Advances in *Actinidia* Research (VI)

猕猴桃研究进展(VI)

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黄宏文 主编

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内 容 简 介

本书收录了国内猕猴桃会议代表提交的论文,以及近两年国外猕猴桃研究的新动态论文,内容涉及从产业、栽培管理技术、病虫害防治、生物技术采后贮藏加工、遗传育种到资源利用的7个主要领域。所录论文是国内外近年来从事猕猴桃研究、管理、开发利用人员的成果或工作积累,以及针对一些产业发展问题和新技术应用提供建议。

本书是供广大从事猕猴桃科研、教学、推广与生产、市场销售等领域人员参考的重要资料,适合科研人员、教师、大中专学生、职业院校及从事果树行业管理的行政部门人员、基层科技人员,以及猕猴桃爱好者阅读。

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《猕猴桃研究进展(VI)》编委会

前　言

（PREFACE）

猕猴桃（*Actinidia chinensis* Planch.）是20世纪初以来驯化栽培成功的水果，至今仅有100余年的历史。猕猴桃以其独特的风味，富含维生素C、饮食纤维、多种矿物营养，以及清肠健胃功效而得到广泛青睐，成为重要的水果种类之一。猕猴桃的驯化栽培被认为是近代由野生到人工商品化栽培最成功的植物驯化范例。

我国自1978年开展全国猕猴桃属植物野生资源普查以来，经过30余年的发展，充分利用自己的资源优势大步追赶世界先进水平，现已成为世界栽培面积第一、产量第二的猕猴桃生产大国。至2010年，我国栽培面积达7.0万hm^2，占世界栽培总面积的45%；产量达49.0万t，占世界产量的28%。我国猕猴桃产业的快速发展进程凝结了我国猕猴桃科技人员、果农及经销者多年的拼搏和不懈的追求！也正是为了这个追求，中国园艺学会猕猴桃分会于2004年开始，每2年召开1次全国学术与产业研讨会，已分别在湖南吉首、广东和平、陕西杨陵和四川蒲江举办了四届，每次会议之后均出版了《猕猴桃研究进展》系列学术论文集，本卷是继《猕猴桃研究进展（V）》之后的第VI卷，是在收集第四届猕猴桃研讨会会议论文的基础上，增加了世界主要生产国的知名专家在猕猴桃上的科研和产业等方面的研究论文，旨在为中外研究人员提供世界猕猴桃研究进展和市场、生产最新信息。

本书共分新品种选育、资源开发与利用、栽培及生理、生物技术、贮藏保鲜与加工、产业与市场6个部分，系统地提供了国内外猕猴桃科研与产业、市场发展趋势，也结合我国猕猴桃发展现状和存在问题，提出了我国猕猴桃科研重点、产业方向和市场策略。

由于能力和水平所限，书中疏漏之处在所难免，恳请大家批评指正。借此机会再次向为本书提供文献的作者和对分会和本书给予支持的领导和同仁们表示衷心的感谢，并希望继续得到你们更多的指导和支持！

中国园艺学会猕猴桃分会

2011.6.10于武汉

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利用多因子分析鉴定二倍体中华猕猴桃品种果实品质相关挥发性物质种类

成灿红[1*]　Alan G. Seal[1]　Elspeth A. MacRae[2]　Mindy Y. Wang[3]

(1 新西兰植物与食品研究院,Te Puke 研究中心,RD 2,Te Puke,New Zealand

2 SCION,Private Bag 3020,Rotorua,New Zealand

3 新西兰植物与食品研究院,Mount Albert 研究中心,Private Bag 92 169,Auckland,New Zealand)

摘　要　在对果实中的糖、酸类物质影响风味进行了很多关注的同时,往往对果实风味与香气同样有重要作用的挥发性物质却难以定义。那些对中华猕猴桃品种风味有重要贡献挥发性物质的鉴定,可以大大提高果实风味改良育种的效率与降低成本。本研究就是利用多因子分析来探讨挥发性化合物、品质鉴评和中华猕猴桃品种果实特性来确定影响风味的主要挥发性物质,以及优良亲本及选择方法。我们从一个育种群体中利用主成分分析(PCA)方法,依据果实特性和风味多样性相关性状为原则选择了24 个基因型进行测试,发现了72 个挥发性物质。事实证明,利用扩展的多因子分析可以从有限的材料中发掘最大量的信息,在主成分分析中,为避免其中单因子相关矩阵分析的缺陷,我们基于挥发性物质表型相关多因子进行聚类分析,成功将这些物质被聚类成4 大家族。根据这些分支,进一步利用PCA 和多因子线性回归分析,探讨了挥发性物质、感官评价和果实性状间的相关性。主成分分析提供了对影响消费者反应的挥发性物质进行综合权衡的依据,13 种对中华猕猴桃品种果实品质有重要影响的挥发性物质被鉴定,'Hort16A'中有5 种脂类对其果实风味和香气有重要决定性作用。与"酸味"、"果实成熟"、"Hort16A 非典型性香气"和"非猕猴桃味(一般指'海沃德')"相关的挥发性物质也鉴定出来。同时,对具备不同挥发性物质的潜在亲本选择以及猕猴桃的风味改良选育的可行性方法也进行了总结。

关键词　猕猴桃　育种　风味　挥发性物质　多因子分析

Identifying Volatile Compounds Associated with Sensory and Fruit Attributes in Diploid *Actinidia chinensis*(kiwifruit) Using Mutivariate Analysis

1　Introduction

Kiwifruit belong to the genus *Actinidia*, which comprises more than 60 species native to large parts of China and some neighbouring countries. All known species are dioecious and grow as long-lived, perennial, woody vines. The familiar green-fleshed kiwifruit of the cultivar 'Hayward' belongs to the species *A. deliciosa*(A. Chev.) Liang et A. R. Ferguson and is grown commercially in many countries. Yellow-fleshed cultivars of *A. chinensis* Planch. are grown widely in China and increasingly in other kiwifruit producing countries. The 'Hayward' cultivar is characteristically described by consumers as having a fresh, sweet and acid flavour, while the commercial yellow-fleshed *A. chinensis* cultivar 'Hort16A' (marketed as ZESPRI® GOLD Kiwifruit) is described as having sweet, banana and blackcurrant-like flavours (Jaeger et al.,2003).

* 通讯作者,E-mail:Canhong. cheng@ plantandfood. co. nz

Flavour is a key aspect of fruit quality and is generally defined as a combination of aroma and taste sensations. While the roles of sugars and acids in taste are well known and described, the volatile compounds that contribute to flavour and odour are more difficult to define. Many of the earlier studies on kiwifruit aroma focused on the composition of volatile compounds. Up to 90 volatiles were identified from fruit of 'Hayward' (Young et al., 1983; Young and Paterson, 1985; Takeoka et al., 1986; Paterson et al., 1991; Young and Paterson, 1995; Young et al., 1995; Perera et al., 1998). Between 40 and 80 volatiles were detected from fruit of *A. arguta* genotypes (Matich et al., 2003). Hex-*E*2-enal was identified as the major component in mature fruit of 'Hayward' but on further ripening, ethyl butanoate began to dominate the profile (Bartley and Schwede, 1989). Young et al. (1995) reported that the sweetness of ripe 'Hayward' kiwifruit was associated with high concentrations of esters, which account for up to 85% of the total volatiles in kiwifruit (Crowhurst et al., 2008). In the yellow-fleshed cultivar 'Hort16A', acetaldehyde, hexanal, hex-*E*2-enal and ethyl butanoate were found to be important contributors to aroma (Friel et al., 2007).

However, because many volatile compounds are not flavour active in a particular product, one of the major difficulties in studying odour is the determination of those compounds that make a real contribution to the flavour of food (Mistry et al., 1997). Odour activity values (OAVs) are used to estimate the odour contribution of individual food volatiles, and are calculated as the ratio between the concentration of an individual substance in a sample and the threshold concentration of that substance (i. e., the minimal concentration that can be detected by the human nose) (Rothe and Thomas, 1963). Buttery (1993) found that of 400 volatiles identified in tomatoes, only 16 reached their OAVs. Hundreds of volatiles have also been identified in apple (Dixon and Hewett, 2000) and strawberry (Dirinck et al., 1981; Pérez et al., 1996), but of these, only a small proportion was classified as 'character impact' volatiles. Of 60 volatiles identified from 'Hayward' kiwifruit using GC/MS-O (gas chromatography/mass spectrometryolfactometry), only 30 were odour-active (Frank et al., 2007). Hex-*E*2-enal ('marzipan, sweet') had the greatest odour impact, followed by 1-penten-3-one ('plastic, herbal, glue, kiwifruit-like') and hexanal ('green, fresh crushed leaves'). In 'Hayward' essence and puree, hex-*E*2-enal was perceived by GC-O as imparting a fruity, strawberry and cherry flavour (Jordán et al., 2002).

Olfactometry is thus a valuable tool as it allows a complex volatile mixture to be separated temporally into individual compounds, but it does not take account of the complexity of human sensory perception (Frank et al., 2007). Sensory evaluation methods offer an organized way to collect information on the sensory attributes of food samples as perceived by the human senses. To take into account the multivariate nature of the data, multivariate chemometric data analysis techniques ought to be applied (Daszykowski et al., 2007). Thus, a complementary approach to studying flavour is to use multivariate statistics to identify associations between sensory and chemical data that may indicate potentially important flavour volatiles that contribute most to the sensory experience. This approach recognizes that while individual flavour components are responsible for taste and odour, the total sensation determined from sensory analysis results from the integration of all the individual flavour stimuli in a mixture (Rouseff and Leahy, 1995; Frank et al., 2007). This type of approach has been used in studies of tomato, where relationships between sensory attributes and volatiles have been identified using multiple linear regression (MLR) (Baldwin et al., 1998; Tandon et al., 2003) and PCA (Krumbein et al., 2004).

Variation has been reported in the quantity of volatiles in thirteen apple cultivars (Young et al., 2004)

and six apricot cultivars (Guillot et al., 2006). López et al. (1998) found that odour components varied in class and quantity in two apple cultivars and were associated with sensory properties characteristic of each cultivar. During fruit ripening, a number of different biosynthetic pathways are involved in volatile synthesis (Dixon and Hewett, 2000; González-Agüero et al., 2009; Zhang et al., 2009). These have not been fully described, but appear to be common to different fruits (Dixon and Hewett, 2000). To date, only a few genes that directly influence fruit flavour biogenesis have been reported, including alcohol dehydrogenase (*ADH2*) in tomato (Speirs et al., 1998), terpene synthases in strawberry (Aharoni et al., 2004), antisense ACC (1-aminocyclopropane carboxylic acid) oxidase and alcohol acyltransferase (AAT) in apple (Schaffer et al., 2007; Souleyre et al., 2005; Li et al., 2006) and lipoxygenase (LOX) in kiwifruit (Zhang et al., 2009).

In an ever more competitive and global market, flavour quality is increasingly important to consumers. Therefore, knowledge of kiwifruit flavour is of utmost importance in developing new cultivars. While studies of the composition of volatile compounds have detected up to 90 volatiles in kiwifruit cultivars and genotypes, there are only a few published studies on 'flavour impact' volatiles related to sensory attributes in 'Hayward' (Frank et al., 2007; Jordán et al., 2002). Moreover, because of the large number of volatiles, the assessment of the volatiles contributing to flavour within breeding populations is especially challenging. Therefore, the main goal of this study was to evaluate multivariate analyses of associations between volatiles, sensory and fruit attributes to identify potential key impact volatiles that make a substantial contribution to the flavour and odour of *A. chinensis* fruit, using 24 genotypes representing the diversity of taste and fruit characteristics in a breeding population. The study also aimed to identify economic and efficient breeding methods for improving flavour volatiles and superior parents for use in our breeding programme.

2 Materials and methods

2.1 Plant material

Twenty-four genotypes of diploid *A. chinensis* were sampled in April 2001 and were numbered from P1 to P24. Twenty-two were seedlings chosen from among 289 females in a population derived from a factorial mating design (3 females × 13 males), planted in 1994 at the Te Puke Research Centre, New Zealand. The other two genotypes were female parents of this population, Female B (P23) and Female C (P24), respectively. The third female parent, Female A, was missing from our study. The three female parents (A, B and C) were selected as testers based on results from previous studies (Cheng et al., 2004). Both Female A and Female B had moderate dry matter (DM) and soluble solids content (SSC). Female C was 'Hort16A', a yellow-fleshed cultivar with large fruit renowned for their good flavour (Jaeger et al., 2003). The 13 male parents were selected randomly from our germplasm collection. The 22 seedling genotypes were selected to be broadly representative of the population, based on the first two principal components (PCs) derived from 14 variables analysed in a previous study (Cheng et al., 2004). The variables included four sugars (fructose, glucose, sucrose and *myo*-inositol), three organic acids (malic, quinic and citric), fruit pH, titratable acidity, vitamin C and four fruit characters (fruit number per vine, fruit weight, DM and SSC at eating ripeness). The two PCs were associated with the traits "sweet taste factor" and "sour taste factor", and accounted for 50.7% of the total variance. The 22 genotypes selected showed extreme differences in combinations of the two PCs. This approach was expected to maximise the discrimination of phenotypes

among vines in this population.

From 50 to 60 fruit were harvested at random from each vine when the mean SSC of a three-fruit sample reached 10%. SSC and DM were measured at harvest as described in Cheng et al. (2004). The remaining fruit were cool stored at 1.5 ℃ before sensory and chemical analyses were performed.

2.2 Fruit sampling and chemical analysis

After 1 month of storage, fruit were removed from the cool store and ripened at 20 ℃ for 3 ~ 5 days. Fifteen fruit (firmness 0.5 ~ 0.7 kgf) from each vine were selected for sensory analysis. Each end of each fruit was sampled for SSC, and a longitudinal slice (1/3) was removed for chemical analysis. One half of this slice was used for sugar (fructose, glucose, sucrose and *myo*-inositol) and acid (malic, quinic and citric acids) analysis and the other half was sampled for volatile analysis. The remaining part of the fruit was used for sensory analysis (see below). The fruit samples for sugar and acid analysis were frozen in liquid nitrogen immediately and stored at −20 ℃ prior to analysis. The individual sugar and acid contents were measured by GLC (gas-liquid chromatographic) analysis as described by Cheng et al. (2004).

For volatile analysis, the methods of Matich et al. (2003) were used with minor modifications. Headspace volatiles released from 1 to 1.5 g of the pulped tissue were collected with a Chromosorb 105 absorbent trap (100 mg per trap) for 15 min at 23 ℃ at a flow rate of 25 ml min^{-1}. The sampled traps were stored at −15 ℃ and analysed using GC-MS (HP5890-VG70) with a DB-wax column (*J&W*, 30 m × 0.25 mm × 0.25 μm), within 2 weeks. Peaks were identified and quantified as described by Matich et al. (2003). All analyses were performed on triplicate tissue samples.

2.3 Sensory analysis

A panel of eight in-house assessors, trained to evaluate the flavour of kiwifruit, was selected (Marsh et al., 2006; Wang et al., 2010). Sensory analysis was performed in a sensory panel room at 20 ℃. Sensory attributes were recorded for sweet taste, sour taste, 'Hort16A'-like flavour and odour and ethyl butanoate flavour and odour. The attribute intensities were recorded on 150 mm unstructured line scales anchored at zero for absent and 150 for extreme, using CompusenseTM 5 sensory software. The attributes were calibrated using 'Hort16A' fruit for both 'Hort16A'-like flavour and odour (100), 20 g l^{-1} and 40 g l^{-1} sucrose solutions for sweet taste (40 and 100, respectively), and 1 g l^{-1} malic acid solution for sour taste (115) as in Marsh et al. (2006). Sensory samples of fruit were presented monadically to panellists stem-end up in coded plastic crème cups. Each sample was cut into three slices. The stem end and middle slice were used for odour and flavour assessment, respectively. The remaining third was used for texture assessment (data not shown). The eight assessors analyzed 24 genotypes of kiwifruit during six sessions. The final sensory data were averaged across the eight assessors.

2.4 Statistical and multivariate analysis

In this study, there were 72 volatile compounds (attributes), but only 24 genotypes (observations). Thus, the dimensions of the data matrix were 24 × 72. For PCA, the correlation matrix for the data was singular and some scoring coefficients were zero (Daszykowski et al., 2007). To overcome this problem, the volatile data were regrouped, using hierarchical clustering of variables as implemented by PROC VARCLUS (SAS Institute Inc., 2003), based on a phenotypic correlation matrix between volatiles estimated by PROC CORR. The VARCLUS procedure divided volatile variables into hierarchical clusters. Four clusters were arbitrarily chosen, to make the number of volatile attributes within the cluster equal to or less than the number of observations (24) (Anderberg, 1973). The four clusters had 19, 13, 20 and 19 volatiles,

respectively(Table 1). In Cluster 1, because four ketones (2-methylpentan-3-one, 4-methyl-2-hexanone, 4-methylpentan-2-one and 5-methylhexan-2-one) and butyl acetate were found only in P2, some scoring coefficients of the PCA were zero because of the singular correlation matrix. Thus, only butyl acetate was included in Cluster 1 for PCA (Table 1). A PCA was carried out on the volatiles in each of the four clusters, using the PRINCOMP procedure of SAS(SAS Institute Inc., 2003). In accordance with Morrison (1990), the analysis was performed on standardised data. The un-rotated structure of each analysis was used to interpret the meaning of the components because the emphasis was on ordination rather than structure explanation. To minimize the number of principal components used for each character ('parsimony') (Morrison, 1990), we set the cut-off at 70 % of the total variance. This meant it was necessary to consider only 14 principal components (PCs) for all four clusters (Table 2).

Multiple linear regressions (MLR) of DM, sugars, acids and sensory attributes were conducted as a function of the 14 PCs, to explore associations between volatile compounds, sensory and fruit attributes and to identify potential key 'flavour impact' volatiles of fruit of *A. chinensis*. MLR was carried out using PROC REG with the STB(standardized β) option of SAS(SAS Institute Inc., 2003). A standardisation for the regression coefficient(STB) is the process whereby raw data are transformed into new variables with a mean of 0 and a standard deviation of 1. DM and SSC were very highly correlated and showed very similar correlations with other taste and fruit characters(Cheng et al. 2004). Therefore, this study reports only the relationship between DM and volatiles. The combined analysis of PCA and MLR enabled 13 potential key 'flavour impact' volatiles in *A. chinensis* fruit to be identified. Another PCA was carried out with only the 13 volatiles, to enable differentiation of genotypes. The parsimony criterion subsequently led to the first two PCs being used. MLRs of sensory attributes were conducted as a function of the two PCs of key volatiles, to specify the relationship between key volatiles and sensory attributes and to identify superior parents for flavour breeding.

Table 1 Occurrence and relative abundance of volatile compounds found in 24 genotypes of *Actinidia chinensis*

Compound	Occurrence (genotypes)	Mean /(ng g^{-1} FW)	Minimum /(ng g^{-1} FW)	Maximum /(ng g^{-1} FW)	'Hort16A' /(ng g^{-1} FW)	Cluster no.
Aldehyde						
Acetaldehyde	15	6.37	0	42.27	5.28	4
Propanal	24	3.97	0.83	7.83	3.60	1
Butanal	22	0.99	0	2.60	0	2
2-methylbutanal	3	0.21	0	2.54	0	1
Hexanal	24	128.95	3.94	616.98	28.24	4
Hex-*E*3-enal	22	4.76	0	23.93	0	4
Hex-*Z*3-enal	21	19.84	0	433.16	0	3
Heptanal	23	2.93	0	11.42	1.82	1
Hex-*Z*2-enal	22	11.74	0	39.01	4.45	4
Hex-*E*2-enal	24	686.48	17.15	2 484.81	123.50	2
Octanal	24	4.87	0.98	22.86	2.06	3
Nonanal	15	10.86	0	34.30	0	2
Decanal	20	17.00	0	78.05	0	3
Benzaldehyde	24	2.26	0.40	19.22	1.01	1

Table 1 Occurrence and relative abundance of volatile compounds found in 24 genotypes of *Actinidia chinensis*

(*Continued*)

Compound	Occurrence (genotypes)	Mean /(ng g^{-1} FW)	Minimum /(ng g^{-1} FW)	Maximum /(ng g^{-1} FW)	'Hort16A' /(ng g^{-1} FW)	Cluster no.
Ethylbenzaldehyde	24	14.36	1.86	180.01	10.99	4
4-Ethylbenzaldehyde	22	8.14	0	155.57	2.60	4
Ester						
Ethyl acetate	23	12.26	0	55.60	2.56	4
Ethyl propanoate	15	7.67	0	51.87	0.39	4
Ethyl 2-methylpropanoate	17	1.16	0	8.51	0.46	2
Methyl butanoate	14	2.46	0	26.49	1.33	4
Ethyl butanoate	21	75.95	0	977.65	42.82	4
Butyl acetate	1	0.03	0	0.73	0	1
Ethyl hexanoate	10	1.41	0	11.53	0.48	4
Methyl benzoate	19	0.68	0	1.86	1.33	3
Ethyl benzoate	21	1.91	0	3.97	3.75	2
Methyl salicylate	13	0.89	0	7.88	0	1
Terpene						
α-Pinene	24	13.92	0.26	57.08	13.80	4
Camphene	7	0.27	0	2.41	0	4
β-Pinene	24	21.03	0.25	99.9	18.56	4
Sabinene	19	2.47	0	13.07	1.42	2
α-Terpinene	5	0.63	0	5.09	0	1
Limonene	16	1.70	0	6.06	0	2
Cineole,1,8-	5	0.53	0	10.76	0	2
γ-terpinene	17	1.64	0	6.05	1.38	1
ρ-Cymene	20	2.82	0	17.35	2.08	3
Terpinolene,iso-	2	0.50	0	6.62	0	1
Geranyl acetone	23	8.88	0	113.96	4.19	3
Hydrocarbon						
Benzene	24	5.45	1.36	13.97	4.76	1
Styrene	24	5.68	0.41	24.81	7.57	4
Toluene	24	9.29	4.52	18.92	12.23	1
Trimethyl benzene	23	2.27	0	5.18	1.47	2
Pentandodecane	22	3.64	0	10.14	0	3
Alcohol						
Methanol	24	11.36	0.85	84.22	45.71	2
Ethanol	24	1 536.83	48.35	4 468.81	2 066.28	4
Propanol	19	13.91	0	256.96	11.43	4
1-Methoxy-propan-2-ol	2	0.25	0	4.26	0	2
Butanol	19	2.49	0	8.41	7.15	3
Penten-3-ol	21	2.602	0	12.17	0.69	3
Pentanol	23	2.19	0	8.03	0.29	3

Table 1 Occurrence and relative abundance of volatile compounds found in 24 genotypes of *Actinidia chinensis*

(*Continued*)

Compound	Occurrence (genotypes)	Mean /(ng g^{-1} FW)	Minimum /(ng g^{-1} FW)	Maximum /(ng g^{-1} FW)	'Hort16A' /(ng g^{-1} FW)	Cluster no.
Hexanol	24	8.81	0.31	38.09	3.25	4
Hex-*Z*3-enol	18	12.84	0	211.41	7.42	3
Hex-*E*2-enol	24	19.71	1.81	68.42	6.57	1
Ether						
Tetrahydrofuran	23	2.31	0	29.60	1.04	1
Acid						
Acetic acid	24	20.74	5.42	56.97	12.72	3
Ketone						
Acetone	24	10.62	4.81	21.17	9.22	3
2-Butanone	23	2.02	0	11.50	0.47	3
3-Methylbutan-2-one	4	0.74	0	15.14	0	1
Butane-2,3-dione	1	0.01	0	0.20	0	3
Hexan-3-one	1	0.05	0	1.12	0	4
2-Methylpentan-3-one[a]	1	0.01	0	0.18	0	1
4-Methyl-2-hexanone[a]	1	0.17	0	4.00	0	1
4-Methylpentan-2-one[a]	1	0.08	0	1.96	0	1
Hexen-3-one	3	0.13	0	1.69	0	1
5-methylhexan-2-one[a]	1	0.23	0	5.41	0	1
3-hydroxybutan-2-one	1	0.013	0	0.31	0	3
6-methylhept-5-en-2-one	23	1.25	0	4.28	0	3
4-hydroxy-4-methylpentan-2-one	9	1.26	0	7.01	2.24	3
Unknowns						
Unknown 3826(1102)	11	2.51	0	15.26	0	2
Unknown 160	11	1.32	0	9.17	0	3
Unknown 3819(1511)	5	0.34	0	2.99	0	2
Unknown 3779(1588)	12	3.22	0	71.13	0	3

[a]volatile not included in PCA

Table 2 Standardized coefficients and percentage of variation of Principal Components(PCs)for four clusters of *Actinidia chinensis* volatiles

Volatile	C1_PC1	C1_PC2	C1_PC3
a. Cluster 1			
Propanal	0.648	-0.114	-0.231
Tetrahydrofuran	0.944	-0.039	0.074
3-Methylbutan-2-one	0.949	0.027	0.049
Benzene	0.458	-0.511	-0.171
2-Methylbutanal	0.413	0.574	-0.604
Toluene	0.614	-0.211	-0.167
Hexen-3-one	0.904	-0.233	0.010

Table 2 Standardized coefficients and percentage of variation of Principal Components(PCs) for four clusters of *Actinidia chinensis* volatiles (*Continued*)

Volatile	C1_PC1	C1_PC2	C1_PC3
Butyl acetate	0.948	-0.011	0.079
α-Terpinene	0.667	0.045	0.439
Heptanal	0.725	-0.080	0.234
γ-Terpinene	0.500	0.186	0.601
Terpinolene,iso-	0.675	0.501	-0.448
Hex-*E*2-enol	0.217	0.819	0.075
Benzaldehyde	0.976	-0.083	-0.011
Methyl salicylate	-0.154	0.448	0.563
Percentage of total variation/%	49.22	12.51	10.82

Volatile	C2_PC1	C2_PC2	C2_PC3	C2_PC4
b. Cluster 2				
Butanal	-0.435	0.700	-0.198	0.271
Methanol	0.543	0.251	0.709	-0.051
Ethyl 2-methylpropanoate	0.336	0.544	-0.592	-0.002
Unknown 3826(1102)	0.705	0.531	0.168	0.136
Sabinene	-0.467	0.263	0.100	-0.359
1-Methoxy-propan-2-ol	0.412	0.325	0.781	0.031
Unknown 3819(1511)	0.642	0.498	-0.370	-0.260
Limonene	-0.672	0.527	0.039	0.289
Cineole,1,8-	-0.501	0.566	-0.010	-0.051
Hex-*E*2-enal	0.277	0.054	-0.009	0.790
Trimethyl benzene	-0.670	0.388	0.308	-0.115
Nonanal	0.677	0.302	-0.160	-0.429
Ethyl benzoate	0.563	-0.074	-0.160	0.354
Percentage of total variation/%	29.98	18.47	14.04	10.19
c. Cluster 3				
Acetone	0.739	0.016	-0.103	-0.354
2-Butanone	0.107	-0.409	-0.263	-0.417
Butane-2,3-dione	-0.188	0.252	0.818	-0.033
Unknown 160	-0.313	0.578	-0.446	0.223
Hex-*Z*3-enal	0.897	0.326	0.062	0.071
Butanol	-0.370	0.627	0.107	-0.254
Penten-3-ol	0.743	0.344	0.097	0.055
Pentanol	0.619	-0.018	-0.001	0.409
ρ-Cymene	-0.066	0.306	0.828	-0.035
Octanal	0.949	0.065	0.093	-0.077
3-Hydroxybutan-2-one-	0.081	-0.118	-0.062	0.830
2-Methylbutanal	0.413	0.574	-0.604	
6-Methylhept-5-en-2-one	0.877	0.038	-0.054	0.092
4-Hydroxy-4-methylpentan-2-one	-0.347	0.743	-0.288	-0.132
Hex-*Z*3-enol	0.858	0.409	0.068	0.056
Acetic acid	0.732	0.007	-0.281	-0.273

Table 2 Standardized coefficients and percentage of variation of Principal Components(PCs) for four clusters of *Actinidia chinensis* volatiles (*Continued*)

Volatile	C3_PC1	C3_PC2	C3_PC3	C3_PC4
Pentandodecane	0.447	-0.213	-0.135	0.201
Decanal	0.871	-0.143	0.031	0.004
Unknown 3779(1588)	0.894	0.328	0.063	0.066
Methyl benzoate	0.601	-0.343	-0.006	-0.116
Geranyl acetone	-0.144	0.655	-0.455	0.013
Percentage of total variation/%	38.88	13.68	10.35	7.24
Volatile	C4_PC1	C4_PC2	C4_PC3	
d. Cluster 4				
Acetaldehyde	0.211	-0.238	-0.152	
Ethyl acetate	0.716	-0.125	0.519	
Ethanol	0.628	-0.308	0.377	
Ethyl propanoate	0.641	-0.023	0.421	
Methyl butanoate	0.895	-0.012	0.279	
Hexan-3-one	-0.098	-0.113	0.218	
α-Pinene	0.782	-0.012	-0.524	
Ethyl butanoate	0.844	-0.031	0.398	
Propanol	-0.164	0.918	0.175	
Camphene	0.809	0.104	-0.390	
Hexanal	0.583	0.284	-0.455	
β-Pinene	0.838	0.032	-0.453	
Hex-*E*3-enal	0.860	0.177	0.093	
Hex-*Z*2-enal	0.505	0.651	-0.331	
Ethyl hexanoate	0.652	-0.069	0.494	
Styrene	0.412	-0.347	-0.189	
Hexanol	0.918	0.262	-0.109	
Ethylbenzaldehyde	-0.150	0.907	0.238	
4-Ethylbenzaldehyde	-0.154	0.921	0.209	
Percentage of total variation/%	40.51	18.09	11.97	

3 Results and discussion

3.1 Chemical analysis

The composition of the volatile compounds from ripe fruit of the 24 genotypes of *A. chinensis* is summarised in Table 1. Seventy-two volatiles (16 aldehydes, 14 ketones, 11 terpenes, 10 esters, 10 alcohols, 5 hydrocarbons, 1 ether, 1 acid and 4 unknowns) were detected and quantified. Seventeen volatiles were found in all genotypes (Table1). Of these, ten were also found in *A. arguta* (Matich et al., 2003) and four in 'Hayward' (Young et al., 1995). Most volatiles of 'Hort16A' were detected in our genotypes, except for methyl carbonate, ethyl pentanoate and ethyl octanoate, which were found at low levels in 'Hort16A' (Wang et al., 2010). Our results confirmed that, as in 'Hayward' (Paterson et al., 1991), ethanol and hex-*E*2-enal were abundant volatiles in mature fruit of *A. chinensis*. Hexanal and ethyl butanoate were other major components in these *A. chinensis* fruit. Ten of eleven major volatile flavour compounds detected in 'Hayward' (*A. deliciosa*) by Young et al.

(1995) were also detected in our *A. chinensis* genotypes. Methyl hexanoate was not found but ethyl hexanoate, which was not a major compound identified by Young et al. (1995), was found in ten genotypes in this study. Our results also confirmed that 'Hort16A' had a trace level of ethyl hexanoate (Wang et al.,2010).

Twelve uncommon volatiles were detected in between one and three genotypes (Table 3) and of these, seven (four ketones and one each of aldehyde, ester and terpene) were found only in genotype P2. The ester butyl acetate, found in P2, occurs in many fruits and is a constituent of apple odours (Surburg and Panten,2006).

3.2 Multivariate analysis of volatiles

3.2.1 *'Flavour impact' volatiles of A. chinensis fruit*

Applying the parsimony criterion of 70 % for PCA meant it was necessary to consider only 14 PCs (Table 2), the first three or four from each of the four clusters (C1 to C4) grouped using VARCLUS based on a phenotypic correlation matrix between volatiles. Therefore, PCA offered a method to reduce the complexity of the multidimensional system, by maximizing the component loadings variance and eliminating less influential components. Moreover, the standardised coefficients of PCA revealed significant patterns in the volatile data (Table 2). In chemometric data analysis, PCA is usually applied as a first step in the analysis and the PCs often serve as the input data for other approaches, such as multivariate regression and discriminant analysis (Daszykowski et al.,2007).

In this study, PCA in combination with MLR made it possible to estimate correlations between volatiles and sensory or fruit data. MLR can establish the relative predictive importance of the independent variables by comparing standardised coefficients. Table 4 shows standardised coefficients of MLR for DM, sugars, acids and sensory data conducted as functions of the 14 PCs. Only two PCs (C2_PC3 and C4_PC1) were positively correlated with 'Hort16A'-like odour (0.456 and 0.482) and 'Hort16A'-like flavour (0.224 and 0.235). They also correlated positively with sweet taste and negatively with sour taste. C4_PC1 was correlated with ethyl butanoate flavour and odour (0.524 and 0.465), whereas C2_PC3 was correlated with ethyl butanoate odour alone (0.307). These results suggest that both PCs were associated with 'flavour impact volatiles'.

Table 3 Twelve uncommon volatiles found in genotypes of *Actinidia chinensis* (ng g^{-1} FW)

	P2	P3	P4	P8	P9	P11	P13	P21	P22
2-Methylbutanal	1.05	2.54	0	0	0	0	0	0	1.51
Butane-2,3-dione	0	0	0	0	0	0	0	0.20	0
Hexan-3-one	0	0	0	0	0	0	0	0	1.12
2-Methylpentan-3-one	0.18	0	0	0	0	0	0	0	0
4-Methyl-2-hexanone	4.00	0	0	0	0	0	0	0	0
4-Methylpentan-2-one	1.96	0	0	0	0	0	0	0	0
Hexen-3-one	1.69	0	0.43	0.93	0	0	0	0	0
Butyl acetate	0.73	0	0	0	0	0	0	0	0
5-Methylhexan-2-one	5.41	0	0	0	0	0	0	0	0
1-Methoxy-propan-2-ol	0	0	0	0	0	4.26	1.69	0	0
Terpinolene, iso-	5.29	6.62	0	0	0	0	0	0	0
3-Hydroxybutan-2-one	0	0	0	0	0.31	0	0	0	0

Table 4 Standardised coefficients for multiple linear regression (MLR) of *Actinidia chinensis* fruit and sensory attributes as a function of the 14 Principal Components (PCs)

Components	DM	Fructose	Glucose	Sucrose	*myo*-Inositol	Malic acid	Quinic acid	Citric acid	TA	Sweet taste	Sour taste	'Hort16A'-like flavour	'Hort16A'-like odour	Ethyl butanoate flavour	Ethyl butanoate odour
C1_PC1	-0.174	-0.193	-0.225	-0.046	0.104	0.235	-0.120	-0.208	0.025	-0.089	0.205	-0.334	-0.289	0.169	-0.106
C1_PC2	-0.074	-0.920	-0.766	0.036	-0.135	-0.736	-0.600	-0.994	0.440	-0.058	0.285	-0.176	-0.203	0.153	-0.249
C1_PC3	0.062	-0.129	-0.180	0.228	-0.165	-[a]	-	-0.513	-0.410	-0.326	0.174	-	-	0.287	-0.559
C2_PC1	-0.081	-0.041	-0.277	0.257	-0.049	0.103	-0.252	-0.690	-0.251	-0.182	0.263	-0.141	-0.425	0.255	0.018
C2_PC2	0.184	0.281	0.262	0.028	0.321	0.143	0.628	-0.015	-0.230	0.278	-0.307	0.065	-0.035	-0.328	0.427
C2_PC3	-0.261	-0.029	0.057	-0.136	-0.140	-0.303	-0.204	0.248	0.004	0.385	-0.311	0.224	0.456	-0.014	0.307
C2_PC4	-0.249	-0.226	-0.293	-0.394	0.637	0.204	0.048	0.236	0.483	-0.684	0.680	-0.192	-0.427	-0.421	0.299
C3_PC1	-0.284	-0.889	-0.831	-0.137	-0.456	-0.371	-0.795	-0.420	0.591	-0.601	0.802	-0.416	-0.216	-	-0.908
C3_PC2	0.096	-0.048	-0.143	0.233	0.115	-0.364	-0.058	-0.619	0.128	0.058	-0.059	0.011	-0.484	-0.077	-0.212
C3_PC3	0.127	0.254	0.170	-0.247	0.067	0.419	-0.193	0.270	0.162	-0.157	0.008	0.094	-0.225	-0.272	0.072
C3_PC4	0.006	0.114	0.110	0.001	-0.229	0.052	0.130	0.005	-0.179	-0.296	0.259	-0.402	-0.398	-0.307	-0.056
C4_PC1	0.630	0.962	0.805	0.182	-0.329	0.657	0.323	0.640	-0.350	0.400	-0.666	0.235	0.482	0.524	0.465
C4_PC2	-0.442	-0.175	-0.251	-0.276	-0.187	-0.034	0.026	0.061	-0.201	-0.067	-0.115	0.019	0.336	0.188	-0.065
C4_PC3	-0.267	-0.613	-0.586	-0.386	0.663	-0.256	-0.044	-0.464	0.521	-0.371	0.590	-0.174	-0.112	-0.118	0.660

TA Titratable acidity

[a] The component did not fit the regression model, e. g. caused some standardised coefficients (absolute values) to be > 1

Table 5 Standardized coefficients, percentage of variation of two Principal Components (PCs) and odour threshold in water for the 13 key *Actinidia chinensis* volatiles

Volatile No	K_PC1	K_PC2	Odour threshold ppb in water
Ethyl acetate	0.109	-0.205	8 000[a]
Ethanol	0.096	-0.211	62 450[a]
Ethyl propanoate	0.096	-0.144	15[a]
Methyl butanoate	0.134	-0.108	31[a]
α-Pinene	0.113	0.206	120[a]
Ethyl butanoate	0.127	-0.156	4.5[a]
Camphene	0.118	0.185	-
Hexanal	0.082	0.262	35[a]
β-Pinene	0.122	0.189	140[b]
Hex-*E*3-enal	0.127	0.011	-
Hex-*Z*2-enal	0.074	0.268	-
Ethyl hexanoate	0.099	-0.193	1.7[a]
Hexanol	0.061	-0.022	1 052[a]
Percentage of total variation/%	52.0	18.0	

[a] Rychlik et al., 1998; [b] Fazzalari, 1978.

Thirteen volatiles in Cluster 4 and two volatiles (methanol and 1-methoxy-propan-2-ol) in Cluster 2 had moderate to high standardised PCA coefficients for C4_PC1 and C2_PC3, respectively (Table 2). C4_PC1 accounted for 40.5% of the total variance in Cluster 4, whereas C2_PC3 accounted for only 14.0% of the total variance in Cluster 2. The first component, as usual in PCA, was the most important, accounting for most of the total variance (Morrison, 1990). The results imply that the potential 'flavour impact volatiles' in Cluster 4 might be more important contributors to 'Hort16A'-like flavour and odour than those in Cluster 2. Methanol and 1-methoxy-propan-2-ol in Cluster 2 are alcohols and 1-methoxy-propan-2-ol was detected in only two genotypes (Table 1). Free and esterified, saturated primary alcohols occur widely in fruits, but their odour is relatively weak (Surburg and Panten, 2006). However, these alcohols could be involved in AAT activities to produce esters (Ke et al., 1994).

The 13 volatiles in Cluster 4 identified as potential 'flavour impact' volatiles for *A. chinensis* fruit included five esters (ethyl acetate, ethyl propanoate, methyl butanoate, ethyl butanoate and ethyl hexanoate), three terpenes (α-pinene, camphene and β-pinene), three aldehydes (hexanal, hex-*E*3-enal and hex-*Z*2-enal) and two alcohols (ethanol and hexanol) (Tables 1, 2). These volatiles together represented 66.0% of the total volatiles produced by these *A. chinensis* fruit. For some genotypes, the concentrations of ethyl propanoate, ethyl butanoate, ethyl hexanoate and hexanal were higher than their odour thresholds (Table 5) (Rychlik et al., 1998). A GC-sniffing study showed that acetaldehyde, hexanal, ethyl butanoate and hex-*E*2-enal had odour activity in macerated fruit of 'Hort16A' (Friel et al., 2007). Young et al. (1983) reported that hex-*E*2-enal, ethyl butanoate, methyl benzoate and hexanal were important contributors to the aroma and flavour of 'Hayward' (*A.*

deliciosa). Studies on the causal effects of selected flavour volatiles on the intensity of sensory attributes in 'Hayward' showed that ethyl butanoate, methyl benzoate and hexanal had positive correlations with sweet aroma and flavour (McMath et al., 1991; Young et al., 1995). The most pronounced effect on 'kiwifruit aroma and flavour' was produced by ethyl butanoate (Gilbert et al., 1996). Paterson et al. (1991) reported that ethyl butanoate, the major ester present in 'Hayward', appeared to be a predictor of flavour acceptability. Our results also showed that ethyl butanoate was the major ester present in these *A. chinensis* fruit. However, high concentrations of this volatile can also contribute to a 'very ripe kiwifruit-like' perception of flavour (Paterson et al., 1991; Gilbert et al., 1996), which is not a desirable attribute (Stec et al., 1989). Thus, the balance of this volatile with others may be a more important characteristic. An integrated approach using GC-MS and GC-O is needed to confirm whether these 13 volatiles play a significant role in contributing to the flavour and odour of *A. chinensis* fruit.

C4_PC2 was also correlated with 'Hort16A'-like odour, but not with 'Hort16A'-like flavour, ethyl butanoate flavour or odour, or sweet or sour tastes (Table 4). Propanol, ethylbenzaldehyde and 4-ethylbenzaldehyde were highly correlated with C4_PC2 (Table 2). This indicated that C4_PC2 identified 'Hort16A'-like odour volatiles'. Turin (1996) found that ethylbenzaldehyde had a typical bitter almond odour and Pennarun et al. (2003) found that 4-ethylbenzaldehyde was characterized by a minty and aniseed odour. Propanol is an alcohol with relatively weak odour and its concentration (Table 1) was below its odour threshold (Rychlik et al., 1998). As C4_PC2 accounted for only 18.1 % of the total variance in Cluster 4, it was not an important component compared with C4_PC1 (40.5 %). However, volatiles represented in this cluster (C4_PC2) could affect the overall perception of flavour or odour by altering the complexity of the experience. Previous studies reported that adding sub-and perithreshold odourant can have a measurable impact on perceived flavour or aroma (Atanasova et al., 2005; Labbe et al., 2007; Miyazawa et al., 2008).

Ethyl 2-methylpropanoate and unknown 3819 in Cluster 2 had negative correlations with C2_PC3. Thus, genotypes with a high concentration of these volatiles had less 'Hort16A'-like flavour and odour, and 'Hort16A' (P24) had a low concentration of these volatiles. Ethyl 2-methylpropanoate was present in 17 genotypes, whereas unknown 3819 (1511) was found in only five genotypes (Table1). The concentrations of ethyl 2-methylpropanoate ranged from 0.23 to 8.51 ng g^{-1} FW, higher than the odour threshold of 0.1 μg l^{-1} in water (Takeoka et al., 1990). Matich et al. (2003) reported that the aroma of fruit of selection A3 (*A. arguta*) was probably the most intense because of the very sweet, fruity odours of ethyl 2-methylpropanoate and ethyl 2-methylbutanoate. Frank et al. (2007), using GC/MS-O, reported that ethyl-2-methylpropanoate ('melon, bubblegum') was the most potent odour-active ester compound in 'Hayward'. Ethyl 2-methylpropanoate was described as a characteristic fruity volatile in pineapple (Tokitomo et al., 2005) and blackberries (Klesk and Qian, 2003). Further study is needed to explore whether ethyl 2-methylpropanoate may contribute different and positive flavours and odours to *A. chinensis* fruit, and therefore be a desirable compound in a breeding population. From a study on consumers' preferences for kiwifruit, Wismer et al. (2005) suggested that fruit breeding should target not only elite fruit that are significantly more like existing successful cultivars, but also unique fruit that could create major new flavour niches.

C4_PC3 had low negative correlations with 'Hort16A'-like flavour odour, but a high positive correlation with ethyl butanoate odour and 'sour taste' (Table 4). This component also had positive moderate correlations with ethyl esters (ethyl acetate, ethyl propanoate, ethyl butanoate and ethyl hexanoate) and an alcohol (ethanol), but negative moderate correlations with terpenes (α-pinene, camphene and β-pinene) and two aldehydes (hexanal and hex-*Z*2-enal) (Table 2). All volatiles correlated with C4_PC3 were found to be potential 'flavour impact volatiles' as in C4_PC1 (as discussed above). Ethyl butanoate was found in much higher concentrations in ripe fruit than in firm fruit of 'Hort16A', and this was shown to be a feature of ripening stage rather than physical maceration of tissue, and hence either enzyme activity or substrate availability (Friel et al., 2007). Marsh et al. (2003) found that a rise in off-flavours and acid taste was consistent with the addition of malate to 'Hayward' pulps. It was suggested that the release of alcohols was associated with a change in acidity (Marsh et al., 2006). In a study of 'Hayward' fruit in an ethylene-free environment, Burdon et al. (2005) suggested that the production of significant esters was driven by alcohol metabolism that developed as fruit reached and passed through the eating firmness range; and Paterson et al. (1991) demonstrated an association between ester concentrations and increasing fruit ethylene production in 'Hayward' fruit. The results indicated that the higher concentrations of esters could relate to over-ripe fruit with strong ethyl butanoate odour and 'sour taste'. Thus, genotypes with overripe fruit would be expected to have a high positive score on C4_PC3. The results suggest that C4_PC3 identified 'kiwifruit fruit ripeness', which is probably a measure of the balance between different components of flavours and odours. The presence of this component may also suggest that AAT enzymes with ethanol may play a key role in the biosynthesis of ethyl esters (Ke et al., 1994), and that alcohol dehydrogenase (ADH) and lipoxygenase (LOX) enzymes could play specific roles in the regulation of odour biosynthesis (Zhang et al., 2009; González-Agüero et al., 2009). Sequence analysis of acyltransferases (ATs) from the *Actinidia* EST database showed that two clades contained enzymes involved in the synthesis of flavour-related esters (Crowhurst et al., 2008). Further study is needed to explore biosynthetic pathways leading to volatile production.

In this study, two PCs (C2_PC4 and C3_PC1) had high correlations with 'sour taste' positively and 'sweet taste' negatively (Table 4). C3_PC1 also had a very high negative correlation with ethyl butanoate odour. This group of volatiles included hex-*E*2-enal in Cluster 2 and hex-*Z*3-enal, octanal, 6-methylhept-5-en-2-one, hex-*Z*3-enol, decanal and the unknown 3779 (1588) in Cluster 3. The mean concentrations of hex-*E*2-enal, hex-*Z*3-enal, octanal and decanal (Table 1) were higher than their odour thresholds (Rychlik et al., 1998). Hex-*E*2-enal was the major volatile component in mature fruit of 'Hayward' (Bartley and Schwede, 1989). Gilbert et al. (1996) studied the consumer perception and acceptability of three volatiles at variable levels in a model base solution. They found that hex-*E*2-enal had a negative effect on the acceptability attributes (i.e., 'overall liking', 'liking of aroma' and 'liking of flavour'), but a positive correlation with the perceived intensity of 'kiwifruit aroma' and 'acid flavour'. Frank et al. (2007) reported that hex-*E*2-enal (perceived as marzipan, sweet) was the odour compound with the greatest impact in 'Hayward'. In apricot, hex-*E*2-enal gave a grassy note, whereas 6-methylhept-5-en-2-one developed a floral note (Guillot et al., 2006). Friel et al. (2007) reported that a low concentration of 6-methylhept-5-en-2-one was found in

the firm fruit of 'Hort16A'. It is well known that the volatiles and sensory properties of kiwifruit are markedly affected by fruit ripeness(Stec et al.,1989). Thus,this group of volatiles could be related to a predominance of aldehydes and associated with less ripe fruit.

C2_PC1 and C2_PC4 had moderate negative correlations with 'Hort16A'-like odour, while C2_PC2 and C2_PC4 had a moderate positive correlation with ethyl butanoate odour but a moderate negative correlation with ethyl butanoate flavour (Table 4). Most PCs for Clusters 1 and 3 had negative correlations with 'Hort16A'-like flavour and odour,and ethyl butanoate flavour and odour, except for C3_PC2 and C3_PC3, which had very low positive correlations with 'Hort16A'-like flavour(Table 4). C3_PC3 also had a very low positive correlation with ethyl butanoate odour. All volatiles in Clusters 1 and 3 had moderate to high correlations with PCs (Table 2). The results showed that most volatiles in Cluster 2 (except for methanol, ethyl 2-methylpropanoate and 1-methoxy-propan-2-ol) were correlated with 'atypical 'Hort16A'-like odour' and those in Clusters 1 and 3 were associated with 'atypical kiwifruit flavour' in *A. chinensis* fruit.

Most of the aldehyde compounds were in Clusters 1 ~3(Table 1),although some were grouped into Cluster 4 as flavour volatiles (hexanal, hex-*E*3-enal and hex-*Z*2-enal) or odour volatiles (ethylbenzaldehyde and 4-ethylbenzaldehyde),as discussed above. The lower fatty aldehydes C_2 ~ C_7 impart fruity characters to flavour compositions(Surburg and Panten,2006). The same authors also reported that octanal had a pungent odour that became citrus-like on dilution,nonanal had a fatty rose-like odour and decanal a strong odour reminiscent of orange peel. While these aldehydes had negative correlations with 'Hort16A'-like flavour and odour,they could contribute to breeding interesting new flavours in *A. chinensis* because their concentrations were higher than their odour thresholds(Rychlik et al.,1998). However,Friel et al. (2007) pointed out that as the volatiles in kiwifruit studies were generally collected over a period of 20 min(15 min for this study),it is unclear whether concentrations of volatile lipid oxidation products (e. g. , aldehydes) would contribute significantly to the odour experienced by a consumer while eating a piece of fruit(i. e. ,over 5 ~15 s).

All ketone compounds were in Clusters 1 and 3,except for hexan-3-one in Cluster 4(Table 1). However,hexan-3-one had low standardized coefficients for the three C4_PCs(Table 2),suggesting that it might not be an important flavour compound in *A. chinensis* fruit. Moreover,only three ketones (acetone,2-butanone and 6-methylhept-5-en-2-one) were common in *A. chinensis*,being detected in 23 or 24 genotypes. The concentrations of acetone and 6-methylhept-5-en-2-one were lower than their odour thresholds for all genotypes(Rychlik et al.,1998;Buttery et al.,1990). 2-Alkanones(C3 ~ C15) have been found in the volatile fractions of many fruits and foodstuffs,but they do not contribute significantly to their odour(Surburg and Panten,2006).

In this study,13 volatiles were successfully identified as potential key 'flavour impact' volatiles for *A. chinensis* fruit from the composition of 72 volatiles by using multivariate analysis,suggesting that it can be possible to measure volatiles in a large breeding population. Moreover,MLR showed that C4_PC1 was highly correlated with other fruit attributes,e. g. ,DM,fructose,glucose,malic and citric acids(Table 4). In breeding,it is sometimes more convenient or effective to select for a correlated character than to select for the desired character itself (Falconer and Mackay,1996). Methods commonly used for identifying volatile compounds,such as GCMS,require expensive equipment and the analysis of large numbers of samples is time consuming. As DM was highly

correlated with C4_PC1, the current study suggests that indirect selection using DM could be a convenient and efficient alternative to direct selection for increased flavour impact volatiles in this *A. chinensis* population. DM is quick and easy to measure on large numbers of samples and had a positive genetic correlation with the amounts of sugars (Cheng et al., 2004). A recent meta-analysis of kiwifruit sensory experiences demonstrated unequivocally that consumers prefer high DM fruit of both 'Hayward' and 'Hort16A' (Harker et al., 2009).

3.2.2 *Differentiation of genotypes associated with flavour impact volatiles*

To refine differentiation between genotypes, the patterns of the 13 potential key 'flavour impact' volatiles (from Cluster 4) for the 24 genotypes were analyzed by PCA. Based on the parsimony criterion, only two PCs were considered. K_PC1 accounted for 52.0% and K_PC2 for only 18.0% of the total variance (Table 5). K_PC1 was positively correlated with all 13 volatiles, suggesting that the first component was associated with 'total key volatiles'. Thus, a genotype with high concentrations of these volatiles would be expected to have high positive scores. Particularly, genotypes with high concentrations of methyl and ethyl butanoates, hex-*E*3-enal and the three monoterpenes (α-pinene, camphene and β-pinene) would have higher positive scores, as indicated by higher coefficients. The results of MLR of the K_PCs as a function of sensory attributes showed that K_PC1 had a high positive correlation with ethyl butanoate flavour and a low correlation with ethyl butanoate odour (Table 6). There was also a contrast between 'Hort16A'-like flavour and 'sour taste', as indicated by negative coefficients for the first PC.

K_PC2 had a negative correlation with five esters (ethyl acetate, ethyl propanoate, methyl butanoate, ethyl butanoate and ethyl hexanoate) and ethanol, while it had a positive correlation with two aldehydes (hexanal and hex-*Z*2-enal) and the three monoterpenes (Table 5). The results of MLR also showed that K_PC2 had a negative correlation with 'sour taste', 'Hort16A'-like odour and flavour and ethyl butanoate flavour, but a positive correlation with ethyl butanoate odour (Table 6). K_PC2 revealed two welldefined groups: one positive and one negative for the flavour impact volatiles of 'Hort16A'-like flavour and odour. Five esters were significant contributors to 'Hort16A'-like flavour and odour. Genotypes with high concentrations of positive 'flavour impact' volatiles would have low negative scores in K_PC2, whereas genotypes with high concentrations of negative 'flavour impact' volatiles would have high positive scores. The results suggest that K_PC2 identified genotypes with ''Hort16A'-like flavour and odour'. However, a lower score for K_PC1 or K_PC2 could relate to over-ripe fruit inducing high 'sour taste' (as discussed above). Thus, genotypes with medium-low scores for K_PC1 and K_PC2 may have high 'Hort16A'-like flavour and odour and yet to some extent a 'sour taste' as well. Jaeger et al. (2003) found that consumers accepted *A. chinensis* fruit because they had high sweet and low acidic flavour.

Table 6 Standardised coefficients for multiple linear regression (MLR) of K_PCs as a function of *Actinidia chinensis* sensory attributes

Sensory attributes	K_PC1	K_PC2	Sensory attributes	K_PC1	K_PC2
Ethyl butanoate odour	0.161	0.109	Sour taste	-0.221	-0.579
'Hort16A'-like odour	0.008	-0.272	Ethyl butanoate flavour	0.444	-0.153
Sweet taste	-0.053	-0.009	'Hort16A'-like flavour	-0.359	-0.259

In this study, PCA has provided some insights into 'kiwifruit fruit ripeness' and an indication of the balance of different volatile components that will affect consumer responses. Those genotypes with a high positive score in K_PC2 could have an imbalance in biosynthetic pathways or lack a critical step for ester formation during fruit ripening. Further study is needed to explore differences in the biosynthetic pathways of volatile production between *A. chinensis* genotypes, to establish a biochemical basis for selecting new cultivars with flavours that appeal to consumers.

Genotypes were highly differentiated by their 'flavour impact' volatiles (Fig. 1). P1, P6, P22 and female parents B and C ('Hort16A') had medium-low scores of K_PC1 and K_PC2, which may result in fruit with high 'Hort16A'-like flavour/odour and to some extent 'sour taste'. Hence, these genotypes may be useful as parents to improve 'Hort16A'-like flavour/odour. P4, P8 and P13 had high concentrations of both positive and negative 'flavour impact' volatiles, whereas P3 had high concentrations of only negative volatiles. Four genotypes (P7, P14, P16 and P20) had low concentrations of total 'impact flavour' volatiles. Other genotypes had either medium-high concentrations of negative 'flavour impact' volatiles or medium-low concentrations of positive volatiles.

In conclusion, extended use of multivariate analysis proved very powerful for extracting maximum information from the limited plant material. In this study, volatiles were successfully grouped, using hierarchical clustering of variables based on phenotypic correlations between volatiles, to avoid a singular correlation matrix of the data in PCA. Based on these groups, using PCA and MLR, correlations between volatiles, sensory and fruit attributes were explored. However, it must be noted that the presence of a correlation does not necessarily mean that a cause and effect relationship exists. In this case, the PCs reflect the influence of different categories of volatile variables of different biological significance. One of the major strengths of PCA is to allow identification of important factors leading to biological interpretations of key processes. In this study, standardized coefficients of MLR enabled the PCs to have meaningful biological interpretations. Consequently, potential key 'flavour impact' volatiles were identified, which may make a substantial contribution to the fruit flavour of these *A. chinensis* genotypes. Five esters were identified that may contribute significantly to 'Hort16A'-like flavour and odour, whereas likely negative 'flavour impact' volatiles included three monoterpenes and two aldehydes. Some volatiles associated with 'sour taste', 'fruit ripeness' 'atypical 'Hort16A'-like odour and 'atypical kiwifruit flavour' were also recognized. Furthermore, the PCs provided a measure of the balance of complex volatile components that is likely to affect consumer responses. The differentiation of genotypes associated with 'flavour impact' volatiles was successfully illustrated, leading to the identification of potentially superior parents for flavour breeding. In addition, the PCA provided some insights into possible biosynthetic pathways of volatile production. However, our results confirmed that indirect selection using DM is a convenient and efficient alternative to direct selection for improving flavour volatiles of fruit (Cheng et al., 2004) providing no new flavour profiles are desired. This fits very well with recent evidence that DM has a desirable and positive influence on consumer acceptance of kiwifruit (Harker et al., 2009). However, a selection strategy based on DM also needs to take into account its positive correlation with some negative 'flavour impact' volatiles. While it is recognised that consumers will

vary in their ability to detect volatile concentrations (Friel et al.,2007), we showed that our *A. chinensis* gene pool contains a variety of flavour and/or odour volatiles useful for breeding kiwifruit cultivars with new flavour characteristics. Based on our results, we can target the testing of new combinations of potential flavour impact volatiles of kiwifruit using in vitro or pulp systems (Marsh et al.,2006).

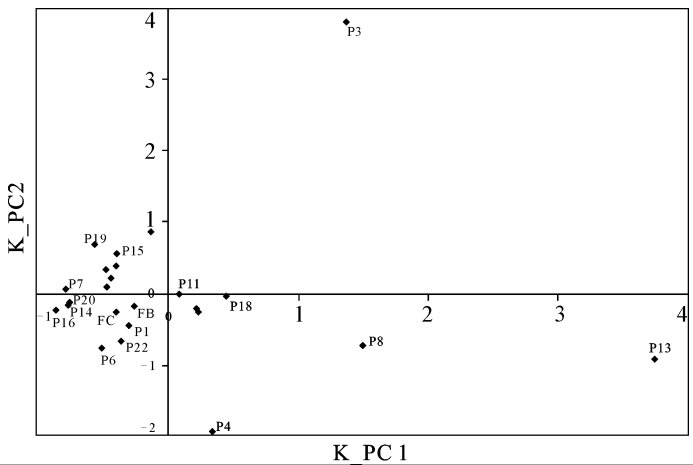

Fig. 1 Scatter plot of K_PC1 against K_PC2 for key 'flavour impact' volatiles in 24 genotypes of *Actinidia chinensis*

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