ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/222156200

Changes in volatile constituents of blackcurrants (Ribes nigrum L. cv. 'Titania') following controlled…

Article *in* Postharvest Biology and Technology · March 2008

DOI: 10.1016/j.postharvbio.2007.08.007

CITATIONS
28

READS
87

3 authors, including:

Jamil Harb
Birzeit University
40 PUBLICATIONS **151** CITATIONS
SEE PROFILE

Riyad Bisharat
GAB Consulting GmbH
2 PUBLICATIONS **37** CITATIONS
SEE PROFILE

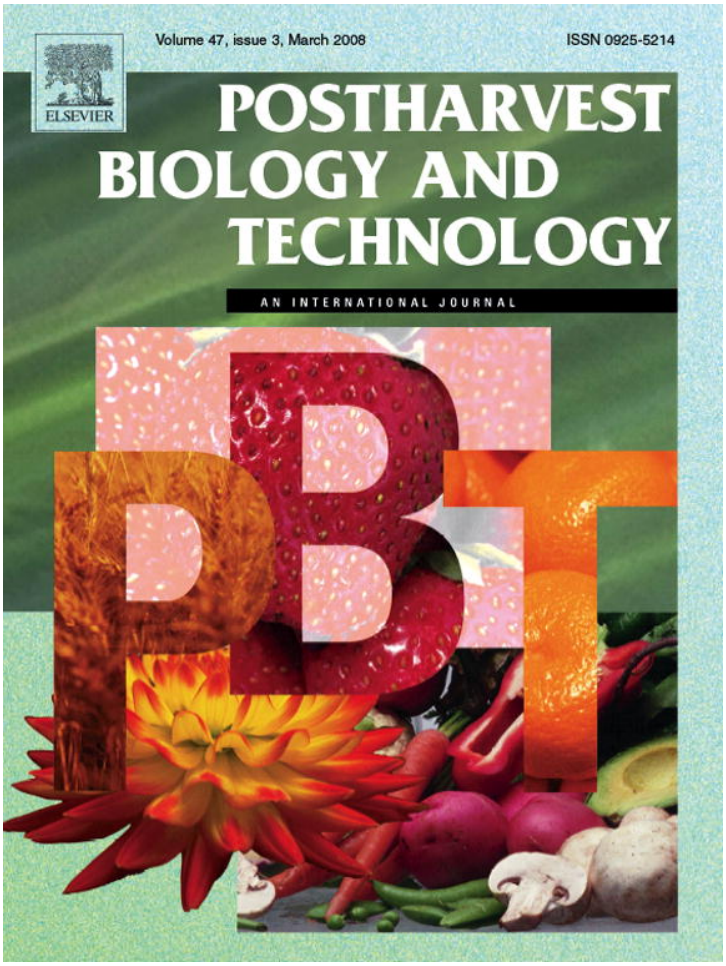
Some of the authors of this publication are also working on these related projects:

Organic Farming in Palestine View project

All content following this page was uploaded by Jamil Harb on 05 January 2017.

The user has requested enhancement of the downloaded file. All in-text references underlined in blue are added to the original document and are linked to publications on ResearchGate, letting you access and read them immediately.

Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article was published in an Elsevier journal. The attached copy is furnished to the author for non-commercial research and education use, including for instruction at the author's institution, sharing with colleagues and providing to institution administration.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

http://www.elsevier.com/copyright

Author's personal copy

Available online at www.sciencedirect.com
ScienceDirect
Postharvest Biology and Technology 47 (2008) 271–279
Postharvest Biology and Technology
www.elsevier.com/locate/postharvbio

Changes in volatile constituents of blackcurrants (*Ribes nigrum* L. cv. 'Titania') following controlled atmosphere storage

Jamil Harb [a,*], Riyad Bisharat [a], Josef Streif [b]

[a] *Department of Biology and Biochemistry, Birzeit University, Palestine*
[b] *Kompetenzzentrum für Obstbau, Schuhmacherhof 6, D88213 Ravensburg, Germany*

Received 26 March 2007; accepted 13 August 2007

Abstract

Blackcurrants (*Ribes nigrum* L. cv. Titania) were stored for 6 weeks under air and the following controlled atmosphere (CA) storage conditions: 12:18; 18:18; 6:2 and 18:2 (kPa CO_2:kPa O_2). The emission of volatiles was assessed after 3 and 6 weeks (prolonged storage) and analyzed by GC/MS. Fifty-three volatile compounds were quantified through calibration curves. Fruit that were stored in air, for either 3 or 6 weeks, did not differ significantly from freshly harvested fruit with respect to total terpene volatiles. However, decreasing O_2 levels and increasing CO_2 levels retarded the capacity of 3-week stored fruit to synthesize terpenes, although prolonged storage under these conditions led to a partial recovery. Differential changes among the various groups of terpenes were more important, where terpene alcohols reached a peak in 6-week air-stored fruit, and storing berries under a high CO_2 level (18 kPa) and/or decreasing O_2 level (2 kPa) resulted, in most cases, in lower biosynthesis of these alcohols compared to 6-week air-stored fruit. Non-terpene compounds, mainly esters and alcohols, were also increased in air-stored fruit. CA storage conditions led to a transitory reduction in the emission of alcohols but a recovery was recorded with prolonged storage. Non-terpene esters differed greatly in storage, in particular the ester ethyl butanoate. Air-stored fruit at both sampling dates synthesized significantly higher amounts of esters than freshly harvested fruit but a significant decline was observed for branched butyl substances (2-methylbutanoate) after 6 weeks storage.
© 2007 Elsevier B.V. All rights reserved.

Keywords: *Ribes nigrum* L.; Aroma volatiles; CA storage

1. Introduction

Blackcurrants (*Ribes nigrum* L.), which belong to the Grossulariaceae family, are common in the Northern Hemisphere (Woodland, 1991), and are considered an excellent source of vitamin C (Stewart, 1996). In blackcurrant juice, terpenes together with esters and alcohols are the major groups of aroma compounds (Nijssen et al., 1996). *In situ* headspace collection of volatiles from blackcurrant flowers revealed 11 compounds that included monoterpene hydrocarbons and monoterpene ethers (Hansted et al., 1994). Changes in the enantiomeric composition of chiral terpenes during ripening of blackcurrants were investigated using solid-phase microextraction–gas chromatography (SPME–GC). This work showed that some terpenes remained constant, while others like β-pinene, limonene, and α-phellandrene exhibited considerable variation (Ruiz del and Dobson, 2002).

CA storage is recommended for a wide range of fresh fruit due to numerous advantages in maintaining fruit firmness, lowering the oxidation of organic acids, and maintaining fruit color. However, changes in volatiles during storage play an important role in the consumer perception of fruit taste. The negative impact of CA storage, in particular ultra-low-oxygen storage, on volatile emission and biosynthesis is well documented, especially for apples (Harb et al., 1993, 2000).

Blackcurrants are cultivated commercially in Europe, and the cultivar 'Titania' is recommended because of its large berry size, and high contents of vitamin C and organic acids. Moreover, this cultivar can also be consumed fresh (Silbereisen, 2002). Usually, blackcurrants designated for fresh consumption are stored in regular atmospheres for short periods (2–3 weeks) and CA storage is needed where these fruit are to be kept for more than 3 weeks. CA storage slows the ripening process, and inhibits decay development. No earlier studies on the impact of CA storage on

* Corresponding author. Tel.: +972 2 2982162; fax: +972 2 2982084.
E-mail address: jharb@birzeit.edu (J. Harb).

0925-5214/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.postharvbio.2007.08.007

Author's personal copy

volatile emission of blackcurrants are available. Consequently, we assessed the influence of CA storage conditions on the levels of various aroma compounds in the blackcurrant cv. Titania using the SPME–GC/MS method.

2. Materials and methods

2.1. Plant material and storage conditions

Blackcurrant fruit (*R. nigrum* L. cv. Titania) were obtained from the Kompetenzzentrum für Obstbau Bodensee experimental orchard located near Lake Constance, Southwest Germany. Fruit were picked and graded for uniformity, absence of decay or external injuries and then stored on the same day. Fruit were stored under air and four CA conditions: 12:18; 18:18; 6:2 and 18:2 (kPa CO_2:kPa O_2). For each treatment there were two storage containers as replicates. The required storage atmospheres were established within 12 h, and the storage temperature was $1\,^{\circ}C \pm 0.5$. At harvest time and after 3 and 6 weeks samples were conditioned at room temperature for 24 h before aroma volatile analysis was conducted.

2.2. Collection of aroma volatiles

Fifty fruit per replicate were immersed in liquid nitrogen and kept at $-30\,^{\circ}C$ until analysis which was conducted within 2 weeks. For analysis, the frozen samples were placed in a prechilled coffee grinder and ground to a coarse powder. Fifteen millilitres of saturated NaCl solution were added to 45 g of the tissue powder and was mixed vigorously. The slurry was then homogenized for 2 min at 20,000 rpm and centrifuged at 14,000 rpm for 15 min at $4\,^{\circ}C$. Twelve millilitres of the clear supernatant were placed in a 25 mL vial for extracting the volatile compounds by headspace solid-phase microextraction (HS-SPME). One microlitre of internal standard mixture (20 μL of benzaldehyde in 100 mL water) was added prior to extraction. Vials were conditioned for 50 min at $30\,^{\circ}C$ and stirred at a constant speed. A manual SPME device (Supelco Co., Bellefonte, PA, USA) equipped with a fused-silica fiber coated with 100 μm polydimethyl siloxane was used. The fiber was inserted into the sample vial and exposed to the headspace for 60 min. For GC analysis, the volatile compounds were thermally desorbed from the SPME fiber for 10 min in the injection port of the gas chromatograph.

2.3. GC/MS conditions

Authentic standard compounds were obtained from Sigma (Sigma–Aldrich Chemie, Munich, Germany) and Roth (Carl-Roth, Karlsruhe, Germany). The identification of compounds was conducted using a gas chromatography–mass spectrometer (GC/MS) by matching their mass spectra with the NIST library of standard compounds and their retention times. The aroma compounds were quantified using external standards (calibration curves) after correction with the internal standards. The calibration curves were obtained with a series of dilutions of a standard mixture which was dissolved in blackcurrant juice obtained from fruit stored in cold storage. Analysis for the calibration curves was conducted under the same conditions as the treatment samples. GC/MS analysis was performed using a Shimadzu gas chromatograph GC-2010 series (Shimadzu, Duisburg, Germany) coupled to a QP2010 mass spectrometer. The separation was achieved using a Zebron capillary column; ZB-WAX, 30 m × 0.25 mm i.d., 0.25 μm film thickness (Phenomenex, Aschaffenburg, Germany). The GC oven temperature was raised from $35\,^{\circ}C$, after a holding time of 5 min, to $180\,^{\circ}C$ at a rate of $5\,^{\circ}C\,min^{-1}$. The helium inlet pressure was 64.3 kPa, linear velocity was $40\,cm\,s^{-1}$, total flow was $2.2\,mL\,min^{-1}$, the column flow was $1.24\,mL\,min^{-1}$, injection temperature was $220\,^{\circ}C$, and injections were splitless. The MS conditions were: ion source temperature = $200\,^{\circ}C$, interface temperature = $190\,^{\circ}C$, solvent cut time = 0.51 min, electron ionization at 70 eV, and mass scan range was 40–250 *m/z*.

2.4. Statistical analysis

The results were subjected to analysis of variance (ANOVA) using the CoStat-software (CoHort Software, Monterey, CA, 1998). Mean separation was calculated by Student–Newman–Keuls range test at $p \leq 0.05$. Correlation tests were also conducted by CoStat-software.

3. Results

The aroma volatiles produced by the blackcurrants are shown in Table 1.

3.1. Terpenes volatiles

Total terpene analysis reveals significant differences between CA-stored, air-stored, and freshly harvested fruit (Table 2). Fruit stored under air conditions, either for 3 or 6 weeks, did not differ significantly from freshly harvested fruit. Compared to prolonged storage (6 weeks storage period) under air, decreasing O_2 levels down to 2 kPa and increasing CO_2 levels up to 12 kPa retarded the capacity of fruit to synthesize terpenes, in particular, after a long-term storage of 6 weeks

3.1.1. Terpene alcohols

The biosynthesis of terpene alcohols reached a peak in air-stored fruit after a prolonged storage of 6 weeks; these fruit contained significantly three times more terpene alcohols as freshly harvested fruit (Table 2). Storing berries under high CO_2 levels (12–18 kPa) and/or decreasing O_2 levels down to 2 kPa resulted, in most cases, in significantly lower biosynthesis of terpene alcohols, mainly terpinen-4-ol, compared to fruit stored under air conditions for 6 weeks. Moreover, it is obvious that lower O_2 levels resulted in a persistent reduction in the biosynthesis of terpene alcohols, irrespective of the CO_2 level. Concerning the specific alcohols, major changes occurred with terpinen-4-ol and eucalyptol (Table 2); air-stored fruit contained the highest amount of terpinen-4-ol after 6 weeks in storage.

Author's personal copy

Table 1
Aroma volatiles of blackcurrant fruit collected in the head space by SPME and determined by GC/MS

Aroma volatile	*m/z*	*RT*
Ethyl acetate	43	3.08
Ethyl butanoate	71	5.61
Ethyl 2-methylbutanoate	57	5.91
Hexanal	44	6.59
3-Carene	93	7.81
β-Pinene	93	8.51
β-Myrcene	41	8.53
Ethyl 2-butenoate	69	8.77
D-Limonene	68	9.29
β-Phellandrene	93	9.55
Eucalyptol	43	9.65
Hexyl formiate	56	10.45
Butyl 2-methylbutanoate	57	10.59
α-Pinene	93	10.66
Ethyl hexanoate	88	10.71
trans-β-Ocimene	93	10.91
β-Cymene	119	11.57
Terpinolene	93	11.72
Hexyl acetate	43	11.85
4-Carene	93	11.87
1-Hexanol	56	14.20
Heptyl acetate	43	14.63
Methyl octanoate	74	15.04
Nonanal	57	15.10
Tetradecane	57	15.22
Butyl hexanoate	56	15.63
Hexyl 2-methylbutanoate	103	15.97
Octyl formiate	70	16.07
Ethyl octanoate	88	16.22
1-Heptanol	70	16.87
Octyl acetate	43	17.28
Heptyl butanoate	71	18.29
Benzaldehyde (IS)[a]	106	18.50
2-Nonenal, (E)-	41	18.73
β-Linalool	71	19.21
Linalool acetate	93	19.32
1-Octanol	56	19.42
β Caryophyllene	41	20.01
Methyl decanoate	74	20.23
Terpinen-4-ol	71	20.41
Hexyl hexanoate	43	20.57
Methyl benzoate	105	20.87
(Z)-β-Farnesene	69	21.15
Ethyl decanoate	88	21.24
Ethyl benzoate	105	21.91
α-Terpineol acetate	121	22.52
Nerol acetate	69	23.30
α-Farnesene	93	23.75
Citronellyl butyrate	81	24.87
Nerol	69	25.01
β-Phenylethyl acetate	104	25.29
Isopropyl laurate	43	25.59
Ethyl dodecanoate	88	25.80
10-Undecen-1-ol	41	27.50
Eugenol	164	32.41

[a] Internal standard.

3.1.2. Terpene esters

As with the terpene alcohols, fruit that were stored for 6 weeks also synthesized high amounts of terpene esters, while fruit stored in the 18:18 atmosphere-synthesized low amounts (Table 2), compared to fruit stored under air for 6 weeks. Furthermore, prolonged air or CA storage led to a significant reduction in the level of the major ester, nerol acetate, when compared to freshly harvested fruit. The exception was fruit stored under the most stressful condition 18 kPa CO_2:2 kPa O_2 which maintained its capacity to synthesize terpene esters.

3.1.3. Monoterpenes

When all monoterpenes are considered together, no major changes can be seen although it is obvious that extending the storage period to 6 weeks led to a reduction in the amount of monoterpenes in most CA-storage conditions, especially in the low O_2 CA-storage treatments (Table 2). However, the changes in individual monoterpenes are more important. Freshly harvested fruit contained higher amounts of 3-carene, α-pinene, and β-myrcene, which were the major monoterpenes, whereas storing fruit for 3 weeks under a moderately high CO_2 level (6 kPa), in combination with a low O_2 level (2 kPa), led to a significant increase in the synthesis of β-pinene, D-limonene, and β-cymene. The synthesis of 4-carene was promoted upon 3 weeks storage period in 12 kPa CO_2:2 kPa O_2. Extending the storage period to 6 weeks led to a significant reduction in the synthesis of 3-carene, α-pinene, and β-myrcene by fruit stored under 12 kPa CO_2:2 kPa O_2, compared to harvest time, and in the synthesis of β-pinene, D-limonene, and β-cymene by fruit from treatments that involved higher O_2 levels, including air storage, compared to 6 kPa CO_2:2 kPa O_2.

3.1.4. Sesquiterpenes

Freshly harvested fruit synthesized significantly higher amounts of these terpenes than fruit stored under most CA-storage conditions (Table 2). The β-caryophyllene level was reduced in all storage conditions. It seems that increasing the CO_2 level to 12 kPa, in combination with a high O_2 level, maintained the fruit's capacity to synthesize caryophyllene, whereas increasing the CO_2 level to 18 kPa was inhibitory. An extended storage period of 6 weeks led to a partial recovery in α-farnesene biosynthesis. When fruit were stored under the most stressful condition, 18 kPa CO_2:2 kPa O_2, the biosynthesis of α-farnesene was significantly reduced compared to freshly harvested fruit.

3.2. Non-terpene volatiles

Non-terpene volatiles are mainly esters, either branched or straight chain followed by alcohols and aldehydes.

3.2.1. Alcohols and aldehydes

The main alcohol produced by the blackcurrants was 1-hexanol followed by 1-octanol (Table 3). Freshly harvested fruit contained high amounts of both alcohols but the level of 1-hexanol then declined in the majority of storage conditions. Most CA storage conditions and air storage did not differ significantly in the resulting alcohol profiles. However, reducing

Author's personal copy

Table 2
Aroma-volatiles (terpenes) of blackcurrant fruit (cv. Titania) at harvest time (0 week) and from regular air (RA) and controlled atmosphere storage (kPa CO_2:kPa O_2) after 3 weeks and 6 weeks

	0 week Harvest	3 weeks RA	6 weeks RA	3 weeks 06:02	6 weeks 06:02	3 weeks 12:02	6 weeks 12:02	3 weeks 18:02	6 weeks 18:02	3 weeks 12:18	6 weeks 12:18	3 weeks 18:18	6 weeks 18:18
Eucalyptol	0.2460 b	1.1720 ab	2.2400 ab	4.9058 a	0.7708 ab	1.6213 ab	0.3578 b	1.7145 ab	0.6973 ab	0.4373 b	1.0060 ab	0.3338 b	0.8410 ab
β-Linalool	0.0635 b	0.2680 ab	0.0650 b	1.7003 a	0.0558 b	0.5695 ab	0.0338 b	0.2725 ab	0.0400 b	0.0406 b	0.0498 b	0.0223 b	0.0485 b
Terpinen-4-ol	12.65 bcde	12.53 bcde	34.82 a	9.02 def	11.49 cdef	8.34 ef	7.87 ef	8.47 def	11.43 cdef	13.48 bcd	17.08 b	7.09 f	16.15 bc
Nerol	0.0280 a	0.0063 a	0.0018 a	0.0149 a	0.0258 a	0.0241 a	0.0385 a	0.0000 a	0.0168 a	0.0094 a	0.0093 a	0.0073 a	0.0078 a
Total terpene alcohols	12.99 bcd	13.98 bcd	37.13 a	15.64 bc	12.34 bcd	10.56 bcd	8.30 cd	10.45 bcd	12.18 bcd	13.97 bcd	18.15 b	7.45 d	17.04 b
Linalool acetate	0.0000 b	0.0031 ab	0.0008 ab	0.0025 ab	0.0018 ab	0.0030 ab	0.0028 ab	0.0020 ab	0.0003 ab	0.0035 ab	0.0008 ab	0.0033 ab	0.0043 a
Nerol acetate	0.0805 a	0.0463 abc	0.0300 bc	0.0393 ab	0.0235 bc	0.0311 abc	0.0023 c	0.0575 ab	0.0460 abc	0.0431 abc	0.0273 bc	0.0379 abc	0.0228 bc
Citronellyl butyrate	0.0000 a	0.1015	0.1335 a	0.1015 a	0.0693 a	0.0335 a	0.0670 a	0.0000 a	0.0665 a	0.0663 a	0.0000 a	0.0663 a	0.0000 a
Total terpene esters	0.0805 ab	0.1509 ab	0.1643 a	0.1433 ab	0.0945 ab	0.0676 ab	0.0720 ab	0.0595 ab	0.1128 ab	0.1129 ab	0.0280 ab	0.1074 ab	0.0270 b
3-carene	2.0719 a	1.0324 ab	0.9384 ab	1.3742 ab	1.1909 ab	0.9625 ab	0.3419 b	1.2970 ab	1.2376 ab	1.2744 ab	0.9225 ab	1.3876 ab	1.4642 ab
β-Pinene	0.9380 b	1.5488 ab	0.7890 b	5.6625 a	0.6465 b	1.9930 ab	0.2335 b	1.7108 ab	0.5308 b	1.1805 b	0.4993 b	0.8338 b	0.9393 b
D-Limonene	0.7345 b	1.2150 b	0.3495 b	5.1083 a	0.5178 b	1.5858 ab	0.1758 b	1.4300 ab	0.4663 b	0.9813 b	0.3970 b	0.6408 b	0.5390 b
α-Pinene	12.9195 a	10.3525 ab	7.9018 abc	5.2950 abc	4.3105 bc	2.4120 bc	1.8870 c	2.9930 bc	7.4193 abc	10.5541 ab	2.8518 bc	5.5645 abc	9.5705 abc
β-Cymene	0.5025 b	1.0338 ab	0.6980 b	3.2890 a	0.5003 b	1.3448 ab	0.1950 b	0.8918 ab	0.4003 b	0.6778 b	0.3998 b	0.4538 b	0.4985 b
−4-Carene	4.3070 ab	6.9460 ab	2.6888 ab	4.3120 ab	2.8678 ab	9.5460 a	0.7663 b	7.4923 ab	2.4475 b	5.6483 ab	2.2718 b	3.6288 ab	3.2300 ab
β-Myrcene	0.5510 a	0.0704 bc	0.0630 bc	0.1483 bc	0.2205 b	0.0470 bc	0.0128 bc	0.0070 c	0.1920 bc	0.1006 bc	0.1068 bc	0.0291 bc	0.0385 bc
Total monoterpene	22.0244 ab	22.1987 a	13.4284 ab	25.1892 a	10.2541 ab	17.8910 ab	3.6122 b	15.8218 ab	12.6936 ab	20.4169 ab	7.4488 ab	12.5382 ab	16.2799 ab
β-Caryophyllene	0.248 a	0.037 d	0.0068 d	0.0473 cd	0.0460 cd	0.0600 bcd	0.0088 d	0.0490 cd	0.0165 d	0.1098 bc	0.1350 b	0.0248 d	0.0408 cd
α-Farnesene	0.312 ab	0.405 a	0.2043 bc	0.2530 abc	0.2650 abc	0.2440 abc	0.2330 abc	0.1090 c	0.2663 abc	0.1593 bc	0.2120 abc	0.2563 abc	0.3070 ab
(Z)-β-Farnesene	0.343 a	0.354 a	0.3263 a	0.2960 a	0.3295 a	0.2013 ab	0.1313 b	0.2807 ab	0.3115 a	0.2914 ab	0.2370 ab	0.3123 a	0.3125 a
Total sesquiterpenes	0.903 a	0.796 ab	0.5373 bc	0.5963 bc	0.6405 abc	0.5053 bc	0.3730 c	0.4387 c	0.5943 bc	0.5604 bc	0.5840 bc	0.5933 bc	0.6603 abc
Total terpenes	35.9949 abc	37.1232 ab	51.2577 a	41.5691 ab	23.3291 bc	29.0237 abc	12.3574 c	26.7765 bc	25.5826 bc	35.0599 abc	26.2098 bc	20.6901 bc	34.0109 abc

Amounts are nmol kg^{-1} fresh fruit. Means within each row followed by different letters indicate significant differences between treatments at $p \leq 0.05$, least significant difference (LSD) test.

Author's personal copy

Table 3
Changes in aroma-volatiles (non-terpenes) of blackcurrants (cv. Titania) at harvest time (0 week) and from regular air (RA) and controlled atmosphere storage (kPa CO_2:kPa O_2) after 3 weeks and 6 weeks

	0 week Harvest	3 weeks RA	6 weeks RA	3 weeks 06:02	6 weeks 06:02	3 weeks 12:02	6 weeks 12:02	3 weeks 18:02	6 weeks 18:02	3 weeks 12:18	6 weeks 12:18	3 weeks 18:18	6 weeks 18:18
Tetradecane	0.0035 a	0.0010 a	0.0050 a	0.0010 a	0.0020 a	0.0010 a	0.0028 a	0.0018 a	0.0018 a	0.0008 a	0.0013	0.0005 a	0.0048 a
10-Undecen-1-ol	0.0105 b	0.0128 b	0.2103 ab	0.0110 b	0.0610 b	0.0863 b	0.3538 a	0.2130 ab	0.2130 ab	0.0175 b	0.1530 ab	0.0175 b	0.1455 ab
1-Octanol	0.3025 ab	0.1518 b	0.0798 b	0.1258 b	0.0743 b	0.3550 a	0.0763 b	0.0738 b	0.0738 b	0.1565 b	0.0765 b	0.1403 b	0.0763 b
1-Hexanol	0.7535 a	0.0995 bc	0.2860 abc	0.1068 bc	0.5028 ab	0.6900 a	0.3548 abc	0.2028 abc	0.2028 bc	0.1943 bc	0.2535 abc	0.0440 c	0.3043 abc
1-Heptanol	0.0310 b	0.0365 b	0.0023 b	0.0440 b	0.0048 b	0.1618 a	0.0300 b	0.0000 b	0.0000 b	0.0460 b	0.0413 b	0.0558 ab	0.0320 b
Eugenol	0.046 b	0.495 b	0.0462 b	0.460 b	0.0242 b	1.832 a	0.000 b	0.572 b	0.000 b	0.7557 ab	0.0482 b	0.5097 b	0.058 b
Total alcohols	1.0975 ab	0.3005 bc	0.5783 abc	0.2875 bc	0.6428 abc	1.2930 a	0.8148 abc	0.4895 abc	0.4895 abc	0.4143 bc	0.5243 abc	0.2575 c	0.5580 abc
Hexanal	2.1780 a	0.6095 cde	0.0898 e	0.4410 de	1.2273 bc	0.3735 de	0.9895 cd	0.6400 cde	0.6400 cde	0.6400 cde	0.7420 cd	0.6345 cde	1.6508 ab
2-Nonenal, (*E*)-	0.0100 a	0.0015 a	0.0030 a	0.0025 a	0.0030 a	0.0098 a	0.0030 a	0.0050 a	0.0050 a	0.0088 a	0.0028 a	0.0045 a	0.0013 a
Nonanal	0.5795 a	1.0780 a	1.0895 a	3.4605	0.5658 a	3.0383 a	0.3085 a	0.8350 a	0.4543 a	0.1988 a	0.4718 a	0.1270 a	0.6018 a
Total aldehydes	2.7675 a	1.6890 a	1.1823 a	3.9040 a	1.7960 a	3.4215 a	1.3010 a	1.1575 a	1.0993 a	0.8475 a	1.2165 a	0.7660 a	2.2538 a
Ethyl butanoate	0.126 f	44.253 bcd	46.524 bcd	3.480 f	23.189 def	31.731 cde	50.946 bc	55.600 bc	111.231 a	12.249 ef	14.040 ef	4.974 f	65.064 b
Heptyl butanoate	0.0000 b	0.0003 ab	0.0000 b	0.0000 b	0.0000 b	0.0000 b	0.0000 b	0.0000 b	0.0000 b	0.0005 b	0.0000 b	0.0000 b	0.0000 b
Hexyl 2-methylbutanoate	0.0000 a	0.0003 a	0.0000 a	0.0000 a	0.0000 a	0.0000 a	0.0000 a	0.0003 a	0.0003 a	0.0003 a	0.0000 a	0.0003 a	0.0000 a
Butyl 2-methylbutanoate	0.0000 b	0.0188 a	0.0000 b	0.0025 b	0.0053 ab	0.0055 ab	0.0000 b	0.0000 b	0.0000 b	0.0003 b	0.0020 b	0.0000 b	0.0000 b
Ethyl 2-methylbutanoate	0.0000 b	0.1038 a	0.0000 b	0.0128 ab	0.0000 b	0.0000 b	0.0085 b	0.0140 ab	0.0140 ab	0.0283 ab	0.0000 b	0.0000 b	0.0000 b
Ethyl 2-butenoate	0.0135 de	0.0940 cd	0.0755 cde	0.0005 e	0.1003 bcd	0.0755 cde	0.1910 b	0.5090 a	0.5090 a	0.0415 cde	0.0350 de	0.0150 de	0.1278 de
Hexyl formiate	0.0080 a	0.0320 a	0.0065 a	0.0175 a	0.0178 a	0.0210 a	0.0118 a	0.0008 a	0.0008 a	0.0260 a	0.0015 a	0.0195 a	0.0173 a
Hexyl acetate	0.4815 ab	0.0865 cd	0.2765 abcd	0.4045 abc	0.5390 ab	0.6368 a	0.1025 cd	0.2203 abcd	0.2203 bcd	0.0190 d	0.2690 abcd	0.0253 d	0.4175 abc
Ethyl hexanoate	0.0060 cd	0.0020 cd	0.0000 cd	0.0570 cd	0.1948 abc	0.0798 bcd	0.0000 d	0.0000 cd	0.0000 cd	0.2678 ab	0.3755 a	0.0915 bcd	0.0000 cd
Butyl hexanoate	0.0000 b	0.0000 b	0.0005 ab	0.0000 b	0.0008 ab	0.0020 a	0.0000 b	0.0000 b	0.0000 b	0.0000 b	0.0000 b	0.0000 b	0.0000 b
Hexyl hexanoate	0.2570 b	0.2035 bc	0.4575 a	0.1445 cd	0.0000 e	0.2233 bc	0.0000 e	0.0000 e	0.0000 e	0.0898 def	0.0000 e	0.1295 cde	0.0000 e
Heptyl acetate	0.0000 a	0.0008 a	0.0003 a	0.0005 a	0.0000 a	0.0003 a	0.0005 a	0.0000 a	0.0000 a	0.0003 a	0.0005 a	0.0000 a	0.0000 a
Methyl octanoate	0.0220 b	0.0070 b	0.0220 b	0.0083 b	0.0545 ab	0.0978 a	0.0363 ab	0.0228 ab	0.0228 ab	0.0078 b	0.0330 ab	0.0073 b	0.0625 ab
Ethyl octanoate	0.0000 d	0.0025 bcd	0.0193 bc	0.0015 cd	0.0048 bcd	0.0155 bcd	0.0208 b	0.0593 a	0.0593 a	0.0008 cd	0.0023 bcd	0.0010 cd	0.0115 bcd
Octyl acetate	0.0000 b	0.0030 ab	0.0013 ab	0.0003 ab	0.0035 ab	0.0048 ab	0.0078 ab	0.0020 ab	0.0020 ab	0.0003 ab	0.0118 a	0.0023 ab	0.0073 ab
Octyl formiate	0.0175 ab	0.0078 b	0.0013 b	0.0025 b	0.0038 b	0.0230 a	0.0030 b	0.0013 b	0.0013 b	0.0035 b	0.0013 b	0.0023 b	0.0020 b
Methyl benzoate	0.5250 bcd	0.8325 abc	0.3098 cd	0.4613 cd	0.4045 cd	1.2718 ab	0.0560 d	0.1568 cd	0.1568 cd	1.2925 a	0.4328 cd	1.0353 abc	0.1768 cd
Ethyl benzoate	0.0045 c	0.1530 a	0.0880 b	0.0190 c	0.0223 c	0.0215 c	0.0248 c	0.0688 b	0.0688 b	0.0815 b	0.0133 c	0.0213 c	0.0238 c
Ethyl decanoate	0.0000 b	0.0763 ab	0.0000 b	0.0740 ab	0.0758 ab	0.1530 ab	0.1548 ab	0.1920 ab	0.1920 a	0.0758 ab	0.0740 ab	0.1498 ab	0.0763 ab
Methyl decanoate	0.1600 a	0.1008 ab	0.0000 c	0.1095 ab	0.1358 ab	0.1470 a	0.1168 ab	0.0478 bc	0.0478 bc	0.0515 bc	0.1320 ab	0.0993 ab	0.1418 a
β-Phenylethyl acetate	0.0090 a	0.0015 c	0.0060 ab	0.0008 c	0.0008 c	0.0005 c	0.0020 c	0.0025 bc	0.0025 bc	0.0028 bc	0.0020 c	0.0005 c	0.0005 c
Ethyl acetate	0.0000 c	6.4568 a	5.4098 ab	1.0213 bc	2.0983 abc	2.0215 abc	3.4353 abc	2.5165 abc	6.9245 a	4.9401 abc	0.7325 bc	2.1288 abc	6.2323 a
Total esters	1.6305 f	52.4365 bcd	53.1985 bcd	5.8188 ef	26.8510 def	35.5205 cde	55.1183 bcd	58.7806 bc	119.4528 a	19.1796 ef	16.1585 ef	8.7025 ef	72.3613 b

Amounts are nmol kg^{-1} fresh fruit. Means within each row followed by different letters indicate significant differences between treatments at $p \leq 0.05$, least significant difference (LSD) test.

Author's personal copy

the O_2 level in storage, in particular with the storage condition 12 kPa CO_2:2 kPa O_2, maintained the fruit's capacity to synthesize these alcohols, even after prolonged storage. Fruit kept under a highly elevated CO_2 level (18 kPa) for 3 weeks showed a significant reduction in alcohol but a recovery was noticed after a storage period of 6 weeks. There were no significant differences in aldehydes.

3.2.2. Non-terpene esters

Freshly harvested fruit synthesized high amounts of the following esters: methyl decanoate, hexyl acetate, methyl benzoate, and hexyl hexanoate (Table 3). Storing fruit in air led to dramatic changes in the profile of non-terpene esters in which new esters were synthesized in higher amounts (compared to freshly harvest fruit), and the major new esters were 'ethyl butanoate', ethyl benzoate and in particular ethyl acetate. Among the volatiles which were quantitatively determined 'ethyl butanoate' was the most emitted compound, although not at harvest time. Freshly harvested fruit contained very low amounts of this compound but the amount contained by air-stored fruit at both sampling dates was significantly much higher than with freshly harvested fruit. Storing fruit in air maintained the fruit's capacity to synthesize these volatiles at a moderate rate even after 6 weeks storage period. However, fruit stored in the most stressful storage condition, 18 kPa CO_2:2 kPa O_2 led to a strong and significant increase in the level of 'ethyl butanoate'. Synthesis of the 2-methylbutanoates: butyl 2-methylbutanoate and ethyl 2-methylbutanoate increased significantly in fruit stored for 3 weeks in air compared to freshly harvested fruit. However, the synthesis of these 2-methylbutanoates declined substantially after an extended storage period of 6 weeks. Fruit from most CA storage conditions synthesized significantly low amounts of butyl 2-methylbutanoate and ethyl 2-methylbutanoate when compared to air storage for 3 weeks. However, fruit stored in the very high CO_2 level (18 kPa) synthesized higher amounts of ethyl 2-butenoate after 6 weeks. Fruit stored in the most stressful storage condition (18 kPa CO_2:2 kPa O_2) maintained their capacity to synthesize ethyl 2-butenoate throughout the entire storage period.

In order to reveal the possible transformations of certain compounds to others, correlation tests were conducted. Significant correlations exist between ethyl butanoate and ethyl octanoate ($r = 0.89$***), the levels of: alcohols and acetate esters ($r = 0.56$**), hexyl esters and of acetate esters (0.86 ***), 1-octanol and 1-hexanol (0.69***), 1-hexanol and octyl esters ($r = 0.78$***). Another interesting finding is that there is a positive correlation between the levels of 1-hexanol and hexyl esters ($r = 0.58$**) which should be coupled with the finding that there is no correlation between the levels of 1-hexanol and hexanoic acid esters ($r = 0.06$ ns). The same trend also exists between the levels of 1-octanol and octyl esters ($r = 0.76$***) but not between 1-octanol and the octanoic acid esters ($r = 0.35$ ns).

Moreover, significant negative correlations exist between the levels of ethyl esters (and butanoic acid esters) and the level of hexanoic acid esters ($r = -0.51$** for both correlations), and between the levels of branched esters and hexanoic acid esters ($r = -0.53$**). Correlations between individual terpenes included positive and significant correlations between eucalyptol and β-linalool (0.95***), eucalyptol and β-cymene (0.95 **), β-linalool and β-pinene (0.97***), β-linalool and β-cymene (0.99***), β-pinene and D-limonene (0.99***) and between D-limonene and β-cymene (0.99***).

4. Discussion

Blackcurrants, as a non-climacteric fruit (Woodhead et al., 1999) are very likely to show a different volatile biosynthesis development to that found in climacteric fruit. The respiration rate of freshly harvested blackcurrants (data not shown) was significantly higher than that of the air-stored fruit, and there were no significant differences between all the storage treatments. The respiration rate in blackcurrants seems not to be directly linked to aroma volatile biosynthesis and it can be presumably excluded as a cause for the differences observed between storage treatments.

Although the variation among replicates was high, clear trends can be seen in this study. But explaining these results further is very difficult, due to the lack of studies that deal with changes in volatiles biosynthesis in blackcurrants, or similar fruit, following either regular air or CA storage.

Our discussion is divided into two parts: non-terpene compounds and terpene compounds. Several trends are clear for the non-terpene compounds. First, air-stored fruit synthesized much more non-terpene esters than freshly harvested fruit. It is well documented that volatile emission by ripe fruit of certain species has been attributed to the action of the enzyme lipoxygenase on lipid metabolism (Baldwin, 2002). Fatty acid ethyl esters are biosynthesized by two enzymatic mechanisms, either by esterification or alcoholysis (Liu et al., 2004). In this respect, Verstrepen et al. (2003) considered the expression level of alcohol acetyltransferases genes as an important factor in controlling volatile acetate ester production in addition to esterases (EC 3.1.1.1). The burst in ester synthesis by fruit stored for 3 or 6 weeks in air may be related directly to the up-regulation of genes providing the enzymes needed either directly or indirectly for ester formation, although an increased level and activity of an enzyme does not always mean a comparable increase in the product (Shalit et al., 2003). Concerning the two most emitted esters 'ethyl acetate' and 'ethyl butanoate', the increase in the level of ethyl acetate is not desirable because of it's solvent-like aroma (Verstrepen et al., 2003), while the increase in 'ethyl butanoate' is welcomed due to its fruity smell (Buettner and Schieberle, 2001). Although we did not quantify the amount of ethanol produced by the fruit, calculations of the peak areas (data not shown) indicate that CA-stored fruit emitted lower amounts of ethanol than air-stored fruit, except for fruit stored for 6 weeks under 18 kPa CO_2:2 kPa O_2. Consequently, an increase in the level of ethyl acetate may reduce or limit the synthesis of other acetate esters, since ethyl acetate and the other acetate esters share acetyl CoA as one of their substrates (Ke et al., 1994). For the biosynthesis of ethyl butanoate, a possible alcohol acyltransferase, designated Eht1 (ethanol hexanoyl transferase I) has been suggested as a candidate ethyl ester synthase in yeast (Mason

Author's personal copy

and Dufour, 2000). Saerens et al. (2006) also identified Eht1 and Eeb1 (for ethyl ester biosynthesis gene 1) as novel acyl-coenzymeA:ethanol *O*-acyltransferases/esterases in yeast. Eht1 preferred *in vitro* short-chain substrates (highest production was for ethyl butanoate), whereas Eeb1 preferred longer chain substrates (highest production was for ethyl octanoate) (Saerens et al., 2006). The high correlation between levels of ethyl butanoate and ethyl octanoate suggests that the expression of both Eth1 and Eeb1, or similar genes, are tightly coupled in blueberries. Knowing that the odor activity values of ethyl butanoate and ethyl octanoate are very high (Qian and Reineccius, 2003), it seems that both compounds are likely to contribute to the characteristic fruity note of blackcurrants.

Furthermore, it seems that lowering the O_2 levels, and/or elevating the CO_2 levels did not suppress the expression of these genes, and the differences recorded between treatments and between sampling dates should be attributed to the substrate availability rather than to the expression of genes. Moreover, the significant correlation values between non-terpene compounds indicate that β-oxidation of fatty acids may play an important role in volatile biosynthesis, as earlier reported with apples (Brackman et al., 1993; Song and Bangerth, 1994). On the other hand, the positive correlation between the levels of 1-hexanol and hexyl esters, coupled with the finding that there is no correlation between the levels of 1-hexanol and hexanoic acid esters, may be related to the preference of an alcohol acyltransferase (MpAAT1) or similar enzymes to produce hexyl esters of C3, C6 and C8 CoAs (Souleyre et al., 2005). Furthermore, the significant negative correlations between the levels of ethyl esters (and butanoic acid esters) and the level of hexanoic acid esters and between the levels of branched esters and hexanoic acid esters indicate competition for the common precursor, probably ethanol and to a lesser extent hexanol. Souleyre et al. (2005) added that for the acetate esters, the MpAAT1 preference depends upon substrate concentration; at low concentrations, the enzyme prefers 2-methylbutanol over hexanol and butanol, while at high concentrations, hexanol can be used at a greater rate.

Our results reveal a differential impact of lowering O_2 and/or increasing CO_2 levels on the emission of volatiles. Changes in the levels of hexanol and hexanal produced by fruit stored under various CA storage conditions suggest that an elevated CO_2 level, and in some treatments a low O_2 level, may slow down the β-oxidation of fatty acids. The amount of hexanal produced by fruit was the highest at harvest time and decreased dramatically and significantly upon storage under the various conditions, although increasing the CO_2 level around fruit partially maintained the capacity of fruit to produce hexanal. The strong and significant reduction in the biosynthesis of hexyl hexanoate and ethyl hexanoate was obvious in fruit subjected to prolonged storage (6 weeks) which may indicate that the required precursors were exhausted over this extended period; it seems that hexanoic acid is exhausted rather than ethanol or hexanol since other ethyl and hexyl esters were synthesizd by the same fruit at higher rates, although competition for ethanol or hexanol cannot be excluded. The differential impact of lowering O_2 and/or increasing CO_2 can be explained by; changes in substrate availability, the competition for the available substrates, differential gene expression and changes in the cellular pH. Siriphanich and Kader (1986) showed a pH reduction with lettuce maintained in a 15 kPa CO_2 condition. Also a 2 kPa O_2 atmosphere resulted, in a lower pH value with persimmon slices (Wright and Kader, 1997). Theoretical calculations and experimental data indicate that CO_2 concentrations >5 kPa will lower intracellular pH (Bown, 1985). In most plant tissues, cytoplasmic pH is ≈7, and CA treatments reduce the pH by 0.2–0.8 units (Ke et al., 1994). Such a pH reduction might cause lower activity for certain enzymes (Frenkel and Patterson, 1977; Kerbel et al., 1988) which may change the substrate availability and result in another set of aroma volatiles.

Our results concerning terpene compounds reveal that air-stored fruit maintained, and even increased with prolonged storage, their capacity to synthesize terpene compounds. Air storage probably allowed the fruit to ripen normally in a manner that terpene synthases are produced at higher levels although the release of terpenes from bound forms cannot be excluded; the presence of a range of aliphatic and aromatic alcohols was identified as glycosidically bound in blackcurrant juice (Varming et al., 2006). The hypothesis that the activities of terpene synthase enzymes are promoted during air storage is supported by the fact that certain CA conditions reduced the cytoplasmic pH as mentioned above. Knowing that the cytoplasmic pH is ≈7, and that monoterpene synthase enzymes exhibit a pH optimum around 7 (Lewinsohn et al., 1992; Savage et al., 1994; Bohlmann et al., 1998; Lücker et al., 2002), it is possible to predict a higher terpene synthesis in air-stored fruit. Terpene emissions were reduced by low O_2 and/or elevated CO_2 levels, despite a partial recovery upon prolonged storage, Bohlmann et al. (1998) reported that the similar characteristics of the monoterpene, sesquiterpene and diterpene synthases are related to the fact that these enzymes carry out similar electrophilic cyclizations involving common steps, i.e., stabilization of highly reactive carbocations and their ultimate quenching by deprotonation or nucleophile capture; lower pH caused by high CO_2 levels around the fruit may make deprotonation more difficult. Lowering O_2, irrespective of CO_2 levels, led generally to lower emission of terpenes. That might be due to a slower delivery of precursors for terpene synthesis. The lower emission rates after 6 weeks storage (6:2 and 12:2) may indicate that the available substrates were depleted at a higher rate than the metabolic machinery could provide. In contrast to this, it appears that an elevated emission rate by fruit stored for 6 weeks under 18:2 and 18:18 conditions may be due to the de-compartmentalization of cellular components. Frenkel and Patterson (1977) reported that elevated CO_2 (5–20 kPa) caused disintegration of plastids, vacuoles, and cytoplasmic matrix in 'Bartlett' pears. It has been speculated (Longhurst et al., 1990) that changes in cytoplasmic pH accompanying changes in cytoplasmic ion concentrations (Vickery and Bruinsma, 1973) may result in membrane leakage in the soft fruit. It was also hypothesized that the formation of monoterpenes in developing caraway fruit may be controlled by subcellular compartmentation of the various enzymes (Gleizes et al., 1983; McCaskill and Croteau, 1995). Another mechanism suggested by Longhurst et al. (1994) stated that an enhanced

Author's personal copy

transcription of the Adh 2 gene, as a consequence of the softening process and in response to a slight lowering of internal O_2 concentrations (Speirs et al., 1998) may enhance this process. Another possible explanation for a reduced terpene emission in most CA treatments at the first sampling date, in particular, may be related to the substrate availability, as mentioned by Bouwmeester et al. (1998).

With regard to the correlations, it is clear that major transformations occurred during storage. The increase in D-limonene is accompanied by a decrease in the levels of other terpenes, in particular terpinen-4-ol, β-myrcene, and caryophyllene which may be related to the lower K_m value of limonene synthases (Lücker et al., 2002). The reduction of β-myrcene after harvest observed in this study may be due to the transformation of this compound to various cyclic monoterpenes such as α-pinene and limonene as reported by Heyen and Harder (2000), who also stated that potential intermediates between myrcene and geranic acid are the hydration products geraniol and linalool. These authors also found that the main conversion product of linalool and limonene in stored model solutions of orange juice was also R-terpineol. Linalool was also found to rearrange to nerol and geraniol (Askar et al., 1973), and under acidic conditions, nerol and geraniol cyclize to form R-terpineol and terpene diols (Baxter et al., 1978). Varming et al. (2006) found, with the thermal treatment of blackcurrant juice, an increase in some of the oxygenated terpenes, including geraniol, terpineol, linalool and eucalyptol, suggesting that these compounds are released from a pool of glycosides or otherwise bound species (Leino and Kallio, 1993; Kollmansberger and Berger, 1994; Varming and Poll, 2003). Release of bound compounds could explain our results with the dramatic increase in terpinen-4-ol during prolonged air storage.

Although the reduction observed in blackcurrant aroma volatile biosynthesis in some CA storage conditions found in this study is not as dramatic as reported with climacteric fruits, further research is needed to elucidate the causes of this reduction, in particular at the molecular level.

Acknowledgements

We wish to thank Mr. Roy McCormick for his thorough review of the manuscript, and the German Academic Exchange Service (DAAD) for their financial support through their short-term fellowship to JH.

References

Askar, A., Bielig, H., Treptow, H., 1973. Aromaveraenderungen von Orangensaft. III. Modellversuche zur Abnahme von Linalool und Limonen bei der Lagerung von Orangensaft in Flaschen und Dosen. Dtsch. Lebensm. Rdsch. 69, 360–364.

Baldwin, E., 2002. Fruit flavor, volatile metabolism and consumer perceptions. In: Knee, M. (Ed.), Fruit Quality and its Biological Basis. Sheffield Academic Press, Sheffield, pp. 89–106.

Baxter, R., Laurie, W., Mchale, D., 1978. Transformations of monoterpenoids in aqueous acids: the reactions of linalool, geraniol, nerol and their acetates in aqueous citric acid. Tetrahedron 34, 2195–2199.

Bohlmann, J., Meyer, G., Croteau, R., 1998. Plant terpenoid synthases: molecular biology and phylogenetic analysis. Proc. Natl. Acad. Sci. 95, 4126–4133.

Bouwmeester, H., Gershenzon, J., Konings, M., Croteau, R., 1998. Biosynthesis of the monoterpenes limonene and carvone in the fruit of caraway. I. Demonstration of enzyme activities and their changes with development. Plant Physiol. 117, 901–912.

Bown, A., 1985. CO_2 and intracellular pH. Plant Cell Environ. 8, 459–465.

Brackman, A., Streif, J., Bangerth, F., 1993. Relationship between a reduced aroma production and lipid metabolism of apples after long-term controlled atmosphere storage. J. Am. Soc. Hortic. Sci. 118, 243–247.

Buettner, A., Schieberle, P., 2001. Evaluation of key aroma compounds in hand-squeezed grapefruit juice (*Citrus paradisi Macfayden*) by quantitation and flavor reconstitution experiments. J. Agric. Food Chem. 49, 1358–1363.

Frenkel, C., Patterson, M., 1977. Metabolic effects of CO_2 in 'Bartlett' pears. In: Dewey, D.H. (Ed.), Controlled Atmospheres for the Storage and Transport of Perishable Agricultural Commodities. Hortic. Rpt. 28. Michigan State Univ., East Lansing, pp. 108–115.

Gleizes, M., Pauly, G., Carde, J., Marpeau, A., Bernard-Dagan, C., 1983. Monoterpene hydrocarbon biosynthesis by isolated leucoplasts of *Citrofortunella mitis*. Planta 159, 373–381.

Hansted, L., Jakonsen, H., Olsen, C., 1994. Influence of temperature on the rhythmic emission of volatiles from *Ribes nigrum* flowers in situ. Plant Cell Environ. 17, 1069–1072.

Harb, J., Streif, J., Bangerth, F., 1993. Synthesis of aroma compounds by controlled atmosphere (CA) stored apples supplied with aroma precursors: alcohols, acids and esters. Acta Hortic. 368, 142–149.

Harb, J., Streif, J., Bangerth, F., 2000. Response of controlled atmosphere (CA) stored "Golden Delicious" apples to the treatments with aroma precursors alcohols and aldehydes as aroma precursors. Gartenbauwiss 65, 154–161.

Heyen, U., Harder, J., 2000. Geranic acid formation, an initial reaction of anaerobic monoterpene metabolism in denitrifying *Alcaligenes defragrans*. Appl. Environ. Microbiol. 66, 3004–3009.

Ke, D., Zhou, L., Kader, A., 1994. Mode of oxygen and carbon dioxide action on strawberry ester biosynthesis. J. Am. Soc. Hortic. Sci. 119, 971–975.

Kerbel, E., Kader, A., Romani, R., 1988. Effects of elevated CO_2 concentrations on glycolysis in intact 'Bartlett' pear fruit. Plant Physiol. 86, 1205–1209.

Kollmansberger, H., Berger, R., 1994. Thermal recovery of blackcurrant flavors, case-study on the industrial-scale. Dtsch. Lebensm. Rdsch. 90, 69–71.

Leino, M., Kallio, H., 1993. Volatile compounds of black-currant juice and wine. Z. Lebensm. Unters. Forsch. 196, 410–414.

Lewinsohn, E., Gijzen, M., Croteau, R., 1992. Wound-inducible pinene cyclase from grand fir: purification, characterization, and renaturation after SDS-PAGE. Arch. Biochem. Biophys. 293, 167–173.

Liu, S., Holland, R., Crow, V., 2004. Esters and their biosynthesis in fermented dairy products: a review. Int. Dairy J. 14, 923–945.

Longhurst, T., Tung, H., Brady, C., 1990. Developmental regulation of the expression of alcohol dehydrogenase in ripening tomato fruits. J. Food Biochem. 14, 421–433.

Longhurst, T., Lee, L., Hinde, R., Brady, C., Speirs, J., 1994. Structure of the tomato *Adh 2* gene and *Adh 2* pseudogenes, and a study of *Adh 2* gene expression in fruit. Plant Mol. Biol. 26, 1073–1084.

Lücker, J., El Tamer, M., Schwab, W., Verstappen, F., van der Plas, L., Bouwmeester, H., Verhoeven, H., 2002. Monoterpene biosynthesis in lemon (*Citrus limon*): cDNA isolation and functional analysis of four monoterpene synthases. Eur. J. Biochem. 269, 3160–3171.

Mason, B., Dufour, J., 2000. Alcohol acetyltransferases and the significance of ester synthesis in yeast. Yeast 16, 1287–1298.

McCaskill, D., Croteau, R., 1995. Monoterpene and sesquiterpene biosynthesis in glandular trichomes of peppermint (*Mentha* × *piperita*) rely exclusively on plastid-derived isopentenyl diphosphate. Planta 197, 49–56.

Nijssen, L., Visscher, C., Maarse, H., Willemsens, L., Boelsens, M., 1996. Black Currants (*Ribes nigrum* L.). In: Volatile Compounds in Food. Qualitative and Quantitative Data, vol. 3., seventh ed. TNO Nutrition, Food Research Institute, Ziest, The Netherlands, pp. 61–71.

Qian, M., Reineccius, G., 2003. Quantification of aroma compounds in parmigiano reggiano cheese by a dynamic headspace gas chromatography–mass spectrometry technique and calculation of odor activity value. J. Dairy Sci. 86, 770–776.

Author's personal copy

Ruiz del, M., Dobson, G., 2002. Influence of stage of ripeness on the enantiomeric distribution of chiral terpenes in blackcurrant fruits (*Ribes nigrum* L.). J. Food Sci. 67, 3284–3288.

Saerens, S., Verstrepen, K., Van Laere, S., Voet, A., Van Dijck, P., Delvaux, F., Thevelein, J., 2006. The *Saccharomyces cerevisiae EHT1* and *EEB1* genes encode novel enzymes with medium-chain fatty acid ethyl ester synthesis and hydrolysis capacity. J. Biol. Chem. 281, 4446–4456.

Savage, T., Hatch, M., Croteau, R., 1994. Monoterpene synthases *of Pinus contorta* and related conifers: a new class of terpenoid cyclase. J. Biol. Chem. 269, 4012–4020.

Shalit, M., Guterman, I., Volpin, H., Bar, E., Tamari, T., Menda, N., Adam, Z., Zamir, D., Vainstein, A., Weiss, D., Pichersky, E., Lewinsohn, E., 2003. Volatile ester formation in roses. Identification of an acetyl-coenzyme A. Geraniol/citronellol acetyltransferase in developing rose petals. Plant Physiol. 131, 1868–1876.

Silbereisen, R., 2002. Die wirtschaftlich wichtigen Obstarten. In: Link, H. (Ed.), 'Lucas' Anleitung zum Obstbau. E. Ulmer, Stuttgart, Germany, pp. 92–145.

Siriphanich, J., Kader, A., 1986. Changes in cytoplasmic and vacuolar pH in harvested lettuce tissue as influenced by CO_2. J. Am. Soc. Hortic. Sci. 111, 73–77.

Song, J., Bangerth, F., 1994. Production and development of volatile aroma compounds of apple fruits at different times of maturity. Acta Hortic. 368, 150–159.

Souleyre, E., Greenwood, D., Friel, E., Karunairetnam, S., Newcomb, R., 2005. An alcohol acyl transferase from apple (cv. Royal Gala), MpAAT1, produces esters involved in apple fruit flavor. FEBS J. 272, 3132–3144.

Speirs, J., Lee, E., Holt, K., Yong-Duk, K., Scott, N., Loveys, B., Schuch, W., 1998. Genetic manipulation of alcohol dehydrogenase levels in ripening tomato fruit affects the balance of some flavor aldehydes and alcohols. Plant Physiol. 117, 1047–1058.

Stewart, K., 1996. Processing in cranberry, blueberry, currant, and gooseberry. In: Somogyi, L., Barrett, D., Hui, Y. (Eds.), Processing Fruits: Science and Technology vol. 2: Major Processed Products. Technomic Publishing, Lancaster, PA, pp. 159–195.

Varming, C., Poll, L., 2003. Aroma recovery during concentration of black currant juice. In: Le Quéré, J.L., Etiévant, P.X. (Eds.), Flavour Research at the Dawn of the Twenty-First Century. Lavoisier, Cachan, France, pp. 741– 744.

Varming, C., Andersen, M., Poll, L., 2006. Volatile monoterpenes in black currant (*Ribes nigrum* L.) juice: effects of heating and enzymatic treatment by α-glucosidase. J. Agric. Food Chem. 54, 2298–2302.

Verstrepen, K., Van Laere, S., Vanderhaegen, B., Derdelinckx, G., Dufour, J., Pretorius, I., Winderickx, J., Thevelein, J., Delvaux, F., 2003. Expression levels of the yeast alcohol acetyltransferase genes *ATF1*, *Lg-ATF1*, and *ATF2* control the formation of a broad range of volatile esters. Appl. Environ. Microbiol. 69, 5228–5237.

Vickery, R., Bruinsma, J., 1973. Compartments and permeability for potassium in developing fruits of tomato (*Lycopersicon esculentum* Mill.). J. Exp. Bot. 24, 1261–1270.

Woodhead, M., Brennan, R., McNicol, R., Davies, H., Taylor, M., 1999. Differential gene expression during ripening in the fruit of blackcurrant (*Ribes nigrum* L.). Acta Hortic. 505, 423–426.

Woodland, D., 1991. Families of Flowering Plants I. Magnoliopsida (Dicots). In: Contemporary plant systematics. Prentice-Hall Inc., Englewood Cliffs, NJ, pp. 141–327.

Wright, K., Kader, A., 1997. Effect of controlled-atmosphere storage on the quality and carotenoid content of sliced persimmons and peaches. Postharvest Biol. Technol. 10, 89–97.

View publication stats