2-hexenal and (*E*)-2-octen-1-al) which were responsible for just 6.6%. The list of compounds statistically significant is given in Table 1.

The formation of volatiles during postharvest storage

In the case of alcohols, phenethyl alcohol and (*E*)-2-hexen-1-ol were the predominant compounds produced during storage, and they accumulated as ripening progressed, providing the substrates for es-

ter formation (Fig. 1). The other primary alcohols, such as *n*-hexan-1-ol, and the branched alcohols (3-methyl butan-1-ol, 3-methyl-(*E*)-2-buten-1-ol and 4-ethylpentan-1-ol) are products of the deamination of the relevant α-amino acids through oxidative reactions which occur under aerobic conditions (Table 1). Aldehydes are an important element in ester synthesis, and they are most abundant in unripe fruit (Mattheis et al. 1991; Defilippi et al. 2005). In terms of individual compounds, *n*-hexanal, (*E*)-2-hexenal and (*E*)-2-octenal were the main aldehydes present in mature and ripe fruit, with a gradual increase in the concentration of (*E*)-2-hexenal throughout the process of ripening (Fig 2). Pentyl butyrate is a common compound present during all stages of senescence, regardless of storage treatment and made up over 90% of the total ester compounds observed, followed by methyl 3-methylbutyrate, butyl 2-methyl butyrate and ethyl (*E*)-3-hexenoate (Table 1).

Influence of higher CO_2 concentrations on the formation of volatiles

Elevated CO_2 concentrations (2–5%), along with reduced O_2 concentrations (1–2%), generally delay ripening in cherries, which are one of the commodity fruits which responds well to elevated CO_2 levels in storage (Remón et al. 2001; Tian et al. 2001; Goliáš et al. 2007). As CA1 and CA2 storage conditions suppressed the synthesis of aroma volatiles (Table 1), it is plausible that the aroma profile of sweet cherries stored in these atmospheres is closer to that of freshly harvested sweet cherries than when sweet cherries were stored in air.

Limonene and other terpenoids

Limonene as a monoterpenic hydrocarbon and α-linalool and (*Z*)-linalool oxide as oxygenated monoterpenes are typically found in sweet cherries throughout storage and are partly responsible for the sweet, fruity flavour of the cvs Kordia and Vanda. They are abundant in the freshly harvested yet not fully-ripe fruit. But after storage at low temperatures and especially when also exposed to controlled gas mixtures, their biogenesis is reduced or even ceases altogether (Figs 3 and 4). When stored at low temperatures without the help of gas mixtures, the biosynthesis of α-linalool recovered in both varieties when the fruit was taken out of stor-

Table 1. Concentrations of volatiles analysed by SPME/GC/MS of the cultivar stored at different atmosphere mixtures

Volatiles (µg/l)	Variety	Treatment/Time (day) – mean (SE)								
		IN	FAN		CA1		CA2		RA	
		0	40	54	40	54	40	54	40	54
Ethan-1-ol	Kordia	427.0 (104.0)	336.6 (66.0)	133.9 (21.8)	8,676 (343)	334.3 (52.9)	19,494 (1,210)	9,870 (2,209)	78.97 (9.21)	169.3 (20.3)
	Vanda	329.0 (19.3)	1,009 (130)	505.4 (24.3)	1,347 (30)	460.8 (77.7)	7,377 (693)	639.0 (109.6)	93.21 (7.13)	234.1 (44.9)
n-pentan-1-ol	Kordia	5.69 (0.52)	6.74 (0.89)	9.12 (0.38)	7.89 (0.52)	8.21 (0.33)	8.32 (0.56)	8.96 (0.28)	5.57 (0.82)	7.64 (1.14)
	Vanda	5.55 (0.35)	3.23 (0.37)	14.73 (1.36)	3.59 (0.14)	12.78 (2.92)	3.64 (0.25)	4.28 (0.31)	3.28 (0.77)	30.06 (2.96)
n-hexan-1-ol	Kordia	48.26 (4.30)	18.96 (1.70)	18.34 (0.50)	47.40 (2.73)	54.47 (7.86)	58.88 (5.82)	74.92 (4.11)	16.59 (2.52)	31.33 (6.71)
	Vanda	66.71 (9.02)	11.30 (1.61)	17.82 (3.51)	19.05 (0.98)	58.18 (8.77)	32.87 (2.37)	37.40 (5.24)	13.59 (1.86)	34.86 (3.36)
3-methyl-2-buten-1-ol	Kordia	5.72 (0.82)	2.48 (0.12)	2.40 (0.38)	1.96 (0.43)	2.09 (0.37)	10.76 (0.65)	2.40 (0.46)	2.56 (0.33)	4.16 (0.53)
	Vanda	6.45 (0.75)	2.33 (0.44)	2.35 (0.14)	3.69 (0.11)	3.51 (0.41)	11.43 (0.60)	2.70 (0.31)	2.63 (0.33)	3.40 (0.46)
3-methyl-1-pentan-1-ol	Kordia	116.7 (13.5)	66.51 (5.68)	53.89 (1.31)	91.40 (4.08)	68.05 (8.59)	80.96 (1.63)	95.02 (17.63)	64.71 (15.66)	77.04 (3.62)
	Vanda	153.1 (12.5)	57.74 (4.49)	45.40 (3.63)	115.9 (12.3)	62.73 (13.50)	274.2 (22.9)	63.99 (4.57)	56.27 (2.96)	72.77 (5.61)
2-heptan-1-ol	Kordia	5.66 (0.52)	2.74 (0.27)	2.27 (0.20)	1.95 (0.36)	2.25 (0.28)	11.41 (0.88)	2.50 (0.71)	3.31 (0.69)	3.99 (0.29)
	Vanda	6.71 (0.81)	2.56 (0.66)	2.69 (0.43)	3.96 (0.15)	4.14 (0.67)	12.11 (1.12)	2.77 (0.32)	2.40 (0.25)	3.53 (0.26)
(*Z*)-2-hexen-1-ol	Kordia	629.1 (73.3)	1,961 (130)	7,155 (107)	1,791 (212)	2,849 (408)	3,676 (57)	3,281 (154)	1,866 (230)	2,932 (97)
	Vanda	666.8 (49.4)	582.5 (95.1)	1,151 (66)	1,002 (147)	1,371 (155)	804.1 (114.8)	863.3 (179.0)	933.6 (70.3)	1,717 (140)
2-methylbutanal	Kordia	4.65 (0.77)	23.34 (1.54)	82.14 (9.22)	5.75 (0.18)	36.03 (3.94)	7.53 (0.62)	17.49 (1.44)	6.01 (0.35)	7.32 (0.34)
	Vanda	3.99 (0.60)	59.21 (10.64)	57.84 (8.26)	5.84 (1.63)	31.85 (4.93)	4.75 (0.49)	7.85 (1.52)	24.35 (0.66)	16.74 (0.56)
Ethyl (*E*)-3-hexenoate	Kordia	25.15 (4.77)	26.12 (2.64)	19.42 (7.19)	34.02 (1.71)	27.22 (3.14)	23.34 (2.63)	20.46 (2.68)	32.33 (1.32)	27.93 (3.88)
	Vanda	32.80 (6.07)	34.47 (1.36)	59.17 (8.08)	44.69 (5.18)	45.87 (4.94)	39.15 (2.02)	38.41 (5.11)	24.16 (2.20)	165.3 (26.7)
(*Z*)-linalool oxide	Kordia	2.28 (0.38)	4.78 (0.27)	5.15 (0.67)	1.23 (0.15)	1.92 (0.26)	1.57 (0.29)	3.13 (0.52)	4.47 (0.56)	2.24 (0.12)
	Vanda	0.90 (0.09)	3.20 (0.29)	4.38 (0.51)	2.14 (0.26)	2.53 (0.23)	1.46 (0.21)	2.37 (0.31)	2.81 (0.25)	2.33 (0.17)

IN – control; FAN – fluctuating atmosphere 0.5–0.6% O_2, 0.2–0.3% CO_2; CA1 – controlled atmosphere with 0.9–1.0% O_2, 9.0–9.2% CO_2; CA2 – controlled atmosphere with 1.5–2.0% O_2, 14.6–15.0% CO_2; RA – regular atmosphere with 20.9% O_2, 0.1% CO_2 for 40 days, and then in air for 15 days, held at +0.5 to +1.0°C; SE – statistical error
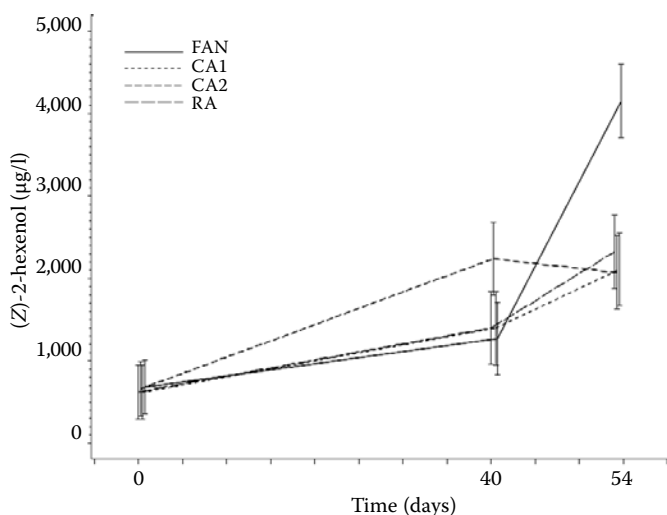
Fig. 1. Concentrations of (*Z*)-2-hexenol analyzed by the SPME metod of the sweet cherry cvs Vanda and Kordia stored in different atmospheres
FAN – fluctuating atmosphere 0.5–0.6% O_2, 0.2–0.3% CO_2; CA1 – controlled atmosphere with 0.9–1.0% O_2, 9.0–9.2% CO_2; CA2 – controlled atmosphere with 1.5–2.0% O_2, 14.6–15.0% CO_2; RA –regular atmosphere with 20.9% O_2, 0.1% CO_2 for 40 days and then in air for 15 days, held at +0.5 to +1.0°C
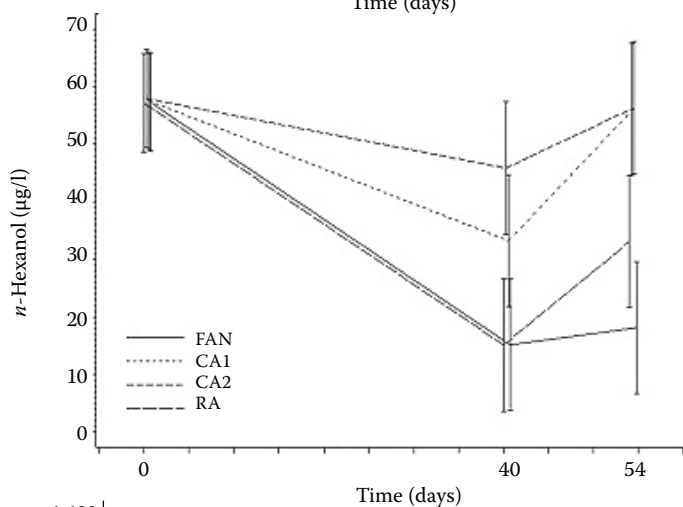

Fig. 2. Concentrations of *n*-hexanol analyzed by the SPME method of the sweet cherry cvs Vanda and Kordia stored in different atmospheres (for explanation see Fig. 1)
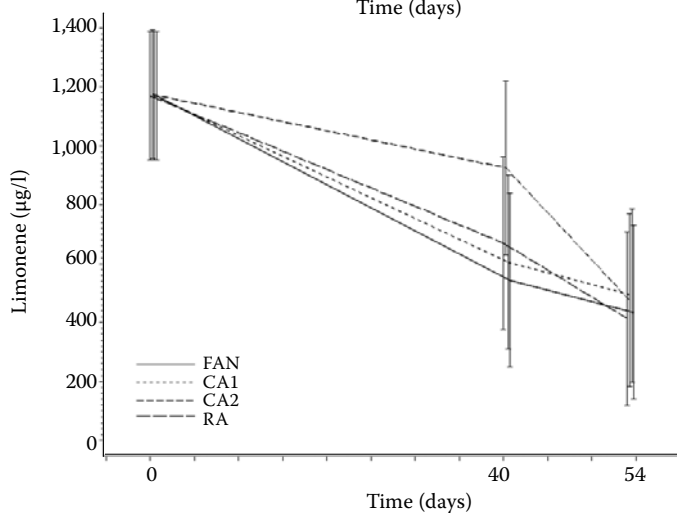

Fig. 3. Concentrations of limonene analyzed by SPME/GC/MS during storage of cvs Vanda and Kordia in controlled atmospheres (for explanation see Fig. 1)

age, but synthesis of (*Z*)-linalool oxide recovered only in the cv. Vanda (Fig. 5). The biosynthesis of limonene, which is the most abundant terpenoid, fell throughout the whole storage period. These two compounds are responsible for floral, green and sweet odours in the fruit (KLESK et al. 2004).

Effect of different atmospheres on fruit storability

The use of CA1, CA2 and FAN atmospheres for storing the cherry cvs Kordia and Vanda effectively maintained the green colour and reduced dessica-
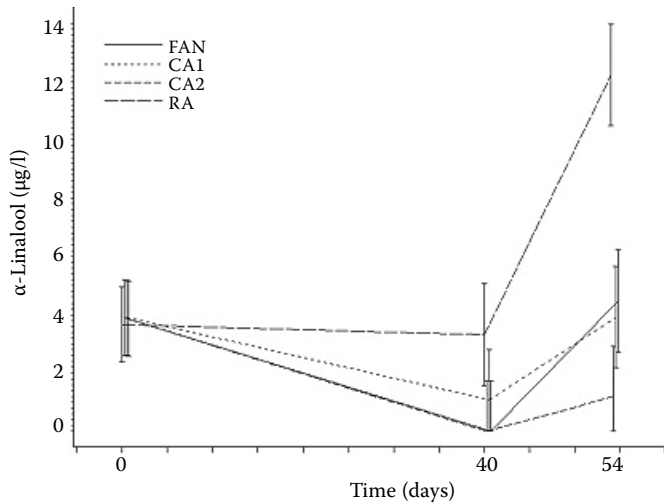
Fig. 4. Concentrations of α-linalool analyzed by SPME/GC/MS during storage of cv. Vanda and cv. Kordia in controlled atmospheres (for explanation see Fig. 1)
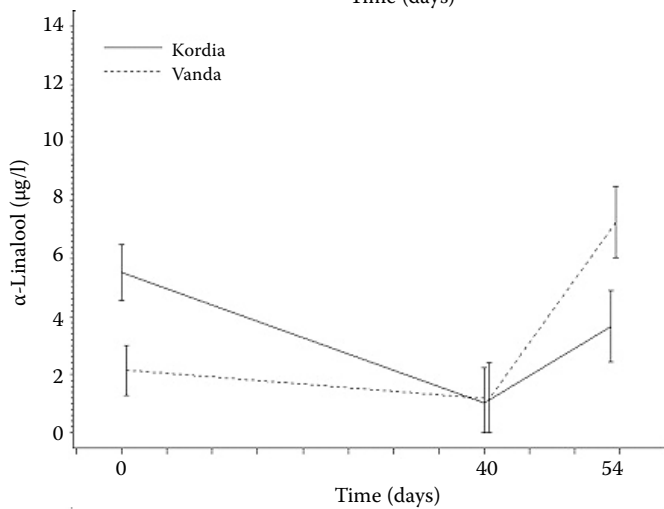

Fig. 5. Concentrations of α-linalool analyzed by SPME/GC/MS during storage of cv. Vanda and cv. Kordia in controlled atmospheres

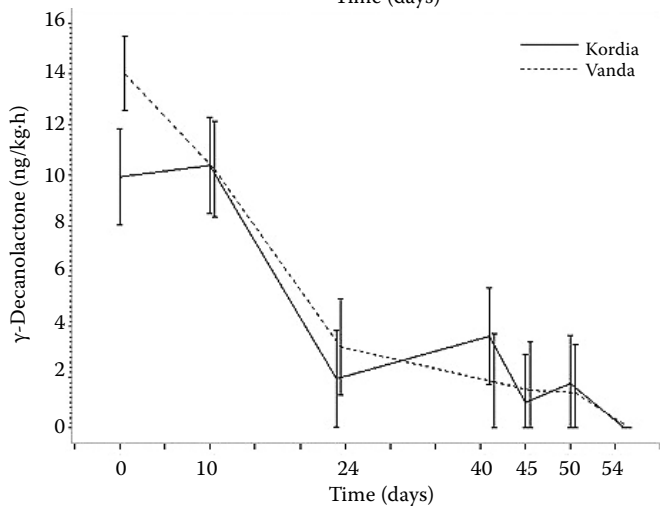

Fig. 6. The amount of γ-decanolactone from fruits of cvs Vanda and Kordia analyzed by headspace analysis, with 95% confidence intervals, of the production by stored in controlled atmospheres

tion in comparison with the RA atmosphere. At the end of the 54-day storage period (of which the last 14 days were in air, at the same temperature), both the higher concentrations of CO_2 (CA1 and CA2) resulted in top quality fruit, with the additional benefit of minimal loss of chlorophyl from the stems – only one tenth of the stems dessicated. The border between affected and nonaffected regions was sharply defined. Due to the high CO_2 concentrations in both atmospheres, 9.0–9.2% CO_2 and 0.9–1.0% O_2 (CA1) and 14.6–15.0% CO_2 and 1.5–2.0% O_2 (CA2), the fruit showed no signs of

Table 2. Production of volatiles (ng/kg·h) evolved from intact fruit stored at 1°C analysed by headspace gas analysis (HP/GC/MS) for treatments RA, FAN, CA1 and CA2, and duration of storage

		Treatment/Time (day) − mean (SE)											
	Cultivar	IN	FAN						CA1					
		0	10	24	40	45	50	56	10	24	40	45	50	56
Ethanol	Kordia	205.2 (13.0)	394.9 (44.3)	84.27 (3.64)	179.7 (47.8)	40.33 (8.61)	18.53 (1.49)	18.53 (1.49)	343.5 (61.3)	241.5 (89.9)	538.0 (140.8)	53.73 (16.20)	34.40 (20.51)	10.34 (0.63)
	Vanda	109.3 (36.8)	381.5 (52.2)	175.6 (42.1)	101.4 (21.6)	247.5 (153.4)	53.00 (12.53)	47.67 (11.69)	319.5 (76.2)	214.2 (38.5)	127.1 (49.1)	31.67 (2.61)	30.27 (4.37)	10.40 (1.50)
Hexanal	Kordia	10.87 (0.81)	129.5 (0.7)	25.00 (13.51)	22.80 (7.58)	1.40 (1.20)	6.47 (0.57)	6.47 (0.57)	52.43 (14.52)	33.87 (0.74)	17.40 (7.60)	8.57 (5.12)	10.44 (0.70)	7.17 (5.63)
	Vanda	6.12 (2.13)	127.2 (9.0)	77.87 (15.38)	31.07 (8.22)	8.10 (3.89)	23.73 (0.93)	14.67 (3.61)	52.57 (20.16)	44.80 (3.56)	27.93 (7.58)	44.87 (9.83)	3.27 (0.27)	5.87 (0.33)
Ethyl (*E*)-3-hexenoate	Kordia	3.27 (0.70)	2.07 (0.27)	1.47 (0.37)	2.27 (0.58)	0.55 (0.43)	– –	– –	6.60 (0.42)	1.40 (0.00)	1.67 (0.13)	1.00 (0.46)	– –	0.60 (0.00)
	Vanda	2.08 (0.65)	2.07 (0.27)	3.93 (1.54)	4.00 (1.06)	1.94 (1.18)	1.73 (0.37)	0.70 (0.45)	5.93 (0.47)	3.40 (1.06)	2.00 (0.61)	2.07 (0.87)	2.00 (0.90)	0.77 (0.15)
Butyl 2-methylbutyrate	Kordia	3.33 (1.31)	13.27 (4.13)	2.40 (0.53)	1.40 (0.40)	– –	– –	– –	6.20 (3.08)	1.47 (0.33)	0.80 (0.20)	0.70 (0.45)	0.37 (0.15)	0.80 (0.00)
	Vanda	4.72 (1.43)	13.27 (4.13)	0.80 (0.60)	2.47 (0.64)	0.80 (0.60)	1.00 (0.42)	0.87 (0.35)	6.20 (3.08)	2.33 (0.67)	1.07 (0.37)	1.47 (0.66)	0.73 (0.53)	0.16 (0.04)
Butyric acid	Kordia	25.33 (24.53)	11.07 (4.93)	5.87 (0.68)	2.20 (1.40)	– –	– –	– –	2.67 (1.19)	5.90 (1.55)	3.50 (0.75)	0.42 (0.19)	– –	0.75 (0.62)
	Vanda	4.04 (2.08)	11.07 (4.93)	2.00 (0.70)	0.77 (0.26)	0.48 (0.36)	– –	– –	2.40 (2.00)	4.53 (2.64)	4.67 (3.01)	0.34 (0.13)	0.80 (0.00)	1.67 (0.29)

		CA2						RA				
		10	24	40	45	50	56	10	24	40	45	50
Ethanol	Kordia	275.9 (86.2)	196.3 (39.4)	1793 (536)	159.3 (25.6)	61.67 (12.26)	93.73 (31.93)	130.9 (24.5)	59.47 (8.07)	26.00 (12.40)	23.67 (6.89)	24.93 (0.93)
	Vanda	241.2 (98.6)	116.7 (35.3)	192.7 (38.9)	34.87 (7.91)	16.27 (3.23)	20.07 (0.87)	109.7 (5.5)	38.67 (5.42)	73.93 (33.79)	24.07 (6.01)	21.73 (5.57)
Hexanal	Kordia	19.40 (6.66)	8.33 (6.45)	4.26 (2.26)	17.60 (4.20)	5.07 (0.64)	6.53 (0.67)	7.67 (2.08)	48.03 (3.45)	137.3 (96.7)	30.27 (17.34)	11.87 (4.27)
	Vanda	19.40 (6.66)	49.87 (10.85)	17.07 (6.91)	5.78 (3.57)	13.07 (4.28)	13.60 (4.22)	56.73 (5.27)	14.00 (7.11)	57.87 (29.82)	20.47 (11.80)	7.11 (3.61)
Ethyl (*E*)-3-hexenoate	Kordia	3.60 (1.64)	1.33 (0.27)	15.53 (2.21)	1.00 (0.40)	0.34 (0.23)	– –	3.47 (0.98)	2.17 (0.12)	1.87 (0.48)	1.00 (0.12)	0.93 (0.13)
	Vanda	3.60 (1.64)	1.73 (0.70)	1.33 (0.85)	0.80 (0.60)	1.03 (0.32)	1.08 (0.57)	2.77 (0.72)	2.17 (0.23)	2.07 (0.18)	1.40 (0.60)	1.01 (0.54)
Butyl 2-methylbutyrate	Kordia	2.41 (0.72)	0.40 (0.12)	0.20 (0.00)	0.60 (0.00)	0.20 (0.10)	0.50 (0.06)	1.67 (0.74)	1.47 (0.13)	1.93 (0.13)	0.53 (0.07)	0.73 (0.07)
	Vanda	2.47 (0.71)	0.67 (0.29)	0.67 (0.24)	0.67 (0.24)	0.47 (0.07)	0.47 (0.18)	4.60 (0.00)	2.33 (0.29)	1.67 (0.75)	0.67 (0.18)	0.67 (0.24)
Butyric acid	Kordia	40.47 (18.87)	3.20 (2.50)	2.53 (1.43)	– –	1.81 (0.75)	– –	2.87 (0.35)	5.93 (0.37)	3.73 (0.81)	1.53 (0.48)	0.80 (0.23)
	Vanda	49.90 (28.30)	2.30 (0.36)	0.38 (0.31)	– –	2.07 (0.77)	– –	0.65 (0.03)	2.27 (1.07)	1.90 (0.55)	1.19 (0.54)	– –

for abbreviations see Table 1

Table 3. *P*-values of main factors for sweet cherry fruit volatiles (μg/l) determined by SPME/GC/MS for treatments RA, FAN, CA1 and CA2, and duration of storage

	Factor			Interactions		
	treatment	time	cultivar	cultivar × treatment	cultivar × time	treatment × time
Ethan-1-ol	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
n-pentan-1-ol	< 0.0001	< 0.0001	0.2902	< 0.0001	< 0.0001	< 0.0001
n-hexan-1-ol	< 0.0001	< 0.0001	0.3228	0.3131	< 0.0001	0.0010
3-methyl-2-buten-1-ol	< 0.0001	< 0.0001	0.1958	0.6748	0.9123	< 0.0001
3-methyl-1-pentan-1-ol	< 0.0001	< 0.0001	0.0191	0.0748	0.0007	0.0002
2-heptan-1-ol	< 0.0001	< 0.0001	0.2472	0.5764	0.6127	< 0.0001
(*Z*)-2-hexen-1-ol	0.0012	< 0.0001	< 0.0001	0.0002	< 0.0001	< 0.0001
2-methylbutanal	< 0.0001	< 0.0001	0.0336	0.1335	0.0001	< 0.0001
Ethyl (*E*)-3-hexenoate	< 0.0001	< 0.0001	0.0217	0.0014	< 0.0001	< 0.0001
(*Z*)-linalool oxide	< 0.0001	< 0.0001	< 0.0001	0.0557	0.0004	< 0.0001

for abbreviations see Table 1

microbiological decay. In contrast, in the FAN atmosphere the stems were green throughout their length and still reasonably firm, but on the surface of some fruit there were the early signs of rot to the 10%. Fruit stored in the RA atmosphere, however, was superficially flaccid, and the stems were completely brown and infected by moulds to a level of 40 to 50% for the cv. Vanda, but the cv. Kordia showed no outward signs of rotting or moulds (*Mucor* spp.). There were no off-flavours present in any of the fruits when they were removed from the various storage atmospheres.

Release of volatiles from whole fruit stored at cool temperatures

At the start of ripening under cool conditions, the various alcohols were produced in significant quantities. The lower production of volatile compounds in the later stages of storage (Table 2), regardless of storage treatment, was caused more by low temperatures than low O_2 and high CO_2 concentrations, since it is a dynamic process influenced by all of the storage conditions. Both the lactones γ-caprolactone and γ-decanolactone, detected by these techniques at the onset of storage (Fig. 6), were later detected at only minimal concentrations. The production of volatiles from intact fruit stored at low temperatures is very low, and cannot be perceived by consumers.

Statistical significance of the formation of volatiles recorded by SPME and headspace analysis methods

Table 3 presents the levels of significance (*P*-values) of the effects of various factors (sweet cherry varieties, storage atmospheres and storage time), and their interactions, detected by an analysis of variance for all selected volatiles. Among cherry fruit volatiles analysed by SPME, the predominant ones are ethan-1-ol, 3-methyl butan-1-ol, (*E*)-2-hexen-1-ol, methyl (*E*)-2-octenoate, (*Z*)-linalol oxide and phenethyl alcohol, and with these it is possible to differentiate the two cultivars (Table 3).

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