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(Methylsulfanyl)alkanoate ester biosynthesis in *Actinidia chinensis* kiwifruit and changes during cold storage

Catrin S. Günther [a,*], Adam J. Matich [b], Ken B. Marsh [a], Laura Nicolau [c]

[a] The New Zealand Institute for Plant and Food Research Ltd., Private Bag 92169 Auckland, New Zealand
[b] The New Zealand Institute for Plant and Food Research Ltd., Private Bag 11600 Palmerston North, New Zealand
[c] Department of Chemistry, The University of Auckland, Private Bag 92019 Auckland, New Zealand

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ABSTRACT

Four 3-(methylsulfanyl)propionate esters, ethyl 3-(methylsulfanyl)prop-2-enoate, two 2-(methylsulfanyl)acetate esters and their possible precursors 2-(methylsulfanyl)ethanol, 3-(methylsulfanyl)propanol and 3-(methylsulfanyl)propanal were quantified from the headspace of *Actinidia chinensis* 'Hort 16A' kiwifruit pulp by GC–MS–TOF analysis. The majority of these compounds were specific for eating-ripe fruit and their levels increased in parallel with the climacteric rise in ethylene, accumulating towards the very soft end of the eating firmness. No ethylene production could be observed after long-term storage (4–6 months) at 1.5 °C and the levels of all methylsulfanyl-volatiles, except methional, declined by 98–100% during that period. This depletion of (methylsulfanyl)alkanoate-esters after prolonged cold storage points towards little flavour impact of these compounds on commercial 'Hort 16A' kiwifruits. However, ethyl 3-(methylsulfanyl)propionate is suggested to be odour active in ripe 'Hort 16A' fruit that has not been stored. Gene expression measured by q-RT PCR of six ripening-specific alcohol acyltransferase (AAT) expressed sequence tags and (methylsulfanyl)alkanoate-ester production of cell-free extracts were also significantly decreased after prolonged cold storage. However, (methylsulfanyl)alkanoate-ester synthesis of cell-free extracts and AAT gene transcript levels could be recovered by ethylene treatment after five months at 1.5 °C indicating that the biosynthesis of (methylsulfanyl)alkanoate-esters in 'Hort 16A' kiwifruit is likely to depend on ethylene-regulated AAT-gene expression. That the composition but not the concentration of (methylsulfanyl)alkanoate-esters in fresh fruit could be restored after ethylene treatment suggests that substrate availability might also have an impact on the final levels of these volatiles.

1. Introduction

Since 1998, the novel kiwifruit cultivar *Actinidia chinensis* 'Hort 16A' has been commercially available under the trade name ZESPRI® GOLD. This cultivar is distinguished from the green variety not only by its yellow flesh colour but also by a sweeter taste with a tropical, fruity aroma reminiscent of melon, mango and banana (Jaeger et al., 2003). (Methylsulfanyl)alkanoate (MeS) esters, in particular methyl and ethyl 3-MeS-propionate as well as ethyl 2-MeS-acetate, display fruity, sweet and tropical odour characteristics (Burdock, 2004) and were reported to occur in several tropical fruits like pineapple (Takeoka et al., 1989), passion fruit (Werkhoff et al., 1998), Asian pear (Takeoka et al., 1992) and durian fruit (Weenen et al., 1996). Wyllie and Leach (1992) assigned these compounds together with MeS-alkyl acetates a significant impact on the characteristic aroma of certain melon cultivars due to their odour activity values (OAV). Moreover, methyl and ethyl 2-MeS-acetate has previously been quantified in *A. deliciosa* 'Hayward' kiwifruit puree by Jordan et al. (2002) and an aroma impact was described for the cooked nut or roasted oil odour of the first ester using GC–O.

General fruit ester production by alcohol acyltransferases (AATs) has been studied in apple (Souleyre et al., 2005; Matich and Rowan, 2007), banana (Wyllie and Fellman, 2000), strawberry (Olias et al., 2002) and melon (El-Sharkawy et al., 2005). It was shown that recombinant *Cucumis melo* AATs were capable of using MeS-alcohols as substrates for ester synthesis with acetyl-CoA (Lucchetta et al., 2007) but (MeS)alkyl-CoAs have never been tested as substrates for ester synthesis. In apple and melon, the gene expression of ripening-specific AATs appeared to be regulated by ethylene (Yahyaoui et al., 2002; Schaffer et al., 2007) and the production of volatile esters has also been correlated with ethylene synthesis in these climacteric fruit (Aubert and Bourger, 2004; Johnston et al., 2009). Kiwifruit in general is known to produce climacteric ethylene during ripening but only very low levels can be detected during cold storage of *A. chinensis* 'Hort 16A' (Patterson et al., 2003) which is the common postharvest practice

* Corresponding author. Tel.: +64 9 9257265; fax: +64 9 9257001.
E-mail address: Catrin.Guenther@plantandfood.co.nz (C.S. Günther).

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to maintain flesh firmness. Kiwifruit firmness is widely used as the defining character for ripeness and the non destructive acoustic firmness sensor (AFS) was suggested as a suitable alternative to destructive devices for monitoring textural changes of 'Hort 16A' kiwifruit during storage (Schotsmans et al., 2008). The AFS measures fruit stiffness in $10^6\,Hz^2\,g^{2/3}$ and to date there is no equation available to express kiwifruit stiffness in *N*. However, 'Hort 16A' fruit are considered as eating-ripe between 10 and $4 \times 10^6\,Hz^2\,g^{2/3}$ using the instrument parameters applied in this study.

The intention of this work was firstly to study the levels of MeS-volatiles during ripening of *A. chinensis* 'Hort 16A' and after cold storage in regards to fruit ethylene production. Secondly, we were aiming to test whether (MeS)alkanoate esters can be synthesised by ripening-specific AATs and if gene expression of these enzymes is influenced by chilling and ethylene. Thirdly, it was of interest to use the quantitative MeS-volatile data to obtain a first indication of their potential impact on the tropical flavour of eating-ripe fruit.

2. Results and discussion

2.1. Methylsulfanyl (MeS)-volatiles accumulate during softening of eating-ripe 'Hort 16A' kiwifruit

Methyl, ethyl, propyl, and butyl esters of 3-MeS-propionic acid, methyl and ethyl 2-MeS-acetate, ethyl 3-MeS-prop-2-enoate, 2-MeS-ethanol, 3-(methylsulfanyl)propanol (methionol) and 3-(methylsulfanyl)propanal (methional) (Fig. 1) were quantified in *A. chinensis* 'Hort 16A' using GC–MS–TOF after dynamic headspace sampling. None of these compounds could be detected in unripe fruit after harvest at $38 \times 10^6\,Hz^2\,g^{2/3}$ and only methyl and ethyl 2-MeS-acetate and methional (Fig. 2B and D) were present, at low concentrations (<0.5 μg kg^{-1}), before 'Hort 16A' kiwifruit had softened to eating firmness. As shown in Fig. 2A–D most MeS-volatiles just appeared to be detectable in eating-ripe fruit after the climacteric rise in ethylene production. In fact, all MeS-compounds increased with fruit softness, except methyl 2-MeS-acetate, which displayed highest levels (0.42 ± 0.01 μg kg^{-1}) in firm

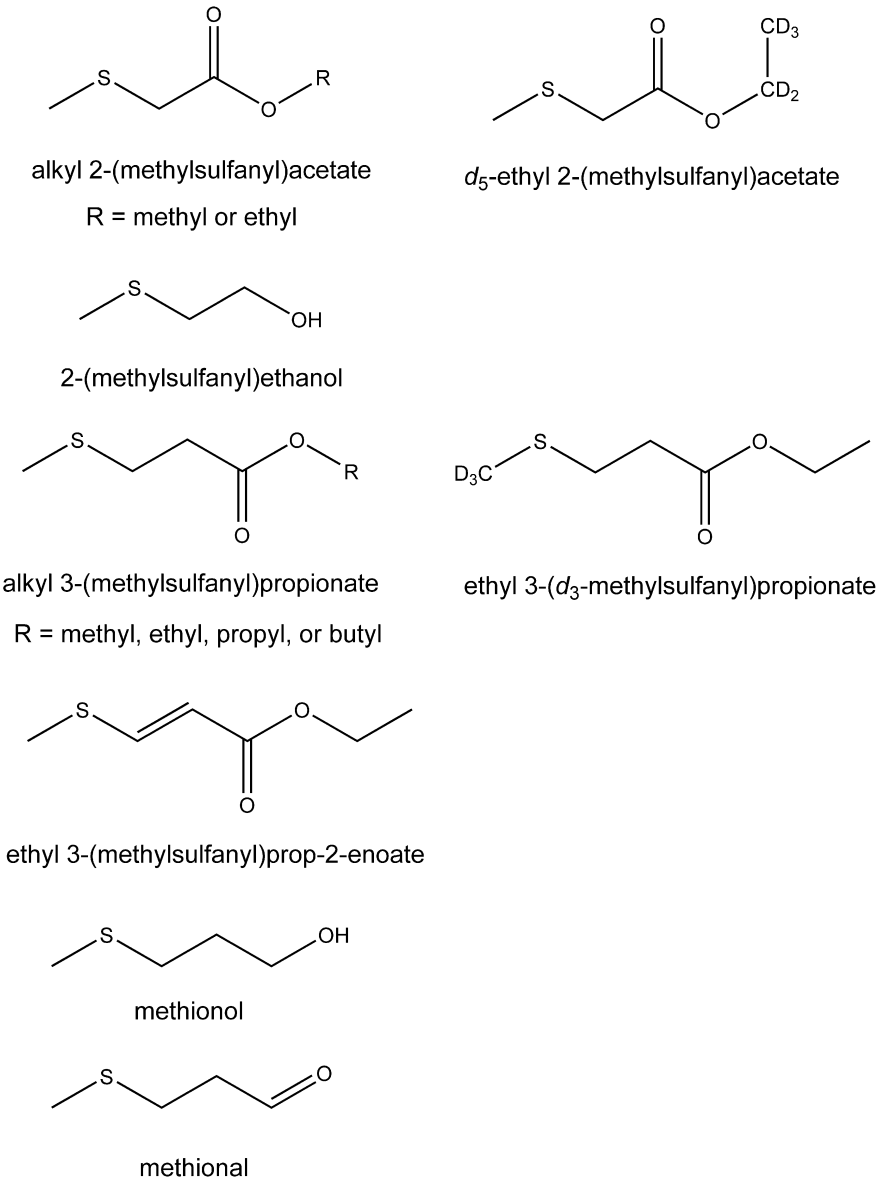

Fig. 1. Structures of MeS-volatiles quantified in *Actinidia chinensis* 'Hort 16A' kiwifruit and the corresponding deuterium-labelled internal standard.

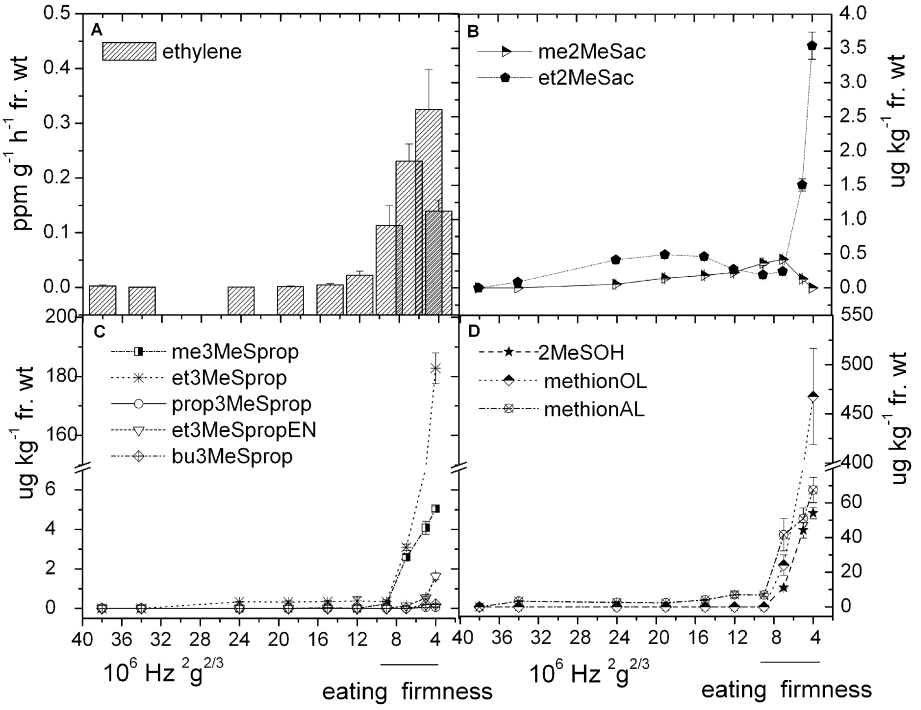

Fig. 2. Ethylene production and direct quantification of MeS volatiles in non-stored 'Hort 16A' with respect to fruit firmness during ripening. Error bars: Standard deviations of means; MeS-volatiles: four replicates; ethylene: six biological replicates; me/et/pr/bu: methyl/ethyl/propyl/butyl; MeS: methylsulfanyl; ac: acetate; prop: propionate; propEN: prop-2-enoate; 2MeSOH: 2-MeS-ethanol; FW: fresh weight.

(8×10^6 Hz^2 g$^{2/3}$) eating-ripe fruit, and dropped to under the detection limit during further ripening (Fig. 2B). Peak levels of MeS-volatiles were quantified in soft fruit (4×10^6 Hz^2 g$^{2/3}$) with ethyl 3-MeS-propionate being the main ester (168 ± 6.7 μg kg^{-1}) and methionol 429 ± 25.8 μg kg^{-1}) being the major MeS-compound in 'Hort 16A' fruit pulp. Friel et al. (2007) reported that the levels of most fruit esters, especially ethyl esters, increased during ripening with high levels in soft 'Hort 16A' fruit and this was also found to be the case for (MeS)alkanoate-esters in this study, indicating that these compounds are specific for eating ripe *A. chinensis* kiwifruit.

2.2. The effect of cold storage on MeS-volatile production

After one month at 1.5 °C, the composition and concentration of all MeS-volatiles in eating-ripe 'Hort 16A' fruit decreased dramatically (Table 1) and only methyl esters of 2-MeS-acetic acid and 3-MeS-propionic acid could be detected in firm, eating-ripe fruit (8×10^6 Hz^2 g$^{2/3}$) with levels corresponding to 10% and 20% of those present in non-stored fruit respectively. After two months of cold storage, methional was the only MeS-volatile detectable at this firmness and its levels remained constant until six months at 1.5 °C when a twofold increase (12.8 ± 2.2 μg kg^{-1}) was observed compared with levels in non-stored fruits (6.6 ± 1 μg kg^{-1}) at 8×10^6 Hz^2 g$^{2/3}$. In addition, volatile levels of ethyl 3-MeS-propionate (0.19 ± 0.01 μg kg^{-1}) and ethyl 2-MeS-acetate (0.11 ± 0.002 μg kg^{-1}) that corresponded to about 56% and 61% of those before storage could be quantified after six months.

The composition of MeS-volatiles in very soft kiwifruit (4×10^6 Hz^2 g$^{2/3}$) after one month at 1.5 °C did not change (Table 1) but their levels were significantly reduced for up to 95% (ethyl 3-MeS-propionate; 8.3 μg kg^{-1}), followed by a further gradual decline with storage time at 1.5 °C. After four months of cold storage, only trace amounts of ethyl 2-MeS-acetate and ethyl 3-MeS-propionate esters remained detectable, at levels less than 3% of those before storage. These results are in good agreement with those of Young and Paterson (1985), who observed that fruit volatiles – especially total esters – increased in *Actinidia deliciosa* 'Hayward' kiwifruit with fruit softness, but decreased with storage time at 0 °C prior to ripening. Methional, however, was less affected by chilling (3–5-fold decrease in concentration), and remained present during the whole storage trial even after methionol concentrations were below detection limit. In lactic acid bacteria methional can act as an intermediate for 3-MeS-propionic acid formation from methionine (Pripis-Nicolau et al., 2004; Landaud et al., 2008; Vallet et al., 2008) and a similar role could be possible in plants. In this situation, methional would act as a biosynthetic precursor for AAT substrates, namely 3-MeSpropionyl- and 2-MeS-acetyl-CoA, from 3-MeS-propionic acid.

2.3. Ethylene production of 'Hort 16A' kiwifruit is altered by cold storage

Interestingly, the pattern of ethylene production was also altered after cold storage (Fig. 3). For instance there was a 25-fold decrease of ethylene levels measured in firm, eating-ripe fruit (8×10^6 Hz^2 g$^{2/3}$) from the first to the second month of cold storage and no ethylene could be detected in eating ripe 'Hort 16A' after four months at 1.5 °C. In non-stored fruit, a climacteric peak of ethylene production was observed within the eating firmness range that appeared in parallel to MeS-volatile production (Fig. 2A), suggesting a link between this hormone and fruit ester production. Low levels (0.023 ± 0.008 ppm g^{-1} h^{-1}) of ethylene were detectable using GC-FID just before the fruit had reached eating firmness (12×10^6 Hz^2 g$^{2/3}$) and ethylene levels increased during softening to peak concentrations (0.33 ± 0.07 ppm g^{-1} h^{-1}) at 5×10^6 Hz^2 g$^{2/3}$, with a further decline towards the very soft end of the eating firmness range (4×10^6 Hz^2 g$^{2/3}$, 0.14 ± 0.02 ppm g^{-1} h^{-1}). The depletion of ethylene production by 'Hort 16A' after cold storage is unlikely to depend on the actual length

Table 1
MeS-volatile concentrations (μg kg^{-1}) in 'Hort 16A' kiwifruit at firmness 8 and 4 × 10^6 Hz^2 g$^{2/3}$ before and after cold storage at 1.5 °C. SD%: Standard deviation of four technical replicates given in%; 0m8…6m8: 0…6 months at 1.5 °C, ripened to 8 × 10^6 Hz^2 g$^{2/3}$; 0m4…6m4: 0…6 months at 1.5 °C, ripened to 4 × 10^6 Hz^2 g$^{2/3}$; 4c8: 4 months of softening to 8 × 10^6 Hz^2 g$^{2/3}$ at 1.5 °C; 6c4: 6 months of softening to 4 × 10^6 Hz^2 g$^{2/3}$ at 1.5 °C; 5et4: ethylene treatment after 5 months at 1.5 °C, ripened to 4 × 10^6 Hz^2 g$^{2/3}$; nd: not detectable. [1] Aubert and Bourger, 2004); [2] Steinhaus et al., 2009; [3] Takeoka et al., 1989; [4] Ferreira et al., 2005; [5] Takeoka et al., 1991.

	Methyl 2-(methyl sulfanyl) acetate		Ethyl 2-(methyl sulfanyl) acetate		Methional		Methyl 3-(methyl sulfanyl) propionate		2-(Methyl sulfanyl) ethanol		Ethyl 3-(methyl sulfanyl) propionate		Propyl 3-(methyl sulfanyl) propionate		Methionol		Ethyl 3-(methyl sulfanyl) prop-2-enoate		Butyl 3-(methyl sulfanyl) propionate	
	C(μg kg^{-1})	SD%	C(μg kg^{-1})	SD%	C(μg kg^{-1})	SD%	C(μg kg^{-1})	SD%	C(μg kg^{-1})	SD%	C(μg kg^{-1})	SD%	C(μg kg^{-1})	SD%	C(μg kg^{-1})	SD%	C(μg kg^{-1})	SD%	C(μg kg^{-1})	SD%
0m8	0.35	10	0.18	5	6.6	15	0.23	12	nd	–	0.34	8	nd	–	nd	–	nd	–	nd	–
1m8	0.03	10	nd	–	5.4	9	0.04	5	nd	–	nd	–	nd	–	nd	–	nd	–	nd	–
2m8	nd	–	nd	–	7.6	28	nd	–	nd	–	nd	–	nd	–	nd	–	nd	–	nd	–
3m8	nd	–	nd	–	2.7	14	nd	–	nd	–	nd	–	nd	–	nd	–	nd	–	nd	–
4m8	nd	–	0.07	2	4.2	7	nd	–	nd	–	0.13	22	nd	–	nd	–	nd	–	nd	–
4c8	nd	–	nd	–	3.3	27	nd	–	nd	–	nd	–	nd	–	nd	–	nd	–	nd	–
5m8	nd	–	0.08	3	6.1	18	nd	–	nd	–	0.09	15	nd	–	nd	–	nd	–	nd	–
6m8	nd	–	0.11	2	12.8	17	nd	–	nd	–	0.19	5	nd	–	nd	–	nd	–	nd	–
0m4	nd	–	3.25	3	62.3	16	4.57	8	42.8	10	168	4	0.06	24	429	6	1.5	4	0.21	8
1m4	0.08	8	0.27	3	18	14	1.17	4	12.4	9	8.4	3	0.04	9	61.3	4	0.12	9	0.15	3
2m4	0.04	32	0.14	6	15.2	13	0.25	15	8.5	10	2.1	9	nd	–	25.7	22	nd	–	0.12	2
3m4	0.02	4	0.37	4	9.8	15	0.12	13	3.6	10	3.4	10	nd	–	15.2	5	0.09	4	nd	–
4m4	0.01	8	0.08	1	9.7	9	0.04	7	nd	–	0.27	9	nd	–	nd	–	nd	–	nd	–
5m4	nd	–	0.08	1	6.8	15	nd	–	nd	–	0.13	10	nd	–	nd	–	nd	–	nd	–
6m4	nd	–	0.08	2	18.9	11	nd	–	nd	–	0.12	12	nd	–	nd	–	nd	–	nd	–
6c4	nd	–	0.06	1	19.9	14	nd	–	nd	–	nd	–	nd	–	nd	–	nd	–	nd	–
5et4	0.02	9	0.28	2	22.9	19	0.14	8	nd	–	3.56	8	nd	–	22.1	13	0.10	7	nd	–
Odour threshold			25 μg kg^{-1} [1]		0.43 μg kg^{-1} [2]		180 μg kg^{-1} [3]				7 μg kg^{-1} [3]				2500 μg kg^{-1} [4]		246 μg kg^{-1} [5]			

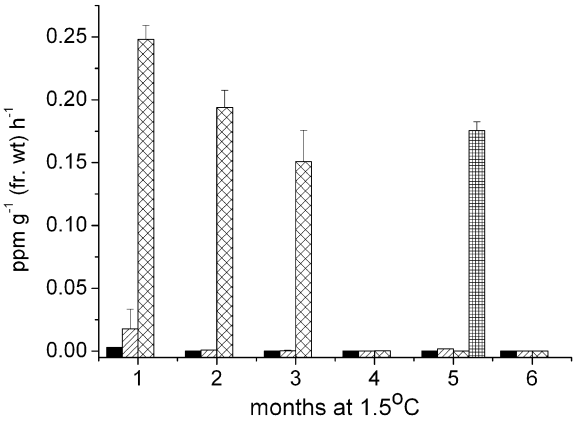

Fig. 3. Ethylene production of cold-stored fruit. ■ not ripened; ▨ ripened to 8×10^6 Hz^2 g$^{2/3}$; ▩ ripened to 4×10^6 Hz^2 g$^{2/3}$; ▦ ethylene-treated and ripened to 4×10^6 Hz^2 g$^{2/3}$. Error bars: Standard deviations of the means of six biological replicates.

of ripening time at 20 °C (data not shown); for example, after two months of chilling, fruit at 8 and 4×10^6 Hz^2 g$^{2/3}$ were both ripened for 13 days but soft, eating-ripe fruit produced 200-fold more ethylene than firm, eating-ripe fruit. After an additional two months at 1.5 °C, fruit were ripened for 11 days to 4×10^6 Hz^2 g$^{2/3}$ but ethylene was not detectable at all. During the six-month storage trial, ethylene production was never observed for fruit that had been taken directly from cold storage without ripening, suggesting that the biosynthesis of this ripening hormone in 'Hort 16A' is inhibited by chilling at 1.5 °C. Ritenour et al. (1999) discovered that ethylene preconditioning of *A. deliciosa* at 0 °C within the first two weeks of cold storage induced fruit softening but at a 2-fold slower rate than observed for fruit which were ethylene treated at 20 °C prior to cold storage. Moreover, the softening pattern of green kiwifruit that were kept for at least three weeks at 0 °C prior to preconditioning was not altered in comparison with non-treated fruit. On the other hand, Antunes and Sfakiotakis (2002) stated that short-term chilling (0–10 °C) for 12 days of 'Hayward' kiwifruit actually enhanced the onset of ethylene production in parallel with its precursor and biosynthetic enzymes after re-warming. However, since the effects of mid and long-term storage were not investigated by these authors, the impact of prolonged chilling on ethylene biosynthesis in kiwifruit remains to be elucidated.

It would be interesting to investigate whether the lack of ethylene production in cold stored 'Hort 16A' fruit is due to increased competition for *S*-adenosyl-methionine (Crowhurst et al.), a common precursor of the ethylene, polyamine and *S*-methyl-methionine biosynthetic pathway, since the later metabolites were shown to increase resistance to cold stress in certain crops (Szego and Horvath, 2007; Cuevas et al., 2008; Groppa and Benavides, 2008).

2.4. Gene expression of putative AATs is regulated by ethylene in A. chinensis 'Hort 16A'

Gene transcription of MpAT1, an AAT that has been previously reported to be of importance for 'Royal Gala' apple fruit flavour (Souleyre et al., 2005), was shown to be regulated by ethylene (Schaffer et al., 2007). Yahyaoui et al. (2002) reported that gene expression of CmAAT1 from melon increased during ripening and furthermore, that transcript levels were reduced in fruit treated with 1-MCP, as well as in antisense amino-cyclopropane-oxidase fruit, which points towards a regulatory role of ethylene for AAT gene transcription in climacteric fruit. Six *Actinidia* Expressed Sequence Tags (ESTs) with putative flavour-related AAT function display high sequence homology to those genes mentioned above and to each other (Crowhurst et al., 2008). The gene transcription of AT15, AT18, AT2, AT17 AT1 and AT16 in *A. chinensis* 'Hort 16A' kiwifruit was measured using quantitative Real-Time PCR. In unripe fruit (38×10^6 Hz^2 g$^{2/3}$), gene expression of these ESTs could not be observed apart from low levels of AT16 (Fig. 4A). Additional transcripts (AT2, AT17, AT1) were detectable before fruit had softened to eating firmness (15×10^6 Hz^2 g$^{2/3}$) and transcripts of AT15 and AT18 occurred only in eating-ripe fruit ($8–4 \times 10^6$ Hz^2 g$^{2/3}$). This suggests an impact of AT2, AT17, AT1, AT15 and AT18 on flavour-related fruit ester production, which could involve MeS-esters. After short-term cold storage for one month, gene expression of AT2, AT17 AT1 and AT16 was significantly reduced in fruit at 8×10^6 Hz^2 g$^{2/3}$ (Fig. 4B), but transcripts of these ESTs in soft, eating-ripe fruits (4×10^6 Hz^2 g$^{2/3}$) displayed similar levels to those before storage, except for AT2 transcripts, which appeared to be twofold reduced. Thus, after five months at 1.5 °C, only transcripts of AdAt2 and AcAT16 with levels < 1% and 50%, respectively could be detected in eating-ripe 'Hort 16A' fruit (8 and 4×10^6 Hz^2 g$^{2/3}$) in comparison with non-stored fruit. However, ethylene treatment of long-term stored fruit prior to ripening was able to increase gene transcription of all ESTs to the levels before storage or after one month at 1.5 °C, suggesting that transcription of those *Actinidia* (ESTs) is regulated by ethylene.

2.5. Ripening-specific AATs produce MeS-esters in 'Hort 16A' kiwifruit

In 'Hort 16A' kiwifruit most MeS volatiles could be restored but to a much lower level by ethylene treatment after five months of

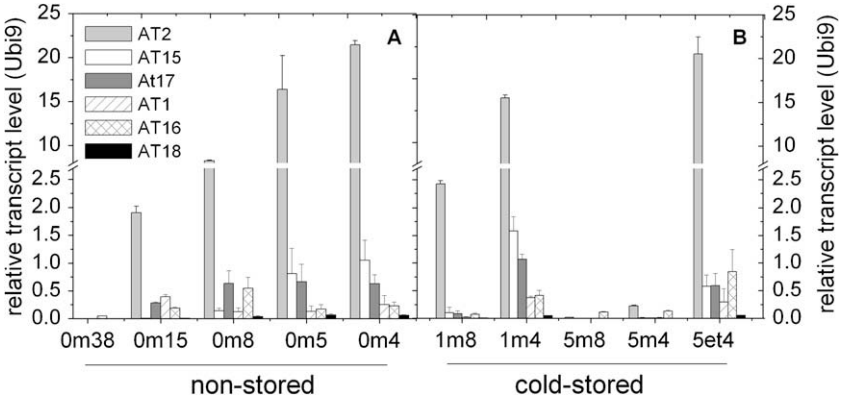

Fig. 4. Relative transcript levels of six *Actinidia* AAT ESTs relative to Ubiquitin9 in non-stored and stored 'Hort 16A' kiwifruit. Error bars: Standard errors of the means of three biological with two technical replicates each. 0m38…4: no storage, $38…4 \times 10^6$ Hz^2 g$^{2/3}$; 1m4…5m4: 1 month and 5 months, ripened to 4×10^6 Hz^2 g$^{2/3}$; 1m8…5m8: 1 month and 5 months, ripened to 8×10^6 Hz^2 g$^{2/3}$; 5et4: 5 months at 1.5 °C, ethylene-treated and ripened to 4×10^6 Hz^2 g$^{2/3}$.

cold storage (Table 1). For example, in soft eating-ripe fruit (4×10^6 Hz^2 g$^{2/3}$), volatile levels of ethyl 2-MeS-acetate could be restored to 8%, methyl 3-MeS-propionate to 3% and ethyl 3-MeS-propionate to 2% compared with those before storage, or 100%, 10% and 40% after one month at 1.5 °C, respectively. Therefore, it was of interest to investigate whether this was caused by reduced enzyme activity or depletion of substrates. We hypothesised that 2-MeS-acetate and 3-MeS-propionate esters are formed by AATs using straight chain alcohols and the corresponding MeS alkyl-CoA that was added to cell-free protein extracts. Ethyl, propyl, butyl 2-MeS-acetates and 3-MeS-propionates were produced by crude extracts from non-stored eating-ripe fruit and after one month at 1.5 °C (Fig. 5), indicating that MeS-esters in 'Hort 16A' are produced by AATs. In addition, there was no MeS-ester production in cell-free extracts from unripe fruit (38×10^6 Hz^2 g$^{2/3}$) and after five months of cold storage (4×10^6 Hz^2 g$^{2/3}$), pointing towards AAT depletion. However, protein extracts from soft eating-ripe fruit (4×10^6 Hz^2 g$^{2/3}$) that had been ethylene-treated after five months at 1.5 °C were able to produce MeS-esters at similar levels to cell-free extracts of 'Hort 16A' fruit after one month of cold storage. This finding correlates with changes in gene expression but not with volatile levels of fresh fruit, suggesting reduced MeS alkyl-CoA or alcohol levels in ethylene-treated kiwifruit after five months of chilling.

2.6. The potential impact of methylsulfanyl (MeS)-volatiles on 'Hort 16A' flavour

In this study MeS-volatiles occurred in trace amounts in 'Hort 16A' kiwifruit pulp. Literature values of odour thresholds for most MeS-volatiles in water or synthetic wine (methionol) are listed in Table 1 and it becomes obvious that methional is the only MeS-compound that exceeds its odour threshold in firm and soft eating-ripe fruit after cold storage for at least six months. This mashed potato or soup-like odorant was reported to be aroma-active in non-processed pink guava (Steinhaus et al., 2008), lychee (Mahattanatawee et al., 2007) and one blackberry cultivar (Klesk and Qian, 2003). However, since methional levels are also above odour threshold in unripe fruit (33×10^6 Hz^2 g$^{2/3}$), its impact on the characteristic flavour of ripe fruit can be questioned. In wine, high OAVs of methional are associated with off-flavours and mainly caused by chemical oxidation of methionol (Escudero et al., 2000). In this study, headspace volatiles were trapped with air for 20 h and oxidation products as artefacts can therefore not be excluded.

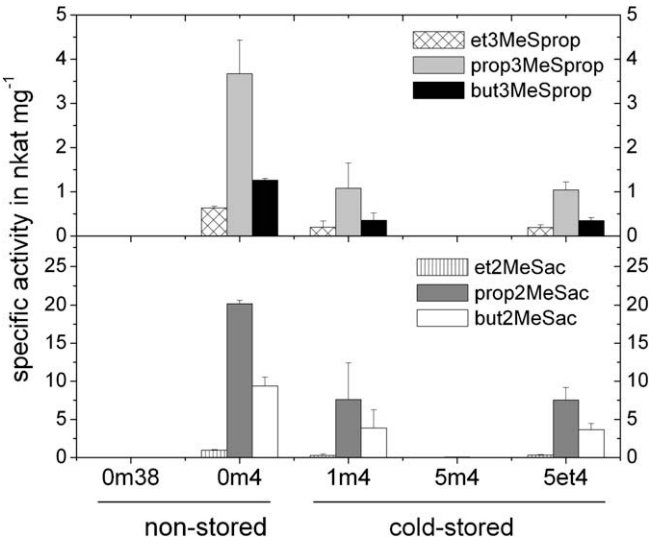

Fig. 5. MeS-ester production of cell-free protein extract from 'Hort 16A' kiwifruit. Error bars: Standard deviations of the average of two biological replicates. 0m38: no storage, 38×10^6 Hz^2 g$^{2/3}$; 0m4: no storage, 4×10^6 Hz^2 g$^{2/3}$; 1m4… 5m4: 1 and 5 months at 1.5 °C, 4×10^6 Hz^2 g$^{2/3}$, 5et4: 5 months at 1.5 °C, ethylene-treated and ripened to 4×10^6 Hz^2 g$^{2/3}$.

Ethyl 3-MeS-propionate on the other hand only exceeds its odour thresholds in soft, eating-ripe (4×10^6 Hz^2 g$^{2/3}$) 'Hort 16A' kiwifruit with an estimated odour activity value (OAV) of 24 in non-stored fruit and an OAV of 1 after one month at 1.5 °C. But the concentration of this tropical, sweet odorant stayed well below odour threshold after further storage at 1.5 °C. However, OAVs that are generally estimated from odour thresholds in a simplified matrix should only be seen as indicative means for the aroma impact of an individual compound. For a better understanding of the impact different volatiles can have on the overall aroma, matrix influence (Marsh et al., 2006) and the interaction between volatile compounds (Escudero et al., 2007) need to be considered. Furthermore, Escudero et al. (2004) demonstrated a key role of low OAV compounds on wine aroma and more recent research showed that volatiles of the same chemical family, which occurred below odour threshold, produced a quite specific odour impression probably due to additive effects (Pineau et al., 2009). These findings make the use of OAVs as critical parameter even more controversial. Whether MeS-volatiles contribute to the tropical flavour of *A. chinensis* 'Hort 16A' kiwifruit would therefore have to be investigated using a systematic GC-O approach and aroma reconstitution studies. However, since commercial 'Hort 16A' kiwifruit is often stored for up to nine months, a contribution of (MeS)alkanoate-esters on the aroma of long-term stored fruits seems very unlikely, since the levels of these compounds markedly decreased below the detection limit with storage time. Therefore, it would be of interest to investigate the impact of cold storage on the general flavour perception of 'Hort 16A'. Also because total ester production is likely to be influenced by decreased AAT gene expression and enzyme activity.

3. Concluding remarks

It can be concluded that most MeS-volatiles specifically occur in eating-ripe *A. chinensis* 'Hort 16A' kiwifruit and increase during softening. Cold storage in general leads to a marked decrease of (MeS)alkanoate-esters and AAT transcript levels, which can be partially restored by ethylene treatment. This finding was confirmed by (MeS)alkanoate-ester production of cell-free extracts with added (MeS)alkyl-CoAs and alcohols. Therefore, we suggest that (MeS)alkanoate-ester production in 'Hort 16A' kiwifruit is likely to depend primarily on gene expression of ripening-specific AATs, which was regulated by ethylene and inhibited by prolonged cold storage. On the basis of estimated OAVs ethyl 3-MeS-propionate appears to be the only odour active MeS-ester primarily in soft, non-stored fruits. But if (MeS)alkanoate-esters contribute to characteristic aroma notes of non or short-term stored 'Hort 16A' – for instance due to cumulative or synergistic effects- will have to be investigated. Finally, the changes in MeS-volatile levels with ripening stage and storage time also demonstrate the impact of postharvest treatment on fruit volatiles and potential flavour abundance.

4. Experimental

4.1. Materials and methods

Actinidia chinensis 'Hort 16A' kiwifruit from three different growers were harvested in 2008 at commercial maturity (Satara Kiwifruit Supply Ltd., Bay of Plenty, New Zealand), colour-conditioned for five days at 5 °C and randomized. A random sample of the fruit was ripened at 20 °C and sampled according to their

firmness using AWETA® acoustic firmness sensor (microphone gain:80, thick power:16). The other fruit were cold stored at 1.5 °C for six months and monthly samples were taken without ripening as well as after ripening to an average firmness of 8 and 4×10^6 Hz^2 g$^{2/3}$, respectively. An additional sample was treated with 100 ppm ethylene for 24 h after five months of cold storage and ripened to 4×10^6 Hz^2 g$^{2/3}$. At each time-point, kiwifruit pulp from 25 to 30 fruit was snap frozen with liquid nitrogen, pulverized with a stone crusher (Rocklabs, New Zealand) and stored at −80 °C prior to sampling.

4.2. Ethylene production

Six fruit per sample were individually placed in an airtight plastic container with rubber seal aperture. In addition, one empty container was prepared as a control. After one hour, 1 ml was taken from the headspace with a syringe and directly injected into a Phillips PU 4500 GC with 1.5 m × 6 mm × 4 mm 80/100 mesh activated alumina F1 column (Pye unicam) and FID-detector (210 °C) that was calibrated with 1 ppm ethylene standard. The injector and column temperature were set at 130 °C.

4.3. Headspace volatile analysis

4.3.1. Standard compounds

Stock solutions (10 mM) of all standards were prepared in isopropanol and stored at −20 °C prior to use. The following reference compounds were purchased from Sigma–Aldrich New Zealand Ltd:

2-(methylsulfanyl)ethanol, 3-(methylsulfanyl)propanol, 3-(methylsulfanyl)propanal methyl and ethyl 2-(methylsulfanyl)acetate, methyl and ethyl 3-(methylsulfanyl)propionate.

Propyl and butyl 3-(methylsulfanyl)propionate were synthesised by reaction of propanol or butanol with 1 equivalent of 3-(methylsulfanyl)propionic anhydride, which was synthesised by stirring 2.3 mmol of dicyclohexylcarbodiimide with 4.2 mmol 3-(methylsulfanyl)propionic acid in 10 ml of dry Et_2O under N_2 for 24 h, and was used without further purification. Propanol or butanol (1 mmol), 3-(methylsulfanyl)propionic anhydride, and *p*-toluene sulfonic acid (10 mg) were stirred for five days in 5 ml of dry Et_2O. Et_2O (40 ml) was added, and the reaction quenched with 2 × 10 ml saturated $NaHCO_3$ (aq.). The organic phase was dried ($MgSO_4$), the solvent removed and the product purified by flash chromatography on silica gel. Propyl 3-(methylsulfanyl)propionate (99% pure); EI–MS, *m/z* (rel. int.): 74 (100), 61 (88), 41 (60), 75 (48), 43 (47), 162 (40, M^+), 47 (32), 103 (27), 55 (20), 73 (18), 120 (15), 77 (14), 119 (10), 102 (7), 89 (7), 147 (1). Butyl 3-(methylsulfanyl)propionate (99% pure); EI–MS, *m/z* (rel. int.): 74 (100), 61 (88), 41 (60), 75 (48), 43 (47), 176 (40, M^+), 47 (32), 103 (27), 55 (20), 73 (18), 120 (15), 77 (14), 119 (10), 102 (7), 89 (7), 120 (1).

Propyl and butyl 2-(methylsulfanyl)acetate were synthesised by reaction of propanol or butanol with 1 equivalent of 2-(methylsulfanyl)acetic anhydride, which was synthesised by stirring 6.3 mmol of dicyclohexylcarbodiimide with 3.33 mmol 2-(methylsulfanyl)acetic acid in 10 ml of dry Et_2O under N_2 for 24 h, and was used without further purification. Propanol or butanol (1.6 mmol), 2-(methylsulfanyl)acetic anhydride (1.67 mmol), and *p*-toluene sulfonic acid (10 mg) were stirred for five days in 10 ml of dry Et_2O. 20 ml of Et_2O was added, and the reaction quenched with 2 × 5 ml saturated $NaHCO_3$ (aq.). The organic phase was dried ($MgSO_4$), the solvent removed, and the product purified by flash chromatography on silica gel. Propyl 2-(methylsulfanyl)acetate (>99% pure); EI–MS, *m/z* (rel. int.): 61 (100), 43 (36), 148 (30, M^+), 41 (19), 42 (11), 45 (11), 60 (11), 35 (9), 47 (7), 63 (4), 62 (4), 149 (4), 102 (4), 77 (4), 106 (4), 46 (3), 89 (3). Butyl 2-(methylsulfanyl)acetate (99% pure); EI–MS, *m/z* (rel. int.): 61 (100), 41 (51), 56 (37), 57 (30), 162 (28, M^+), 106 (21), 42 (15), 60 (12), 35 (12), 62 (11), 45 (10), 46 (10), 39 (9), 47 (7), 43 (7).

The internal standard, ethyl 3-([D3]-methylsulfanyl)propionate (Fig. 1), was synthesised by reaction of CD_3I with ethyl 3-mercaptopropionate according to Sen and Grosch (1991), and was purified (97.5%) by distillation at 65 °C under reduced pressure. EI–MS, *m/z* (rel. int.): 77 (100), 64 (71), 151 (58, M^+), 78 (48), 106 (20), 43 (19), 55 (16), 49 (13), 46 (12), 80 (11), 59 (11), 50 (10), 45 (10).

For the synthesis of [D5]ethyl 2-(methylsulfanyl)acetate (Fig. 1), 1.58 mmol of 2-(methylsulfanyl)acetic anhydride (5.74 mmol 2-(methylsulfanyl)acetic acid and 3.15 mmol dicyclohexylcarbodiimide reacted as described above) were stirred with 1.75 mmol d_6-ethanol and *p*-toluene sulfonic acid (5 mg) for 3 h under N_2. Pentane (10 ml) was added to the reaction mixture, which was then quenched with 2 × 10 ml saturated $NaHCO_3$ (aq.). The aqueous layer was extracted twice with 5 ml pentane before combining and drying ($MgSO_4$) the organic fractions. After the solvent was removed, the product was purified (>99%) by distillation at 50 °C under reduced pressure. EI–MS, *m/z* (rel. int.): 61 (100), 139 (82, M^+), 93 (56), 63 (9), 45 (6), 74 (5), 140 (5), 46 (4), 141 (4), 73 (4).

4.3.2. Dynamic headspace sampling

Four replicates, each containing 10 g of pulverized 'Hort 16A' fruit pulp, 1 µl of each deuterated internal standard (10 mM), and 0.1 mM of Paraoxon (Sigma–Aldrich) were prepared in 250 ml Erlenmeyer flasks (Quick Fit™). The addition of the carboxyesterase inhibitor Paraoxon significantly increased peak areas of MeS-esters as well as [D5]-ethyl 2-(methylsulfanyl)acetate and was therefore crucial for quantification. After sealing the equipment hermetically, dry air (BOC) was purged through the system with a flow rate of 30 ml min^{-1} and headspace volatiles were trapped with 100 mg Tenax®-TA resin for 20 h at room temperature. Prior to sampling, the Tenax®-filled direct thermal desorption (DTD) vials (ATAS GL International) were conditioned for two hours at 235 °C with a nitrogen flow of 20 ml min^{-1}.

4.3.3. GC–MS–TOF analysis

For DTD injection, the Focus (ATAS GL) autosampler was operated via the PAL cycle composer software 1.5.4. The headspace volatiles were cryo-focused at −120 °C and thermally desorbed at 175 °C after heating the cryogenic trap with a ramp rate of 50 °C min^{-1} using an Optic 3 thermal desorption system (ATAS GL). The initial injector temperature of 35 °C was increased to 220 °C with a rate of 16 °C s−1. Three minutes after the first split mode (15 ml min^{-1}), a second split of 25 ml min^{-1} was introduced and the volatiles were transferred onto a 30 m × 0.25 mm × 0.25 µm film thickness DB-Wax (J&W Scientific, Folsom, CA, USA) capillary column in a HP6890 GC (Agilent Technologies). A linear GC-programme of 3 °C min^{-1} from 30 °C for 1 min to 220 °C for 2.3 min was applied with a column flow of 1 ml min^{-1}. Peaks were identified by time-of-flight mass spectrometry (TOF-MS, Leco Pegasus III, St. Joseph, MI, USA). Helium was used as carrier gas, the transfer line temperature was set to 220 °C and a detector voltage of 1700 V was applied. The ion source temperature was kept at 200 °C and ionisation energy of 70 eV was used for electron impact ionisation. Ion spectra from 26 to 250 amu were collected with a data acquisition rate of 20 Hz s^{-1}.

4.3.4. Qualitative and quantitative analysis

Retention times and mass spectra of reference compounds were used to identify methylsulfanyl-volatiles, except for ethyl 3-(methylsulfanyl)prop-2-enoate whose spectrum was matched to National Institute of Standards and Technology (NIST) library. The peak area of the corresponding molecular ion peaks were integrated manually using the LECO chromaTOF software and the

critical limit for integration was set at a signal–noise-ratio of 20. For quantitative analysis, peak areas of 3-(methylsulfanyl)propionic acid derivatives were corrected against ethyl 3-([D3]-methylsulfanyl)propionate and 2-(methylsulfanyl)acetic acid derivatives against [D5]ethyl 2-(methylsulfanyl)acetate. Standard curves with a correlation coefficient $R^2 > 0.995$ were acquired by standard dilution series of each compound in a 'Hort 16A' model solution (5.5 g l^{-1} malic acid, 11.2 g l^{-1} citric acid, 20.6 g l^{-1} quinic acid, 1.3 g l^{-1} myo-inositol, 15 g l^{-1} sucrose, 39.3 g l^{-1} glucose 49.6 g l^{-1} fructose, pH 4.5) and dynamic headspace sampling at the parameters described. Ethyl 3-(methylsulfanyl)prop-2-enoate concentrations were expressed in equivalents of ethyl 3-(methylsulfanyl)propionate.

4.4. Quantitative real-time PCR analysis

Three RNA extractions were made from each sample according to Lopez-Gomez and Gomez-Lim (1992) and quantified with NanoDrop™ (Thermo scientific). 1 μg RNA of each replicate was treated with DNase I amplification grade (Invitrogen) prior to cDNA synthesis. First-strand cDNA was synthesised using the SuperScript™ VILO cDNA synthesis-kit (Invitrogen) according to the manufacturer's instructions and diluted 100-fold prior to use. The same procedure without addition of reverse transcriptase was applied for each replicate to test for gDNA contamination. Real-time gene transcription analysis of *Actinidia* AAT ESTs (Crowhurst et al., 2008) relative to the reference gene Ubiquitin9 were performed on a LightCycler® 480 platform using LightCycler® 480 SYBR Green master mix in a reaction volume of 5 μl. Two technical replicates of each biological replicate per sample were used and cDNA was replaced by water to test for false amplification. A standard dilution series with plasmid DNA was generated to evaluate the primer efficiencies of each primer, which were > 1.85. The results were analysed using the LightCycler 480 software (Roche) and quantified according to Pfaffl (2001). Programme: 1 min at 95 °C; 40 cycles of 10 s at 95 °C, 10 s at 60 °C, and 12 s at 72 °C; followed by melting curve analysis: 95 °C 5 s, 65 °C 60 s then ramping at 0.18 °C s^{-1} to 95 °C. The following primer pairs were used for real-time PCR (size of PCR-product is given in brackets):

Ubi 9 (109 bp): 5'- CCATTTCCAAGGTGTTGCTT -3' (forward); 5'- TACTTGTTCCGGTCCGTCTT-3'(reverse); AT15 (142 bp): 5'- ACAA CAGCAACAGCATGACC -3'(forward), 5'- AGTGCGGCATCTCCATAAAC -3'(reverse); AT2*:* 5'- CTCCCTCCATCACATTCCAG -3' (forward); 5'- C GGCATCTCCATAAACACG -3' (reverse); AT18 (107 bp)*:* 5'- ACCCCGT CACAGTCATCAGAG-3' (forward); 5'- GCAATCCACCACAAGTTTTCG-3' (reverse); AT16 (133 bp)*:* 5'- CCATCACATTCCAGCATCAC-3' (forward); 5'- GTGTGGTGGAAAATGGCTTC-3' (reverse);

AT17 (160 bp): 5'- TGGCAGAGGGAGTTTCTAGC-3' (forward); 5'- TGGCTTCGAATGGCTCTTAT-3' (reverse); AT1 (198 bp): 5'- TCCC TTCATCCCATTCCAG-3' (forward); 5'- CGGCATCTCCATAAACACG-3' (reverse).

4.5. (Methylsulfanyl)alkanoate- ester production of cell free extract

All chemicals were purchased by Sigma–Aldrich New Zealand LTD unless stated otherwise.

4.5.1. (Methylsulfanyl)alkyl-CoA synthesis

2-(methylsulfanyl)acetyl-CoA (99% purity) and 3-(methylsulfanyl)propionyl-CoA (purity 99.5%) were synthesised by reaction between the corresponding MeS-anhydride (see 2.3.1) and Coenzyme A Lithium salt according to Goldman and Vagelos (1961). The crude product was purified by RP-HPLC after Pourfarzam and Bartlett (1991), and quantified using the hydroxamate assay, modified from Lipmann and Tuttle (1945).

4.5.2. Protein crude extraction

0.5 g of pulverized tissue was extracted with 1 ml of 0.25 M potassium phosphate buffer (pH 7) containing Complete™ protease inhibitor (Roche Applied Science), 1% Triton and PVPP. The supernatant was desalted with a NAP-5 column (Illustra™) and eluted in assay buffer (0.25 M potassium phosphate buffer, pH 8, 10% glycerol, complete™ protease inhibitor tablet). Total protein concentrations were quantified using the Quick start™ Bradford protein assay (Bio-Rad) and BSA-standard curve. Two replicates were extracted per time point.

4.5.3. AAT assay

10 ml glass vials with pierced Teflon liners were used to mix 600 μg of crude protein, assay buffer, 3 mM methanol, ethanol, propanol and butanol, 1 μM of each deuterated internal standard and 300 μM of each (methylsulfanyl)alkyl-CoA in a reaction volume of 2 ml. After one hour at 33 °C, a 65 μm polydimethylsiloxane-divinylbenzene (PDMS-DVB) HS-SPME fibre (Supelco) was injected into the headspace of the sample and volatiles were adsorbed onto the fibre for 15 min. The same GC-MS-TOF equipment was utilised as described above but HS-SPME fibres were injected manually with an injector temperature of 220 °C in splitless mode. A GC-oven programme of 90 °C for 1 min, 5 °C min^{-1} to 200 °C, and hold for 1 min was applied. The spectra were compared with those obtained with boiled and substrate-free protein extracts and only additional peak areas were considered for integration.

(Methylsulfanyl)alkanoate-esters were identified according to reference compounds and quantified in equivalents of the corresponding deuterated internal standard.

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References

Antunes, M.D.C., Sfakiotakis, E.M., 2002. Chilling induced ethylene biosynthesis in 'Hayward' kiwifruit following storage. Scientia Horticulturae 92, 29–39.

Aubert, C., Bourger, N., 2004. Investigation of volatiles in *Charentais cantaloupe* melons (*Cucumis melo* Var. Cantalupensis). Characterization of aroma constituents in some cultivars. J. Agric. Food Chem. 52, 4522–4528.

Burdock, G.A., 2004. Fenaroli's Handbook of Flavor Ingredients, fifth ed.. CRC Press, Boca Raton.

Crowhurst, R.N., Gleave, A.P., MacRae, E.A., Ampomah-Dwamena, C., Atkinson, R.G., Beuning, L.L., Bulley, S.M., Chagne, D., Marsh, K.B., Matich, A.J., Montefiori, M., Newcomb, R.D., Schaffer, R.J., Usadel, B., Allan, A.C., Boldingh, H.L., Bowen, J.H., Davy, M.W., Eckloff, R., Ferguson, A.R., Fraser, L.G., Gera, E., Hellens, R.P., Janssen, B.J., Klages, K., Lo, K.R., MacDiarmid, R.M., Nain, B., McNeilage, M.A., Rassam, M., Richardson, A.C., Rikkerink, E.H., Ross, G.S., Schroeder, R., Snowden, K.C., Souleyre, E.J., Templeton, M.D., Walton, E.F., Wang, D., Wang, M.Y., Wang, Y.Y., Wood, M., Wu, R., Yauk, Y.K., Laing, W.A., 2008. Analysis of expressed sequence tags from *Actinidia*: applications of a cross species EST database for gene discovery in the areas of flavor, health, color and ripening. BMC Genom. 9, 351.

Cuevas, J.C., Lopez-Cobollo, R., Alcazar, R., Zarza, X., Koncz, C., Altabella, T., Salinas, J., Tiburcio, A.F., Ferrando, A., 2008. Putrescine is involved in Arabidopsis freezing tolerance and cold acclimation by regulating abscisic acid levels in response to low temperature. Plant Physiology 148, 1094–1105.

El-Sharkawy, I., Manriquez, D., Flores, F.B., Regad, F., Bouzayen, M., Latche, A., Pech, J.C., 2005. Functional characterization of a melon alcohol acyl-transferase gene family involved in the biosynthesis of ester volatiles. Identification of the crucial role of a threonine residue for enzyme activity. Plant Mol. Biol. 59, 345–362.

Escudero, A., Hernandez-Orte, P., Cacho, J., Ferreira, V., 2000. Clues about the role of methional as character impact odorant of some oxidized wines. J. Agric. Food Chem. 48, 4268–4272.

Escudero, A., Gogorza, B., Melus, M.A., Ortin, N., Cacho, J., Ferreira, V., 2004. Characterization of the aroma of a wine from maccabeo. Key role played by compounds with low odor activity values. J. Agric. Food Chem. 52, 3516–3524.

Escudero, A., Campo, E., Farina, L., Cacho, J., Ferreira, V., 2007. Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. J. Agric. Food Chem. 55, 4501–4510.

Ferreira, V., Pet'ka, J., Cacho, J., 2005. Intensity and persistence profiles of flavor compounds in synthetic solutions. Simple model for explaining the intensity and persistence of their aftersmell.. J. Agric. Food Chem. 54, 489–496.

Friel, E.N., Wang, M., Taylor, A.J., MacRae, E.A., 2007. In vitro and in vivo release of aroma compounds from yellow-fleshed kiwifruit. J. Agric. Food Chem. 55, 6664–6673.

Goldman, P., Vagelos, P.R., 1961. The specificity of triglyceride synthesis from diglycerides in chicken adipose tissue. J. Biol. Chem. 236, 2620–2623.

Groppa, M.D., Benavides, M.P., 2008. Polyamines and abiotic stress: Recent advances. Amino Acids 34, 35–45.

Jaeger, S.R., Rossiter, K.L., Wismer, W.V., Harker, F.R., 2003. Consumer-driven product development in the kiwifruit industry. Food Qual. Pref. 14, 187–198.

Johnston, J.W., Gunaseelan, K., Pidakala, P., Wang, M., Schaffer, R.J., 2009. Co-ordination of early and late ripening events in apples is regulated through differential sensitivities to ethylene. J. Exp. Bot. 60, 2689–2699.

Jordan, M.J., Margaria, C.A., Shaw, P.E., Goodner, K.L., 2002. Aroma active components in aqueous kiwi fruit essence and kiwi fruit puree by GC–MS and multidimensional GC/GC–O. J. Agric. Food Chem. 50, 5386–5390.

Klesk, K., Qian, M., 2003. Aroma extract dilution analysis of cv. Marion (*Rubus* spp. hyb) and cv. Evergreen (*R-laciniatus* L.) blackberries. J. Agric. Food Chem. 51, 3436–3441.

Landaud, S., Helinck, S., Bonnarme, P., 2008. Formation of volatile sulfur compounds and metabolism of methionine and other sulfur compounds in fermented food. Appl. Microbiol. Biotechnol. 77, 1191–1205.

Lipmann, F., Tuttle, C., 1945. A specific micromethod for the determination of acyl phosphates. J. Biol. Chem. 159, 21–28.

Lopez-Gomez, R., Gomez-Lim, M., 1992. A method for extracting intact RNA from fruits rich in polysaccharides using ripe mango mesocarp. Hortscience 27, 440–442.

Lucchetta, L., Manriquez, D., El-Sharkawy, I., Flores, F.B., Sanchez-Bel, P., Zouine, M., Ginies, C., Bouzayen, M., Rombaldi, C., Pech, J.C., Latche, A., 2007. Biochemical and catalytic properties of three recombinant alcohol acyltransferases of melon. Sulfur-containing ester formation, regulatory role of CoA-SH in activity, and sequence elements conferring substrate preference. J. Agric. Food Chem. 55, 5213–5220.

Mahattanatawee, K., Perez-Cacho, P.R., Davenport, T., Rouseff, R., 2007. Comparison of three lychee cultivar odor profiles using gas chromatography–olfactometry and gas chromatography-sulfur detection. J. Agric. Food Chem. 55, 1939–1944.

Marsh, K.B., Friel, E.N., Gunson, A., Lund, C., MacRae, E., 2006. Perception of flavour in standardised fruit pulps with additions of acids or sugars. Food Qual. Pref. 17, 376–386.

Matich, A., Rowan, D., 2007. Pathway analysis of branched-chain ester biosynthesis in apple using deuterium labeling and enantioselective gas chromatography-mass spectrometry. J. Agric. Food Chem. 55, 2727–2735.

Olias, R., Perez, A.G., Sanz, C., 2002. Catalytic properties of alcohol acyltransferase in different strawberry species and cultivars. J. Agric. Food Chem. 50, 4031–4036.

Patterson, K., Burdon, J., Lallu, N., 2003. 'Hort 16A' kiwifruit: progress and issues with commercialisation. Acta Horticulturae 610, 267–273.

Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29 (9), e45.

Pineau, B., Barbe, J.C., Van Leeuwen, C., Dubourdieu, D., 2009. Examples of perceptive interactions involved in specific "red-" and "black-berry" aromas in red wines. J. Agric. Food Chem. 57, 3702–3708.

Pourfarzam, M., Bartlett, K., 1991. Products and intermediates of the beta-oxidation of [U-14C]hexadecanedionoyl-mono-CoA by rat liver peroxisomes and mitochondria. Biochem. J. 273 (Pt 1), 205–210.

Pripis-Nicolau, L., de Revel, G., Bertrand, A., Lonvaud-Funel, A., 2004. Methionine catabolism and production of volatile sulphur compounds by *Oenococcus oeni*. J. Appl. Microbiol. 96, 1176–1184.

Ritenour, M.A., Crisosto, C.H., Garner, D.T., Cheng, G.W., Zoffoli, J.P., 1999. Temperature, length of cold storage and maturity influence the ripening rate of ethylene-preconditioned kiwifruit. Postharvest Biol. Technol. 15, 107–115.

Schaffer, R.J., Friel, E.N., Souleyre, E.J.F., Bolitho, K., Thodey, K., Ledger, S., Bowen, J.H., Ma, J.H., Nain, B., Cohen, D., Gleave, A.P., Crowhurst, R.N., Janssen, B.J., Yao, J.L., Newcomb, R.D., 2007. A Genomics approach reveals that aroma production in apple is controlled by ethylene predominantly at the final step in each biosynthetic pathway. Plant Physiol. 144, 1899–1912.

Schotsmans, W.C., Mackay, B., Mawson, A.J., 2008. Temperature kinetics of texture changes in *Actinidia chinensis* 'Hort 16A' during storage. J. Horticult. Sci. Biotechnol. 83, 760–764.

Sen, A., Grosch, W., 1991. Synthesis of 6 deuterated sulfur-containing odorants to be used as internal standards in quantification assays. Z. Lebensm. Unters. For. 192, 541–547.

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

Steinhaus, M., Sinuco, D., Polster, J., Osorio, C., Schieberle, P., 2008. Characterization of the aroma-active compounds in pink guava (*Psidium guajava* L.) by application of the aroma extract dilution analysis. J. Agric. Food Chem. 56, 4120–4127.

Steinhaus, M., Sinuco, D., Polster, J., Osorio, C., Schieberle, P., 2009. Characterization of the key aroma compounds in pink guava (*Psidium guajava* L.) by means of aroma re-engineering experiments and omission tests. J. Agric. Food Chem 57, 2882–2888.

Szego, D.K., Horvath, E., 2007. Role of S-methylmethionine in the plant metabolism. Acta Agron. Hung. 55, 491–508.

Takeoka, G., Buttery, R.G., Flath, R.A., Teranishi, R., Wheeler, E.L., Wieczorek, R.L., Guentert, M., 1989. Volatile constituents of pineapple (*Ananas-Comosus* [L.] Merr). ACS Symp. Ser. 388, 223–237.

Takeoka, G.R., Buttery, R.G., Teranishi, R., Flath, R.A., Guentert, M., 1991. Identification of additional pineapple volatiles. J. Agric. Food Chem. 39, 1848–1851.

Takeoka, G.R., Buttery, R.G., Flath, R.A., 1992. Volatile constituents of Asian pear (*Pyrus serotina*). J. Agric. Food Chem. 40, 1925–1929.

Vallet, A., Lucas, P., Lonvaud-Funel, A., de Revel, G., 2008. Pathways that produce volatile sulphur compounds from methionine in *Oenococcus oeni*. J. Appl. Microbiol. 104, 1833–1840.

Weenen, H., Koolhaas, W.E., Apriyantono, A., 1996. Sulfur-Containing Volatiles of Durian Fruits (*Durio zibethinus* Murr.). J. Agric. Food Chem. 44, 3291–3293.

Werkhoff, P., Guntert, M., Krammer, G., Sommer, H., Kaulen, J., 1998. Vacuum headspace method in aroma research: flavor chemistry of yellow passion fruits. J. Agric. Food Chem. 46, 1076–1093.

Wyllie, S.G., Fellman, J.K., 2000. Formation of volatile branched chain esters in bananas (*Musa sapientum* L.). J. Agric. Food Chem. 48, 3493–3496.

Wyllie, S.G., Leach, D.N., 1992. Sulfur-containing-compounds in the aroma volatiles of melons (*Cucumis melo*). J. Agric. Food Chem. 40, 253–256.

Yahyaoui, F.E., Wongs-Aree, C., Latche, A., Hackett, R., Grierson, D., Pech, J.C., 2002. Molecular and biochemical characteristics of a gene encoding an alcohol acyl-transferase involved in the generation of aroma volatile esters during melon ripening. Eur. J. Biochem. 269, 2359–2366.

Young, H., Paterson, V.J., 1985. The effects of harvest maturity, ripeness and storage on kiwifruit aroma. J. Sci. Food Agric. 36, 352–358.