Characterisation of the bound volatile compounds of kiwifruit

Coralia V. Valdizon Garcia

The University of Auckland, February 2013

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in Food Science

ABSTRACT

The genus *Actinidia,* collectively known as "kiwifruit", includes over 70 species, of which only a few are commercially important. Kiwifruit is the subject of this research.

At present, the knowledge of kiwifruit aroma is limited to the free volatile portion, and concentrated on the 'Hayward' cultivar. Information on the volatiles of other species/cultivars is limited, and information concerning the bound volatiles of kiwifruit consisted, until now, of one article published more than 15 years ago. Therefore, this study aimed to provide new knowledge on kiwifruit aroma by focusing on the bound volatile portion of four kiwifruit species, three of them being commercially important (*A. deliciosa* 'Hayward', *A. chinensis* 'Hort16A', *A. arguta*), plus one with low aroma (*A. eriantha*).

Initially, a preliminary investigation of the bound volatiles of kiwifruit was conducted to select the best way to isolate and hydrolyse the glycosides, and extract the released aglycones.

The bound volatile content and composition of the four kiwifruit species mentioned was elucidated, and the important odorants were identified by GC-MS/olfactometry. It was found that benzenoids, alcohols and terpenoids predominated in the bound volatile extracts of all the fruits analysed. The information gathered was compared with information currently available on the free volatiles of kiwifruit, to gain a deeper understanding of kiwifruit aroma. In *A. arguta*, 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF, furaneol) was the predominant bound volatile compound, as well as the bound volatile with the strongest smell. The DMHF glycoside was identified by LC-MS as DMHF-β-D-glucopyranoside. Glucose was identified as the major sugar moiety in *A. arguta* glycosides. The bound volatile composition of *A. eriantha* was unexpectedly diverse, considering the bland, green aroma of this fruit, with

odorants such as 2-phenylethanol, (*E*)-isoeugenol and vanillin having been found. The bound volatile profiles of ‘Hayward’ and ‘Hort16A’ were similar, but compounds were found at different concentrations. Bound volatiles with green notes were found in both ‘Hayward’ and ‘Hort16A’, although they were more predominant in ‘Hayward’.

Finally, the effect of ripening on the bound volatiles of ‘Hayward’ and ‘Hort16A’ was evaluated, finding that bound volatiles tend to increase in the ripe stage, and then decrease in over-ripe fruit.

ACKNOWLEDGEMENTS

I would like to thank my supervisors at the University of Auckland, Siew-Young Quek and Ralph Stevenson, as well as my supervisor at the New Zealand Institute for Plant & Food Research, Robert Winz, for their advice, support and input.

I very much appreciate the expert advice and help provided by Ross Atkinson, William Laing, Mindy Wang, Xiuyin Chen, Adam Matich, Dave Greenwood, Chen Goh, Yar-Khing Yauk, Ken Marsh and Mark Wohlers.

I am also grateful to Ringo Feng and Anne White for their advice in the postharvest handling of kiwifruit and evaluation of ripening.

I further wish to thank Sreeni Pathirana and Chris Nipper for technical support with the GC-MS system.

I am very grateful for the scholarship I received from Education New Zealand (NZIDRS scholarship), as well as for the funding and support provided by the University of Auckland and the New Zealand Institute for Plant & Food Research. The scholarship extension provided by the University of Auckland is also appreciated.

Lastly, I am grateful to all other individuals who provided me with support and encouragement throughout this study.

TABLE OF CONTENTS

ABSTRACT I

ACKNOWLEDGEMENTS III

TABLE OF CONTENTS IV

ABBREVIATIONS IX

DECLARATION XI

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW 1

1.1 Overview and objectives 2

1.2 Kiwifruit 4

1.2.1 Cultivation 4

1.2.2 Fruit maturation 5

1.2.3 Composition 7

1.2.4 Processing and potential uses 8

1.2.5 Economical significance 9

1.3 Flavour 11

1.3.1 Flavour development in fruits 13

1.3.2 Glycosidic precursors of flavour compounds 16

1.4 Analysis of aroma compounds 20

1.4.1 Sample preparation 20

1.4.2 Isolation 21

1.4.3 Separation and identification 24

1.4.4 Quantification 25

1.4.5 Gas chromatography-olfactometry (GC-O) 26

1.5 Analysis of glycosidic aroma precursors 27

1.5.1 Isolation of precursors 28

1.5.2 Analysis of aglycones 29

1.5.3 Analysis of glycoconjugates 33

1.6 Review of literature concerning the aroma compounds of kiwifruit 34

1.6.1 Free aroma compounds of kiwifruit 34

1.6.2 Bound aroma compounds of kiwifruit 42

1.7 Conclusions 42

CHAPTER 2: PRELIMINARY STUDY OF BOUND VOLATILE COMPONENTS FROM KIWIFRUIT 44

2.1 Introduction 45

2.2 Materials and methods 46

2.2.1 Plant material 46

2.2.2 Reagents and standards 46

2.2.3 Isolation of glycosidic precursors 47

2.2.4 Release of bound volatile compounds 48

2.2.5 Solid phase microextraction (SPME) 49

2.2.6 GC-MS analysis 49

2.3 Results and discussion 53

2.3.1 Hydrolysis method 53

2.3.2 Aglycones extraction method 60

2.4 Conclusions 65

CHAPTER 3: ANALYSIS OF BOUND VOLATILES AND GLYCOSIDES OF *ACTINIDIA ARGUTA* (KIWIBERRY) 66

3.1 Introduction 67

3.2 Materials and methods 68

3.2.1 Plant material 68

3.2.2 Standards and solvents 68
3.2.3 Bound volatile analysis 69
3.2.4 Glycoside analysis 73
3.2.5 Analysis of sugar moieties 76
3.3 Results and discussion 77
3.3.1 Bound volatile analysis 77
3.3.2 Glycoside analysis 87
3.3.3 Analysis of sugar moieties 89
3.4 Conclusion 90
CHAPTER 4: ANALYSIS OF BOUND VOLATILES COMPOUNDS OF *ACTINIDIA ERIANTHA* 92
4.1 Introduction 93
4.2 Materials and methods 94
4.2.1 Plant material 94
4.2.2 Standards and solvents 94
4.2.3 Isolation and hydrolysis of glycosidic precursors 95
4.2.4 GC-MS analysis 95
4.2.5 GC-MS/O analysis 95
4.3 Results and discussion 95
4.4 Conclusion 105
CHAPTER 5: CHANGES IN THE BOUND VOLATILE PROFILES OF 'HAYWARD' AND 'HORT16A' KIWIFRUIT DURING RIPENING 107
5.1 Introduction 108
5.2 Materials and methods 108
5.2.1 Plant material 108
5.2.2 Evaluation of ripening 109

5.2.3 Standards and solvents 109

5.2.4 Isolation and hydrolysis of glycosidic precursors 109

5.2.5 GC-MS analysis 109

5.2.6 GC-MS/O analysis 109

5.2.7 β-Glucosidase assay 110

5.2.8 Statistical analysis 111

5.3 Results and discussion 112

5.3.1 Bound volatiles in 'Hayward' kiwifruit 112

5.3.2 Bound volatiles in 'Hort16A' kiwifruit 116

5.3.3 Differences between the bound volatile profiles of 'Hayward' and 'Hort16A' kiwifruit 124

5.3.4 Changes in the bound volatile composition of 'Hayward' kiwifruit during ripening 125

5.3.5 Changes in the bound volatile composition of 'Hort16A' kiwifruit during ripening 126

5.3.6 Results of GC-MS/O analysis 127

5.3.7 Effect of ripening on β-glucosidase activity 130

5.4 Conclusion 132

CHAPTER 6: GENERAL DISCUSSION AND CONCLUSIONS 133

6.1 General discussion 134

6.2 Concluding remarks 138

6.2.1 Analysis methodology of bound volatiles 138

6.2.2 Bound volatiles and glycosides of *Actinidia arguta* 139

6.2.3 Bound volatiles of *Actinidia eriantha* 139

6.2.4 Changes in the bound volatiles of *Actinidia deliciosa* 'Hayward' and *A. chinensis*

'Hort16A' during ripening 140

6.3 Future prospects 141

REFERENCES 143

PUBLICATIONS AND PRESENTATIONS 152

ABBREVIATIONS

AEDA	Aroma extract dilution analysis
ANOVA	Analysis of variance
DMHF	2,5-Dimethyl-4-hydroxy-3(2H)-furanone
DMMF	2,5-Dimethyl-4-methoxy-3(2H)-furanone
DMS	Dimethyl sulfide
EI	Electron impact
FID	Flame ionisation detector
fob	Free on board
GC	Gas chromatography
GC-FTIR	Gas chromatography-Fourier transform infrared spectroscopy
GC-MS	Gas chromatography-mass spectrometry
GC-MS/O	Gas chromatography-mass spectrometry/olfactometry
GC-O	Gas chromatography-olfactometry
HPLC	High-performance liquid chromatography
HS-SPME	Headspace-solid phase microextraction
LC-ESI-MS	Liquid chromatography-electrospray ionisation mass spectrometry
LC-MS	Liquid chromatography-mass spectrometry
MALDI-TOF-MS	Matrix assisted laser desorption ionisation time-of-flight mass spectrometry
MeS	Methylsulfanyl
NMR	Nuclear magnetic resonance
OAV	Odour activity value
PDMS-DVB	Polydimethylsiloxane/divinylbenzene

PTLC	Preparative TLC
RI	Retention index
SPE	Solid phase extraction
SPME	Solid phase microextraction
SSC	Soluble solids content
TIC	Total ion current
TLC	Thin layer chromatography
TOF-MS	Time-of-flight-mass spectrometry
UV	Ultraviolet

DECLARATION

This is to certify that this thesis comprises only my original work. The experimental methodology, data generation and collection, interpretation of results and preparation of the manuscript, including all Figures and Tables, were my own work, except where indicated below.

Chapter 3:

The LC-MS measurement (described in 3.2.4.5) was carried out by Dave Greenwood. The chromatographic run of sugar samples and collection of raw data (described in 3.2.5) was performed by Chen Goh.

Chapter 1

Introduction and literature review [1]

[1] This chapter is based on the manuscript Garcia et. al, Trends in Food Science and Technology, **2011**, 24, 82-91.

1.1 Overview and objectives

The genus *Actinidia*, which is native of China, contains more than 70 species, of which only a few are of commercial importance. *Actinidia deliciosa* and *A. chinensis* are the most important commercial species. The former includes the 'Hayward' cultivar (Green Kiwifruit), which has large cylindrical fruits with a skin covered with hairs; the latter includes the 'Hort16A' cultivar (Gold Kiwifruit), with smaller, hairless fruits (Perera et al., 1998). *A. arguta* is a smooth skinned, grape-sized kiwifruit native to North East Asia (Matich et al., 2003). All *Actinidia* species are perennial and mainly deciduous, and their fruit appears single or in bunches in the vine, displaying diverse size, shape, skin hairiness, colour and texture of skin and flesh characteristics (Ferguson, 1984) (Fig. 1-1).

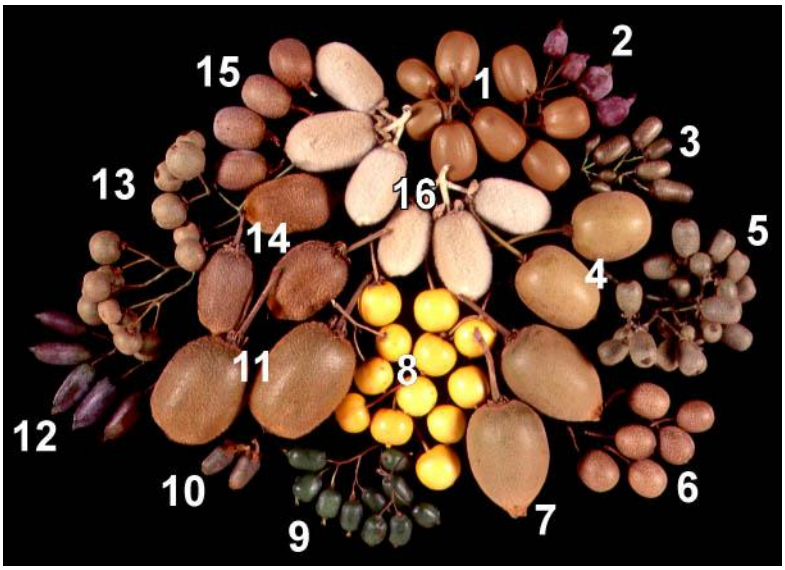

Fig. 1-1 Fruit diversity in *Actinidia.* Taken from (Ferguson, 1999).
1, *A. rufa*; 2, *A. melanandra*; 3, *A. glaucophylla*; 4, *A. chinensis*; 5, *A. latifolia*; 6, *A. indochinensis*; 7, *A. chinensis* 'Hort16A'; 8, *A. macrosperma*; 9, *A. arguta*; 10, *A. fulvicoma*; 11, *A. deliciosa* 'Hayward'; 12, *A. arguta* var. *purpurea*; 13, *A. guilinensis*; 14, *A. setosa*; 15, *A. chrysantha*; 16, *A. eriantha.*

Kiwifruit are botanically classified as berries. Mature 'Hayward' and 'Hort16A' kiwifruit weight an average of 100 g, while fruit of *A. arguta* weights around 15 g. In 'Hayward', both

the outer and inner pericarps contain chloroplasts, which give the green colour to the flesh. Kiwifruit seeds are embedded in the inner pericarp, which surrounds the white core, made of parenchyma cells which lack chlorophyll (Beever and Hopkirk, 1990).

Kiwifruit was known in China since the Tang dynasty (7th century A.D.), where it was called “mihoutao” (monkey peach) (Huang and Ferguson, 2007). In 1904, Isabel Fraser, who was the principal of the Wanganui Girls’ College, brought the first seeds to New Zealand, which were planted and bore fruit in 1910. Fourteen years later, Auckland grower Hayward Wright developed the green variety that bears his name, which is the most cultivated around the world. The first commercial shipment, to England, was exported in 1952, and consisted of 13 tonnes of fruit. Until this time, the fruit was called “Chinese gooseberry”, and was renamed briefly as “melonette”; but in 1959, exporters decided a more appealing name was needed, and coined the name “kiwifruit”. ‘Hayward’ kiwifruit was the only variety exported until 1998, when Gold Kiwifruit was introduced to the world market (Patterson et al., 2003).

Kiwifruit is appreciated for its flavour and nutritional qualities. The volatile compounds that contribute to kiwifruit flavour are produced through metabolic pathways, and are dependent on the variety, stage of maturity, environmental conditions, and post-harvest treatments. Kiwifruit is the subject of this research.

At present, the knowledge of kiwifruit aroma is limited to the free volatile portion, and concentrated on the ‘Hayward’ cultivar. Information on the volatiles of other species/cultivars is limited, and information concerning the bound volatiles of kiwifruit consisted, until now, of one article published more than 15 years ago (Young and Paterson, 1995), showing that there is an important gap in the knowledge about kiwifruit aroma, that the present study intends to fill.

This study aimed to provide new knowledge on kiwifruit aroma, by focusing on the bound volatile portion of four kiwifruit species, three of them being commercially important

('Hayward', 'Hort16A', *A. arguta*), plus one with low aroma (*A. eriantha*). The goals were to elucidate the differences in the bound volatile content and composition of the various kiwifruit species. The information gathered was compared with information currently available on the free volatiles of kiwifruit, to gain a deeper understanding of kiwifruit aroma. Furthermore, this study aimed to explain how aroma compounds are produced from glycosidic precursors. To achieve this, the bound volatile compounds were identified and quantified, and the important aroma compounds identified by olfactometry analysis. The effect of ripening on the bound volatiles of kiwifruit was evaluated.

The interest in the study of bound volatiles is justified by the importance of these compounds in the flavour potential of a fruit, since their proportion tends to be much greater than that of free volatiles, but their role as potential flavour compounds is poorly understood. It is not surprising, then, that the bound volatiles of grape and other fruits of economic value have been studied, and bound volatiles have caught the attention of kiwifruit researchers.

The results obtained can provide a basis for applications such as quality control of the fruits during post-harvest and processing, enhancement of fruit beverages by the release of flavour precursors, and recovery of flavour compounds from by-products of fruit processing.

The methodology developed is not limited to the study of the aroma of kiwifruit, as it can also be applied to other volatile compounds of plant origin, as well as to other flavour research applications such as research of volatiles derived from lipids, synthesis of flavour compounds, and development of encapsulated products.

1.2 Kiwifruit

1.2.1 Cultivation

Kiwifruit are deciduous, temperate plants which require a period of winter chilling for

bud break and flowering; however, they are susceptible to frosts and require a frost-free growing period of at least 220 days. The plants need around 800 to 1200 mm of water during the growing season, and protection from winds (Ferguson and Stanley, 2003). To support the vines, a T-bar fence or overhead pergolas are used; vines should be pruned to control their growth, and prevent diseases and pests (Perera et al., 1998).

Kiwifruit is a dioecious plant, that is, the male and female flowers occur in different plants, making it necessary for orchards to contain both. A 1:6 ratio of male to female plants is recommended. 'Matua' is the male plant commonly used in New Zealand. 'Bruno' seedlings have been the traditionally preferred rootstock in New Zealand; but TR2 is an alternative shown to increase yields of 'Hayward' kiwifruit (Perera et al., 1998).

Kiwifruit vines are in full flower in mid to late spring, two months after bud break, 'Hayward' being the last cultivar to flower. Bees are important pollinators of kiwifruit flowers. The fruits are ready to harvest in late autumn (Perera et al., 1998).

1.2.2 Fruit maturation

The terms maturity and ripening should not be confused. Maturity can be defined as the developmental stage in which the fruits can be removed from the parent plant and be able to develop the eating qualities. Ripening is the development of the eating qualities of a fruit (softness, flavour, higher sugar content, etc.), which can occur on or off the plant (Beever and Hopkirk, 1990). Although there are differences in the ripening process of fruits according to the species, there are several points in common, such as a changes in colour and texture, and changes in the content and composition of sugars, acids and volatiles. Climacteric fruits are those which experience an increase in ethylene production and respiration at the beginning of ripening, while non-climacteric fruits do not experience these changes (Giovannoni, 2004).

The volume and weight of kiwifruit increase significantly during the first 10 weeks after

anthesis; final fruit size depends on the cultivar, the number of seeds, the crop load and growing conditions. There are no evident signs of maturation in kiwifruit, as the shape and colour of the fruits do not change; furthermore, individual fruits in a vine will ripen at different times. However, there are notable changes in the sugar content as the fruits ripen (Beever and Hopkirk, 1990).

The stage of maturity at which the fruit are harvested influences their storage behaviour as well as their eating quality; fruit harvested too early do not develop their full aroma and tend to manifest storage disorders. Fruit are harvested at a minimum SSC of 6.2%; but fruit harvested at higher SSC, of 8 to 9%, and in some cases up to 12%, result in good eating quality fruit. In New Zealand, kiwifruit are stored in packs surrounded by polyliner, to reduce water loss and obtain a homogeneous ripening of the fruit (Beever and Hopkirk, 1990, Perera et al., 1998).

At the early stages of ripening, the levels of soluble solids increase, at the same time acids decrease, and the fruit softens; later, the production of ethylene and respiration increase, and flavour compounds are produced; but this process is slowed if the fruit are stored in cold rooms. If the fruit are going to be sent to the markets soon after harvest, it is useful to ripen them with ethylene (Perera et al., 1998). Kiwifruit are climacteric fruits; their respiration rate decreases slowly as they ripen, then increases sharply when flesh firmness is around 1.0 kgf, before decreasing again. Ethylene production also increases during the later stages of softening (Beever and Hopkirk, 1990).

A reliable indicator of kiwifruit ripeness is flesh firmness; fruit for export should have a firmness of 1.0 kgf or more. A kiwifruit is considered to be ready to eat when the firmness is 0.5-0.8 kgf (McDonald, 1990).

‘Hayward’ kiwifruit can be stored for up to 7 months before being marketed, if the right conditions are applied. For long-term storage, kiwifruit should be kept at 0 ± 0.5 °C, with a

relative humidity above 95%, and ethylene levels under 0.03 ppm. If controlled atmosphere (CA) is applied, the recommended atmosphere is 2% O_2 and 5% CO_2 for storage of up to 16 weeks longer than in air; however, an ethylene scrubber is necessary in these conditions. The end of the storage life is determined by the breakdown of tissues (McDonald, 1990).

1.2.3 Composition

The non-edible portion of kiwifruit (skin) represents 5% of the fresh weight. As with other fruits, water is the main component, representing around 83% of the fruit weight (Beever and Hopkirk, 1990).

The main soluble sugars of kiwifruit are glucose, fructose and sucrose. Sugars, in particular glucose and fructose, increase with ripening, while starch declines. Ethylene treated fruit contain higher levels of sucrose and starch than naturally ripened fruit. Soluble solids content in ripe kiwifruit is 14-16%. The pectin content also decreases with maturity, being less than 1% at ripeness; half of the pectin in ripe kiwifruit is water-soluble (Beever and Hopkirk, 1990, Perera et al., 1998).

The protein content of kiwifruit is around 0.11 to 1.2% of its weight; the large range may indicate differences in the methods of analysis. The main kiwifruit protein is actinidin, a proteolytic enzyme (Beever and Hopkirk, 1990). Calcium oxalate crystals are present in the flesh of kiwifruit, and are responsible for the irritating effects some people experience when eating the fruit (Perera et al., 1998).

Kiwifruit contains a large amount of ascorbic acid, ranging from 0.4 to 2 mg/g in ripe fruit; it also contains citric (7-9 mg/g), quinic (5-6 mg/g) and malic (1-1.5 mg/g) acids. Phenolic compounds have been also found in kiwifruit, including glycosidic derivatives of quercetin and kaempferol, chlorogenic acid, leucocyanidin and leucodelphinidin. The levels of both acids and phenolics decrease with maturity (Perera et al., 1998).

The volatile compounds of kiwifruit are mainly esters, aldehydes and alcohols, and are responsible for the characteristic flavour of the fruit (Young et al., 1983). The flavour of 'Hayward' kiwifruit is described as fresh, sweet, acid, while the flavour of 'Hort16A' is described as sweet and fruity (Marsh et al., 2006).

Kiwifruits are nutrient dense fruits, with high contents of vitamin C, potassium and fibre; the fruit is also a good source of magnesium and vitamin E (Stanley et al., 2007). Kiwifruit has also shown therapeutic potential. It has been reported that kiwifruit has the capacity to lower the levels of blood triglycerides and platelet activity (Duttaroy and Jørgensen, 2004), and improve lung and bowel function (Forastiere et al., 2000, Rush et al., 2002). The anti-carcinogenic effects of kiwifruit have also been reported (Motohashi et al., 2002).

1.2.4 Processing and potential uses

Kiwifruit is mainly commercialised as a fresh fruit, since the characteristic colour and flavour are lost during processing. Flavour compounds important for kiwifruit, such as hexanal and (*E*)-2-hexenal react in the presence of heat and oxygen; the green colour of chlorophyll also degrades in such conditions, and even during frozen storage (Stanley et al., 2007).

The amount of low grade fruit that could be processed represents 2-8% of the crop (Stanley et al., 2007). Some of the possibilities for kiwifruit processing include beverages, dried kiwifruit, and pulp (Perera et al., 1998).

Pulp is produced by mashing the fruit and removing the seeds, and is commercialised frozen. Frozen pulp is stored at -18 °C, and is used by the industry to make juices, nectars, jams, yogurts, etc. Kiwifruit nectar can be made by adding water, sugar and citric acid to the pulp, so that the final product contains 40% pulp and 14 °Bx. Kiwifruit juice is produced by pressing the fruit, and adding enzymes to remove the pectin; the juice is pasteurized and

pectinase is added again, as well as bentonite, to clarify it. The juice can be concentrated to 65-70 °Bx. Concentrates are stored at -20 °C to prevent browning. Wine from kiwifruit is made using clarified juice adjusted to 18 °Bx, and fermented to less than 1% residual sugar. The production of dried kiwifruit presents difficulties due to the loss of colour and vitamin C, and the development of off-flavours. Dried kiwifruit products include kiwifruit leather, produced by the mixture of apple and kiwifruit pulps, and candied kiwifruit (Perera et al., 1998).

Potential uses for kiwifruit include the production of aromatic extracts, deionised juice base, ready to eat sliced kiwifruit, and seed oil (Perera et al., 1998). Other market opportunities include products such as shampoo, soap and cosmetics, which can take advantage of the image of freshness and health connoted by kiwifruit (Stanley et al., 2007).

1.2.5 Economical significance

Kiwifruit is one of the newest fruit crops to gain international commercial importance. In New Zealand, the first commercial plantations of *A. deliciosa* were established around 1930 (Ferguson and Stanley, 2003).

Kiwifruit is produced in temperate areas located between the latitudes 25° and 45°; China, Italy, New Zealand, Chile and Greece are the biggest producers in the world. The production of these five countries accounts for 87% of the world kiwifruit production (Belrose Inc., 2011). Around 85-90% of the New Zealand kiwifruit is exported. Around 90% of the kiwifruit in the global market belongs to the 'Hayward' cultivar. The Chinese cultivar 'Qinmei' is the second most widely planted, but it is mainly commercialised in the Chinese domestic market. The 'Hort16A' cultivar was the first *A. chinensis* to be grown commercially outside China, and is now being produced in New Zealand, where it was originally bred, and also in Italy (Ferguson and Stanley, 2003).

Kiwifruit is New Zealand's second most important horticultural export (Fig. 1-2.). In the last three years, New Zealand exported more than 360,000 metric tons/year of kiwifruit, mainly of the 'Hayward' cultivar, with a value over NZ$1 billion, the main destinations being the European Union and Japan. The New Zealand kiwifruit production (Zespri) for the period 1997-2010 increased considerably, and also experienced diversification, with new types of kiwifruit being produced (Fig. 1-3). The average yield was over 30 metric tons/ha, and there are more than 12,500 planted hectares. New Zealand's Zespri is the leading world marketer of kiwifruit, representing 30% of the global trade (Plant & Food Research, 2010, Belrose Inc., 2011).

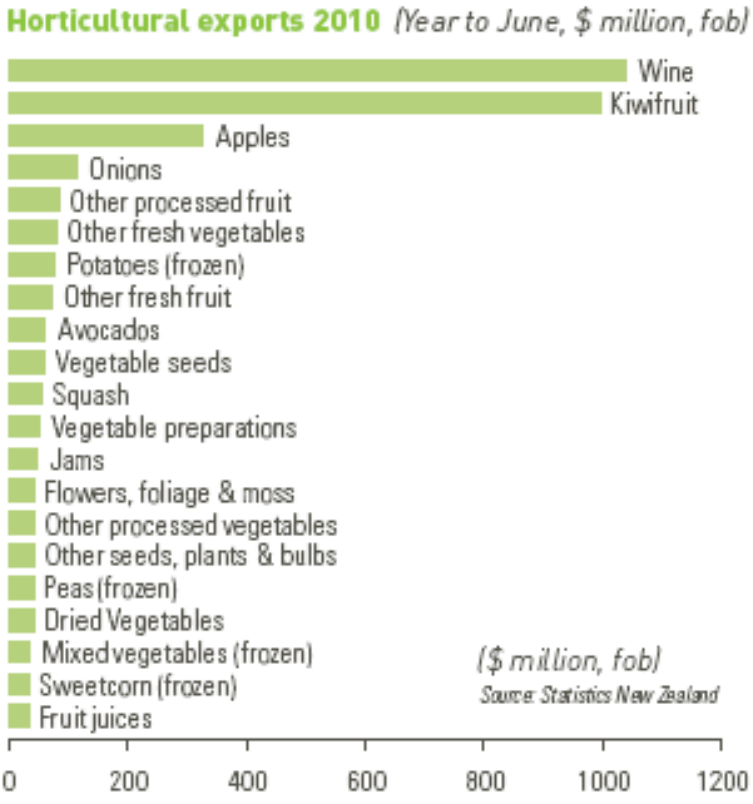

Fig. 1-2 Horticultural exports in 2010 (fob = free on board). Taken from (Plant & Food Research, 2010).

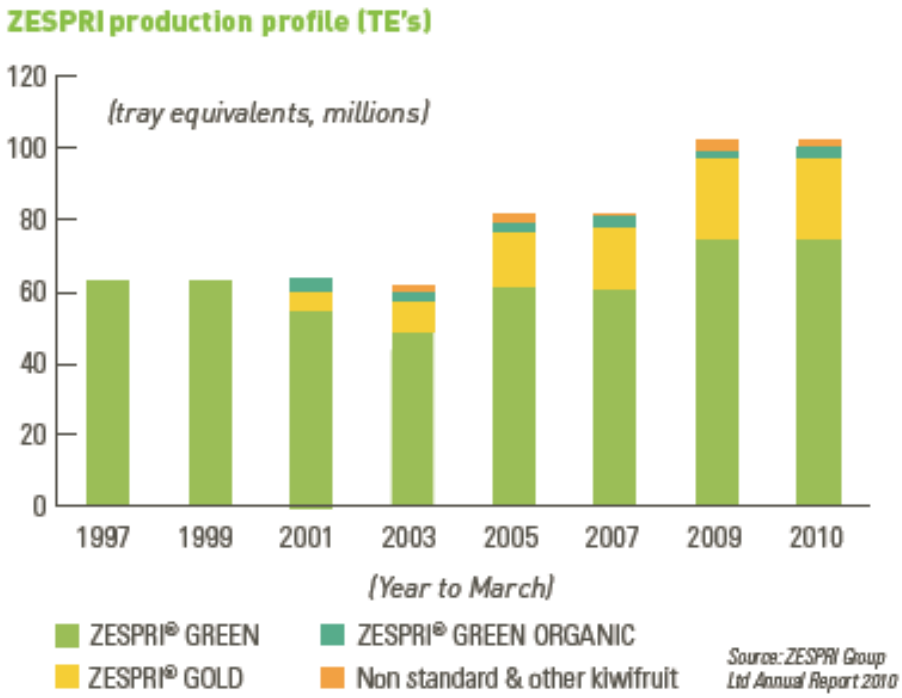

Fig. 1-3 Kiwifruit production 1997-2010 (Zespri). Taken from (Plant & Food Research, 2010).

1.3 Flavour

Flavour is the sum of sensations produced by a food in the mouth, including smell and taste, as well as tactile and temperature sensations (Flath et al., 1981).

Flavour includes two main components, as emphasised by this definition: taste and smell. Taste is produced by the non-volatile components of food. Smell is the olfactory sensation produced by sniffed volatiles (aroma), and volatiles released from the mouth and perceived retronasally (flavour) (Lawless and Lee, 1993). The word "odour" is also used to describe the retronasal smell of food (Acree, 1993).

Food volatiles are characterised by the complexity of their mixtures, their low concentrations, their chemical instability, their generally high polarity, their wide range of boiling points, the considerable presence of water, the differences in thresholds, and the close relationship between the quantitative composition of a mixture and its sensory properties

(Bemelmans, 1981).

Aroma compounds represent only a minuscule part of the composition of food, being present at the ppm or ppb level. However, the concentration of a compound does not determine its odour activity, because compounds with high concentrations may contribute little to the aroma of a food (Acree, 1993). The contribution of a compound to flavour has to be determined by sensory characterisation (Forss, 1981). One of the most common methods to assess the flavour activity is the use of flavour units, which can be expressed as follows:

Flavour activity = 1/ threshold × concentration

A flavour unit value of 1 or more indicates the compound has flavour impact (Acree, 1993).

Aromatic extracts from natural sources are complex mixtures, often containing hundreds of volatiles belonging to many groups, such as terpenes, aldehydes, alcohols, ketones, and esters. Sometimes one or a few “character impact” compounds predominate in the flavour of a food (Buttery, 1981), but more frequently, flavour is the result of the presence of a multitude of compounds (Forss, 1981). The quantitative ratios of compounds are generally more important for the aroma than the individual contributions of the compounds, as a certain aroma impression is the result of a mixture of compounds present at determinate proportions (Maarse and Belz, 1981).

The three most important attributes of an aroma compound are: odour intensity, quality, and attractiveness (Forss, 1981). Odour thresholds, or the minimum amount of compound that can be detected, vary greatly. As an example, the threshold of ethanol is 100 mg/kg, while that of 2-methoxy-3-isobutylpyrazine is 0.002 μg/kg (Flath et al., 1981). Character impact compounds typically have low thresholds, of a few μg/kg (Buttery, 1981).

Odour quality, or the sensory description of a compound, may change with concentration. At 0.1 μg/kg, (*E*)-2-nonenal smells woody, but at 1000 μg/L in aqueous solution, it smells of cucumbers (Forss, 1981).

The complexity of the mixtures of volatiles, and the low concentrations at which the aroma compounds are present, make their analysis difficult, considering the researcher not only needs to isolate the compounds, but to ensure they are spectrometrically and sensorially pure (Flath et al., 1981).

Furthermore, foods are dynamic systems, and their volatile composition may change during storage, or during the preparation of the sample (Reineccius, 2006b). Aroma compounds are highly reactive, and susceptible to chemical changes of various kinds. Oxidations, rearrangements, isomerisations and polymerisations can change the organoleptic characteristics of a food, a point to consider when selecting a sample for flavour study (Flath et al., 1981).

1.3.1 Flavour development in fruits

A large number of compounds are common in fruits, but they occur in different proportions in different products (Buttery, 1981). Maintaining the quality of the flavour of fruits is key for their commercial success, but the mechanisms leading to the formation of their flavour are still largely unknown (Taylor, 1996).

The four main types of compounds responsible for the flavour of fruits are organic acids, sugars, phenols, and volatiles. Citric and malic are the most common acids, followed by tartaric, malonic, fumaric, gluconic, and ascorbic, and traces of benzoic, salicylic, shikimic, quinic, formic and acetic acids. Sugars are mainly glucose, fructose, and sucrose. Phenolic compounds are responsible for the sensation of astringency common in unripe fruits, and their levels decrease with ripening (Taylor, 1996).

Flavour development in fruits occurs during a short ripening period, when their metabolism turns to catabolism, and the cell walls start to degrade, allowing enzymes to act on substrates previously unavailable to them. The enzymatic action generates simple sugars, acids, and volatiles (Reineccius, 2006b). Both the content of sugar and the pH increase (Wang et al., 1996). Numerous volatile compounds are generated through different metabolic pathways, and are dependent on the variety, stage of maturity, environmental conditions, and post-harvest treatments of the fruits (Sampaio and Nogueira, 2006). In climacteric fruits, the rise in respiration coincides with the increase in the formation of flavour compounds, a process which continues until senescence is reached (Taylor, 1996).

Other volatiles are produced as by-products of tissue damage, through the action of enzymes, while some others are generated when the components of the plant tissue break down during cooking (Buttery, 1981).

The aroma composition during ripening is related to biochemical reactions involving precursors and their products (Gholap et al., 1986). The composition and concentration of the precursors, and their enzymatic conversion into volatiles during ripening, determine the aroma profile of the fruit. Since the enzymatic activity is controlled by genes, this may explain the differences in the aroma profiles of fruits from different cultivars (Wang et al., 1996).

There are also important differences in the volatile profiles from fruits attached to the tree, compared to picked fruits. In peaches, the volatiles responsible for their characteristic aroma develop after picking, while the low boiling point compounds typical of the attached fruit are lost (Mookherjee et al., 1989).

In fruits, the metabolism of fatty acids generates straight chain compounds, including alcohols, aldehydes, ketones, esters and lactones. Some volatiles, such as sulfur-containing and methyl-branched compounds, are produced via amino acid metabolism. Aromatic

compounds are generated by cinnamic acid metabolism, while terpenoids are produced by both carbohydrate and lipid metabolism (Reineccius, 2006b).

Esters are the most important compounds for the aroma of fruits, and are responsible for many of their characteristic flavours. Carbonyls are important contributors for the aroma of most fruits, including benzaldehyde in stone fruits, acetaldehyde in oranges, and furfural in strawberries. Lactones give apricots and peaches their characteristic flavour (Taylor, 1996).

The precursors of aroma compounds and their corresponding volatiles are shown in Table 1-1.

Table 1-1 Precursors of aroma compounds.

Precursor	**Aroma compound**
Carbohydrates	Organic acids Esters Short chain primary alcohols Short chain aldehydes Monoterpenes Strecker degradation aldehydes [a] Pyrazines [a] Maltol, furanones [b]
Amino acids	Methyl-branched compounds Aromatic aldehydes Sulfur-containing compounds Strecker degradation aldehydes [a] Pyrazines [a]
Fatty acids	Aliphatic aldehydes, alcohols and ketones
Carotenoids	Norisoprenoids
Lignin-related compounds	Vanillin, eugenol, guaiacol
Glucosinolates	Isothiocianates Cyanides

[a] Products of the Maillard reaction
[b] Products of thermal degradation of sugars
Sources: Buttery (1981); Reineccius (2006b)

1.3.2 Glycosidic precursors of flavour compounds

In plant tissues, flavour compounds accumulate as non-volatile, flavourless glycoconjugates (Stahl-Biskup et al., 1993, Sarry and Günata, 2004). Glycosylated compounds are low reactive, water soluble species, and they are considered to be storage compounds (Sarry and Günata, 2004). Glycosylation of volatiles such as terpenoids, alcohols and phenols may also be a defensive mechanism, to prevent these lipophilic compounds from destroying the cell membranes (Stahl-Biskup et al., 1993, Lücker et al., 2001).

In fruits, glycosides of aroma compounds are mainly *O*-β-D-glucosides and *O*-diglycosides but triglycosides have also been identified. The aglycone is always attached to β-D-glucopyranose. In diglycosides, the rest of the molecule may include one the following sugars: α-L-arabinofuranose, α-L-arabinopyranose, α-L-rhamnopyranose, β-D-glucopyranose, β-D-apiofuranose, and β-D-xylopyranose. Aglycones include alcohols, terpenoids, norisoprenoids, and phenyl propane derivatives (Fig. 1-4) (Sarry and Günata, 2004, Stahl-Biskup et al., 1993).

The proportion of glycosidically bound volatiles is greater than that of free volatiles, by as much as 10:1, making them an important potential source of flavour compounds. The odorous aglycones may be released from the sugar moiety during maturation, processing and storage, or by enzymes, acids or heat (Reineccius, 2006b). The levels of glycosidic precursors change during development. It was reported that the contents of both free and bound terpenols in grapes increased with maturity, but while the content of free volatiles tended to decrease in overripe grapes, that of glycosides continued increasing (Günata et al., 1985b).

β-glucosidase is a crucial enzyme for the release of volatiles from glycosidic precursors. This enzyme is not only found in plants, but in bacteria and fungi as well (Sarry and Günata, 2004). The optimum conditions for plant β-glucosidases are a pH of 4.0 to 6.0 and a temperature of 40 to 50 °C (Sarry and Günata, 2004). The enzymatic release of flavour

compounds in fruits tends to be slow, because of their acidic pH and high glucose content, which inhibit the action of the native enzymes (Reineccius, 2006b). The structure of the aglycones attached to the β-D-glucoside also has an effect on the action of the enzymes, which are highly substrate-specific. For this reason, when using exogenous enzymes, the volatiles released may change depending on the source of the enzyme used (Sarry and Günata, 2004).

The release of volatiles from diglycosides occurs as a one-step or two-step process (Fig. 1-5). If the hydrolysis occurs in one step, the products will be the disaccharide and the corresponding aglycone (Ogawa et al., 1997). If the hydrolysis occurs in two steps, the first step is the cleavage of the sugar-sugar bond, followed by the release of the aglycone from the β-D-glucoside, by the action of β-glucosidase (Günata et al., 1988). Most of the fungi-derived enzymes are capable of both β-glucosidase action and hydrolysis of the sugar-sugar link in diglycosides (Williams, 1993). One of the enzymes isolated from *Aspergillus niger* is capable of yielding the aglycone and leaving the disaccharide intact; this enzyme has been used to increase the concentration of volatiles in wine and passion fruit juice (Shoseyov et al., 1990).

The direct cleavage of volatiles from glycoconjugates is not the only pathway for the hydrolytic release of flavour compounds. Occasionally, the hydrolysis of monoterpene glycoconjugates produces polyhydroxylated volatile precursors, with little aroma. Further hydrolysis of the polyols by acid releases the volatile compounds (Williams, 1993). In addition, plants also store volatile compounds by binding them with non-glycosidic moieties, e.g.: volatile thiols bound as glutathione- or cysteine-S-conjugates, so the analysis of glycoconjugates may not represent the whole picture of the bound volatile content (Williams et al., 1989).

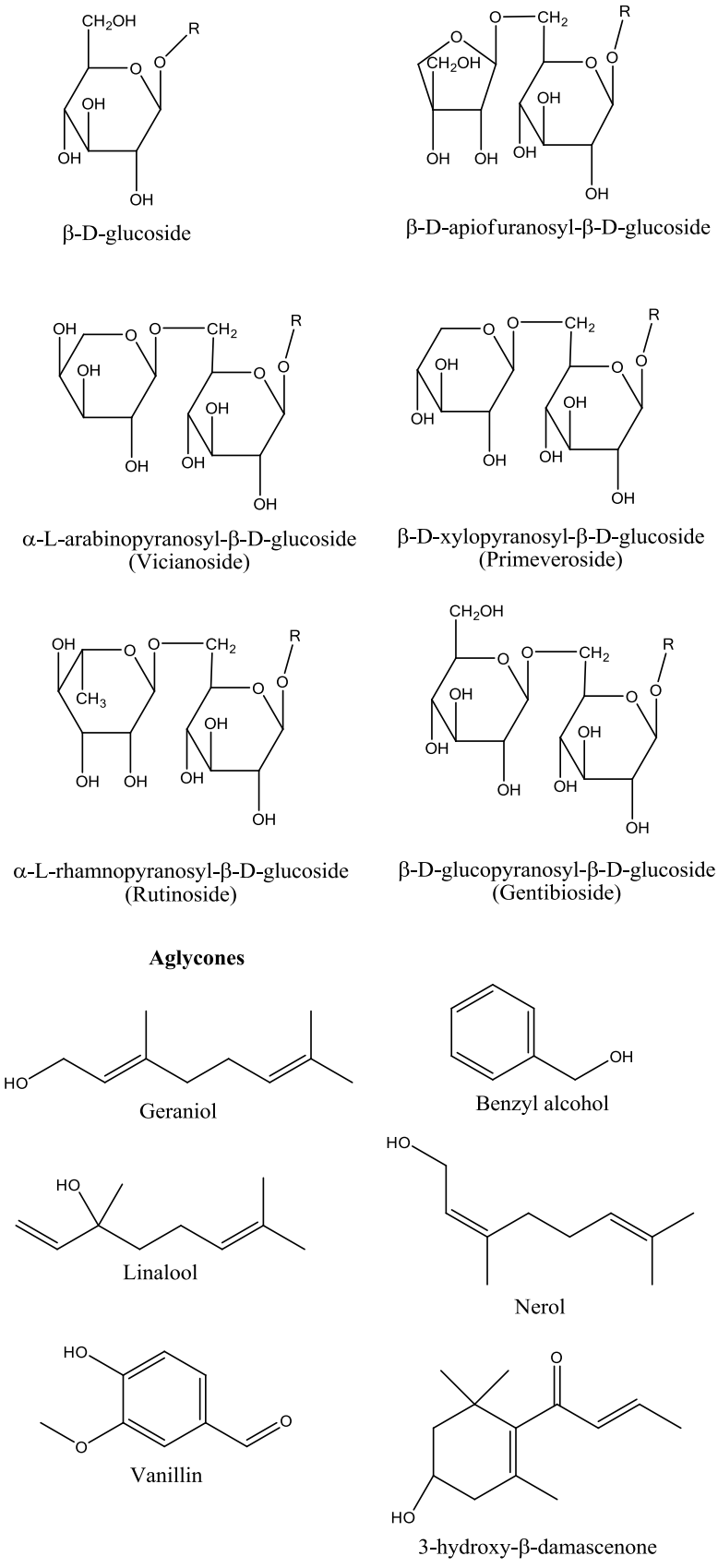

Fig. 1-4 Structure of some glycosidic aroma precursors and their aglycones, where R represents the aglycone. Source: Sarry & Günata (2004).

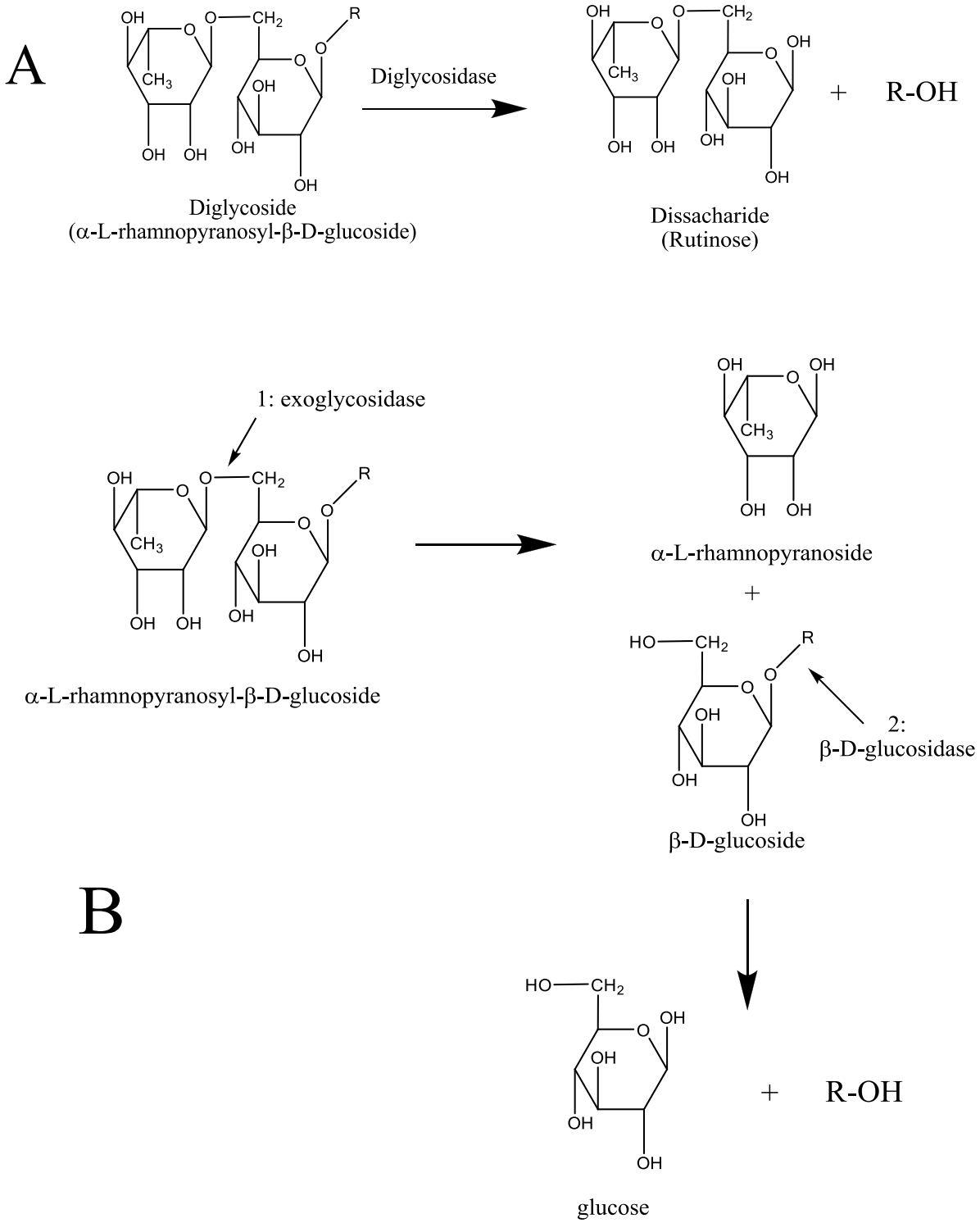

Fig. 1-5 One-step (A) and two-step enzymatic (B) hydrolysis of glycoconjugates. Source: Sarry & Günata (2004).

1.4 Analysis of aroma compounds

The goal of aroma analysis is to elucidate which compounds make the aroma profile of a food and in what proportions they are found, as well as to identify which of them actually contribute to the flavour (Werkhoff et al., 2002). To achieve this, the flavour researcher must achieve a consistent isolation of flavour compounds. Some challenges for the analysis of odorants are the low concentration of volatiles in food, the large number and variety of compounds, the low thresholds of odorous compounds, the complexity of the food matrix, the relatively low sensitivity of the instrumentation currently available, and the instability of the compounds (Reineccius, 2006b).

1.4.1 Sample preparation

Sample preparation is at least as important as the identification of the odorous compounds (Werkhoff et al., 2002). Samples should be representative of the flavour to be studied. The samples selected should have the typical flavour of the product, and their aroma should be intense. However, this is a difficult goal to achieve in practice, since there is no definition of what is the typical flavour of a given product, and the perception of aroma is subjective (Reineccius, 2006b). In the case of fruits, samples should be selected according to ripening stage, and variations due to cultivar, season, picking time, and storage conditions should be considered (Sugisawa, 1981, Teranishi and Kint, 1993). For example, the ripening stages of mango have been determined according to colour, texture, aroma and flavour (Gholap et al., 1986). The total soluble solid content, which is expressed as degrees Brix (ºBx), is a good indicator of fruit ripeness, as it is closely correlated with the volatile content, as well as pH and total sugars (Wang et al., 1996). Another ripeness indicator is the firmness of the flesh, measured by the use of a penetrometer, and expressed as kilogram-force (kgf) (Young and

Paterson, 1985, Wan et al., 1999).

The amount of sample needed is determined by the concentration of the volatiles, the type of analysis and the sensitivity of the analytical equipment (Teranishi and Kint, 1993).

The volatiles should be isolated as fully as possible, avoiding artefacts and contaminants during the process. To avoid contaminants, labware containing plastic and rubber parts, antifoaming agents, and grease should not be used. Water and solvents should also be of the highest purity (Reineccius, 2006b).

1.4.2 Isolation

Volatile isolation methods can be classified into two main categories: methods based on solubility, and methods based on volatility. The methods based on solubility involve direct contact of the sample with a solvent or an adsorbent, their disadvantage is the co-extraction of contaminating high-boiling point compounds, such as fatty acids and pigments. Methods based on volatility do not have this disadvantage, but they require the use of high volumes of water (distillation), or extract only the most volatile portion of the aroma (headspace) (Chaintreau, 2001). In some cases, due to the selectivity of the volatile extraction techniques, a combination of them is used (Teranishi and Kint, 1993).

No single method produces a whole picture of the volatile composition of a sample, because different compounds respond better to different techniques. For example, in a study of the volatiles of *Actinidia arguta*, the aromatic profiles obtained by headspace and solvent extraction were significantly different, with the lower molecular weight, lower boiling point compounds being poorly represented in the latter (Matich et al., 2003). Water-soluble compounds are difficult to extract because of their low solubility in organic solvents and because their vapour pressure decreases when dissolved (Teranishi and Kint, 1993, Reineccius, 2002).

The criteria used to select an isolation method include the volatility and boiling point of the compounds of interest, their polarity and stability, the type of analysis (qualitative or quantitative), the concentration and distribution of volatiles in the product sample, and the composition of the product itself. In practice, the most useful criteria are the purpose of the analysis, the physical state and composition of the product, and the distribution of volatiles in it (Bemelmans, 1981). Since the composition and sensory properties of an aromatic extract will depend on the isolation method employed, the researcher has to ensure that the method chosen will produce an extract that is organoleptically representative of the product being analysed (Werkhoff et al., 2002).

1.4.2.1 Methods based on solubility

Solvent extraction is better used with samples containing no lipids, since the solvent extracts lipids as well. The solvent should be of the highest grade, to avoid contaminants. To increase the efficiency of the solvent extraction, the aqueous solution may be saturated with sodium chloride or other salt. Adding the salt after the solvent is recommended, to avoid loss of volatiles (Johnson et al., 1968). The application of salt is also helpful to reduce the formation of emulsions (Bemelmans, 1981). One of the disadvantages of solvent extraction is the bias toward higher molecular weight compounds, which due to their low volatility may not be important contributors to the aroma (Matich et al., 2003).

The use of supercritical CO_2 to extract volatiles is a higher cost technique, but has the advantage of avoiding artefacts in the extract (Sugisawa, 1981, Reineccius, 2002).

The solvents used for aroma extraction include hydrocarbons, chlorinated hydrocarbons and ethers. Hydrocarbons are useful when avoiding water and ethanol is a must, for example, when analysing samples of alcoholic beverages where the high concentration of ethanol would likely interfere with the detection of odorants. Diethyl ether is cheap and highly

efficient, but also extracts water and ethanol. Chlorinated solvents are useful for separatory funnel extractions (Sugisawa, 1981, Teranishi and Kint, 1993).

1.4.2.2 Methods based on volatility

The methods based on volatility include distillation, static and dynamic headspace, direct injection, and solid-phase microextraction. These methods extract compounds with low to medium volatility, and low solubility in water, or oil for lipid-rich foods (Reineccius, 2002).

Steam distillation is a common technique for the extraction of volatile compounds, but since the distillate containing the volatiles also contains a large amount of water, an extraction step is necessary. The distillation process can be affected by the composition of the food sample, which, apart from water, contains carbohydrates, lipids and fats. Carbohydrates may generate foaming and gels, and proteins may denature if the temperatures are higher than 50 °C (Teranishi and Kint, 1993).

In flavour research, distillation under vacuum is preferred, due to the lower temperatures involved. Steam distillation at atmospheric pressure can also be used to isolate volatiles from products such as grains and spices. The main disadvantage of distillation is the potential generation of artefacts (Sugisawa, 1981).

Headspace analysis is an easy and fast technique, which extracts the compounds which actually contribute to the aroma of a product (Matich et al., 2003), but has the disadvantage of co-extracting large amounts of water from aqueous-based samples. To avoid the presence of water in the extracts, adsorbent traps have been used, although the problem of air contaminants still remains (Sugisawa, 1981). Another disadvantage of headspace analysis is the low reproducibility of results, since it is difficult to collect the same amount of volatiles from one experiment to another (Teranishi and Kint, 1993).

Static headspace extracts low amounts of volatiles, which are usually not sufficient for

analysis, making a concentration step or the enrichment of the headspace necessary. Since only the compounds in the headspace are extracted, determining the actual concentrations of the compounds in the sample is very difficult (Reineccius, 2002).

In dynamic headspace, or purge and trap methods, the sample is flushed with an inert gas, and the volatiles are collected in cold, activated coal, or polymer traps (Reineccius, 2002). To eliminate impurities when polymers such as Tenax are employed, a preconditioning step should be done before use by flushing out with solvents, followed by inert gas, and heating. The trapped volatiles can be desorbed with solvents, or thermally, in equipment furnished with a direct thermal desorption unit. Isopentane is the best desorption solvent to obtain extracts with representative aromas, although it does not produce the highest yields (Teranishi and Kint, 1993). Dynamic headspace tends to generate an aroma profile intermediate of those generated by static headspace and solvent extraction; it is generally used for identification purposes (Schaefer, 1981).

Solid phase microextraction (SPME) is an equilibrium technique based on the adsorption of volatiles by a fibre coated with an adsorbent. The volatiles are later desorbed in a GC port for analysis (Reineccius, 2002).

1.4.3 Separation and identification

Gas chromatography (GC) is the most important method for aroma analysis. Even though GC capillary columns make compound separation very efficient, the process could be improved by separating the starting material into its acidic, neutral and basic fractions (Teranishi and Kint, 1993, Mussinan, 1993).

GC is in essence a separation technique, which can be used for qualitative and quantitative purposes, as well as for identification when coupled to a mass spectrometer (MS). In the electron-impact (EI) mode, the spectra produced are dependent on the

fragmentation patterns of the compounds, which are related to their chemical structure. High degree of purity of the sample and good separation of the compounds are key to good spectra and successful identification (Mussinan, 1993).

The retention time of a compound does not change if the GC conditions are constant; however, in practice the retention times are not always reliable and relative retention times are used. The Kovats index is a system in which the retention times of the compounds are expressed relative to the retention times of an alkane series (Mussinan, 1993).

If there is a high certainty of the identity of a compound, spiking may be done by adding a pure standard to the extract. If the peak of the compound of interest increases, the identity of the compound is confirmed (Mussinan, 1993).

For the identification of compounds which have low thresholds and are generally present in trace amounts, such as sulfur compounds, the use of selective detectors is advisable (Mussinan, 1993).

For non-volatile compounds, separation is generally achieved by high-performance liquid chromatography (HPLC). HPLC is also useful to isolate larger amounts of volatiles for sensory evaluation or spectroscopic identification. In LC-MS, the eluting compounds can be identified; this setup is also useful for the analysis of thermally labile substances. Nuclear magnetic resonance (NMR) provides an alternative for structure elucidation; however, a relatively large amount of the pure compound is necessary (Mussinan, 1993).

1.4.4 Quantification

The concentrations of volatiles in a sample are in many cases reported only as percentage of peak area; however, this does not represent their true concentration due to the different responses of the compounds to the detector (Reineccius, 2006a).

A better way to quantify volatiles is the use of internal standards, which should be stable

compounds that do not overlap the peaks of the sample (Buttery, 1993). Multiple internal standards are sometimes used, each one representing a class of compounds present in the sample. After choosing the internal standards, the relative recoveries and response factors of the compounds relative to the internal standards should be obtained (Reineccius, 2006a).

Another quantification technique consists in spiking the samples with a known amount of each volatile of interest, and calculating their concentration by the increase in the peak areas after spiking. The quantification of key aroma compounds may be done by Isotope Dilution Assay, which consists in the addition to the sample of isotopically labelled analogs of the compounds of interest (Reineccius, 2006a).

1.4.5 Gas chromatography-olfactometry (GC-O)

Gas chromatography-olfactometry (GC-O) is a technique consisting in the separation of the compounds of an extract by GC, while at the same time their odour is being assessed by sniffing the eluate (Acree, 1993).

GC-O is a very useful method to recognize the important compounds for the aroma of a food. It should be remembered that the number of compounds in an aromatic extract can be in the hundreds, but only a tiny fraction is responsible for the aroma (Buttery, 1993). It has been reported that the aroma of fresh tomato can be reproduced with only 10 compounds (Buttery et al., 1987).

Sniffing the effluent is also helpful for the identification of the compounds. If the sniffer is skilled, co-eluting compounds may be identified in one GC peak, and isomers may be distinguished by their odour (Mussinan, 1993).

However, the data produced by just smelling the effluent does not measure the relative odour intensity of compounds during a run. To produce quantitative data on odour response, techniques such as CharmAnalysis and Aroma Extract Dilution Analysis (AEDA) have been

developed (Acree, 1993).

Both Charm and AEDA are dilution techniques that involve the splitting of the eluate between a sniffing port and a detector such as a flame ionisation detector (FID). The aroma extract is serially diluted by a factor of two, in the case of AEDA, or (generally) three, for Charm, until no odour is detected. The dilutions are analysed by GC-O, and the odours and retention indices, registered. By comparing all the runs, the greatest dilution at which an odour is detected is determined; this dilution value is the odour activity of the compound. In AEDA aromagrams, taller peaks have larger dilution values. Charm measures the dilution value during the time the compounds elute, generating an aromagram whose peaks show heights and areas. The difference between Charm and AEDA is that Charm integrates and sums the dilution values and odour durations, whereas AEDA only determines the maximum dilution value. Reproducibility of the GC is critical for both techniques (Acree, 1993).

The use of odour units is helpful to recognise the important aroma compounds. An odour unit (or odour activity value, OAV) can be defined as follows:

OAV = compound concentration / odour threshold

If the ratio is greater than 1, the compound is above its threshold and contributes directly to the aroma. The use of odour units can therefore assist in the selection of the compounds to be further analysed (Buttery, 1993).

1.5 Analysis of glycosidic aroma precursors

The study of flavour precursors could offer new possibilities for the food processing industry, including applications such as flavour enhancement, development of new products capable of a slow release of flavour compounds, selection of plant cell cultures which

accumulate flavour glycosides efficiently, recovery of flavour compounds through hydrolysis of precursors available in plant by-products (Williams, 1993).

1.5.1 Isolation of precursors

To determine if volatile precursors are present in a sample, the free volatiles are removed, and the material is then subjected to hydrolysis. The presence of precursors is inferred if volatiles are released (Williams, 1993).

The use of C-18 reversed phase silica gel and XAD-2 resin is widely popular with researchers because these adsorbents allow the isolation of glycoconjugates without interfering substances like protein, sugars, and organic acids (Williams, 1993). To obtain the glycosides, polar compounds such as sugars are removed by washing the adsorbent with water, followed by elution of the glycosides by an organic solvent (Maicas and Mateo, 2005).

C-18 has been used to isolate glycosides from grape juice or de-alcoholised wine; the process involves washing the column with water and acetic acid, followed by elution of the glycoconjugates with diluted acetic acid and methanol (Williams et al., 1982b). C-18 is especially good for the extraction of terpene glycosides (Williams et al., 1995). Solid phase extraction cartridges containing C-18 reversed phase have made the process easier; however, their disadvantage is that separation differs depending on the commercial source of the cartridges (Maicas and Mateo, 2005).

XAD-2 resin is used in a similar way as C-18 reversed phase, and has a similar extraction capacity. Another advantage of XAD-2 is the larger size of the particles, allowing it to be used in preparative columns (Maicas and Mateo, 2005); however, the disadvantage of this resin, and the similar XAD-16, is that free glucose is also retained in addition to the glycoconjugates (Williams et al., 1995).

As with C-18, the adsorbent is washed with water to eliminate interfering substances, and

then solvents used to elute the free and bound volatiles. The XAD-2 resin method has been applied to isolate free and glycosidically bound monoterpenes from grape by subsequent elution with pentane and ethyl acetate (Günata et al., 1985a). Glycosidic volatile precursors from apple have been recovered by elution with methanol (Schwab and Schreier, 1988).

A method using microwaves to extract glycosides has been proposed. This procedure consisted in covering grapes and juice with ethyl acetate, and extracting in a microwave oven. The resulting extract was dried and purified by passing through an Amberlite XAD-2 column. This method has as advantages the possibility of using intact plant material, avoiding the maceration of tissues, and the short time required for the experiment (Bureau et al., 1996).

If several classes of glycosides are present in a sample, they could be fractionated by the use of C-18RP columns and solvents of increasing polarity. Williams et al. (1982b) reported the separation of C10 and C13 glycosides of wine by elution with acetic acid and methanol. Strauss et al. (1987) obtained C10, C13 and aromatic fractions of grape glycosides by using C-18RP columns followed by counter-current chromatography. Fractions of glycosides corresponding to grape terpenes, norisoprenoid alcohols and shikimate-pathway compounds were obtained by Mateo et al. (1997) by the use of C-18RP columns and eluted by increased percentages of methanol.

Once the precursors have been isolated, they can be studied directly by HPLC and NMR among other techniques, or indirectly, by hydrolysing them and then analysing the liberated aglycones, or by derivatisation of the precursors (Williams, 1993).

1.5.2 Analysis of aglycones

The analysis of the liberated aglycones provides qualitative and quantitative information about the glycosides in the extract. The volatile profile of the hydrolysate should be compared to the volatile profile of the original extract. GC-O analysis of the hydrolysate can

show the potential contribution of the bound volatiles to the aroma (Williams, 1993).

1.5.2.1 Acid hydrolysis

Hydrolysis should be done at around the same pH of the fruit, because strong acidic conditions tend to produce undesirable changes in the volatile composition, such as rearrangements (Fig. 1-6) (Williams et al., 1982a). It was reported that the volatile profiles generated by hydrolysis of grape glycosides at pH 1.0 and 3.0 were significantly different, with the former having more compounds at a higher oxidation level and resembling the aroma of heated juice (Williams et al., 1982a).

The products of acid hydrolysis do not necessarily correspond to the composition of the aglycones; for example, the grape glycosides contained mainly geranyl, linalyl and neryl derivatives, but the liberated aglycones at pH 3.0 were mainly linalool and α-terpineol. The formation of α-terpineol, and other monoterpenes during the maturation of wine can be ascribed to acid catalysed rearrangements (Maicas and Mateo, 2005).

1.5.2.2 Enzymatic hydrolysis

The volatiles released by enzymatic hydrolysis depend on the nature of the sugar moiety, and the specificity of the enzymes (Williams, 1993). However, the enzymatic hydrolysis of glycosides produces a flavour profile more resembling the original product, in contrast with the one generated by acid hydrolysis. The enzymes used could be of plant or microbial origin (Maicas and Mateo, 2005). The properties of some commercial glycosidases are shown in Table 1-2.

To determine if glycoconjugates still remain after the enzymatic hydrolysis, the residue of the hydrolysate may be subjected to acid hydrolysis, followed by examination for the presence of volatiles (Günata et al., 1988).

For industrial purposes, some disadvantages of the use of enzyme preparations to hydrolyse glycosides include the possible combined action of enzymes, which liberates undesirable volatiles (e.g.: vinylphenols in wines) (Maicas and Mateo, 2005), and the colour change caused by the action of enzymes on anthocyanins (Blom, 1983). The generation of oxidation artefacts by fungal glycosidases, when used in high concentrations, has also been reported (Sefton and Williams, 1991).

Table 1-2 Properties of glycosidases.

Enzyme source	Optimum pH	Inhibition by glucose	Activity [a]	Aglycon alcohol glycoside substrates
Plant				
Almond	5.0	10% at 50 mM	β-gluc	primary
Fungi				
Aspergillus niger	na	50% at 280 mM	β-gluc, α-arab, α-rham	primary, tertiary
A. niger (Hemicellulast REG2)	5.0	na	β-gluc, α-arab, α-rham	primary, tertiary
A. niger (Pectinol VR)	na	na	β-gluc, α-arab, α-rham	primary, tertiary
A. niger (Hesperidinase)	3.8	na	β-gluc, α-rham	primary, secondary, phenolic
Others				
Glucanase	na	na	β-gluc	primary, tertiary
Rohapect C	6.0	80% at 50 mM	β-gluc, α-arab	primary, tertiary

[a] gluc is glucosidase, arab is arabinosidase, and rham is rhamnosidase
na means data not available
Source: Williams (1993)

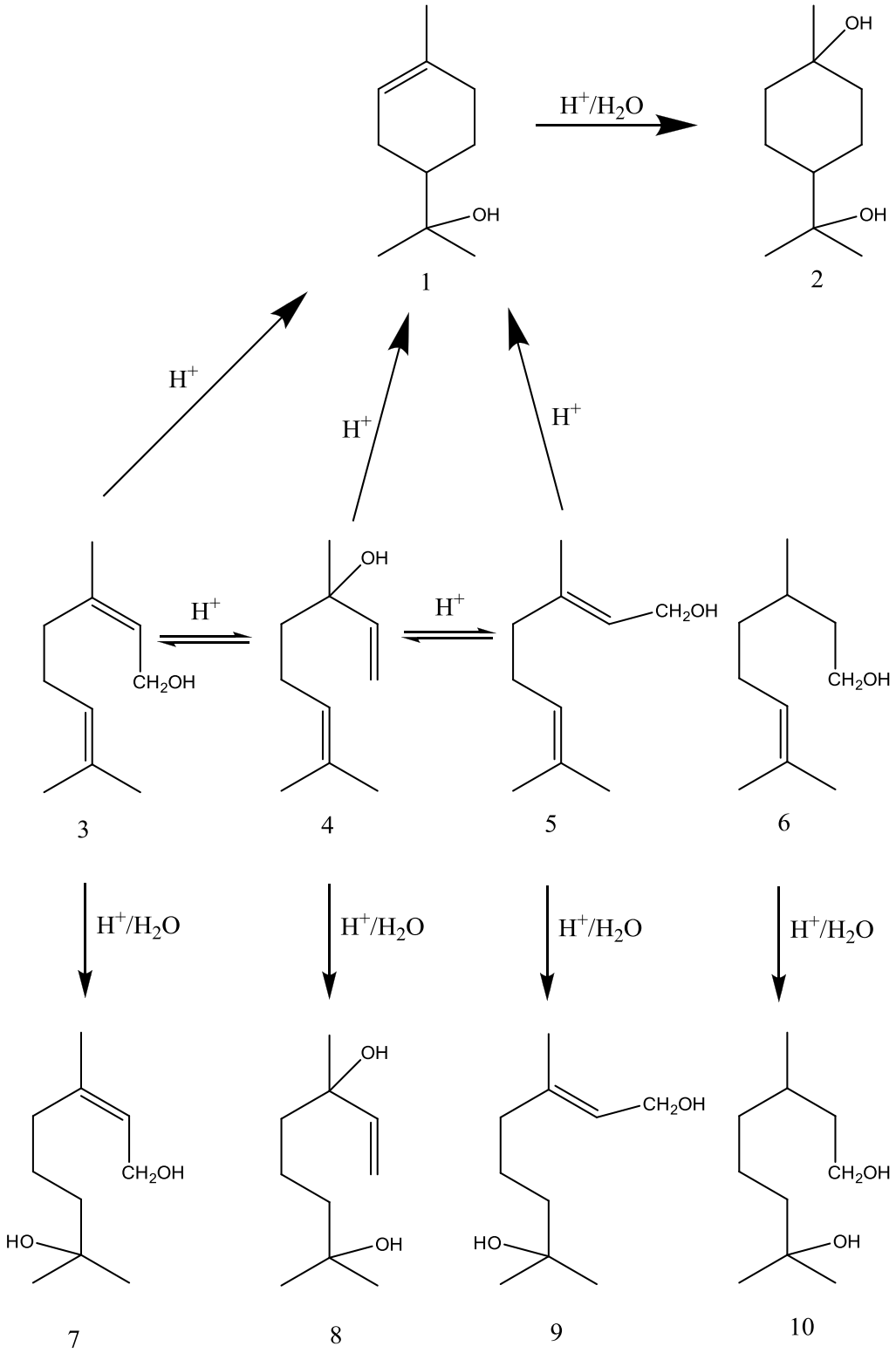

Fig. 1-6 Acid-catalysed rearrangements of monoterpenes: 1, α-terpineol; 2, (*Z*)-1,8-terpine; 3, nerol; 4, linalool; 5, geraniol; 6, citronellol; 7, hydroxynerol; 8, hydroxylinalool; 9, hydroxygeraniol; 10, hydroxycitronellol.
Source: Maicas & Mateo (2005).

1.5.3 Analysis of glycoconjugates

Elucidating the structures of the glycoconjugates is another approach, which has the potential to generate information about how different glycosides respond to enzymatic hydrolysis, and how the properties of a metabolite change when it is substituted by different moieties (Williams, 1993).

TMS-derivatives of glycoconjugates have been analysed by GC-MS (Bitteur et al., 1989, Nasi et al., 2008).

Bitteur et al. (1989) also performed direct analysis of the glycosides by HPLC, and concluded that better results are obtained with this technique when using C18-bonded silica column and acetonitrile compared to other stationary phases and solvents; however, a preliminary separation of the glycosides is always needed.

To achieve a preliminary separation, the application of silica gel chromatography followed by flash chromatography, and subsequent analysis of the glycosides has been reported (Salles et al., 1990). LC-ESI/MS and MALDI-TOF-MS have been applied as direct methods to analyse the glycoconjugates (Nasi et al., 2008).

To improve separation of glycoconjugates, counter-current chromatography has also been applied, followed by GC-MS (Strauss et al., 1987, Winterhalter et al., 1990) and LC-MS analysis (Schwab and Schreier, 1990).

1.6 Review of literature concerning the aroma compounds of kiwifruit

1.6.1 Free aroma compounds of kiwifruit

1.6.1.1 'Hayward' kiwifruit (*Actinidia deliciosa* C.F. Liang and A.R. Ferguson var. *deliciosa*)

Most of the studies on kiwifruit aroma involved the analysis of 'Hayward' (green-fleshed) kiwifruit, the major cultivar. The volatile composition of other cultivars has been less studied. Different analytical techniques have been used to analyse kiwifruit, including simultaneous distillation extraction (Shiota, 1982), vacuum distillation (Young et al., 1983, Takeoka et al., 1986), dynamic headspace (Young and Paterson, 1985, Bartley and Schwede, 1989) and SPME (Wan et al., 1999).

Shiota (1982) used simultaneous distillation extraction to isolate the volatiles, and identified ethyl butanoate, (*E*)-2-hexenal, (*E*)-2-hexenol, methyl and ethyl benzoate as the major compounds of kiwifruit aroma. In the experiment by Young et al. (1983) the pulp of ripe 'Hayward' kiwifruit was subjected to vacuum distillation, and the volatiles collected in cold traps, concentrated and analysed by GC-MS. The components identified included alkyl and alkenyl esters, alcohols, aldehydes, ketones, and methyl benzoate. Ethyl butanoate, hexanal and (*E*)-2-hexenal were identified as important odorants of kiwifruit (Young et al., 1983). Other authors identified 48 aroma compounds in an extract of ripe 'Hayward' kiwifruit, obtained by vacuum distillation. Over 90% of the volatiles reported were lipid degradation products such as C6 aldehydes and alcohols; esters and terpenoids were also found (Takeoka et al., 1986). Additional compounds, including two aldehydes, two esters, one lactone and one ketone, were identified in the headspace of ripe kiwifruit flesh analysed

by SPME (Wan et al., 1999).

Kiwifruit, like any other fruit, contains small amounts of lipids (5 g/kg for 'Hayward'), which are important volatile precursors (Ferguson and Stanley, 2003). Linolenic acid, linoleic acid, oleic acid, and stearic acid are the major fatty acids in both 'Hayward' and 'Hort16A' kiwifruit, and are mainly contained in the inner pericarp. Overall, 50-90% of the fatty acids in both kiwifruit cultivars are unsaturated, while 6-38% are saturated, with the former being more important as aroma precursors. 'Hayward' has been reported to contain significantly more linolenic acid than 'Hort16A', which agrees with its high levels of green note compounds. However, the levels of fatty acids in both cultivars did not correlate directly with those of (*E*)-2-hexenal and hexanal, although the ratio of linolenic acid to linoleic acid was similar to the ratio of (*E*)-2-hexenal to hexanal (Wang, 2007). Lipoxygenase activity has been reported to increase in kiwifruit stored at 20 ºC. At the same time, significant changes in the unsaturated fatty acid content were observed, with the proportions of linoleic acid and linolenic acid decreasing at first and then increasing quickly (Chen et al., 1999).

At present, over 80 volatile compounds have been reported in analyses of kiwifruit aroma, the major components being methyl and ethyl butanoate, hexanal, (*Z*) and (*E*)-hexenal, hexanal, (*Z*) and (*E*)-3-hexenol, and methyl benzoate. The aroma of eating-ripe 'Hayward' has been described as grassy and sulfurous with a melon and sweet candy flavour (Wang et al., 2011). Sulfurous compounds from green kiwifruit include methyl-2-(methylthio) acetate and ethyl (methylthio) acetate (Jordán et al., 2002), dimethyl disulfide (Bartley and Schwede, 1989), hydrogen sulfide (Young and Paterson, 1990, Paterson et al., 1991), and dimethyl trisulfide; this latter compound is characterised by a garlic-like smell and was identified as an odour-active compound in 'Hayward' by GC-MS/O (Frank et al., 2007).

(*E*)-2-hexenal has been reported in some publications as the most abundant compound in ripe kiwifruit (Young et al., 1983, Takeoka et al., 1986, Bartley and Schwede, 1989, Frank et

al., 2007). Interestingly, (*E*)-2-hexenal did not appear in the chromatogram of kiwifruit extract to which an enzyme inhibitor (stannous chloride) was added, but the content of esters was not affected (Bartley and Schwede, 1989); a similar result was obtained by Paterson et al. (1991), who reported that the major odorous compounds of kiwifruit aroma were esters.

Young and Paterson (1985) evaluated the effects of harvest maturity, ripening and storage on kiwifruit aroma, using the pulp of 'Hayward' kiwifruit as sample, and extracting the volatiles by dynamic headspace. Harvest maturity was determined by soluble solids content at the time of harvest, and ripeness was correlated to flesh firmness. These authors found that the levels of aroma compounds of kiwifruit increased dramatically during a short ripening period, as confirmed by Bartley and Schwede (1989) and Wang et al. (2011). Similar results have been observed in other fruits, and seem to be related to the increase in the production of ethylene (Paterson et al., 1991). In freshly harvested fruit, the levels of esters were greater in fruit harvested at higher soluble solids (SS) levels than in fruit harvested at lower SS levels, while the opposite occurred with aldehydes, explaining the green odour of the latter (Young and Paterson, 1985). The reduced levels of aldehydes are probably due to a diminished lipoxygenase activity in ripe fruit (Bartley and Schwede, 1989).

According to Young and Paterson (1985) and Wan et al. (1999), the aroma of under-ripe kiwifruit had a green note, which changed to fruity with ripening; they attributed this change to the decrease of aldehydes, responsible for the green aroma, and the increase of esters, responsible for the fruity aroma. A similar result was obtained by Bartley and Schwede (1989), who found that ethyl butanoate increased drastically, at the same time (*E*)-2-hexenal decreased; the former, being a powerful odorant, had a remarkable effect on the flavour of ripe kiwifruit. In contrast, in another study in which the volatiles of sliced kiwifruit were isolated by dynamic headspace, no relationship between the aldehyde content and the degree of ripeness was found, and only a slight increase in the aldehyde content of stored fruit was

observed (Paterson et al., 1991). This result may indicate that the high levels of aldehydes reported by Young and Paterson (1985) and Bartley and Schwede (1989) could be generated during sample preparation, which involved maceration of tissues, an optimal condition for the action of lipoxygenases (Paterson et al., 1991) or a higher availability of fatty acid precursors (Wang et al., 2011).

The effect of storage on kiwifruit has been studied, and differences in the volatile profiles of stored fruit harvested at different maturities have been observed; but generally, long term storage resulted in a diminished volatile content (Young and Paterson, 1985, Paterson et al., 1991). The decrease of volatiles may be due to a slow ripening process occurring during storage, which not only results in softening and increase of sugars, but in the loss of volatiles (Young and Paterson, 1985). In the study by Young and Paterson, only fruit harvested at 8.0 °Bx showed a slight increase in the volatile content after nine weeks, but the volatiles decreased considerably after 21 weeks. Therefore, the short term storage of fruit harvested at 8.0 °Bx was recommended (Young and Paterson, 1985).

Paterson et al. (1991) found that stored kiwifruit had ethyl acetate as the most abundant ester, in contrast to ethyl butanoate, the major ester in freshly harvested kiwifruit. Methyl butanoate was another important ester contributing to the aroma. Mature fruit that had been stored also had higher levels of α and β-pinenes (Paterson et al., 1991). It was observed that short term storage (4-6 weeks) of kiwifruit at 0 °C had positive effects such as the decrease of green notes, acidity and off-flavour, and the increase in flavour intensity, particularly in early picked fruit; however, long term storage had negative effects on kiwifruit volatiles, as observed in fruit that had been stored at 0 °C for 12 weeks or 4 °C for 4 weeks, which was less sweet and had more off-flavours than freshly harvested fruit (MacRae et al., 1990). Furthermore, kiwifruit does not only lose flavours during long-term storage, but colour as well (Perera et al., 1998). A study done on kiwifruit covered with polyliner and ripened in an

incubator for five months, however, reported that the total volatile content of kiwifruit increased, even after five months of storage; but admitted that the difference may be due to the different handling of the sample, which involved softer fruit, longer ripening times and storage at a lower temperature; furthermore, the extraction of volatiles was done by SPME (Wan et al., 1999) instead of the more commonly used solvent extraction. This contrasts with the findings of Young and Paterson (1985), MacRae et al. (1990), and Paterson et al. (1991).

Ethanol has been reported as the major volatile in kiwifruit that had been stored prior to ripening, but due to its high threshold, it is not an important contributor to the aroma (Paterson et al., 1991); however, it has been suggested that ethanol may affect ester production (Burdon et al., 2005). Ethanol has also been reported as the major component of overripe and stored mango, so it may be an indicator of fermentation and off-flavour in fruit (MacLeod and Snyder, 1985).

The volatiles of kiwifruit stored in controlled atmosphere conditions also change depending on the CO_2 control method applied. Controlled atmosphere (CA) consists of the control of O_2 and/or CO_2 in gas-tight cool rooms, and its purpose is to extend the storage life of fruits. The control of CO_2 is achieved by an activated carbon scrubber, hydrated lime scrubber, or nitrogen purge; whose application changes the volatile composition of the room, and in turn affects the volatile production of the fruit. In an experiment comparing fruit stored in different CA conditions during two seasons, it was found that the fruit from activated carbon rooms had a higher content of esters than the fruit stored in air (Burdon et al., 2005). The increase of volatiles in CA conditions contrast with the decrease observed in air stored kiwifruit, but it should be noted that in this experiment the kiwifruit was ripe before CA storage. Differences were observed according to the CO_2 control used, but the levels of the three main volatiles associated with kiwifruit aroma, hexanal, (*E*)-2-hexenal and ethyl butanoate, were similar for the three CA treatments (Burdon et al., 2005).

Sensory evaluation of a kiwifruit juice model was performed to evaluate the contribution of the aroma compounds to consumer acceptability. Ethyl butanoate, (*E*)-2-hexenal and hexanal increased the perceived intensity of kiwifruit aroma. Ethyl butanoate had positive effects as it was associated with the characteristic kiwifruit flavour, but increasing levels of (*E*)-2-hexenal were negative for the acceptability of kiwifruit flavour (Gilbert et al., 1996, Ball et al., 1998).

Sensory evaluation has also been done on modified kiwifruit pulps, to which sugars and acids have been added. It was found that flavour intensity is not affected by the change in soluble solids or acidity, but the overall liking increased with increasing Brix (Rossiter et al., 2000). Banana-like flavour increased with added sugar and decreased with added acid (Marsh et al., 2006), which may be due to sugar and acid interactions in the mouth, but the increased release of some volatiles with the addition of sugar may be another possible explanation (Friel et al., 2000).

Frank et al. (2007) identified the most important odorants for 'Hayward' by GC-MS/O. The major contributors to the aroma were (*E*)-2-hexenal, 1-penten-3-one, hexanal, ethyl-2-methylpropanoate, dimethyl trisulfide, (*E*)-3-hexenol and 1-octen-3-one. In contrast with the findings of Young et al. (1983), ethyl butanoate was not identified by Frank et al. (2007) as a major odorous compound.

Processed kiwifruit products, including puree (Fischböck et al., 1988, Jordán et al., 2002), juice (Young et al., 1992), essence (Jordán et al., 2002) and wine (Craig, 1988) have also been studied.

Processed kiwifruit products generally have strong off-odours, which have been described as old cut grass, hay, and cooked. (*E*)-3-hexenal has been reported as the compound responsible for the hay off-odour of kiwifruit juice (Young et al., 1992). The aroma compounds of deep-frozen kiwifruit puree, an ingredient used by the food industry in the

production of ice cream, jam, etc., were analysed by GC-FTIR, and it was found that the presence of the terpene esters linalyl acetate, isobornyl acetate, and α-terpinyl-acetate was responsible for the overripe off-flavour in stored puree (Fischböck et al., 1988). On the other hand, the main odorants of fresh puree included 3-penten-2-ol, ethyl butanoate, (*E*)-2-hexenal, 6-methyl-5-hepten-2-one, 1-octen-3-ol, methyl benzoate, and hexyl hexanoate; α-terpineol was also an important odorant (Jordán et al., 2002).

The major contributors to the aroma of aqueous kiwifruit essence were 3-penten-2-ol, (*E*)-2-pentanal, (*E*)-2-hexanal, (*E*)-2-hexenol, heptanal, 6-methyl-5-hepten-2-one, carvone and hexyl hexanoate (Jordán et al., 2002).

The aroma of kiwifruit wine does not resemble the aroma of fresh kiwifruit. Ethyl acetate, ethyl propanoate, ethyl 2-methylpropanoate, ethyl 2-methyl butanoate, ethyl 3-methylbutanoate and fenchone were reported as important compounds for the aroma of kiwifruit wine (Craig, 1988).

1.6.1.2 Other kiwifruit cultivars (*Actinidia chinensis* 'Hort16A', *A. arguta*, *A. eriantha*)

Actinidia chinensis Planch var. *chinensis* 'Hort16A', also known as Gold Kiwifruit, has been described as having sweet, banana and blackcurrant flavours (Jaeger et al., 2003). The aroma of this cultivar differs from that of the 'Hayward' kiwifruit in that it contains less green aromas and more tropical notes (Friel et al., 2007).

The most important odorants of 'Hort16A' have been identified as acetaldehyde, hexanal, ethyl butanoate, (*E*)-2-hexenal and dimethyl sulfide (DMS). DMS has not been reported in 'Hayward' kiwifruit, and may be one of the key components that differentiate the aroma of both cultivars. The levels of esters increased with ripening, but the content of C6 aldehydes did not change significantly. Firm fruit (0.71-0.82 kgf) contained some compounds that overripe fruit did not, including 6-methyl-5-hepten-2-one, methyl pentanoate, *p*-cymene, β-

pinene and sabinene. For both ripening stages, ethanol was the most abundant volatile. Eucalyptol was also found at significant levels (Friel et al., 2007).

Ethyl butanoate has been reported as the major ester in 'Hort16A', contributing to the sweet and fruity flavour, however, its marked increase at the over-ripe stage is responsible for the sweet-vomit notes characteristic of over-ripe fruit (Wang et al., 2011).

Methylsulfanyl (MeS) compounds are reported to increase considerably with ripening. As these compounds have been detected in trace amounts, their role in 'Hort16A' flavour is not clear yet, although their generally low odour thresholds (e.g.: methional,0.43 μg/kg; ethyl-3-MeS-propionate, 7 μg/kg) suggest that they may contribute to the tropical nuances (Zhang et al., 2009).

Actinidia arguta (kiwiberry or baby kiwifruit) has been described as having tropical, fruit candy, and green flavours different from those of the 'Hayward' kiwifruit. The aroma compounds of *A. arguta* are mainly esters, aldehydes, and alcohols, and some monoterpenes (Matich et al., 2003).

The volatiles in the headspace of *A. arguta* have been isolated, and the main contributors to the aroma have been identified as esters, the most abundant of them being ethyl butanoate and ethyl hexanoate. Products of lipid degradation, such as (*E*)-2-hexenal, hexanal and (*Z*)-3-hexenal were also important contributors to the aroma. The solvent extract of *A. arguta* also contained methyl and ethyl benzoate, the compounds responsible for the aroma of feijoa (Crowhurst et al., 2008), as well as camphor.

Yang et al. identified the odour-active compounds in *A. arguta* by using GC-MS/O. These compounds included 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF), 1-penten-3-one, (*E*)-2-hexenal, pentanal, hexanal, ethyl butanoate and methyl benzoate (Yang et al., 2010).

Actinidia eriantha Benth. is a species that can be used to generate new, peelable cultivars, and is also known for its high vitamin C content and low flavour (Atkinson et al., 2009). The

volatile content of *A. eriantha* is lower than in other *Actinidia* cultivars. The dominant ester in this fruit is butyl acetate, a compound with a pineapple-like aroma (Crowhurst et al., 2008).

1.6.2 Bound aroma compounds of kiwifruit

It is known that glycoconjugates constitute a major portion of the aroma compounds of fruits (Sarry and Günata, 2004) and there have been studies done on grape (Williams et al., 1982a, Williams et al., 1982b, Günata et al., 1985b, Günata et al., 1985a), passionfruit (Chassagne et al., 1999), and tomato (Buttery et al., 1990) among others. In a previous study, the juice of ripe 'Hayward' kiwifruit was used to isolate the glycoconjugates by adsorption onto an Amberlite XAD-2 column, followed by enzymatic hydrolysis by β-glucosidase (Young and Paterson, 1995). Twenty nine volatiles were identified in the hydrolysate, including new compounds not previously found in kiwifruit, such as 3-octanol, camphor, 4-methylbenzaldehyde, 2-hydroxybenzaldehyde, neral, geranial, methyl 2-hydroxybenzoate, nerol, geraniol and 2-phenylethanol. The major compounds in the hydrolysate were (*E*)-2-hexenal and benzaldehyde; terpenoids and alcohols were also found. (*E*)-2-Hexenal was also found in a control experiment in which no enzyme was added, hence, it is probably a product of non-enzymatic hydrolysis. Many of the compounds found have been reported in previous analyses of kiwifruit aroma, except esters, which are not stored as glycosides (Young and Paterson, 1995). β-Damascenone was also found, and may be responsible for the heated kiwifruit aroma of the hydrolysate, as this compound has a heated apple-like aroma, which is also similar to the aroma of heated kiwifruit juice (Young and Paterson, 1995).

1.7 Conclusions

The genus *Actinidia* (kiwifruit) includes a large number of species, although only three of

them are economically important (*A. deliciosa*, *A. chinensis*, and *A. arguta*). Kiwifruit is appreciated for its flavour and nutritional qualities. Flavour is the result of the presence of volatile and non-volatile compounds, and is a key factor for consumer acceptance. In fruits, flavour develops during a short ripening period when volatile compounds are produced from precursors, including aroma glycosides. Glycosidically bound volatiles are thus a potential source of flavour in fruits.

To elucidate the aroma profile of a fruit, it is necessary to isolate and analyse its volatile compounds. Olfactometry analysis is often required to identify the true contributors to the aroma. The free volatile composition of several kiwifruit species has been studied, including *A. deliciosa* 'Hayward' (Green Kiwifruit), *A. chinensis* 'Hort16A' (Gold Kiwifruit), *A. arguta*, and the non-commercial *A. eriantha*. Most studies have focused on 'Hayward' because this is the predominant cultivar in the market. A large number of volatiles have been identified, including mainly esters, aldehydes and alcohols. 'Hort16A' and *A. arguta* have different volatile profiles, including minor but significant components like sulfur compounds and terpenoids, and their flavour is clearly different from that of 'Hayward'. *A. eriantha* is known for its low volatile content and poor flavour. The bound volatile fraction of these four kiwifruit species has largely been ignored, showing that there is an important knowledge gap that the present study intends to fill by elucidating their bound volatile profiles, identifying the odour-active bound volatiles, and assessing the effects of ripening on the bound volatile composition.

Chapter 2

Preliminary study of bound volatile compounds from kiwifruit

2.1 Introduction

In fruits, aroma compounds accumulate as non-volatile, flavourless, water soluble glycosides, which, due to their low reactivity, may act as storage compounds. Fruit glycosides are mainly *O*-β-D-glucosides and *O*-diglycosides.

To isolate bound aroma compounds, adsorption on hydrophobic adsorbents such as C18 reversed phase and Amberlite XAD-2 is the technique of choice. The retained analytes are then eluted with an appropriate solvent and analysed by TLC, GC or HPLC, either directly or after hydrolysis with acids or enzymes (Sarry and Günata, 2004).

For acidic hydrolysis, hydrochloric, perchloric or sulfuric acid can be used, and the pH adjusted (pH 1.0 and 3.0 are typical values). Acidic hydrolysis can induce molecular rearrangements, and thus may not accurately reflect the composition of the aglycones (Maicas and Mateo, 2005).

For enzymatic hydrolysis, several enzymes from diverse origins can be used. β-Glucosidases from plant sources tend to have low activity on terpene glucosides. β-Glucosidases from yeast have been used in oenology with satisfactory results. Preparations of fungal glycosidases containing a mixture of β-D-glucopyranosidase, α-L-arabinofuranosidase, α-L-rhamnopyranosidase, and β-D-apiofuranosidase are recognised as the best for winemaking processes (Maicas and Mateo, 2005).

The release of bound volatiles of kiwifruit by acid and enzymatic hydrolysis was investigated in this chapter. In addition, an experiment comparing the volatile profiles of solvent extract vs. SPME was conducted.

2.2 Materials and methods

2.2.1 Plant material

Actinidia deliciosa 'Hayward' and *A. chinensis* 'Hort16A' were used. Fruits were obtained from the Plant & Food Research orchard in Te Puke, New Zealand, in July 2009, and kept in cool storage at 0 °C until required.

Fruits were at eating-ripe stage, with average flesh firmness of 0.59 kgf for Hayward and 0.60 kgf for Hort16A, measured with a GUSS Fruit Texture Analyser FTA (GUSS Manufacturing, Strand, South Africa). A refractometer model N-20E (0-20% Brix, ATAGO, Tokyo, Japan) was used to measure soluble solids (SS) content, obtaining values of 15.1 °Bx for Hayward and 15.65 °Bx for Hort16A.

'Hort16A' kiwifruit was used to assess the suitability of acid hydrolysis versus enzymatic hydrolysis. 'Hayward' kiwifruit stored for 5 months at 0 °C was used to compare the efficacy of solvent extraction versus solid phase microextraction (SPME), as well as to compare the hydrolysis efficiency of almond β-glucosidase versus Rapidase AR2000, and evaluate several hydrolysis times. Fruit for these experiments were chosen based on availability.

2.2.2 Reagents and standards

All chemicals used were of analytical grade or better. Diethyl ether was obtained from Rhône-Poulenc (Perth, Australia). Pentane was obtained from Scharlau (Barcelona, Spain), and re-distilled before use.

Almond β-glucosidase was purchased from Sigma-Aldrich (St. Louis, MO, USA). Amberlite XAD-2 was purchased from Supelco (Bellefonte, PA, USA). Rapidase AR2000 was obtained from DSM (Delft, Netherlands).

Stock standard solutions of cyclohexanone (Sigma-Aldrich) were prepared in ether, with a

concentration of 1 mg/mL and 0.1 mg/mL and stored at 4 °C. Laboratory distilled (RO) water was used in all experiments.

2.2.3 Isolation of glycosidic precursors

The glycosidic precursors were isolated by adsorption onto an Amberlite XAD-2 column, which was prepared at room temperature according to the manufacturer's instructions at least one day prior to use, to allow it to reach equilibrium, and washed sequentially with two bed volumes of water, acetone, water, 3 M HCl, and abundant water until Cl^- free. The bed volumes were calculated using the formula:

Bed volumes = $\pi \times r^2 \times$ bed length, where r is the radius.

The fruit flesh was scooped out and the juice extracted using a BJE 200 juicer (Breville, Australia), followed by pressing in a Medio wine press (Ferrari Group, Italy) lined with cheesecloth. The fruit flesh and juice were kept over ice. The juice was filtered over Celite 545 (VWR, Haasrode, Belgium), under vacuum.

The clear juice obtained was passed down the previously prepared Amberlite XAD-2 column at room temperature, followed by elution with 3 bed volumes of water, pentane and methanol, in that order. Both the water fraction, containing free sugars and other polar compounds, and the pentane fraction, containing residual free volatiles, were discarded. The methanol fraction, containing the glycosidic aroma precursors, was evaporated to dryness using a rotary evaporator (Buchi, Flawil, Switzerland) and further dried under vacuum at room temperature for 2 h. The dried glycosidic extract obtained was kept at -20 °C until required.

2.2.4 Release of bound volatile compounds

To release the glycosidically bound volatiles, both acid hydrolysis under different pH conditions, and enzymatic hydrolysis were performed, as described below.

2.2.4.1 Acid hydrolysis

Acid hydrolysis was conducted based on previously published methods (Williams et al., 1982b, Ananthakumar et al., 2006), with modifications. The dried glycosidic extract was dissolved in 1 M HCl, and extracted with pentane to remove residual free volatile compounds. After which, hydrolysis was carried out in strong acidic conditions (pH 0), or after adjusting the pH with 4 M NaOH to pH 3 or pH 5.

Acid hydrolysis was carried out in a closed vessel, in a water bath at 80 °C for 60 min. The hydrolysate was cooled over ice, before extracting the released aglycones with a mixture of pentane:ether (1:1 v/v). The extract was dried over anhydrous sodium sulfate, filtered, pre-concentrated in a Kuderna-Danish apparatus, and further concentrated by slow static distillation to a final volume of 50 µL.

2.2.4.2 Enzymatic hydrolysis

The citrate/phosphate buffer for the hydrolysis was prepared by dissolving 4.2 g of citric acid and 2.84 g of di-sodium hydrogen phosphate in 100 mL distilled water, and adjusting to pH 5 with 4 M NaOH, as described by Young and Patterson (1995).

The dried glycosidic extract was dissolved in the buffer, and extracted with pentane to remove residual free volatiles.

Almond β-glucosidase, dissolved in buffer, was added, and hydrolysis was carried out at 37 °C for 42 h (Young and Paterson, 1995). The hydrolysate was cooled over ice, and the released aglycones were extracted with a mixture of pentane:ether (1:1 v/v). The extract was

dried over anhydrous sodium sulfate, filtered, pre-concentrated in a Kuderna-Danish apparatus, and further concentrated by slow static distillation to a final volume of 50 μL. A sample with no enzyme added (control) was treated in the same way. Concentration of the solvent extracts in a rotary evaporator, over an ice bath, followed by further concentration under a gentle stream of nitrogen to a final volume of 100 μL was also evaluated. These steps were chosen for further experiments, as they were less time consuming than using a Kuderna-Danish apparatus, and gave satisfactory results.

The commercial enzyme Rapidase AR2000, from *Aspergillus niger*, was also tested by comparison to the almond β-glucosidase, conducting hydrolysis in the same conditions.

The effect of the duration of the hydrolysis was evaluated by comparing the chromatograms obtained after 24, 42 and 48 h of hydrolysis at 37 °C.

Cyclohexanone (45 μL of a 1 mg/mL solution) was added as an internal standard before hydrolysis, to estimate the amount of aglycones released.

2.2.5 Solid phase microextraction (SPME)

The use of SPME for the isolation of the released volatiles was evaluated. Fibres coated with 65 μM PDMS-DVB (blue, Supelco) were used. Fibres were preconditioned by heating in a GC injector at 250 °C for 30-60 min. Hydrolysis was carried out at 37 °C for 42 h, and the fibre was exposed to the released volatiles during the whole duration of the hydrolysis. Fibres containing the adsorbed volatiles were stored at -20 °C until analysis. Only qualitative analysis was performed for SPME samples.

2.2.6 GC-MS analysis

An Agilent 6890 GC coupled with a LECO Pegasus II TOF-MS was used. The column was a DB-Wax (Phenomenex, Torrance, CA; 30 m length × 0.25 mm i.d. × 0.25 μm film

thickness).

The temperature programme for solvent-extracted samples was as follows: kept at 50 °C for 2 min, raised to 150 °C at a rate of 5 °C/min, raised to 200 °C at a rate of 10 °C/min, raised to 247 °C at a rate of 20 °C/min, and kept at 247 ºC for 10 min. Sample volume was 1 μL, injected by autosampler into the GC-MS instrument in split mode with a split ratio of 5. The total run time was 39.35 minutes.

For the SPME samples, the column temperature was kept at 45 °C for 2 min, then raised to 100 °C at a rate of 3 °C/min, raised to 160 °C at a rate of 6 °C/min, raised to 200 °C at a rate of 10 °C/min, raised to 245 °C at a rate of 20 °C/min, and kept at 245 ºC for 10 min. The sample was injected in split mode with a split ratio of 1. The total run time was 46.583 min.

The injector temperature was 250 °C for liquid injection and 240 °C for SPME, and the ion source temperature was 230 °C, the ionising voltage was 70 eV, the flow rate of the carrier gas (He) was 1.5 mL/min for liquid injection and 2.0 mL/min for SPME. A higher flow rate was necessary for SPME samples to prevent peak tailing.

The scanning range was *m/z* 33-450 for liquid injection and *m/z* 31-400 for SPME.

Data analysis was performed using the ChromaTOF software v. 3.34 (LECO Corporation, St. Joseph, MI, USA). The mass spectral libraries of the GC-MS system (Wiley 7th ed. 2001, NIST 2005) were used for identification purposes.

The experiments comparing almond β-glucosidase vs. Rapidase AR2000, and the duration of hydrolysis were conducted using a Shimadzu 2010 GC coupled with a Shimadzu 2010 QPlus MS (Kyoto, Japan) fitted with a Stabilwax column (Restek, Bellefonte, PA; 30 m length × 0.25 mm i.d. × 0.25 μm film thickness). The GC settings were the same as detailed above for liquid injection.

Kovats indices were obtained by injecting a C8-C23 linear alkane mixture, in the same conditions as the analysed samples, and using the retention index feature of the software

(ChromaTOF) to calculate them.

Tentative identification of the compounds was achieved by comparing their mass spectra and retention times with those available in the GC-MS libraries and the compound database of Plant & Food Research, or published in the literature (NIST Chemistry WebBook, http://webbook.nist.gov/chemistry/).

Semi-quantification of aroma compounds was performed by comparing the peak areas of the volatiles in the sample to the peak area of an internal standard of cyclohexanone (45 μL of a 1 mg/mL solution), and expressing the result as percentage of the peak area of the internal standard. The equation for quantification is shown below:

Volatile content = peak area of unknown / peak area of internal standard × 100

The procedure for the analysis of kiwifruit bound volatiles is shown in Fig. 2-1.

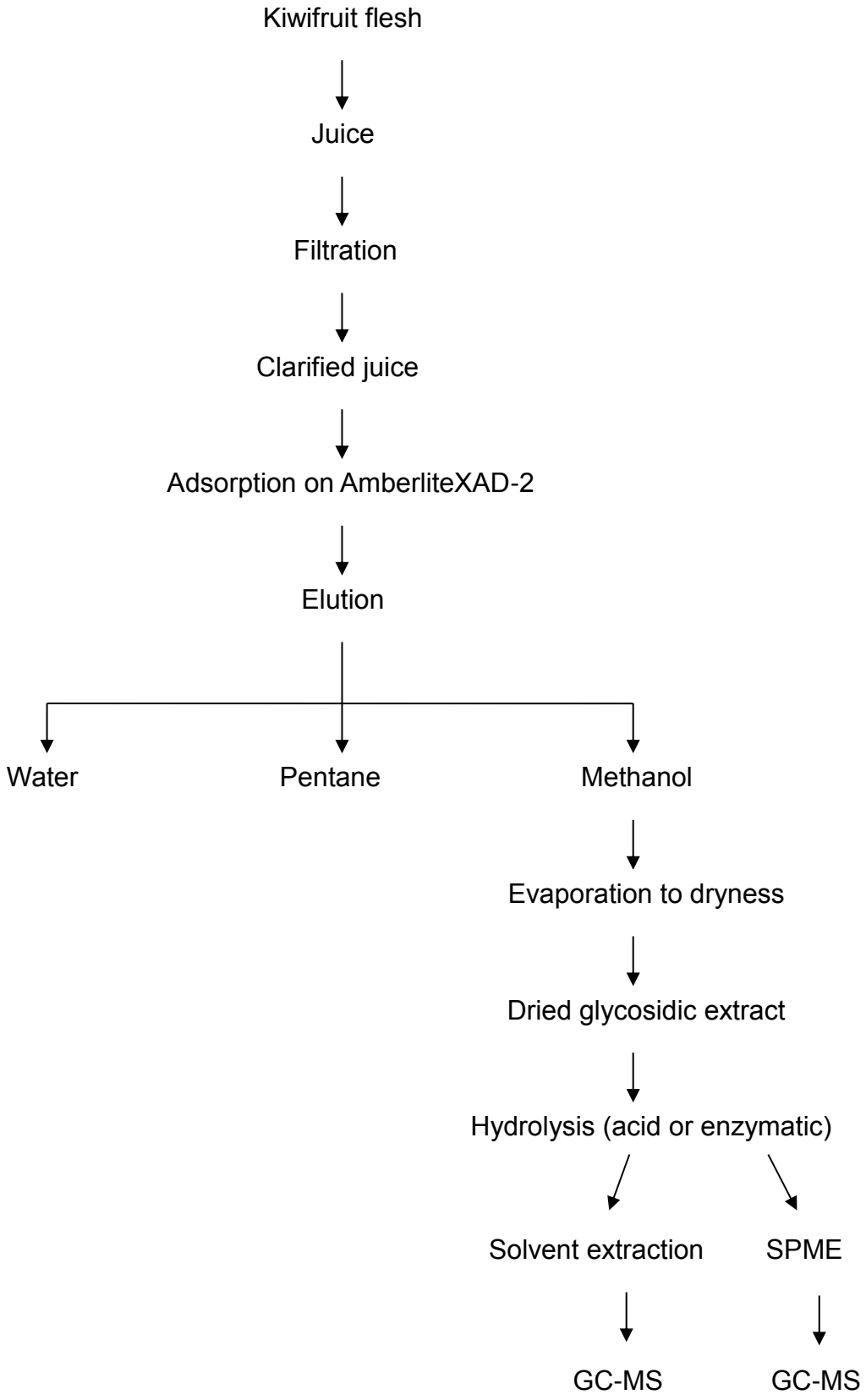

Fig. 2-1 Analysis of kiwifruit bound volatiles.

2.3 Results and discussion

2.3.1 Hydrolysis method

The volatile composition and the chromatograms of the hydrolysed samples of 'Hort16A' kiwifruit are shown in Table 2-1 and Figs. 2-2 and 2-3, respectively. All compounds were tentatively identified by MS only.

The hydrolysis of the glycosidic extracts of 'Hort16A' and 'Hayward' kiwifruit confirmed the presence of bound volatiles (Tables 2-1 and 2-2). The volatiles released included mainly alcohols, aldehydes, and terpenoids. The composition of the aroma profiles differed according to the cultivar, pH conditions during acid hydrolysis, the application of enzyme, and the extraction technique.

The volatiles released by harsh acid hydrolysis of 'Hort16A' glycosides at pH 0 included 42 compounds, mainly alcohols and terpenoids, a number of which were not found in hydrolysates obtained at higher pHs, or by enzymatic action. Some of these compounds, such as isocineole, eucalyptol, *p*-cymene, *p*-cymenol and phellandral are powerful odorants, which may explain the strong, spicy smell of the hydrolysed solution. The presence of a wide range of terpenoids suggests that molecular rearrangements occur in strong acidic conditions, as reported by other researchers (Maicas and Mateo, 2005, Williams et al., 1982a). The products of hydrolysis with HCl at pH 3 and 5 included 15 and 13 compounds respectively, including several carboxylic acids as well as the norisoprenoids β-damascenone (obtained at pH 3) and 3-hydroxy-β-damascone (obtained at pH 3 and 5) (Table 2-1).

Hydrolysis of the glycosidic isolates of 'Hort16A' kiwifruit with almond β-glucosidase produced an aroma more resembling kiwifruit or apple juice. Among the 32 volatile compounds of the enzymatic hydrolysate, alcohols were predominant, followed by carboxylic acids, and terpenoids. Some of the aroma compounds detected are (*E*)-2-hexenal, 1-hexanol, benzaldehyde, α-terpineol, geraniol, 2-phenylethanol, 3-hydroxy-β-damascone, and vanillin.

The untreated sample (control) contained many carboxylic acids, but few aroma compounds (Table 2-1). The presence of the green note compounds (*E*)-2-hexenal and 1-hexanol, indicates that these compounds may not only be produced by glycosidase action, as stated by Young and Patterson (1995).

Hydrolysis of 'Hayward' glycosides with Rapidase AR2000 resulted in a chromatogram very similar to that generated by hydrolysis with almond β-glucosidase (Fig. 2-4). Basically, the same compounds were released, but peaks tended to be larger and sharper in the former. This, coupled with the ability of Rapidase AR2000 to release aglycones from diglycosides (Sarry and Günata, 2004) and its lower cost, were the reasons for the choice of Rapidase AR2000 for this study.

A comparison of the peak areas obtained after different incubation times of 'Hayward' glycosidic extracts is shown in Fig. 2-5. The length of the incubation time affected the hydrolysis of the glycosides. After adding Rapidase AR2000, and incubating at 37 ºC for 24, 42 and 48 h, solvent extracts were obtained and analysed. It was observed that a longer incubation time tended to produce larger peaks. Thus, 48 h was chosen as the length of time for the hydrolysis.

Table 2-1 Bound volatile compounds from 'Hort16A' kiwifruit, obtained by acid and enzymatic (almond β-glucosidase) hydrolysis.

Peak	Name [a]	CAS	R.T. (min) [b]	pH			Enzymatic [c]	
				0	3	5	C	E
1	Propyl acetate	109-60-4	5.58				o	
2	2-Pentanone	107-87-9	5.80	o				
3	2-Butanol	78-92-2	6.72	o				
4	2-Methylpropanol	78-83-1	8.21	o				
5	2-Methyl-2-butenal	1115-11-3	8.33	o				
6	2-Pentanol	6032-29-7	8.88					o
7	4-Methyl-3-penten-2-one	141-79-7	9.31	o	o		o	o
8	Butanol	71-36-3	9.57	o			o	o
9	4-Methyl-2-pentanol	108-11-2	10.13	o				
10	Isocineole	470-67-7	10.54	o				
11	4-Methyl-2-heptanone	6137-06-0	11.20		o		o	
12	3-Methylbutanol	125-51-3	11.24	o				o
13	Eucalyptol	470-82-6	11.39	o				
14	(*E*)-2-Hexenal	6728-26-3	11.52				o	o
15	2,2-Dimethyl-6(secbut-2-ene) tetrahydrofuran		12.22	o				
16	Pentanol	71-41-0	12.38	o				
17	*p*-Cymene	99-87-6	12.93	o				
18	3-Methyl-2-buten-1-ol	556-82-1	14.30			o		
19	Hexanol	111-27-3	15.12	o	o		o	o
20	4-Hydroxy-4-methyl-2-pentanone	123-42-2	15.50	o	o	o	o	o
21	(*E*)-2-Hexen-1-ol	928-95-0	16.52					o
22	Isophorone	78-59-1	16.59	o				
23	(*E*)-Linalool oxide	5989-33-3	17.57	o				
24	Acetic acid	64-19-7	17.61			o	o	o
25	2-Furancarboxaldehyde	98-01-1	17.97	o				
26	(*Z*)-Linalool oxide	34995-77-2	18.29	o				
27	2-Ethylhexanol	104-76-7	18.62	o		o		
28	Benzofuran	271-89-6	19.10	o				
29	Benzaldehyde	100-52-7	19.57	o	o			o
30	2-Methylpropanoic acid	79-31-2	20.57				o	o
31	Terpinen-1-ol	586-82-3	20.84	o				
32	4-Hydroxy-5-methyl-2-hexanone	38836-21-4	20.91			o	o	o
33	3,5,5-Trimethyl-2(5H)-furanone	50598-50-0	21.06				o	
34	(*E*)-2-Octen-1-ol	18409-17-1	21.63	o				
35	2-Furanmethanol	98-00-0	22.72					o
36	α-Terpineol	98-55-5	23.58					o
37	Carvenone	499-74-1	24.09	o				
38	Phellandral	21391-98-20	24.16	o				
39	Pentanoic acid	109-52-4	24.23		o		o	
40	2-Hydroxycineole	18679-48-6	24.24	o				
41	Exo-2-hydroxycineole	92999-78-5	24.26					o
42	β-Damascenone	23726-93-4	25.80	o	o			
43	Hexanoic acid	142-62-1	26.01			o	o	o

Table 2-1 (Continued)

44	Geraniol	106-24-1	26.08						o
45	*p*-Cymenol	1197-01-9	26.13	o					
46	Benzyl alcohol	100-51-6	26.58	o		o			o
47	2-Phenylethanol	60-12-8	27.10	o				o	o
48	Heptanoic acid	111-14-8	27.50						o
49	2-Hexenoic acid	13419-69-7	27.70						o
50	Phenol	108-95-2	28.14	o	o	o			o
51	δ-Pantolactone	599-04-2	28.51	o					
52	3,4-Dihydro-2,5-dimethyl-2H-pyran-2-carboxaldehyde	1920-21-4	28.64	o					
53	Octanoic acid	124-07-2	28.65						o
54	4-Methylphenol	106-44-5	28.90	o					
55	Cumin alcohol	536-60-7	29.13	o					o
56	2-Phenoxyethanol	122-99-6	29.52					o	
57	Nonanoic acid	112-05-0	29.56		o			o	o
58	Eugenol	97-53-0	29.69	o					
59	(*Z*)-1,8-Terpin	89923-77-3	29.77	o					
60	4-Vinylguaiacol	7786-61-0	29.95			o			
61	3,4,5-Trimethylphenol	527-54-8	31.35	o					
62	Hexadecyl-2-ethylhexanoate	59130-69-7	31.76		o				
63	Benzoic acid	65-85-0	31.94			o		o	o
64	Tridecanoic acid	638-53-9	32.24		o				
65	3-Hydroxy-β-damascone	102488-09-5	32.49	o	o	o			o
66	Vanillin	121-33-5	33.46		o			o	o
67	Octadecyl 2-ethylhexanoate	59130-70-0	34.11						o
68	3-Oxo-β-ionol		34.31						o
69	2-Hydroxy-benzeneethanol	7768-28-7	35.48	o					
70	Hexadecanoic acid	57-10-3	36.41		o				
71	4-Hydroxy-benzeneethanol	501-94-0	37.26						o
72	Heptadecanoic acid	506-12-7	37.59						o

[a] Compounds were tentatively identified (MS only)
[b] Retention times on DB-Wax column
[c] C, control (untreated); E, experimental (enzyme added)
o indicates the presence of the compound

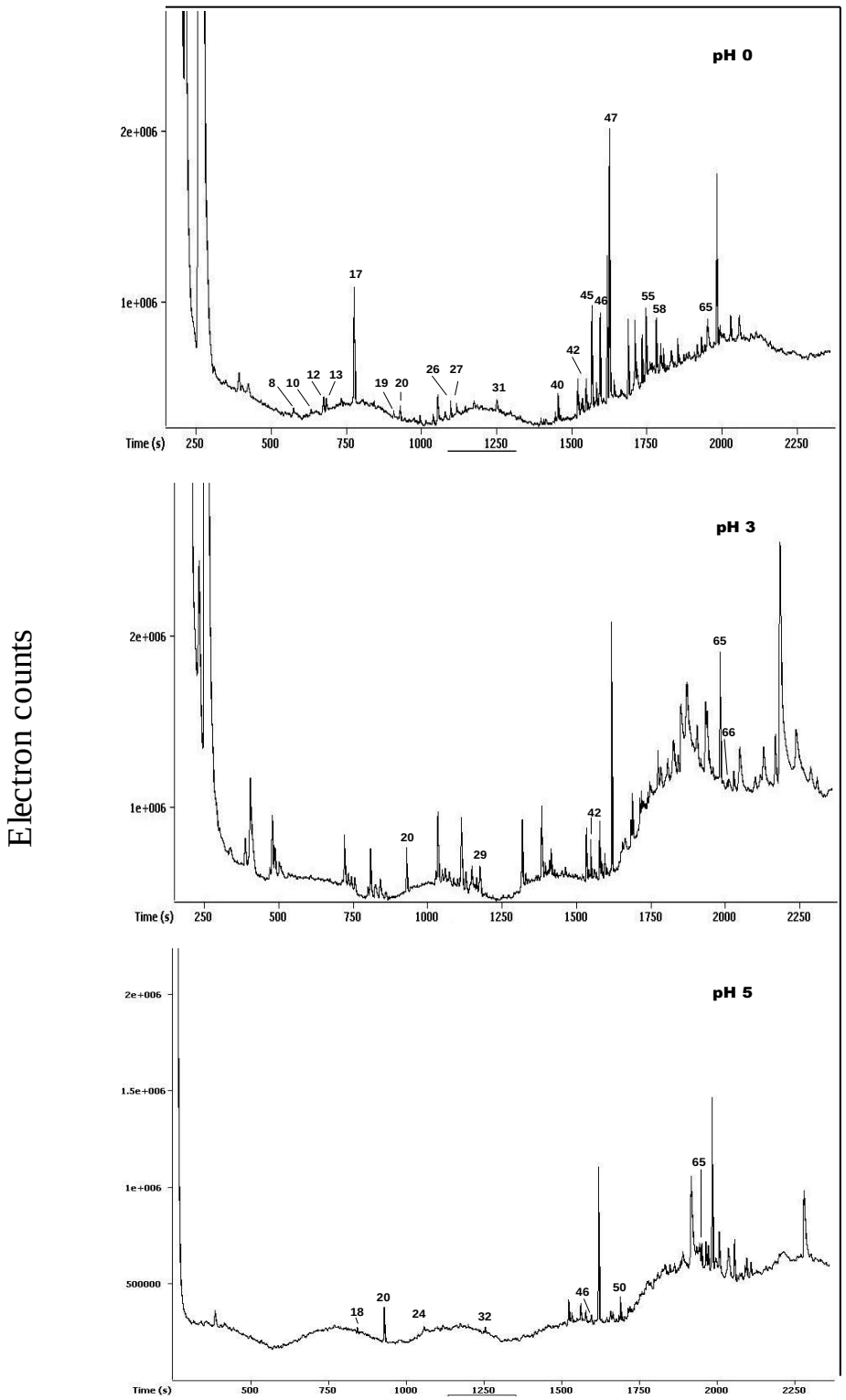

Fig. 2-2 Gas chromatograms (TIC) of the 'Hort16A' kiwifruit bound volatiles released by hydrolysis with HCl at pH 0, 3 and 5. Peak numbers corresponding to the compounds are listed in Table 2-1.

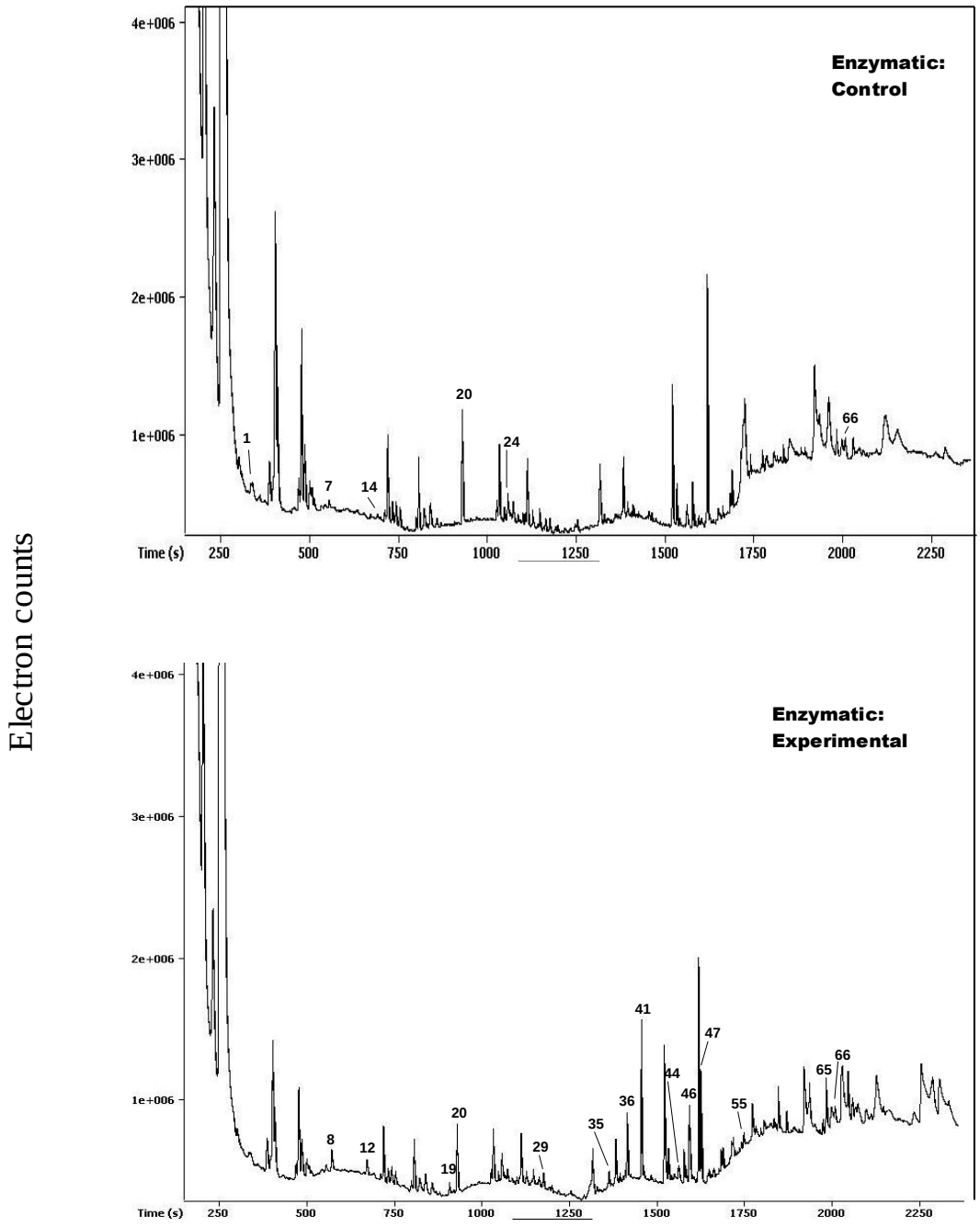

Fig. 2-3 Gas chromatograms (TIC) of the 'Hort16A' kiwifruit bound volatiles released by enzymatic hydrolysis using almond β-glucosidase. Chromatograms of control (no enzyme added) and experimental samples are shown. Peak numbers corresponding to the compounds are listed in Table 2-1.

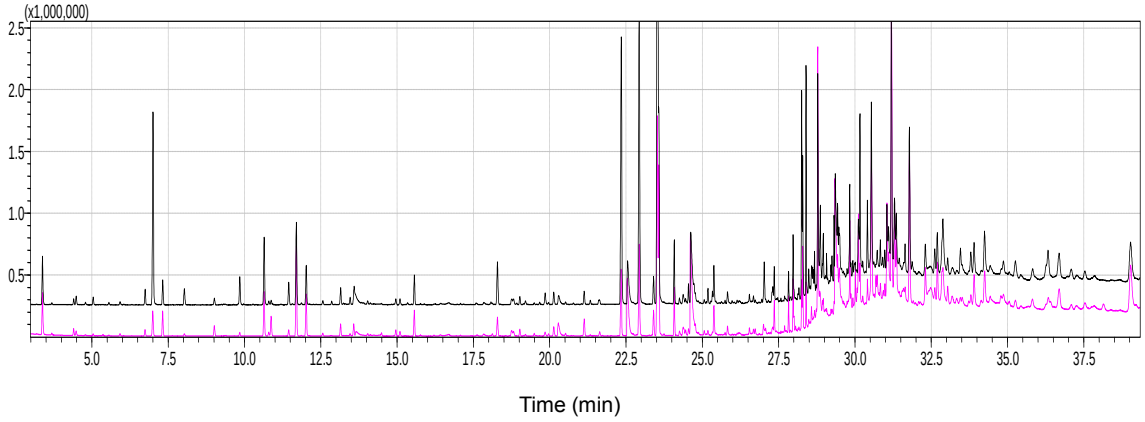

Fig. 2-4 Comparison of the chromatograms (TIC) obtained after the hydrolysis of the glycosidic extract from 'Hayward' kiwifruit with almond β-glucosidase (magenta) and Rapidase AR2000 (black).

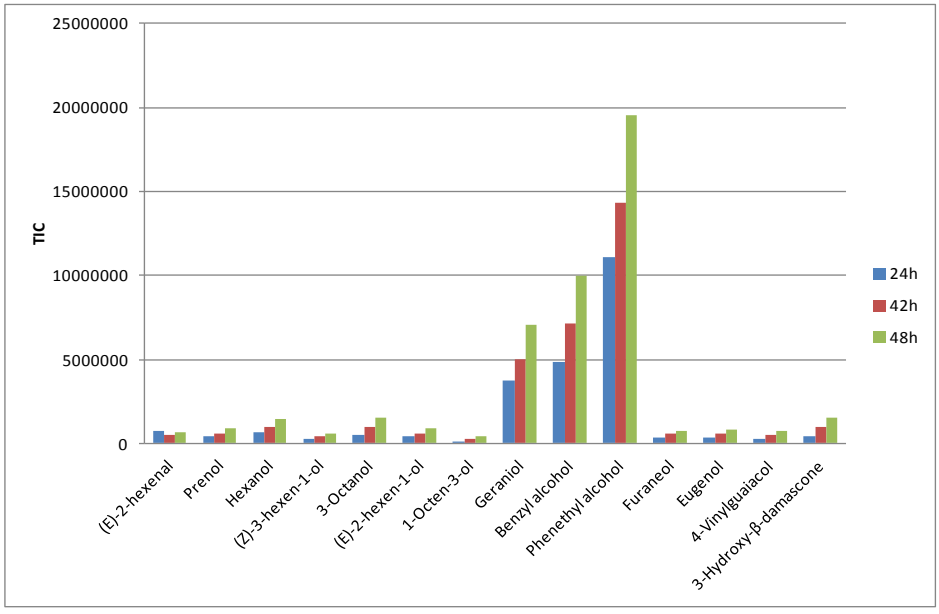

Fig. 2-5 Comparison of the peak areas of representative bound volatiles from 'Hayward' kiwifruit after hydrolysis with Rapidase AR2000 for 24, 42 and 48 h.

2.3.2 Aglycone extraction method

The method used to extract volatile compounds from a matrix influences the composition of the aromatic profile. Some techniques, such as headspace, tend to extract the most volatile portion, while others, such as solvent extraction, can co-extract unwanted components (Reineccius, 2006b). Solvent extraction has been used by many researchers to isolate the volatiles released after glycoside hydrolysis (Young and Paterson, 1995, Günata et al., 1985a, Mateo et al., 1997, Aubert et al., 2003), while headspace-solid phase microextraction (HS-SPME) has been previously applied to the analysis of kiwifruit volatiles (Wan et al., 1999). SPME has as advantages the fast preparation of the sample and the high sensitivity towards highly volatile compounds, so this technique is suitable for the screening of the volatile composition of extracts and for automated analysis (Harker et al., 2009). However, SPME is an equilibrium technique, and as such, is prone to analyte competition and sampling errors (Reineccius, 2006b). Achieving good reproducibility with SPME is challenging, so SPME is not recommended for volatile quantification (Harker et al., 2009). Furthermore, SPME is known to be poorly effective for the extraction of some important odorants such as DMHF and DMMF (Siegmund and Leitner, 2010).

The compounds identified in solvent and SPME extracts of Hayward bound volatiles are shown in Table 2-2, and the TIC chromatograms are shown in Fig. 2-6. The effect of the extraction technique was evaluated by hydrolysing glycoconjugates of Hayward kiwifruit with almond β-glucosidase, and extracting the released volatiles with solvent (pentane:ether, 1:1 v/v) and HS-SPME. A total of 83 compounds were detected (Table 2-2). The identified compounds included mainly alcohols, terpenoids and aldehydes. A larger number of volatile compounds, in particular terpenoids and alcohols, were detected in the SPME sample.

Of the 83 compounds found, there were 30 compounds common to the solvent and SPME

extracts, including most green note alcohols and aldehydes, such as hexanal, 3-hexanol, (*E*)-2-hexenal, 1-hexanol, (*Z*)-3-hexen-1-ol and (*E*)-2-hexen-1-ol, but the relative concentrations were higher in the SPME sample. Other odorants, including geraniol, 3-octanol, 1-octen-3-ol and linalool also showed higher concentrations in the SPME sample. Myrcene, α-pinene, (*E*)-β-ocimene, nerol, rose and nerol oxides were only found in the SPME extract, but other important compounds, including β-damascenone, 3-hydroxy-β-damascone, 3-oxo-α-ionol and vanillin were only found in the solvent extract.

It is possible that the SPME fibre chosen had an effect on the volatile compounds detected, as 65 μM PDMS/DVB is recommended for volatile polar analytes, such as alcohols and amines, but for a larger range of flavour compounds, including volatiles and semi-volatiles, 50/30 μM DVB/Carboxen on PDMS would have probably been a better choice. Other option would have been 100 μM PDMS fibres, which are recommended for volatiles (http://www.sigmaaldrich.com/analytical-chromatography/sample-preparation/spme/selecting-spme-fiber.html). Nevertheless, a comprehensive comparison of different SPME fibres was out of the scope of this study, and the fibres were chosen based on availability.

The differences in the aroma profiles obtained by solvent extraction and SPME indicate that no single extraction technique produces a complete picture of the released bound volatiles; therefore, both techniques could be complementary. However, since important odorants known to occur in bound form such as vanillin and DMHF are difficult to extract using SPME, and this technique is prone to error, solvent extraction was chosen to isolate the bound volatiles.

Table 2-2 Bound volatile compounds from 'Hayward' kiwifruit obtained by solvent extraction and SPME after hydrolysis with almond β-glucosidase.

Peak	Name	CAS	Retention Index [b]	Concentration (%) SPME	Concentration (%) solvent extract	ID method
1	2-Ethylfuran	3208-16-0	962	0.18	nd	MS, RI
2	2-Pentanone	107-87-9	976	nd	3.26	MS, RI
3	2-Butanol	78-92-2	1034	11.28	32.54	MS, RI
4	Unknown [a]		1070	0.94	nd	
5	Hexanal	66-25-1	1084	6.15	1.26	MS, RI
6	Isobutanol	78-83-1	1089	1.18	4.69	MS, RI
7	3-Pentanol	584-02-1	1106	nd	1.23	MS, RI
8	2-Pentanol	6032-29-7	1119	nd	2.81	MS, RI
9	4-Methyl- 3-penten-2-one	141-79-7	1136	1.41	1.27	MS, RI
10	Butanol	71-36-3	1151	3.13	5.43	MS, RI
11	1-Penten-3-ol	616-25-1	1157	nd	1.51	MS, RI
12	Myrcene	123-35-3	1161	26.65	nd	MS, RI
13	2-Hexanol	626-93-7	1194	0.80	nd	MS, RI
14	3-Hexanol	623-37-0	1201	5.52	2.07	MS, RI
15	3-Methyl-1-butanol	123-51-3	1213	18.38	19.20	MS, RI
16	(*E*)-2-Hexenal	6728-26-3	1221	17.00	2.99	MS, RI
17	2-Pentylfuran	3777-69-3	1230	8.70	0.36	MS, RI
18	α-Pinene [a]	80-56-8	1239	7.23	nd	MS
19	4-Methyl-3-penten-2-one	141-79-7	1248	nd	0.54	MS
20	(*E*)-β-Ocimene [a]	3779-61-1	1257	10.18	nd	MS, RI
21	Terpinolene [a]	586-62-9	1287	3.08	nd	MS, RI
22	Isocyanatocyclohexane	3173-53-3	1289	nd	0.06	MS
23	Unknown [a]		1329	2.17	nd	
24	(*Z*)-Rose oxide [a]	3033-23-6	1355	2.46	nd	MS
25	3-Methyl-2-buten-1-ol	556-82-1	1323	nd	5.84	MS, RI
26	Hexanol	111-27-3	1361	62.91	24.13	MS, RI
27	4-Hydroxy-4-methyl-2-pentanone	123-42-2	1372	nd	23.11	MS, RI
28	(*Z*)-3-Hexen-1-ol	928-96-1	1390	2.51	2.33	MS, RI
29	3-Octanol	589-98-0	1399	405.81	30.59	MS, RI
30	(*E*)-2-Hexen-1-ol	928-95-0	1412	30.78	23.26	MS, RI
31	Acetic acid	64-19-7	1437	nd	28.74	MS, RI
32	1-Octen-3-ol	3391-86-4	1456	139.13	28.74	MS, RI
33	Nerol oxide	1786-08-9	1470	5.20	nd	MS, RI
34	(*Z*)-Linalool oxide	5989-33-3	1482	15.98	12.16	MS, RI
35	2-Ethyl-1-hexanol	104-76-7	1495	4.39	0.37	MS, RI
36	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	10230-62-3	1527	nd	2.77	MS
37	Benzaldehyde	100-52-7	1533	17.79	9.13	MS, RI
38	ξ-Fenchene			6.42	nd	MS
39	Linalool	78-70-6	1552	33.59	3.17	MS, RI
40	1-Octanol	111-87-5	1565	2.54	nd	MS, RI
41	2-Methylpropanoic acid	79-31-2	1569	nd	3.46	MS, RI
42	4-Hydroxy-5-methyl-2-hexanone	38836-21-4	1589	nd	1.42	MS
43	α-Methyl-γ-butyrolactone [a]	1679-47-6	1596	nd	0.50	MS
44	Hotrienol	29957-43-5	1615	6.01	5.91	MS, RI
45	Butanoic acid	107-92-6	1631	nd	4.48	MS, RI
46	Ethyl decanoate [a]	110-38-3	1641	2.91	nd	MS, RI

Table 2-2 (Continued)

47	2-Furanmethanol	98-00-0	1669	7.65	48.05	MS, RI
48	Nona-3,5-dien-2-one	152522-81-1	1683	9.98	nd	MS
49	Isovaleric acid	503-74-2	1705	nd	7.11	MS, RI
50	α-Terpineol	98-55-5	1711	21.79	3.85	MS, RI
51	γ-Hexalactone	695-06-7	1725	nd	1.14	MS, RI
52	4-Ethylbenzaldehyde	4748-78-1	1726	9.00	nd	MS
53	3-(Methylthio)-1-propanol	505-10-2	1741	nd	1.13	MS, RI
54	Pentanoic acid	109-52-4	1751	nd	9.76	MS, RI
55	(*Z*)-Linalool oxide (pyranoid)	14049-11-7	1756	nd	2.95	MS
57	Unknown [a]		1765	3.56	nd	
56	Epoxylinalol	60047-17-8	1771	nd	2.90	MS
58	Methyl salicylate	119-36-8	1797	19.63	1.51	MS, RI
59	β-Methyl-δ-valerolactone	1121-84-2	1802	nd	2.48	MS, RI
60	Nerol [a]	106-25-2	1805	13.60	nd	MS, RI
61	β-Damascenone	23726-93-4	1843	nd	2.53	MS
62	Hexanoic acid	142-62-1	1854	9.17	14.27	MS, RI
63	Geraniol	106-24-1	1859	316.96	19.74	MS, RI
64	Benzyl alcohol	100-51-6	1894	13.42	67.07	MS, RI
65	2-Phenylethanol	60-12-8	1936	117.74	149.18	MS, RI
66	α-Calacorene [a]	21391-99-1	1942	8.45	nd	MS, RI
67	3-Hexenoic acid	1775-43-5	1972	nd	70.63	MS
68	Unknown		1981	nd	13.00	
69	1,3,7,7-Tetramethyl-9-oxo-2-oxabicyclo[4.4.0]dec-5-ene [a]		2136	15.87	nd	MS
70	2,5-Dimethyl-4-hydroxy-3(2H)-furanone (DMHF)	3658-77-3	2055	51.30	306.02	MS, RI
71	Eugenol	97-53-0	2116	10.57	6.91	MS, RI
72	4-Vinylguaiacol	7786-61-0	2222	38.58	61.28	MS, RI
73	Methyl hexadecanoate [a]	112-39-0	2239	3.61	nd	MS, RI
74	Ethyl hexadecanoate [a]	628-97-7	2256	36.94	nd	MS, RI
75	Coumaran	496-16-2	2419	nd	23.03	MS
76	Indole	120-72-9	2514	8.28	15.54	MS, RI
77	Phenacyl acetate	2243-35-8	2518	nd	2.31	MS
78	Methoxyeugenol	6627-88-9	2614	nd	0.91	MS
79	3-Hydroxy-β-damascone	102488-09-5	2626	nd	35.89	MS
80	Vanillin	121-33-5	2682	nd	5.11	MS
81	Unknown			nd	13.71	
82	3-Oxo-β-ionol		2797	nd	38.00	MS
83	2-Hydroxy-benzeneethanol	7768-28-7	3195	nd	22.97	MS

[a] Retention index obtained by extrapolation
[b] Retention index on DB-Wax column
nd means not detected

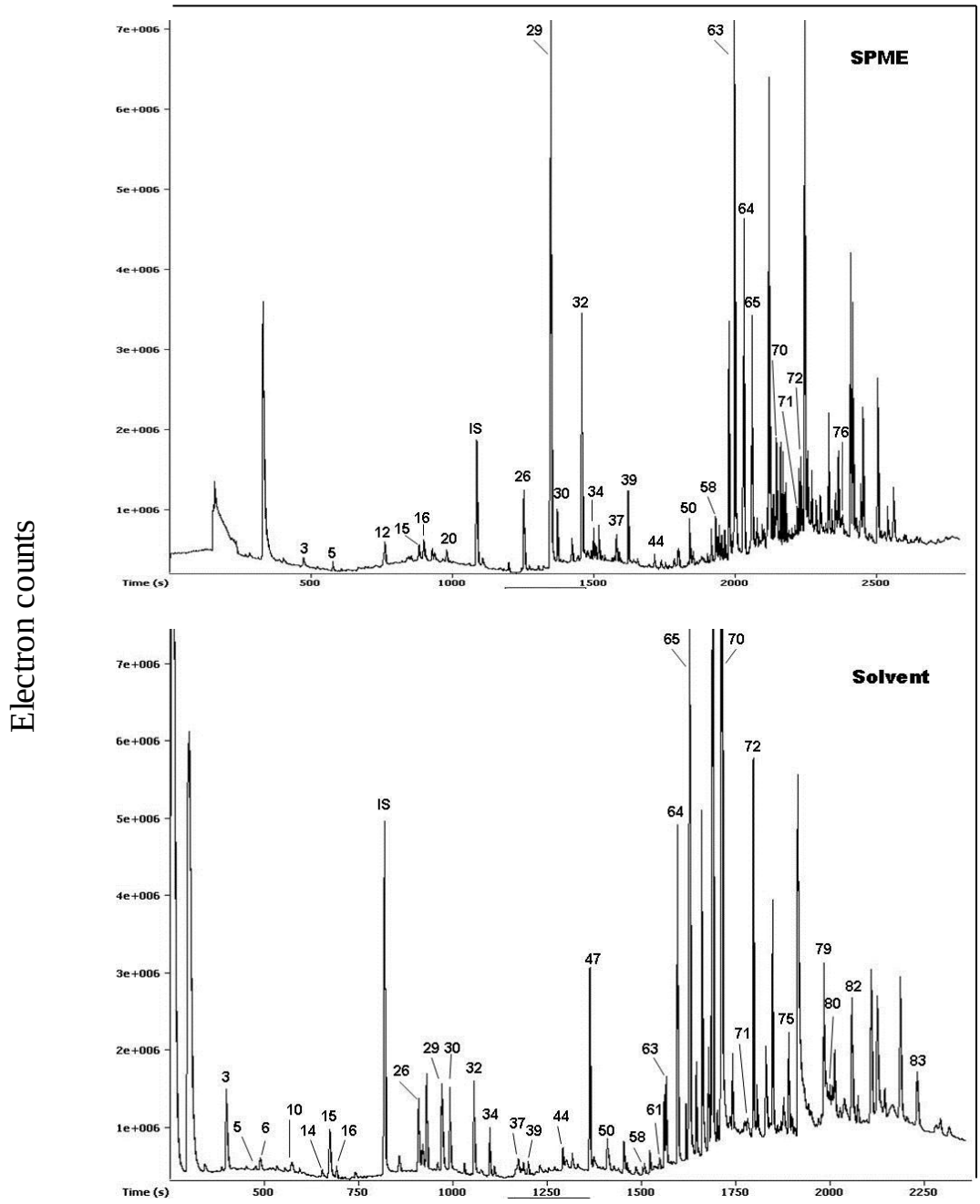

Fig. 2-6 Gas chromatograms (TIC) of the 'Hayward' kiwifruit bound volatiles released by hydrolysis with almond β-glucosidase, and extracted using solvent or SPME (PDMS-DVB fibre). Peak numbers corresponding to the compounds are listed in Table 2-2.
IS = internal standard.

2.4 Conclusions

Several conditions for the isolation of glycosidically bound volatiles from kiwifruit were evaluated. Enzymatic hydrolysis produced an extract with an odour more resembling kiwifruit than acid hydrolysis. Almond β-glucosidase and Rapidase AR2000 produced similar results, but the latter produced larger peaks and had a lower cost, so it was chosen for the study. The longer the duration of the enzymatic hydrolysis, the larger the peaks tended to be, so 48 h was chosen as the length of the hydrolysis for this study. Acid hydrolysis at low pHs tended to generate artefacts. Neither SPME nor solvent extraction produced a complete picture of the bound volatile composition, but solvent extraction was chosen because it is a more reproducible technique and is able to extract particularly important odorants such as DMHF.

Chapter 3

Analysis of bound volatiles and glycosides of *Actinidia arguta* (kiwiberry) [2]

[2] Part of this chapter is based on the manuscript Garcia et. al, *Journal of Agricultural and Food Chemistry*, **2011**, 59, 8358-8365.

3.1 Introduction

Actinidia arguta (Sieb et Zucc.) Planch. ex Miq. var. *arguta* is widespread in North-East Asia, being found in Siberia, Korea, Japan and northern China. This species has a high resistance to cold, which is the reason why it is sometimes called "hardy kiwifruit". Other common names for this fruit are "baby kiwi" and "kiwiberry" (Nishiyama, 2007, Williams et al., 2003). The fruit are small and hairless, weighing 5-15 g, with edible skin. *A. arguta* tends to be sweeter than *A. deliciosa* or *A. chinensis*, but has the notable disadvantage of a much shorter storage and shelf life. Commercially available cultivars include 'Ananasnaya' in the USA and Chile, 'Kosui' in Japan, 'Chiak' in Korea, and 'Hortgem Tahi', 'Hortgem Toru', 'Hortgem Wha' and 'Hortgem Rua' in New Zealand (Nishiyama, 2007).

The aroma of baby kiwi has been described as banana, floral, fruit candy, grassy, green, melon and tropical (Matich et al., 2003). It is because of this rich aromatic flavour that *A. arguta* is an attractive kiwifruit species, despite its short storage life and difficulty to grow (Williams et al., 2003).

The volatile composition of *A. arguta* fruit has been revealed by using headspace and solvent extraction techniques (Matich et al., 2003). The main compounds were identified as the esters ethyl butanoate and ethyl hexanoate, followed by several lipid-derived volatiles, such as (*E*)-2-hexenal, hexanal and (*Z*)-3-hexenal. From the solvent extract, additional major compounds including camphor, methyl and ethyl benzoate were detected (Matich et al., 2003). A study applying GC-MS/O identified the odour-active compounds in *A. arguta*, which included fruity esters such as ethyl butanoate and methyl benzoate, the caramel-like 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF), several green smelling compounds such as (*E*)-2-hexenal and hexanal, and some odorants with floral notes (Yang et al., 2010).

The objective of this chapter was to identify the bound volatile compounds of *A. arguta* fruit, in view of the information gap on this kiwifruit species. The presence of potential

flavour compounds was also determined.

3.2 Materials and methods

3.2.1 Plant material

Actinidia arguta (Sieb et Zucc.) Planch. ex Miq. var. *arguta* 'Hortgem Tahi' fruit were harvested from the Plant & Food Research orchard in Te Puke, New Zealand. Fruit for bound volatile analysis were harvested during March 2010, and fruit for glycoside and sugar analysis were harvested during March 2011. Fruit were kept in cool storage at 2 ºC until required, but for under a month. Fruit were taken out of storage, repacked into standard export trays for kiwifruit, covered with a plastic liner (polyliner) for even ripening, and held at 20 ºC until ripe.

The fruit were analysed at the eating-ripe stage, having an average flesh firmness of 1.28 Instron (0.13 kgf) and a soluble solids content of 15 ºBx.

The flesh firmness was evaluated using an Instron model 4301 compression tester (Instron, Canton, MA, USA). The Instron was chosen to measure the firmness as this equipment operates by compressing the fruit with a flat-tipped probe (diameter 7.9 mm), without penetrating it, so it is useful for small fruits. A handheld refractometer model N-20E (0-20% Brix, ATAGO, Tokyo, Japan) was used to measure soluble solids content.

3.2.2 Standards and solvents

All chemicals used were of analytical grade or better. Methanol was purchased from Merck (Darmstadt, Germany); diethyl ether, acetone, and pentane were obtained from Scharlau (Barcelona, Spain). Pentane was re-distilled before use.

The C7-C30 saturated alkanes standard mixture, for calculating retention indices, was purchased from Supelco (Bellefonte, PA, USA).

Pure volatile standards were obtained from the following suppliers: 4-vinylguaiacol (2-methoxy-4-vinylphenol), from Endeavour (Daventry, UK); 2-pentanol, eugenol, benzoic acid, acetic acid, methyl salicylate, cinnamic acid, geraniol, benzyl alcohol, isovaleric acid and butanol were from VWR (Poole, UK); cyclohexanone, 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF, furaneol), (*E*)-2-hexenal, 2-phenylethanol, benzaldehyde, 3-(methylthio)-1-propanol, raspberry ketone (4-(4-hydroxyphenyl)butan-2-one), coniferyl alcohol, 1-octen-3-ol, 2-ethylhexanol, *p*-hydroxyphenethyl alcohol, hexanal, hexanol, vanillin, isoamyl alcohol, (*Z*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol, linalool, nerol, α-terpineol and furfuryl alcohol were from Sigma-Aldrich (St. Louis, MO, USA); *cis*- and *trans*-linalool oxide (furanoid) and β-damascenone were from Shiono Koryo Kaisha (Osaka, Japan).

Isobutanol, 3-pentanol, 3-methyl-2-butenal, cyclopentanol, prenol (3-methyl-2-buten-1-ol), 3-octanol, *trans*-carveol, (*E*)-8-hydroxylinalool, and *trans*-isoeugenol were obtained from the standards collection at Plant & Food Research.

3.2.3 Bound volatile analysis

3.2.3.1 Isolation and hydrolysis of glycosidic precursors

The glycosidic precursors were isolated by adsorption onto an Amberlite XAD-2 column (Supelco, Bellefonte, PA, USA), according to the method of Young and Paterson (1995) with some modifications. The 55 mL column was prepared at least one day prior to use and by washing sequentially with 110 mL each of Milli-Q water, acetone, water, 3 M HCl, and abundant water until chloride free (same pH value as distilled water). The column was then stored in water at room temperature until use.

Fruit (2.3 kg) were cut in half and the juice extracted with a BJE 200 juicer (Breville, Australia), followed by pressing in a wine press (Medio model, Ferrari Group, Italy) lined with cheesecloth. The procedure was done at 4 ºC in a cold room. The juice was clarified by

vacuum filtration through Celite 545 (VWR, Haasrode, Belgium) in a Büchner funnel.

The clear juice obtained was passed down the previously prepared Amberlite XAD-2 column at room temperature, followed by elution with 160 mL each of water, pentane and methanol, in that order. The water and pentane eluents were discarded. The methanol fraction, containing the glycosidic aroma precursors, was collected and then divided into four portions and evaporated to dryness in a rotary evaporator, and further dried under vacuum at room temperature for 2 h. The dried glycosidic extracts obtained were kept at -20 °C until required.

The citrate/phosphate hydrolysis buffer was prepared by dissolving 4.2 g of anhydrous citric acid (AppliChem, Darmstadt, Germany) and 2.84 g of anhydrous Na_2HPO_4 (Scharlau, Barcelona, Spain) in 100 mL of Milli-Q water, and adjusting to pH 5 with 4 M NaOH, as described by Young and Patterson (1995).

Each portion of the dried glycosidic extract (1/4 of the total) was dissolved in 2 mL of buffer, and extracted three times with 1 mL of pentane to remove residual free volatiles.

Enzymatic hydrolysis was carried out using Rapidase AR2000 (DSM Food Specialties, Delft, Netherlands). The enzyme was dissolved in hydrolysis buffer and used at a concentration of 74.4 mg enzyme/kg pulp. After adding 30 μL of a 0.1 mg/mL solution of cyclohexanone in ether as internal standard, the tube was closed and placed in a dry bath (AccuBlock D1200, Labnet, Woodbridge, NJ, USA) at 37 °C for 48 h. Three replicates and a control (no enzyme added) were prepared. The hydrolysate was cooled over ice, and the released aglycones were extracted five times with 0.80 mL of a mixture of pentane:ether (1:1 v/v). The extract was dried over anhydrous Na_2SO_4, pre-concentrated in a rotary evaporator, over an ice bath, and then further concentrated under a gentle stream of nitrogen to a final volume of 100 μL.

3.2.3.2 GC-MS analysis

Analysis of the bound volatile extract was performed on a Shimadzu QP2010 Plus gas chromatograph-mass spectrometer (Kyoto, Japan) equipped with a CombiPAL auto-sampler (CTC Analytics, Switzerland). The column used was a Stabilwax (Restek, Bellefonte, PA, USA) (30 m length × 0.25 mm i.d. × 0.25 μm film thickness). Helium was used as the carrier gas, at a flow rate of 1.5 mL/min. A 1 μL sample was injected in split mode (split ratio of 5). The operating conditions were as follows: injector, 250 ºC; ion source, 230 ºC; interface, 250 ºC. The GC oven temperature was programmed at 50 °C for 2 min, increased at 5 °C/min to 150 °C, 10 °C/min to 200 ºC, and 20 °C/min to 247 ºC, and then held at 247 ºC for 10 min. The total analysis time was 39.35 min. The mass spectrometer's source was operated in electron impact ionization (EI) mode, at 70 eV. Compounds were analysed in SCAN mode, and the total ion current (TIC) in the range 40-400 m/z was acquired.

Kovats indices were obtained by injecting a C_7-C_{30} straight-chain alkane mixture, under the same conditions as the analysed samples, and using the retention index feature of the software to calculate them.

Identification of the compounds was achieved by comparing their mass spectra and retention times with those available in the GC-MS libraries (NIST08, FFNSC1.3) and the Compound Database of Plant & Food Research, or published in the literature (NIST Chemistry WebBook, http://webbook.nist.gov/chemistry/). When available, authentic standards were used for a positive identification. Compounds for which no standards were available are reported as tentatively identified.

Semi-quantification of aroma compounds was obtained from the total TIC, and was performed by comparing the peak areas of the volatiles in the sample to the peak area of an internal standard of cyclohexanone. Response factors were determined by the analysis of solutions containing a known concentration of the reference compounds with cyclohexanone

as internal standard. When the authentic standard for a compound was not available, the response factor of a representative compound was used as follows: 1-hexanol for alcohols, linalool for terpenoids, (*E*)-2-hexenal for aldehydes, acetic acid for carboxylic acids, vanillin for phenolic compounds, and β-damascenone for norisoprenoids.

3.2.3.3 GC-MS/O analysis

Ripe *Actinidia arguta* glycosides were used for the analyses. Five GC-olfactometry (GC-O) runs were performed. A sample volume of 1 μL was injected into the Shimadzu QP2010 GC-MS system described above, which was fitted with a Phaser OP275 olfactory detection port (ODP; ATAS/GL Sciences, Tokyo, Japan). The effluent was split 6:1 between the MS and the sniffing port. The port was heated at 250 ºC. Helium was used as the auxiliary gas, at a flow rate of 5 mL/min. Moist air was pumped into the sniffing port at a rate of 50 mL/min. A VI277 Voice Chromatogram Interface (ATAS/GL Sciences) was used to record voice and olfactory intensity data. The voice recognition software was trained and a series of standards were run to calibrate the system. A diagram of the GC-O system is shown in Fig. 3-1.

The operating conditions were similar to those of GC-MS analysis, but the final temperature of 247 ºC was kept for 15 min, for a total analysis time of 44.35 min. The column flow was 2.50 mL/min and the split ratio was 12.

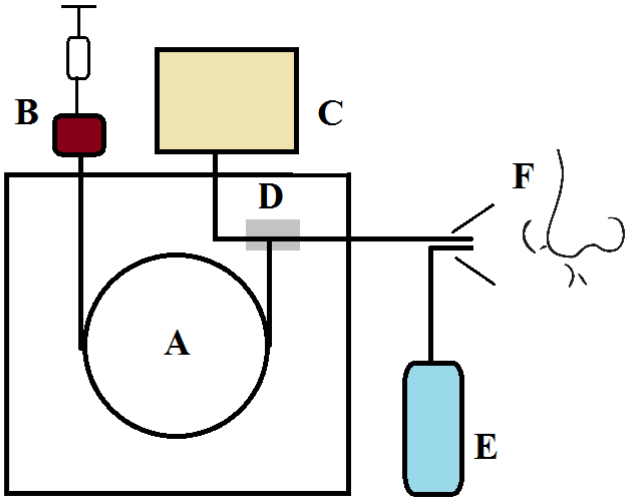

Fig. 3-1 Diagram of the GC-O system: A, column; B, injector, C, MS detector; D, splitter; E, air humidifier; F, olfactory port.

3.2.4 Glycoside analysis

3.2.4.1 Isolation of glycosides

Glycosidic precursors were isolated from juice as described above. The dried glycosidic extract from ~2 kg pulp was dissolved in 10 mL water, extracted three times with 3 mL pentane and fractionated using solid phase extraction (SPE) SEP-PAK C18 cartridges (5 g; Waters, Milford, MA, USA) and a gradient of methanol:water as a solvent (50 mL of each 20:80, 30:70, 40:60, and 25 mL of 100:0, v/v) (Mateo et al., 1997). Seven fractions (25 mL each) were collected, evaporated to dryness and stored at -20 ºC until required.

3.2.4.2 Identification and purification of the fraction containing DMHF

The fraction containing DMHF was selected for further analysis, based on the high concentration of this compound in the bound volatile extract of *A. arguta*. In addition, DMHF is known to be a potent odorant, and has been identified as one of the most important compounds for the aroma of *A. arguta* (Yang et al., 2010). To detect the presence of DMHF,

the glycosidic fractions were hydrolysed and analysed by GC-MS as previously described.

Fraction 1 had a distinct sweet, pineapple-like aroma, as a result of the presence of DMHF, identified by GC-MS. This compound was present at the trace level or was absent in the other fractions. As a result, fraction 1 was selected for further analysis.

To isolate the DMHF glycoside, fraction 1 was re-fractionated using the method of Mateo et al. (1997), with modifications. C18 cartridges (1 g; Alltech, Deerfield, IL, USA) were used. Methanol:water (20:80 v/v) was used as the mobile phase, collecting six 1 mL fractions. Fractions containing DMHF glycoside were combined, and purified by re-fractionation as previously described, but collecting ten 0.5 mL fractions. Fractions containing DMHF glycoside were combined and used for the analysis.

To check for the presence of the DMHF glycoside, thin-layer chromatography (TLC) was carried out using aluminium sheets coated with silica gel 60 F_{254} (Merck, Darmstadt, Germany) and ethyl acetate:isopropanol:water (65:30:15 v/v/v) as a solvent. Plates were developed using Nediac reagent (N-1-(naphthyl)ethylendiamine dihydrochloride) and heating (Sakho et al., 1997). The fractions containing the DMHF glycoside were combined and analysed by HPLC, preparative TLC and LC-MS.

3.2.4.3 HPLC analysis

A Hewlett Packard 1100 (Palo Alto, CA, USA) high performance-liquid chromatograph (HPLC) fitted with a Hewlett Packard 1050 autosampler was used. The column was a Symmetry C18 (particle size 5 μm; 4.6 mm × 250 mm; Waters). A Symmetry C18 column guard (particle size 5 μm; 3.9 mm × 20 mm; Waters) was also used. Solvent A was water with 0.1% trifluoroacetic acid (TFA) and solvent B was acetonitrile. Flow rate was 1 mL/min and injection volume was 3 μL. The detector was operated at UV 280 nm. The solvent program was as follows: 0-2 min, 0% B; 2-15 min, increase to 40% B; 15-25 min, increase to 70% B;

26-30 min, increase to 90% B; 30-35 min, increase to 100% B, and finally 100% B for the last 5 min. Samples were analysed in triplicate.

Chromeleon 6.80 software (Dionex, Sunnyvale, CA, USA) was used to analyse the data. A DMHF standard was used to detect the presence of released DMHF in the glycosidic sample. The results of HPLC analysis indicated the presence of DMHF and its glycoside (Fig. 3-2).

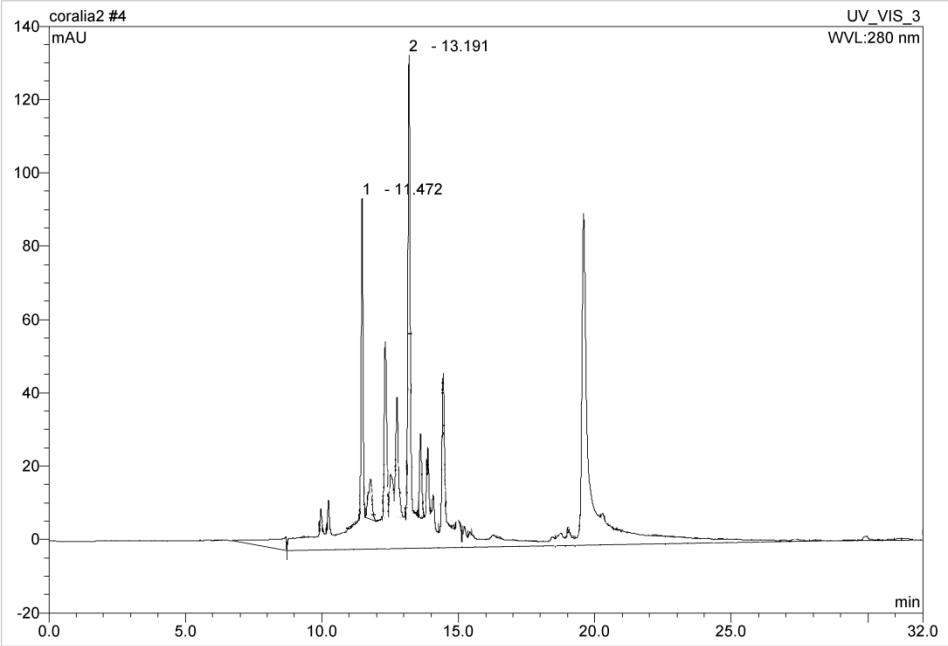

Fig. 3-2 Chromatogram of fraction containing DMHF (fraction 1). Peaks 1 and 2 correspond to DMHF and its glycoside, respectively.

3.2.4.4 Preparative TLC (PTLC)

Glass-backed TLC plates coated with silica (0.22 mm thick; Merck) were used. The solvent system was ethyl acetate:isopropanol:water (65:30:15 v/v/v). A portion of fraction 1 was dissolved in water and applied as a band. After the chromatographic run, plates were revealed under UV light. Four bands were observed, with the band at Rf = 0.68 being much

darker than the others. This band was scraped with a spatula, suspended in the same solvent, filtered, and the filtrate evaporated to dryness and subjected to LC-MS analysis.

3.2.4.5 LC-MS analysis

An LTQ FT Ultra hybrid mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) with electrospray (ES) ionisation source was used. The solvent was methanol:water (1:1, v/v) and the flow rate was 3 μL/min. Samples were analysed in positive and negative modes.

Two types of samples were analysed: the DMHF glycoside fraction obtained directly after fractionation and the band containing DMHF glycoside obtained by PTLC.

3.2.5 Analysis of sugar moieties

Glycosidic fractions 2-7 (section 3.2.4.1) were used for sugar analysis. Fractions were combined, evaporated to dryness, dissolved in 8 mL of 1 M HCl, and extracted three times with 3 mL of pentane to remove residual volatiles. The combined sample was divided into two portions. The pH of one of the samples was adjusted to 3.0 by the addition of 4 M NaOH, while the other untreated sample remained at pH 0. Hydrolysis was conducted at 80 ºC for 60 min. Samples were cooled, neutralised with 4 M NaOH and extracted four times with 2 mL of pentane:ether (1:1 v/v). The aqueous portion was filtered and evaporated to dryness in a rotary evaporator over a 40 ºC water bath, and further dried for 2 h in a vacuum desiccator at room temperature, then stored at -20 ºC until required.

The sugar samples hydrolysed at pH 0 and 3 were analysed using a Dionex ICS 3000 unit (Thermo Fisher Scientific), fitted with a CarboPac PA20 carbohydrate column (Thermo Fisher Scientific). Elution was carried out in isocratic conditions with 4.5 mM KOH solution as the mobile phase. Samples were rehydrated and filtered prior to analysis.

3.3 Results and discussion

3.3.1 Bound volatile analysis

The glycosidically bound volatile compounds of *A. arguta* were released by enzymatic hydrolysis using Rapidase AR2000. This commercial enzyme preparation contains several glycosidase activities, including β-glucosidase (5.6 nkat/mg), α-arabinofuranosidase (9.2 nkat/mg), α-rhamnosidase (0.32 nkat/mg) and β-apiosidase (1.08 nkat/mg), which has the advantage of hydrolysing most glycosides of aroma compounds including *O*-β-D-glucosides, and *O*-diglycosides.

A total of 89 compounds were detected in the bound volatile extract of ripe *A. arguta* fruit. There were few compounds present in the control sample (without enzyme). However, small amounts of (*E*)-2-hexenal, benzyl alcohol, 2-phenylethanol, DMHF, 3-hydroxy-β-damascone (tentatively identified) and some carboxylic acids were found, which can be explained by the acidity of the buffer (pH 5.0), because flavour glycosides are also known to be hydrolysed by acid (Williams et al., 1982b). The compounds isolated in the bound volatile extract of *A. arguta* are shown in Table 3-1.

Alcohols (22 compounds), terpenoids (17 compounds) and benzenoids (21 compounds, including 14 phenols and phenylpropanoids) were the most numerously represented compound classes; whereas norisoprenoids and aromatic compounds were the most abundant (Fig. 3-3). Compounds with the highest concentrations were DMHF, benzyl alcohol, 3-hydroxy-β-damascone, hexanal, and (*Z*)-3-hexen-1-ol. On the other hand, the free volatile compounds of *A. arguta* have been reported to mainly consist of esters, alcohols and aldehydes, with ethyl butanoate, ethyl hexanoate, (*E*)-2-hexenal, hexanal and (*Z*)-3-hexenal being the main contributors (Matich et al., 2003).

Previous studies have shown that the concentration of C6 alcohols and aldehydes as free volatiles varies according to the cultivar, ripening stage and isolation technique, with samples analysed by headspace showing the highest levels. The reported levels of these compounds as free volatiles were generally higher than the bound levels observed (Matich et al., 2003). This is because most aldehydes are derived from alcohols and cannot be directly glycosylated. C6 green note compounds are generally more abundant as free volatiles, as they are synthesised directly from fatty acids.

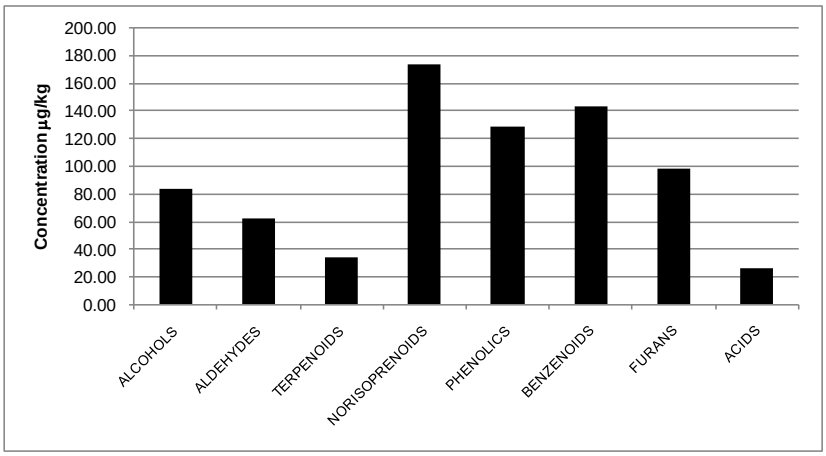

Fig. 3-3 Levels of bound volatile compounds of ripe *Actinidia arguta* fruit.

Table 3-1 Compounds found in the bound volatile fraction of ripe *Actinidia arguta* fruit.

Compound	CAS	RI [a]	μg/kg	Std. dev.	ID method [b]
Alcohols					
2-Butanol	78-92-2	1022	5.17	0.57	MS, RI
Isobutanol	78-83-1	1091	2.26	0.22	MS, RI, Std
3-Pentanol	584-02-1	1107	0.45	0.02	MS, RI, Std
2-Pentanol	6032-29-7	1119	0.97	0.02	MS, RI, Std
Butanol	71-36-3	1143	0.31	0.02	MS, RI, Std
1-Penten-3-ol	616-25-1	1160	0.59	0.02	MS,RI
Isoamyl alcohol	125-51-3	1208	13.25	0.26	MS, RI, Std
3-Methyl-3-buten-1-ol	763-32-6	1250	1.25	0.02	MS,RI
Cyclopentanol	96-41-3	1302	0.21	0.03	MS, RI, Std
3-Methyl-1-pentanol	589-35-5	1328	0.09	0.01	MS,RI
Hexanol	111-27-3	1354	1.27	0.10	MS, RI, Std
(*Z*)-3-Hexen-1-ol	928-96-1	1386	41.96	2.75	MS, RI, Std
4-Methyl-3-penten-1-ol	763-89-3	1390	0.16	0.02	MS,RI
3-Octanol	589-98-0	1395	0.38	0.03	MS, RI, Std
(*E*)-2-Hexen-1-ol	928-95-0	1408	3.70	0.41	MS, RI, Std
2-Methyl-3-hexanol	617-29-8	1437	0.48	0.03	MS
1-Octen-3-ol	3391-86-4	1454	4.85	0.42	MS, RI, Std
2-Ethylhexanol	104-76-7	1492	0.76	0.08	MS, RI, Std
2,3-Butanediol	513-85-9	1542	0.13	0.02	MS,RI
1,2-Pentanediol	5343-92-0	1706	2.12	0.19	MS
4,5-Octanediol	22607-10-9	1735	0.98	0.13	MS
1,7-Octadien-3-ol	30385-19-4	2087	1.86	0.08	MS
Aldehydes					
Hexanal	66-25-1	1086	54.92	1.59	MS, RI, Std
3-Methyl-2-butenal	107-86-8	1204	1.54	0.08	MS, RI, Std
(*E*)-2-Hexenal	6728-26-3	1221	4.51	0.06	MS, RI, Std
Ketones					
3-Penten-2-one	625-33-2	1128	0.13	0.03	MS,RI
3-Ethyl-3-buten-2-one	4359-77-7	1133	0.11	0.02	MS
4-Hydroxy-4-methylpentan-2-one	123-42-2	1363	3.72	1.97	MS,RI
4-Hydroxy-5-methyl-2-hexanone	38836-21-4	1578	0.34	0.03	MS
5-Hydroxy-4-octanone	496-77-5	1748	10.18	0.48	MS

Table 3-1 (Continued)

Terpenoids					
2,3-Dehydro-1,8-cineole	92760-25-3	1189	0.57	0.09	MS
Prenol	556-82-1	1323	4.64	0.21	MS, RI, Std
(*Z*)-Linalool oxide	5989-33-3	1442	0.04	0.01	MS, RI, Std
Linalool	78-70-6	1551	0.16	0.02	MS, RI, Std
α-Terpineol	98-55-5	1698	1.17	0.15	MS, RI, Std
α-Phellandren-8-ol	1686-20-0	1729	0.98	0.12	MS
Nerol	106-25-2	1802	0.46	0.11	MS, RI, Std
Geraniol	106-24-1	1852	2.21	0.29	MS, RI, Std
p-Cymen-8-ol	1197-01-9	1855	2.64	0.36	MS,RI
(*E*)-Carveol	1197-06-4	1870	0.28	0.04	MS, RI, Std
(*E*)-2,6-Dimethyl-3,7-octadiene-2,6-diol	13741-21-4	1951	0.67	0.01	MS,RI
Perilla alcohol	536-59-4	1999	0.31	0.07	MS,RI
(*E*)-1-(Ethenyloxy)-3,7-dimethyl-2,6-octadiene		2167	5.16	1.00	MS
(*Z*)-8-Hydroxylinalool	103619-06-3	2278	2.18	0.21	MS,RI
(*E*)-8-Hydroxylinalool	64142-78-5	2317	6.03	0.60	MS, RI, Std
8-Hydroxy-carvotanacetone		2343	3.07	3.10	MS
Geranic acid	459-80-3	2356	4.10	2.21	MS,RI
Acids					
Acetic acid	64-19-7	1471	9.90	0.51	MS, RI, Std
Isobutyric acid	79-31-2	1586	0.63	0.15	MS,RI
Isovaleric acid	503-74-2	1686	1.98	0.75	MS, RI, Std
Pentanoic acid	109-52-4	1755	2.22	0.05	MS,RI
Hexanoic acid	142-62-1	1864	8.51	1.41	MS,RI, Std
(*E*)-2-Hexenoic acid	13419-69-7	1990	1.70	0.58	MS,RI
Octanoic acid	124-07-2	2075	1.90	0.45	MS,RI
Sulfur compounds					
3-(Methylthio)-1-propanol	505-10-2	1720	0.36	0.03	MS, RI, Std
Furans					
Furfuryl alcohol	98-00-0	1666	3.03	0.13	MS, RI, Std
DMHF	3658-77-3	2041	95.36	5.17	MS, RI, Std
Phenols and phenylpropanoids					
Methyl salicylate	119-36-8	1777	6.85	0.64	MS, RI, Std
Phenol	108-95-2	2017	0.86	0.07	MS,RI
Eugenol	97-53-0	2178	1.02	0.27	MS, RI, Std

Table 3-1 (Continued)

4-Vinylguaiacol	7786-61-0	2209	6.00	0.61	MS, RI, Std
(*E*)-Isoeugenol [c]	5932-68-3	2365	9.84		MS, RI, Std
Vanillin	121-33-5	2597	11.51	1.67	MS, RI, Std
Vanillyl alcohol	498-00-0	2831	1.40	1.39	MS
o-Hydroxyphenethyl alcohol	7768-28-7	2867	4.21	0.61	MS
Cinnamic acid	140-10-3	2911	36.47	5.61	MS, RI, Std
Homovanillic acid	306-08-1	3008	5.41	0.38	MS,RI
Raspberry ketone	5471-51-2	3023	0.81	0.37	MS, RI, Std
p-Hydroxyphenethyl alcohol	501-94-0	3038	31.83	1.52	MS, RI, Std
Rhododendrol	501-96-2	3113	1.08	0.02	MS
Coniferyl alcohol	458-35-5	3441	11.02	1.03	MS, RI, Std
Benzenoids					
Benzaldehyde	100-52-7	1526	1.45	0.10	MS, RI, Std
3-Ethylbenzaldehyde	34246-54-3	1709	0.39	0.03	MS
Benzyl alcohol	100-51-6	1883	69.48	5.46	MS, RI, Std
2-Phenylethanol	60-12-8	1915	18.88	2.13	MS, RI, Std
2,4-Dimethylphenethyl alcohol	6597-59-7	2024	0.95	0.06	MS
Benzoic acid	65-85-0	2478	26.85	3.26	MS, RI, Std
Phenylacetic acid	103-82-2	2603	25.12	3.64	MS,RI
Norisoprenoids					
3-Hydroxy-β-damascone	102488-09-5	2551	56.43	5.90	MS,RI
3-Oxo-α-ionol	34318-21-3	2658	40.63	5.04	MS,RI
3-Oxo-7,8-dihydro-α-ionol	36151-02-7	2738	21.44	3.83	MS,RI
3-Hydroxy-7,8-dihydro-β-ionol	172705-13-4	2757	26.87	3.85	MS,RI
4-(3-Hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one	52210-15-8	2862	4.87	0.25	MS
4-Hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-2-cyclohexen-1-one	7070-24-8	3213	23.92	2.06	MS
Miscellaneous					
Unknown		1539	0.19	0.07	
δ-Octalactone	698-76-0	1971	0.88	0.15	MS,RI
3-Acetoxy-4-(1-hydroxy-1-methylethyl)-1-methyl-cyclohexene		2126	4.26	0.15	MS
Coumaran	496-16-2	2411	2.08	0.74	MS,RI
Hexahydro-3-(2-methylpropyl)-pyrrolo[1,2-a]pyrazine-1,4-dione	5654-86-4	3316	2.75	0.72	MS

[a] RI, retention indices on Stabilwax column
[b] Identification methods: MS, RI, Std (authentic standard)
[c] Detected only in one of the replicates

DMHF has been identified as a key odorant for strawberry and pineapple (Buechi et al., 1973), and is known to occur in glycosylated form in strawberry (Mayerl et al., 1989), pineapple (Wu et al., 1990), raspberry (Pabst et al., 1991), tomato (Krammer et al., 1994) and blackberry (Du et al., 2010). This compound is also known as pineapple ketone or strawberry furanone, and is characterised by a strong sweet, caramel-like odour (Buechi et al., 1973), and a very low odour threshold (0.04 μg/kg in water), indicating its importance as an odorant. A carbohydrate, 6-deoxy-D-fructose, is considered to be the key precursor in the biosynthesis of DMHF and its glycoside (Siegmund and Leitner, 2010).

Another furan, 2,5-dimethyl-4-methoxy-3(2H)-furanone (DMMF, mesifurane), has an odour described as fermented fruit, and its odour threshold (0.03 μg/kg) is also very low. Both DMHF and DMMF have been reported as important odorants in strawberry (Siegmund and Leitner, 2010). DMMF is a derivative of DMHF, and is generated through methylation by an *O*-methyl-transferase (Wein et al., 2002). DMMF has been reported as a free volatile in solvent extracts of *A. arguta* fruit, but at a low concentration of 5.65 μg/kg (Plant & Food Compound Database, data not published), which contrasts with the high concentration of DMHF found as bound volatile in this experiment (95.36 μg/kg). Yang et al. (2010) reported DMHF as free volatile in *A. arguta*, but did not determine its concentration because of the lack of sensitivity of HS-SPME for this compound.

The high concentration of DMHF in bound form justifies further studies on the *A. arguta* aroma glycosides, because there is an obvious source of potential flavour stored within the fruit cells.

Benzyl alcohol and 2-phenylethanol were also detected in significant amounts in bound form (69.48 and 18.88 μg/kg respectively); 2-phenylethanol is reported for the first time in *A. arguta* fruit. Benzyl alcohol has sweet, flowery notes, while 2-phenylethanol has been described as honey, spice, and rose (http://flavornet.org).

Some aromatic compounds such as benzoic acid, cinnamic acid and coniferyl alcohol, are important precursors of odorous compounds, including benzoate esters and eugenol (Koeduka et al., 2006, Reineccius, 2006b) and were detected as bound volatiles, although they have not been reported as free volatiles in kiwifruit. Eugenol, raspberry ketone and 4-vinylguaiacol were found for the first time in the fruit of an *Actinidia* species. Eugenol is the compound responsible for the aroma of cloves, 4-vinylguaiacol has a similar clove-like smell, and raspberry ketone, as the name indicates, has a strong raspberry aroma (http://flavornet.org).

A small number of acids were found, probably bound as glycosidic esters. Fatty acids have been reported to be important components of the bound volatiles of mango (Lalel et al., 2003).

There were a number of tentatively identified norisoprenoid compounds in the bound volatile extract, including 3-hydroxy-β-damascone, 3-oxo-α-ionol, 3-oxo-7,8-dihydro-α-ionol and 3-hydroxy-7,8-dihydro-β-ionol, which are reported for the first time in *A. arguta*. These compounds, with the exception of 3-hydroxy-β-damascone, are being reported for the first time in an *Actinidia* species. Interestingly, norisoprenoids have not been found in *A. arguta* free volatile extracts. 3-Hydroxy-β-damascone is a major component of grape glycosides (Günata et al., 2001); this flavourless compound is related to β-damascenone, a potent odorant (http://leffingwell.com/odorthre.htm). The labile (3S, 9R)- and (3S, 9S)-megastigma-6,7-dien-3,5,9-triol have generated these compounds upon heating under acidic conditions, and are considered to be the progenitor compounds. The suggested biosynthetic pathway generates both β-damascenone and 3-hydroxy-β-damascone as products (Suzuki et al., 2002). The aroma of 3-oxo-α-ionol is described as spicy (http://flavornet.org); interestingly, this compound has been described as an insect pheromone (Ishida et al., 2008).

Terpenoids found in bound form include linalool, (*Z* and *E*)-8-hydroxylinalool, nerol,

geraniol and prenol, among others. 8-Hydroxylinalool has been reported in glycosidic form in several species of fruit; whereas linalool, geraniol and nerol have been detected as bound volatiles in kiwifruit (Young and Paterson, 1995). Linalool, nerol and geraniol are floral compounds, while prenol is a simple terpenoid having an herb-like aroma (http://flavornet.org). 8-Hydroxylinalool is a product of the hydroxylation of linalool, and a precursor of lilac aldehydes and alcohols (Kreck et al., 2002).

A comparison of the odour-active compounds in the free volatile portion of *A. arguta* vs. *A. deliciosa* 'Hayward' is shown in Table 3-2. Yang et al. identified ethyl butanoate, DMHF, 1-penten-3-one, (*E*)-2-hexenal, 1-octen-3-ol, pentanal, hexanal, methyl benzoate and pentanol as the most important compounds for the aroma of *A. arguta* (Yang et al., 2010). On the other hand, Frank et al. (2007) determined that the seven most important compounds for the aroma of 'Hayward' are (*E*)-2-hexenal, 1-penten-3-one, hexanal, ethyl 2-methylpropanoate, dimethyltrisulfide, (*E*)-3-hexenol and 1-octen-3-one. It is noticeable that sweet-smelling compounds are predominant in *A. arguta*, while green-smelling compounds predominate in 'Hayward', which agrees with the sensory descriptions of these fruit. In particular, the presence of DMHF as one of the most important odorants for *A. arguta* is of interest, as this compound was found in high concentration in glycosylated form. Hexanal, 1-octen-3-ol and (*E*)-2-hexenal were reported as odorants by Yang et al. (2010) and also found in the bound volatile portion of *A. arguta.*

Table 3-2 Aroma components of ripe *Actinidia arguta* and *A. deliciosa* 'Hayward' identified in the free volatile extract by GC-O. The main compounds ranked by odour intensity (determined by Osme value in *A. arguta* and time-intensity profiling in 'Hayward').

A. arguta [a]		*A. deliciosa* 'Hayward' [b]	
Compound	**Descriptor**	**Compound**	**Descriptor**
Ethyl butanoate	fruity, sweet	(*E*)-2-Hexenal	green, marzipan, sweet
DMHF	caramel	1-Penten-3-one	herbal, kiwifruit-like
1-Penten-3-one	cut grass	Hexanal	green, crushed leaves
(*E*)-2-Hexenal	green, fruity	Ethyl 2-methylpropanoate	melon, bubblegum
1-Octen-3-ol	mushroom	Dimethyl trisulfide	garlic, rotten
Pentanal	green, earthy	(*E*)-3-Hexenol	herbal, green
Hexanal	cut grass	1-Octen-3-one	mushroom
Methyl benzoate	fruity, sweet		
Ethyl benzoate	floral, celery		
Pentanol	tropical fruit		

[a] Adapted from Yang et al. (2010), compounds were identified by MS, RI and odour
[b] Adapted from Frank et al. (2007), compounds were identified by MS, RI and odour

In this study, 11 odour-active compounds were found in the bound volatile extract of ripe *A. arguta* (Table 3-3). The chromatogram displaying the odour descriptors and intensities is shown in Fig. 3-4. Most odour-active compounds were found at moderate to low concentrations, with the exception of DMHF and 2-phenylethanol. DMHF was the compound with the highest concentration and it also was the odorant perceived with the strongest intensity by GC-MS/O; this compound was perceived with a sweet, caramel or pineapple-like smell. DMHF is known to be an important contributor to the aroma of *A. arguta* (Yang et al., 2010). 2-Phenylethanol and vanillin (co-eluted with phenylacetic acid) were also perceived with strong intensities. The former had a rose-like aroma, while the latter had a sweet, characteristic vanilla smell. 4-(4-Hydroxyphenyl)-2-butanone (raspberry ketone), which had the characteristic aroma of raspberries, was perceived with intermediate intensity despite its low concentration. This is the first time raspberry ketone has been reported as an odour-active compound in *A. arguta*. Traces of β-damascenone were present, although a peak could not be

observed; this compound was identified by comparison of RI and odour with an authentic standard.

Table 3-3 Aroma compounds perceived by GC-MS/O in the bound volatile extract of ripe *Actinidia arguta.*

Compound	CAS	RI [a]	Detection frequency [b]	Intensity [c]	Odour description
Isovaleric acid	503-74-2	1684	1	weak	sweat
β-Damascenone	23726-93-4	1832	5	weak	apple juice
2-Phenylethanol	60-12-8	1924	5	middle	rose
DMHF	3658-77-3	2046	5	strong	pineapple, sweet, caramel
Eugenol	97-53-0	2185	5	weak	spicy, flower, sweet
4-Vinylguaiacol	7786-61-0	2218	4	weak	spicy, smoke
Unknown		2262	5	weak	sweet, flower
Phenylacetic acid/vanillin	103-82-2/ 121-33-5	2611	5	strong	sweet, vanilla
Raspberry ketone	5471-51-2	3030	5	middle	raspberry
Unknown		3173	5	weak	mild

[a] RI, retention indices on Stabilwax column
[b] Detection frequency in 5 GC-O runs
[c] Intensities are described relative to the compound perceived with the strongest smell (DMHF)

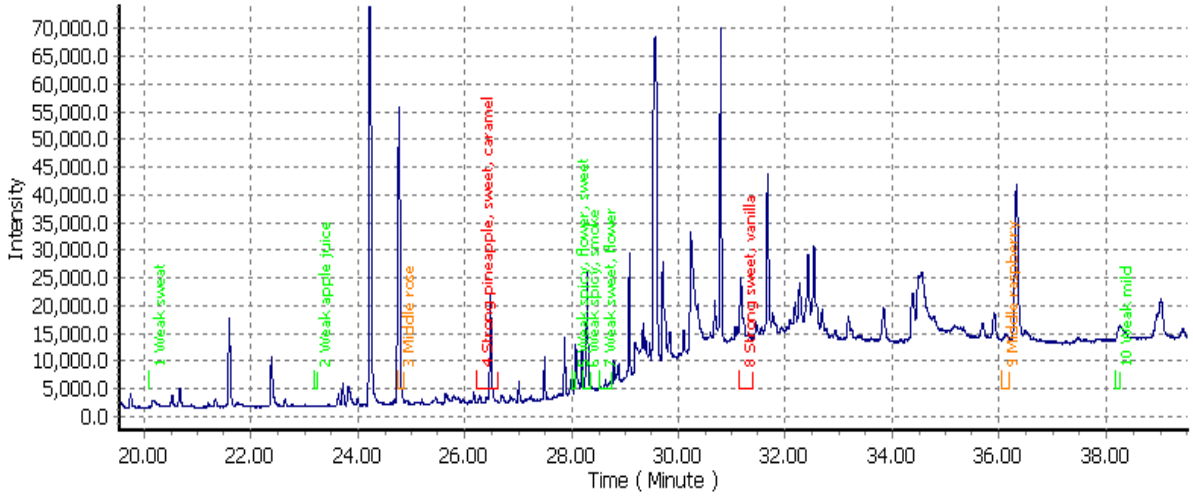

Fig. 3-4 Chromatogram of bound volatiles from ripe *Actinidia arguta* showing the aromas detected and their intensities.

3.3.2 Glycoside analysis

Seven glycosidic fractions were obtained, and the fraction containing DMHF was selected for the analysis (Fig. 3-5).

LC-MS analysis in positive mode of the sample purified by PTLC suggested the presence of a sodiated form of the DMHF glucoside (*m/z* 313). However, the sample was weak and a clear mass spectrum (MS) could not be obtained, suggesting that the silica in the plate adsorbed most of the compound. Consequently, the fraction containing DMHF was analysed directly after further purification by solid-phase extraction (SPE). The LC-MS chromatogram is shown in Fig. 3-6 (A).

The fraction purified by SPE was dissolved in methanol:water (1:1 v/v), to reach a concentration of 1 μg/mL, and its analysis in positive mode resulted in a clear MS, with a large peak at *m/z* 313, which corresponds to a sodiated form of DMHF-β-D-glucopyranoside ($C_{12}H_{18}O_8Na$) (Fig. 3-6 (B)). Other peaks showing the presence of DMHF glucoside were observed at *m/z* 285 (sodiated DMHF glucoside minus CO), 185 (sodiated sugar moiety) and 151 (sodiated DMHF) (Fig. 3-6 (C) and (D)). Thus, the formula of the compound was determined to be $C_{12}H_{18}O_8$ (Fig. 3-7).

DMHF-β-D-glucopyranoside has been identified in analyses of the glycoconjugates from strawberry (Mayerl et al., 1989), pineapple (Wu et al., 1991), tomato (Krammer et al., 1994), and mango (Sakho et al., 1997).

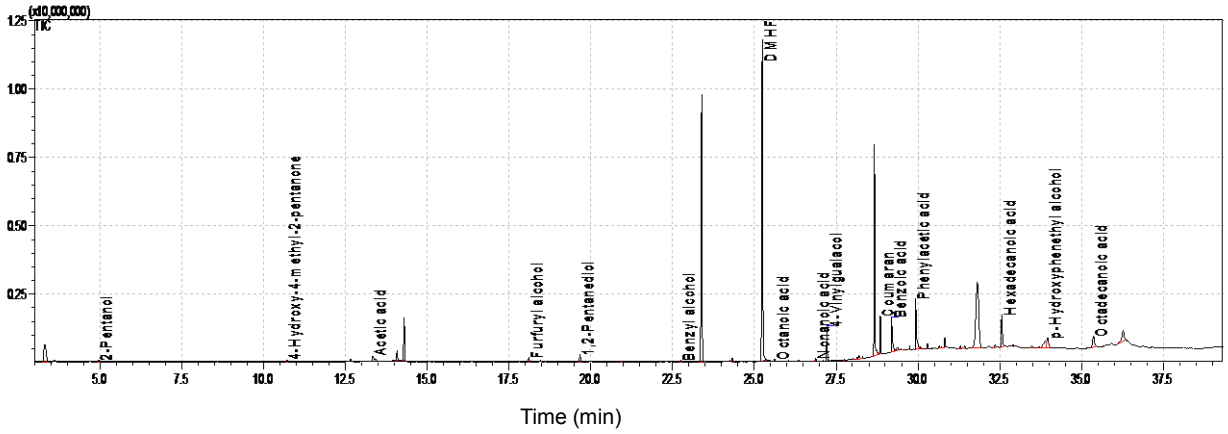

Fig. 3-5 GC-MS chromatogram (TIC) of fraction 1 of the bound volatile extract from ripe *Actinidia arguta.*

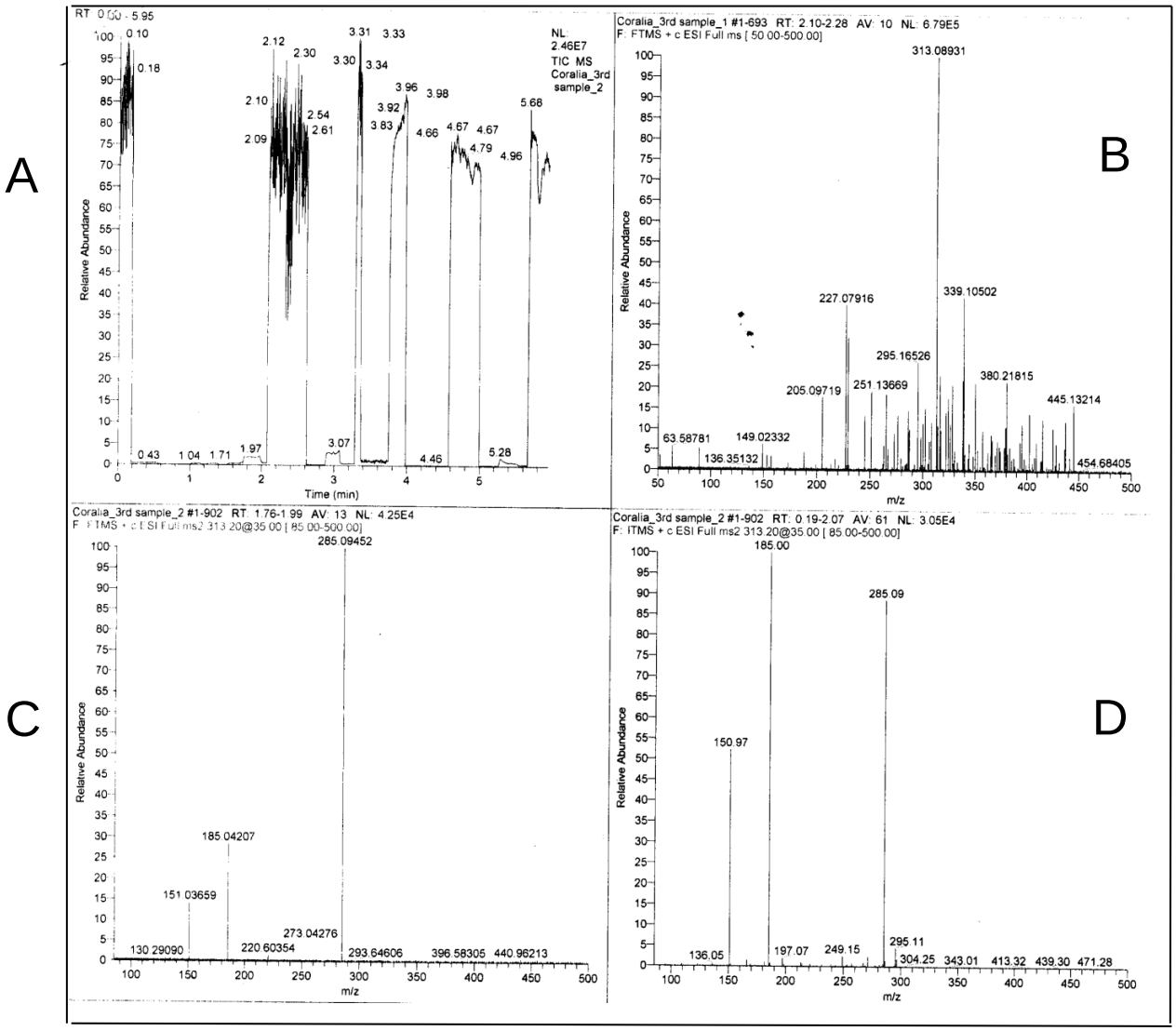

Fig. 3-6 Results of LC-MS analysis (positive mode) of the fraction containing DMHF. (A) LC-MS chromatogram; (B), MS of sodiated DMHF-β-D-glucopyranoside; (C), MS of sodiated DMHF glucoside minus CO; (D) peaks corresponding to the sodiated sugar moiety (*m/z* 185) and sodiated DMHF (*m/z* 151).

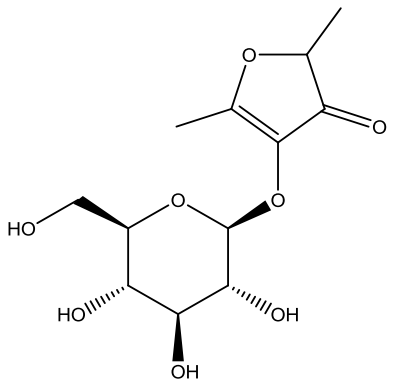

Fig. 3-7 Structure of DMHF-β-D-glucopyranoside.

3.3.3 Analysis of sugar moieties

The sugar content of the glycosidic samples hydrolysed at pH 0 and 3 is shown in Table 3-4. There were differences between the samples hydrolysed at pH 0 and 3. The former had a sweet smell and a darker colour, and its sugar content was also higher, suggesting a complete hydrolysis of the glycosides. Glucose was the predominant sugar, which agrees with the literature on fruit glycosides (Sarry and Günata, 2004, Maicas and Mateo, 2005). It is known that glycosides of aroma compounds are mainly *O*-β-D-glucosides and *O*-diglycosides, with the aglycone always attached to β-D-glucopyranose (Sarry and Günata, 2004). Glucose represented over 68% of the sugar content of the sample hydrolysed at pH 0. Arabinose, xylose and galactose were also found, representing 7, 3.5 and 3% of the sugar content. These sugars suggest the presence of some diglycosides in *A. arguta*. Stachyose, a tetrasaccharide consisting of two α-D-galactose units, one α-D-glucose unit and one β-D-fructose unit, which is common in numerous plants, was also found. Several unknowns were present, which may be intact diglycosides, sugars for which there was no standard (e.g.: apiose) or contaminants.

Table 3-4 Sugars found in the *Actinidia arguta* glycosidic extracts hydrolysed at pH 0 and 3.

	pH 0		pH 3	
Sugar	**μg/mL**	**% Total sugars**	**μg/mL**	**% Total sugars**
Glucose	27.41	68.57	3.20	31.40
Stachyose	3.91	9.78	4.69	46.04
Arabinose	2.80	7.01	0.10	0.98
Xylose	1.41	3.53	0.01	0.10
Galactose	1.21	3.02	0.04	0.38
Unknown 7	0.94	2.35	1.19	11.73
Unknown 8	0.53	1.33	0.00	0.00
Rhamnose	0.49	1.22	0.00	0.00
Unknown 4	0.31	0.77	0.17	1.67
myo-Inositol	0.29	0.72	0.01	0.10
Unknown 3	0.23	0.58	0.20	1.96
Arabitol	0.21	0.53	0.01	0.13
Unknown 1	0.13	0.32	0.47	4.64
Unknown 5	0.08	0.21	0.00	0.00
Unknown 2	0.01	0.03	0.01	0.10
Unknown 6	0.01	0.03	0.00	0.00

3.4 Conclusion

Compounds with aromatic rings, derived from the shikimate pathway, as well as C_{13} norisoprenoids derived from the degradation of carotenoids (Reineccius, 2006b, Winterhalter and Skouroumounis, 1997) showed the highest concentrations in the bound volatile extract. According to other reports, norisoprenoids, in particular 3-hydroxylated and 3-oxo derivatives, such as the ones found in this study, are an abundant component of the bound volatile portion of fruits. Norisoprenoid glycosides have been considered to be important flavour precursors, although further degradation is often needed after enzymatic release to generate odorous compounds (Winterhalter and Skouroumounis, 1997). There is also a great diversity in the structures of shikimate derivatives reported as glycosides, including important odorants such as eugenol, 4-vinylguaiacol and vanillin, which were also found in this study. Several compounds reported as important odorants for *A. arguta* were found in bound form;

in particular, the high level of DMHF in bound form was interesting.

GC-O analysis revealed the compounds important for the aroma of the bound volatile extract. Sweet odorants predominated. DMHF was not only the compound with the highest concentration, but also the compound perceived with the strongest intensity. Vanillin (co-eluted with phenylacetic acid), 2-phenylethanol and raspberry ketone were also identified as components important for the aroma.

To elucidate the structure of the DMHF glycoside, the corresponding fraction was purified and analysed by LC-MS. On the basis of the MS obtained, the glycoside was identified as DMHF-β-D-glucopyranoside, which is the most common form of DMHF glycoside (Zabetakis et al., 1999).

Analysis of the sugar moieties after acid hydrolysis revealed that glucose was the most abundant sugar, with arabinose, xylose and galactose also having been found. This confirms what is currently known about fruit glycosides, in particular, the fact that aglycones are found as β-D-glucosides and diglycosides, with the aglycone always attached to β-D-glucopyranose (Sarry and Günata, 2004), making glucose the most abundant monosaccharide in fruit glycosidic extracts.

Chapter 4

Analysis of bound volatile compounds of *Actinidia eriantha* [3]

[3] This chapter is based on the manuscript Garcia et. al, *Food Chemistry*, **2012**, 134, 655-661.

4.1 Introduction

Actinidia eriantha Benth. is a kiwifruit species which produces fruit with a bland and unpalatable flavour, which is covered in fine, white hairs, but can be easily peeled, and thus, has the genetic potential to generate new kiwifruit cultivars with convenient attributes (Atkinson et al., 2009). One cultivar of *A. eriantha*, 'Bidan', has been released in Korea (Jo et al., 2007) and another one, 'White', in China (Wu et al., 2009). Wild *A. eriantha* fruit weighs 25 g on average, while the average weight of 'White' is 94 g. 'White' has been described as having a good eating quality flesh, but with little juice. This fruit is also known as the "banana kiwifruit" because of its peelability (Wu et al., 2009).

Kiwifruit is appreciated for its flavour and nutritional qualities; however, it is perceived by consumers as inconvenient since the fruit has to be peeled with a knife or cut in half before scooping out the flesh with a spoon. The generation of new kiwifruit cultivars that can be peeled by hand would thus combine the positive attributes of flavour, health and convenience. Among *Actinidia* species, *A. eriantha* is the only one producing easy to peel fruit when ripe, (Atkinson et al., 2009) although *A. arguta* fruit have edible skins.

A. eriantha has a high vitamin C content, which is significantly higher than that of commercial species, and can reach concentrations up to 8 mg/g (Atkinson et al., 2009). As in several *Actinidia* species, the roots of *A. eriantha* have also been reported to contain triterpenes such as ursolic acid, a compound with anti-inflammatory, anti-tumour, and anti-microbial activities (Huang et al., 1988, Liu, 2005).

The volatile content of *A. eriantha* is lower than in other kiwifruit species, explaining its bland flavour. Solvent and headspace analyses of this fruit revealed few odorous compounds, with butyl acetate, an ester with a pineapple-like aroma, being the major compound. Other esters included ethyl hexadecanoate and ethyl acetate, although they were found at low concentrations. Apart from esters, the volatile fraction from *A. eriantha* was found to contain

several C6 alcohols, known for their green notes, including (*E*)-2-hexenol, (*Z*)-3-hexenol and hexanol (Crowhurst et al., 2008).

Glycosidically bound aroma compounds have been reported in many fruit, including 'Hayward' kiwifruit and baby kiwifruit (*A. arguta*), but there are no journal publications on the free or bound volatile composition of *A. eriantha* (Young and Paterson, 1995, Burdon et al., 2005).

The objective of this chapter was to elucidate the bound volatile profile of *A. eriantha*. Furthermore, the question of this fruit containing hidden flavour potential is addressed.

4.2 Materials and methods

4.2.1 Plant material

Actinidia eriantha fruit (line 11-02-16a) were harvested during April 2010 from the Plant & Food Research orchard in Te Puke, New Zealand, and kept in cool storage at 0 ºC until required. Fruit were kept in storage for less than a month. Prior to sampling, fruit were taken out of storage, repacked into kiwifruit trays covered with a polyliner, and held at 20 ºC until ripe.

The fruit were analysed at eating-ripe stage, having an average flesh firmness of 0.76 kgf, determined using a GUSS Fruit Texture Analyzer FTA (GUSS Manufacturing, Strand, South Africa), and soluble solids content of 11.5 ºBx, determined using a handheld refractometer model N-20E (0-20% Brix, ATAGO, Tokyo, Japan)

4.2.2 Standards and solvents

Chemicals were purchased according to section 3.2.2.

4.2.3 Isolation and hydrolysis of glycosidic precursors

The glycosidic precursors were isolated and hydrolysed as described in section 3.2.3.1. The sample consisted of 1.6 kg of fruit, peeled.

4.2.4 GC-MS analysis

Analysis of the bound volatile extract was performed as described in section 3.2.3.2.

4.2.5 GC-MS/O analysis

GC-MS/O analysis of the bound volatile extract was performed as described in section 3.2.3.3. The sample consisted of ripe *A. eriantha* fruit.

4.3 Results and discussion

A total of 91 compounds were detected in the bound volatile extract of ripe *A. eriantha* fruit. The control sample (no enzyme added) contained few volatiles, including (*E*)-2-hexenal, *p*-cymen-8-ol, benzoic acid and vanillin, which may have been released from their glycosides by the acidity of the buffer (Williams et al., 1982b).

Alcohols (24 compounds), terpenoids (16 compounds) and phenolics (15 compounds) were the most numerously represented compound classes in the *A. eriantha* extract. Alcohols, benzenoids and phenolics were the most abundant (Fig. 4-1). 2-Phenylethanol, furfuryl alcohol, (*Z*)-3-hexen-1-ol, coniferyl alcohol, isoamyl alcohol and linolenic acid showed the highest concentrations.

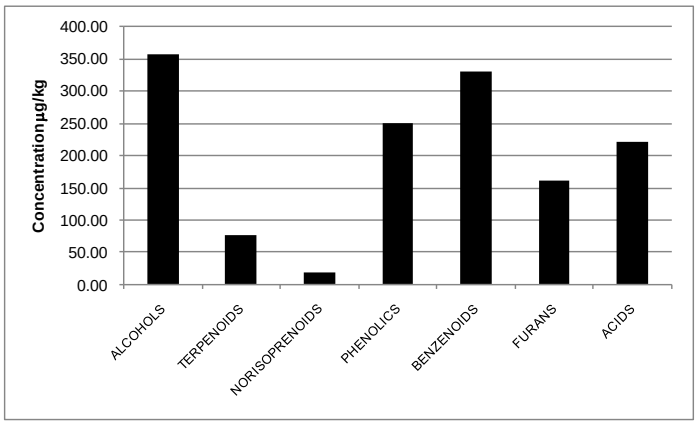

Fig. 4-1 Levels of bound volatiles, categorised as class of compound, of ripe *Actinidia eriantha* fruit.

It was particularly interesting to find high levels of aroma precursors in bound form in *A. eriantha*, especially fatty acid precursors of green note compounds (Table 4-1), considering that this fruit has been described as having a bland flavour (Atkinson et al., 2009). This indicates that the low flavour of this fruit is not the result of a lack of precursors, but probably of a lack of gene expression or enzymatic activity to metabolise precursors into flavour compounds, or insufficient ethylene production, which is needed to regulate aroma production (Schwab et al., 2008, Defilippi et al., 2004, Defilippi et al., 2005). Furthermore, it is possible that some aroma compounds remain in glycosidic form in *A. eriantha*, and are not released during ripening, as happens with *A. deliciosa* or *A. chinensis*. An example of this is (*Z*)-3-hexen-1-ol, a green note compound that had a high concentration in bound form in *A. eriantha*, but it is absent in the free volatile portion (Crowhurst et al., 2008).

Table 4-1 Compounds found in the bound volatile fraction of ripe *Actinidia eriantha* fruit.

Compound	CAS	RI [a]	µg/kg	Std. dev.	ID method [b]
Alcohols					
2-Butanol	78-92-2	1022	26.50	3.25	MS, RI, Std
2-Methyl-3-buten-2-ol	115-18-4	1037	0.83	0.07	MS, RI, Std
Isobutanol	78-83-1	1091	14.08	1.23	MS, RI, Std
3-Pentanol	584-02-1	1107	0.73	0.11	MS, RI, Std
2-Pentanol	6032-29-7	1119	0.90	0.21	MS, RI, Std
Butanol	71-36-3	1143	0.42	0.03	MS, RI, Std
1-Penten-3-ol	616-25-1	1160	0.98	0.08	MS, RI
4-Methyl-2-pentanol	108-11-2	1165	0.21	0.05	MS, RI
3-Hexanol	623-37-0	1198	0.63	0.06	MS, RI
Isoamyl alcohol	123-51-3	1208	102.09	5.08	MS, RI, Std
3-Methyl-3-buten-1-ol	763-32-6	1250	3.67	0.20	MS, RI
Cyclopentanol	96-41-3	1302	0.51	0.07	MS, RI, Std
3-Methyl-1-pentanol	589-35-5	1328	0.14	0.04	MS, RI
Hexanol	111-27-3	1354	5.82	0.53	MS, RI, Std
(*Z*)-3-Hexen-1-ol	928-96-1	1386	137.61	7.52	MS, RI, Std
3-Octanol	589-98-0	1395	1.33	0.21	MS, RI, Std
(*E*)-2-Hexen-1-ol	928-95-0	1408	25.78	1.97	MS, RI, Std
Cyclopentanemethanol	3637-61-4	1446	0.15	0.00	MS
1-Octen-3-ol	3391-86-4	1453	21.93	3.19	MS, RI, Std
Sulcatol	1569-60-4	1465	0.89	0.10	MS, RI, Std
2-Ethylhexanol	104-76-7	1492	0.44	0.06	MS, RI, Std
1,2-Pentanediol	5343-92-0	1735	7.05	0.14	MS
7-Methyl-4-octanol	33933-77-6	1995	2.79	3.73	MS
1,3-Octanediol	23433-05-8	2135	1.21	0.63	MS
Aldehydes					
Hexanal	66-25-1	1086	3.52	1.42	MS, RI, Std
(*E*)-2-Hexenal	6728-26-3	1221	1.23	0.16	MS, RI, Std
Ketones					
(*E*)-3-Penten-2-one [c]	3102-33-8	1128	0.02		MS, RI
4-Methyl-3-penten-2-one [c]	141-79-7	1134	0.06		MS, RI
4-Hydroxy-4-methylpentan-2-one	123-42-2	1363	10.29	3.45	MS, RI
4-Hydroxy-5-methyl-2-hexanone	38836-21-4	1578	0.95	0.02	MS
Terpenoids					
Prenol	556-82-1	1323	12.41	0.44	MS, RI, Std
cis-Linaloloxide	5989-33-3	1441	0.19	0.04	MS, RI, Std

Table 4-1 (Continued)

trans-Linaloloxide	34995-77-2	1470	0.88	0.39	MS, RI, Std
Linalool	78-70-6	1551	4.62	0.42	MS, RI, Std
α-Terpineol	98-55-5	1698	10.93	0.70	MS, RI, Std
Nerol	106-25-2	1802	1.85	0.20	MS, RI, Std
p-Cymen-8-ol	1197-01-9	1855	17.24	0.28	MS, RI
2,6-Dimethyl-3,7-octadiene-2,6-diol	13741-21-4	1951	1.04	0.08	MS, RI
6,7-Dihydro-7-hydroxylinalool	29210-77-3	1981	1.69	0.22	MS, RI
Perilla alcohol	536-59-4	2007	0.96	0.13	MS, RI
Geranyl vinyl ether	17957-93-6	2167	1.61	0.09	MS
(*Z*)-8-Hydroxylinalool	103619-06-3	2278	1.46	0.06	MS, RI
(*E*)-8-Hydroxylinalool	64142-78-5	2316	10.53	0.47	MS, RI, Std
Geranic acid	459-80-3	2355	3.25	0.63	MS, RI
Spathulenol	77171-55-2	2589	5.88	0.23	MS
(*E*,*E*)-2,6-Dimethyl-2,6-octadiene-1,8-diol	26488-97-1	2639	2.87	0.92	MS
Acids					
Acetic acid	64-19-7	1472	7.98	1.57	MS, RI, Std
Isobutyric acid	79-31-2	1586	0.91	0.16	MS, RI
Isovaleric acid	503-74-2	1685	3.61	0.25	MS, RI, Std
2-Ethylbutanoic acid	88-09-5	1689	3.08	0.05	MS, RI
Hexanoic acid	142-62-1	1864	7.43	0.41	MS, RI
trans-2-Hexenoic acid	13419-69-7	1988	18.80	2.99	MS, RI
Octanoic acid	124-07-2	2075	3.66	0.21	MS, RI
Nonanoic acid	112-05-0	2181	0.23	0.36	MS, RI
Hexadecanoic acid	57-10-3	2918	42.38	7.54	MS, RI, Std
Octadecanoic acid	57-11-4	3169	11.11	1.89	MS, RI, Std
Linoleic acid	60-33-3	3312	53.06	16.46	MS, RI, Std
Linolenic acid	463-40-1	3453	68.65	18.42	MS, RI, Std
Sulfur compounds					
2-(Methylthio)-ethanol	5271-38-5	1532	0.14	0.04	MS, RI, Std
3-(Methylthio)-1-propanol	505-10-2	1719	2.73	1.61	MS, RI, Std
Furans					
Dihydro-3,5-dimethyl-2(3H)-furanone	5145-01-7	1608	0.25	0.00	MS, RI
Furfuryl alcohol	98-00-0	1666	162.00	3.35	MS, RI, Std
Phenols and phenylpropanoids					
Methyl salicylate	119-36-8	1777	4.63	0.59	MS, RI, Std
Phenol	108-95-2	2016	1.06	0.07	MS, RI
p-Cresol	106-44-5	2092	2.03	0.21	MS, RI

Table 4-1 (Continued)

Eugenol	97-53-0	2177	4.38	0.22	MS, RI, Std
4-Vinylguaiacol	7786-61-0	2208	1.41	0.12	MS, RI, Std
4-Hydroxy-3-methylacetophenone	876-02-8	2216	0.17	0.03	MS, RI
cis-Isoeugenol	5912-86-7	2270	4.05	2.68	MS, RI, Std
(*E*)-Cinnamyl alcohol	4407-36-7	2295	5.54	7.66	MS, RI, Std
trans-Isoeugenol	5932-68-3	2365	60.71	3.74	MS, RI, Std
Vanillin	121-33-5	2595	19.81	10.27	MS, RI, Std
2-Methoxyhydroquinone [c]	824-46-4	2994	9.10		MS
Homovanillic acid	306-08-1	3008	4.81	0.59	MS, RI
p-Hydroxyphenethyl alcohol	501-94-0	3036	4.49	1.47	MS, RI, Std
3,4,5-Trimethoxyphenol	642-71-7	3100	10.20	0.24	MS, RI
Coniferyl alcohol	458-35-5	3440	117.44	2.83	MS, RI, Std
Benzenoids					
Benzaldehyde	100-52-7	1526	1.82	0.30	MS, RI, Std
3-Ethylbenzaldehyde	34246-54-3	1709	0.41	0.08	MS
4-Ethylbenzaldehyde	4748-78-1	1739	0.40	0.15	MS
Benzyl alcohol	100-51-6	1882	68.47	2.29	MS, RI, Std
2-Phenylethanol	60-12-8	1915	169.26	4.85	MS, RI, Std
2,4-Dimethylphenethyl alcohol	6597-59-7	2023	1.32	0.03	MS
o-Methoxybenzyl alcohol	612-16-8	2171	0.42	0.12	MS
Coumaran	496-16-12	2412	30.97	9.91	MS, RI
Benzoic acid	65-85-0	2476	37.98	3.04	MS, RI, Std
Phenylacetic acid	103-82-2	2600	18.22	9.45	MS, RI
Norisoprenoids					
1-(1a,2,3,5,6a,6b-Hexahydro-3,3,6a-trimethyloxireno[g]benzofuran-5-yl)-ethanone	80114-25-6	2453	10.03	0.70	MS
3-Hydroxy-β-damascone	102488-09-5	2552	9.11	0.46	MS, RI
Unidentified compounds					
Unknown 1		1360	2.52	0.31	
Unknown 2		2809	4.17	0.54	

[a] RI, retention indices on Stabilwax column
[b] Identification methods: MS, RI, Std (authentic standard)
[c] Not detected in all replicates

The oxidative degradation of linoleic and linolenic acids gives origin to many aroma compounds, including alcohols, aldehydes, esters and ketones (Reineccius, 2006b). The mechanism of lipid oxygenation via lipoxygenase has been studied in several plants including tomatoes and involves the conversion of linoleic and linolenic acids to hydroperoxides, of which 95% corresponds to the 9-hydroperoxide, and 5% to the 13-hydroperoxide. Surprisingly, the 13-hydroperoxide is readily converted to C6 aldehydes through cleavage by a hydroperoxide lyase, making it the greater contributor to aroma (Galliard and Matthew, 1977). The 9-hydroperoxide, on the other hand, is not further metabolised by enzymes, and degrades slowly to form volatile compounds (Reineccius, 2006b). Aldehydes are also reduced to alcohols by alcohol dehydrogenase and (*Z*)-3-hexenal is isomerised to (*E*)-2-hexenal by an isomerisation enzyme (Hatanaka, 1993).

Linoleic acid is the precursor of hexanal, while linolenic acid is the precursor of (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, and (*E*)-2-hexen-1-ol (Fig. 4-2) (Hatanaka, 1993). All of these compounds were found in the bound volatile portion of *A. eriantha* (Table 4-1). The high levels of linoleic and linolenic acids may suggest an abundance of these precursors in the fruit tissue, and therefore explain the predominantly green aroma of *A. eriantha*. Hexanal and (*Z*)-3-hexen-1-ol have a grassy smell, (*E*)-2-hexenal is described as having an apple/green aroma and (*E*)-2-hexen-1-ol has a green leaf-like smell (http://flavornet.org). Of these compounds, (*Z*)-3-hexen-1-ol had the highest concentration in the bound volatile extracts (137.61 μg/kg).

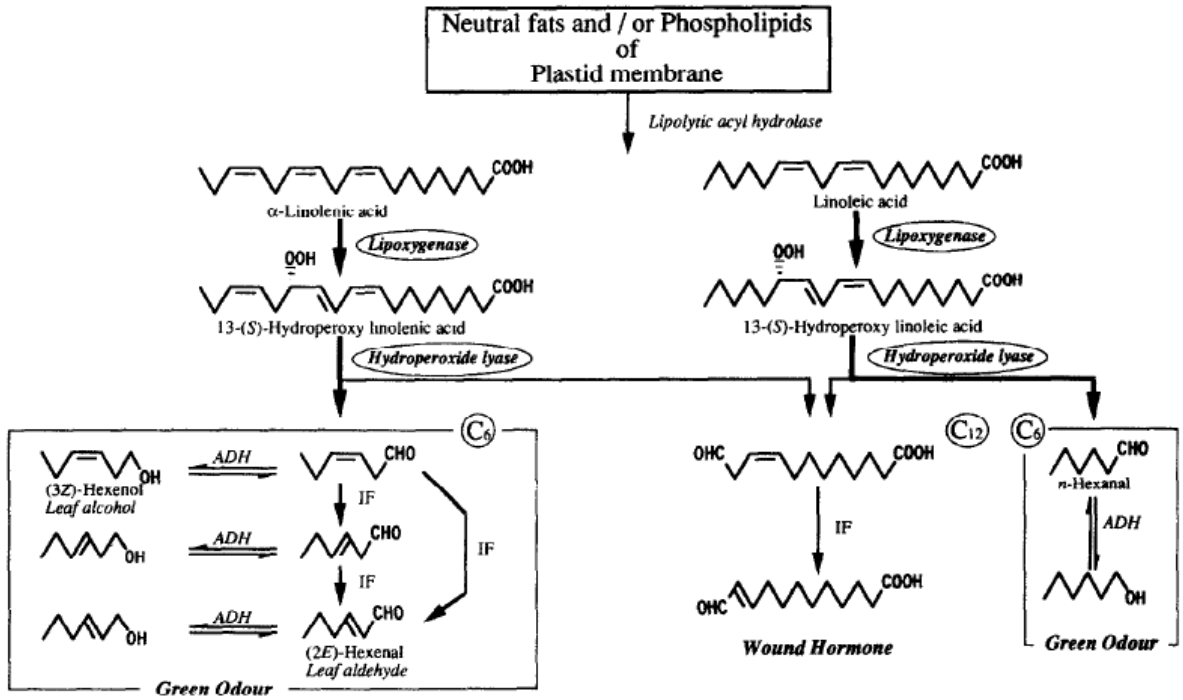

Fig. 4-2 Biosynthesis of green odour compounds. ADH: alcohol dehydrogenase. IF: isomerisation factor. Taken from (Hatanaka, 1993).

Among the alcohols detected, (*Z*)-3-hexen-1-ol, isoamyl alcohol, 2-butanol, (*E*)-2-hexen-1-ol and 1-octen-3-ol showed the highest concentrations (Table 4-1). (*Z*)-3-Hexen-1-ol and (*E*)-2-hexen-1-ol are green-aroma compounds, as previously described. 1-Octen-3-ol has a mushroom aroma. Isoamyl alcohol and 2-butanol are weak, winey odorants. Isoamyl alcohol has been reported to be a precursor of the banana-smelling ester isoamyl acetate, with both compounds being derived from the amino acid leucine (Reineccius, 2006b, Myers et al., 1970).

Benzyl alcohol, 2-phenylethanol and phenylacetic acid were found in the bound volatile extract (Table 4-1). These compounds have sweet, flowery notes and their presence may partially explain the flowery notes perceived in the bound volatile extract of *A. eriantha* after enzymatic hydrolysis, which was unexpectedly different from the leafy smell of the fresh fruit. Benzyl alcohol and 2-phenylethanol are common components of the aroma of flowers, including flowers of *Actinidia* (Crowhurst et al., 2008). Benzoic acid, a precursor of benzyl

esters, benzaldehyde and benzyl alcohol, (Reineccius, 2006b) was also found.

Coniferyl alcohol was the phenylpropanoid with the highest concentration in the *A. eriantha* extract (Table 4-1). This compound is a precursor of eugenol and isoeugenol, which are spicy odorants found in cloves and allspice, respectively, (Koeduka et al., 2006) and which were also present in the bound volatile extract of *A. eriantha*. For the biosynthesis of eugenol and isoeugenol, coniferyl alcohol first reacts with NADPH and acetyl Co-A to generate coniferyl acetate, which is reduced by enzymatic action to produce the phenylpropene odorants (Koeduka et al., 2006). Cinnamyl alcohol, a compound with a pleasant sweet-floral aroma resembling balsam or hyacinth, and vanillin were also found. Both compounds have been reported in previous analyses of *A. chinensis* but not in *A. eriantha* (Plant & Food Compound Database, data not published).

A number of terpenoids were also present in the *A. eriantha* extract, among which the predominant compounds were *p*-cymen-8-ol, prenol, α-terpineol and (*E*)-8-hydroxylinalool (Table 4-1). *p*-Cymen-8-ol is also known as cherry propanol, and has a cherry-like, fruity, citrusy aroma, prenol has a green herb-like smell, while the aroma of α-terpineol resembles mint or anise. Linalool and 8-hydroxylinalool were also detected; 8-hydroxylinalool is a precursor of lilac aldehydes and alcohols, and is generated through the hydroxylation of linalool (Kreck et al., 2002).

Two sulfur compounds were detected in the *A. eriantha* extract, the meaty 2-(methylthio)-ethanol and 3-(methylthio)-1-propanol (Table 4-1), which smells of cooked potatoes (http://flavornet.org).

The major bound volatile compounds of *A. eriantha* and *A. arguta* are shown in Table 4-2. The major compounds in the bound volatile extract of *A. eriantha* were mainly aroma precursors including coniferyl alcohol, linoleic, linolenic and benzoic acids, as well as some floral compounds such as 2-phenylethanol, benzyl alcohol and (*E*)-isoeugenol, and the grass-

like (*Z*)-3-hexen-1-ol. On the other hand, the *A. arguta* extract had higher concentrations of aromatic compounds and norisoprenoids. The powerful sweet odorant 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF) was the major bound volatile of this extract, followed by benzyl alcohol, 3-hydroxy-β-damascone and the green note compounds hexanal and (*Z*)-3-hexen-1-ol. The *A. eriantha* hydrolysate had a green-floral smell, while the *A. arguta* hydrolysate had a strong pineapple or caramel-like aroma, reflecting its high DMHF content.

Table 4-2 Comparison of the major compounds in the bound volatile extracts of ripe *Actinidia eriantha* and *A. arguta*.

A. eriantha		***A. arguta***	
Compound	**μg/kg**	**Compound**	**μg/kg**
2-Phenylethanol	169.26	DMHF	95.36
Furfuryl alcohol	162.00	Benzyl alcohol	69.48
(*Z*)-3-Hexen-1-ol	137.61	3-Hydroxy-β-damascone	56.43
Coniferyl alcohol	117.44	Hexanal	54.92
Isoamyl alcohol	102.09	(*Z*)-3-Hexen-1-ol	41.96
Linolenic acid	68.65	3-Oxo-α-ionol	40.63
Benzyl alcohol	68.47	Cinnamic acid	36.47
(*E*)-Isoeugenol	60.71	*p*-Hydroxyphenethyl alcohol	31.83
Linoleic acid	53.06	3-Hydroxy-7,8-dihydro-β-ionol	26.87
Hexadecanoic acid	43.28	Benzoic acid	26.85
Benzoic acid	37.98	Phenylacetic acid	25.12
2-Butanol	26.50	3-Oxo-7,8-dihydro-α-ionol	21.44
(*E*)-2-Hexen-1-ol	25.78	2-Phenylethanol	18.88
1-Octen-3-ol	21.93	4-Vinylguaiacol	6.00
Vanillin	19.81	1-Octen-3-ol	4.85

In this study, 15 odour-active compounds were found in the bound volatile extract of ripe *A. eriantha* (Table 4-3). The chromatogram showing the odour descriptors and intensities is shown in Fig. 4-3. Many of the compounds important for the aroma were present at very low levels, with only 2-phenylethanol, (*E*)-isoeugenol and vanillin being among the major components of the bound volatile extract. The number and type of odorants found was surprising, considering the low aroma of this fruit and its green, leaf-like odour. Only one

compound in the extract had a green smell, 6-methyl-5-hepten-2-ol (sulcatol). Compounds with floral, sweet and spicy notes predominated. Vanillin (co-eluted with phenylacetic acid) and 2-phenylethanol were perceived with strong intensities. The smoke-like guaiacol was detected as a very small peak. It was surprising to find β-damascenone, DMHF and raspberry ketone, as these compounds showed no peaks because of their very low concentrations, so they were identified by comparison of odour and RI with authentic standards. A compound with a plastic-like odour and another with a floral/spicy smell could not be identified.

Table 4-3 Aroma compounds detected by GC-MS/O in the bound volatile extract of ripe *Actinidia eriantha*.

Compound	CAS	RI [a]	Detection frequency [b]	Intensity [c]	Odour description
Sulcatol	1569-60-4	1467	5	weak	green, green beans
Linalool	78-70-6	1551	2	weak	floral
Unknown		1695	4	weak	plastic
β-Damascenone	23726-93-4	1833	3	weak	floral, apple
Guaiacol	90-05-1	1872	2	weak	smoke
2-Phenylethanol	60-12-8	1920	5	strong	rose
DMHF	3658-77-3	2046	5	weak	pineapple, sweet, caramel
1,3-Octanediol	23433-05-8	2135	4	weak	flower, spicy
Eugenol	97-53-0	2186	4	weak	flower, spicy
4-Vinylguaiacol	7786-61-0	2216	5	weak	smoke, spicy
Unknown		2261	5	weak	sweet, flower, spicy
(*E*)-Isoeugenol	5932-68-3	2375	2	weak	spicy, woody
Phenylacetic acid/vanillin	103-82-2/ 121-33-5	2612	5	strong	sweet, vanilla
Raspberry ketone	5471-51-2	3029	5	weak	raspberry

[a] RI, retention indices on Stabilwax column
[b] Detection frequency in 5 GC-O runs
[c] Intensities are described relative to the compound perceived with the strongest smell (2-phenylethanol)

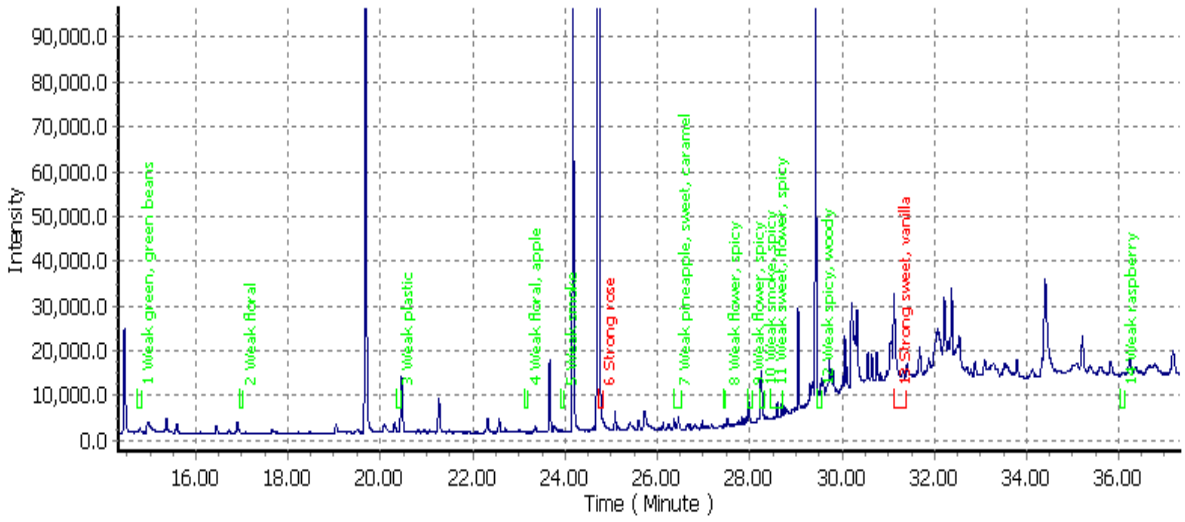

Fig. 4-3 Chromatogram of bound volatiles from ripe *Actinidia eriantha* showing the aromas detected and their intensities.

4.4 Conclusion

Compounds found with high concentrations in the bound volatile extract of *A. eriantha* included important aroma precursors such as linoleic and linolenic acids, which are metabolised to generate straight-chain C6 alcohols and aldehydes. These C6 compounds are characterised by their green, grassy smells and are an important component of the aroma profile of this fruit. A number of terpenoids were also detected. Phenylpropanoids, derived from the shikimate pathway, were also an important component of the bound volatile profile of *A. eriantha* fruit. Important phenylpropanoids found in the bound volatile extract include eugenol, isoeugenol, and vanillin. Coniferyl alcohol, a precursor of eugenol and isoeugenol, was the phenylpropanoid with the highest concentration.

There were notable qualitative and quantitative differences between the bound volatile profiles of *A. eriantha* and *A. arguta*. For instance, DMHF was an important component in *A. arguta*, but in *A. eriantha* no DMHF peak was observed, although its presence was detected by smell. GC-O analysis revealed the compounds important for the aroma of the bound volatile extract. These compounds included 2-phenylethanol, (*E*)-isoeugenol and vanillin,

which were also among the compounds with the highest concentrations, as well as the green-smelling sulcatol, and some powerful odorants found at the trace level, such as DMHF, β-damascenone and raspberry ketone.

Many compounds found in the bound volatile extract of *A. eriantha* have not been previously reported as free volatiles in this fruit. Notable exceptions are benzyl alcohol, 1-octen-3-ol, 2-phenylethanol, linalool, benzaldehyde and α-terpineol, as well as green note compounds such as (*Z*)-3-hexen-1-ol, hexanol, hexanal and (*E*)-2-hexenal, which may also be synthesized *de novo* in the fruit from their fatty acid precursors. Nevertheless, the reported levels of these compounds as free volatiles are generally lower than the levels found as bound volatiles in this study. This may indicate that there is a pool of potential odorants that remain as glycosides within the fruit cells, and are not released during ripening. This phenomenon has significance for the understanding of the biosynthetic pathways of aroma volatiles in *Actinidia*, and may be helpful to explain the lack of flavour in *A. eriantha*, justifying further research in the area. Experiments involving the feeding of labelled precursors may be needed to clarify the biosynthetic pathways to the aroma compounds, and determine the role of glycosylated compounds in aroma production.

A. eriantha has been reported to contain low levels of free volatiles compared to other *Actindia*, in particular, it contains almost no esters, which are important for fruity notes and which are synthesized from alcohol and acid precursors by acyltransferases, suggesting that these enzymes may be fairly inactive or not expressed in *A. eriantha*, although it is also possible that substrate availability is the crucial factor for the low production of esters in this fruit (Crowhurst et al., 2008, Souleyre et al., 2011). In addition, *A. eriantha* has a green, bland flavour, and green note compounds have been reported in the free volatile extract, indicating the action of lipoxygenase on fatty acid precursors (Crowhurst et al., 2008, Hatanaka, 1993).

Chapter 5

Changes in the bound volatile profiles of 'Hayward' and 'Hort16A' kiwifruit during ripening [4]

[4] This chapter is based on the manuscript Garcia et. al, *Food Chemistry*, **2013**, 137, 45-54.

5.1 Introduction

'Hayward' (*Actinidia deliciosa* (A. Chev.) C.F. Liang and A.R. Ferguson var. *deliciosa*) and 'Hort16A' (*Actinidia chinensis* Planch. var. *chinensis*) are the most widely planted kiwifruit cultivars, and the most commercially important.

The volatile composition of 'Hayward' kiwifruit has been analysed, and the major components have been identified as methyl and ethyl butanoate, (*Z*) and (*E*)-2-hexenal, hexanal, (*Z*) and (*E*)-3-hexenol, and methyl benzoate (Garcia et al., 2012). On the other hand, 'Hort16A' has been less studied, but the presence of diverse sulfur compounds has been noticed (Garcia et al., 2012).

So far, there is only one report on the bound volatiles of ripe 'Hayward' kiwifruit (Young and Paterson, 1995), and no reports on the bound volatiles of 'Hort16A' kiwifruit, indicating an important research gap that needs to be addressed. Therefore, this chapter aimed to investigate the changes in the bound volatile composition of 'Hayward' and 'Hort16A' kiwifruit at different ripening stages, as well as to identify odour-active aglycones derived from the bound volatile extracts.

5.2 Materials and methods

5.2.1 Plant material

'Hayward' (green-fleshed kiwifruit) and 'Hort16A' (gold-fleshed kiwifruit) were harvested at commercial maturity during May 2010, from the Plant & Food Research orchard in Te Puke, New Zealand. Fruit for measuring β-glucosidase activity were collected during July 2012 from the Plant & Food Research orchard in Kerikeri, New Zealand. Soluble solids content at harvest were 8.9 ºBx for 'Hayward' and 15.2 ºBx for 'Hort16A'. Fruit were kept in cool storage at 0 ºC ('Hayward') or 1 ºC ('Hort16A') until required, but for less than a month.

Prior to sampling, fruit were taken out of storage, repacked into kiwifruit trays covered with a polyliner, and held at 20 ºC until reaching the desired ripening stage.

5.2.2 Evaluation of ripening

Flesh firmness was evaluated using a GUSS Fruit Texture Analyzer FTA (GUSS Manufacturing, Strand, South Africa), and a handheld refractometer model N-20E (Brix 0-20%, ATAGO, Tokyo, Japan) was used to measure soluble solids content. Fruit were placed into the following ripening classes: 0.8-1.2 kgf for under-ripe, 0.5-0.79 kgf for ripe, and 0.3-0.49 kgf for over-ripe fruit.

5.2.3 Standards and solvents

Chemicals were purchased according to section 3.2.2. *p*-Nitrophenyl-β-D-glucopyranoside was obtained from Sigma-Aldrich (St. Louis, MO, USA).

5.2.4 Isolation and hydrolysis of glycosidic precursors

The glycosidic precursors were isolated as described in section 3.2.3.1. The samples consisted of flesh from ‘Hayward’ and ‘Hort16A’ kiwifruit at the three ripening stages mentioned above.

5.2.5 GC-MS analysis

Analysis of the bound volatile extract was performed as described in section 3.2.3.2.

5.2.6 GC-MS/O analysis

GC-MS/O analysis of the bound volatile extracts was performed as described in section

3.2.3.3. Flesh from ripe ‘Hayward’ and ‘Hort16A’ kiwifruit was used for the analyses.

5.2.7 β-Glucosidase assay

β-Glucosidases are widely distributed among plants and microorganisms, and are considered a key enzyme for flavour release. The optimum temperature of plant and microbial β-glucosidases is in the range 40-50 ºC. Their optimum pH is in the range 4.0-6.0, so they do not achieve their maximum activity in the acidic environment of fruits. Nevertheless, the β-glucosidase and exoglycosidases from *A. niger* show good stability at an acidic pH. β-Glucosidase activity is inhibited by glucose, with the enzymes derived from yeast and fungi being more sensitive than those from plant sources. Although β-glucosidase activity tends to increase with ripening, there is no clear evidence yet of a direct relationship with the release of glycosylated odorants. The activities of glucosidases differ according to their origin, and the aglycone structures may also influence their activity. Plant and microbial β-glucosidases hydrolyse β-glucosides of primary and secondary alcohols, while fungal β-glucosidases prefer β-glucosides of tertiay alcohols (Pogorzelski and Wilkowska, 2007, Sarry and Günata, 2004).

β-Glucosidase assay was performed according a published method (Orruño et al., 2001), with modifications. Flesh of kiwifruit at the three ripening stages was chopped, frozen in liquid nitrogen and kept at -80 ºC until use. The crude enzymatic extract was prepared by homogenising 5 g of kiwifruit tissue with 5 mL of 0.1 M citrate-0.2 M phosphate buffer (pH 4.0), followed by centrifugation at 5000 rpm and 4 ºC for 20 min. The supernatant was filtered through muslin cloth and kept on ice.

For the enzymatic assay, 100 μL of crude extract was mixed with 500 μL of 40 mM *p*-nitrophenyl-β-D-glucopyranoside solution and 400 μL of 0.1 M citrate-0.2 M phosphate

buffer (pH 4.0), followed by incubation at 40 ºC for 30 min. Then, 2 mL of 3 M sodium carbonate solution was added to stop the reaction. The resulting mixture was transferred into a 96-well microplate and read at 405 nm against 0.1 M citrate-0.2 M phosphate buffer as a blank, using a SPECTRAmax PLUS 384 spectrophotometer (Molecular Devices Corporation, Sunnyvale, CA, USA). The protein content of the extracts was determined with a NanoDrop ND-1000 spectrophotometer (Wilmington, DE, USA). The specific enzyme activity was calculated using the following equation:

$$Specific\ enzyme\ activity\ \left(\frac{U}{mg\ protein}\right) = \frac{OD\ \times total\ V\ (mL)}{\varepsilon \times min \times protein\ (mg)}$$

Where OD is the absorbance at 405 nm, total V is the total reaction volume, ε is the millimolar extinction coefficient for *p*-nitrophenol (18.35) (Orruño et al., 2001), min is the incubation time, and protein is the amount (mg) of enzyme added. A unit is defined as the amount of enzyme liberating 1 μmol of *p*-nitrophenol/min.

Results (specific activities) were expressed in mU/mg, as means of triplicate experiments.

5.2.8 Statistical analysis

Analyses were conducted in triplicate for each ripening stage. The concentrations of the compounds from fruit at each ripening stage were averaged and analysed applying analysis of variance (ANOVA) using GenStat (12th ed., VSN International, U.K.). For mean comparisons, Tukey's Least Significant Difference procedure was performed. Data were considered to be statistically significant at $P < 0.05$.

5.3 Results and discussion

5.3.1 Bound volatiles in 'Hayward' kiwifruit

The aroma of eating-ripe 'Hayward' kiwifruit has been described as grassy and sulfurous, with a melon and sweet candy flavour (Wang et al., 2011). The most important components of the aroma have been identified as (*E*)-2-hexenal, 1-penten-3-one, hexanal, ethyl-2-methylpropanoate, dimethyl trisulfide, (*E*)-3-hexenol and 1-octen-3-one (Frank et al., 2007). Of these, (*E*)-2-hexenal and hexanal have been reported as being among the major components of the free volatile fraction (Young et al., 1983) and were also found in the bound volatile extracts.

In this study, a total of 95 compounds were detected in the bound volatile extracts of ripe fruit, while a slightly lower number of compounds were present in the extracts of under and over-ripe fruit. This represents a considerable increase over the number of compounds reported previously as bound volatiles in 'Hayward', which was only 29 (Young and Paterson, 1995). Alcohols, benzenoids and terpenoids were the most abundant (Fig. 5-1). (*Z*)-3-Hexen-1-ol, 2-phenylethanol, 3-oxo-α-ionol, benzyl alcohol, geraniol and 3-hydroxy-β-damascone showed the highest concentrations.

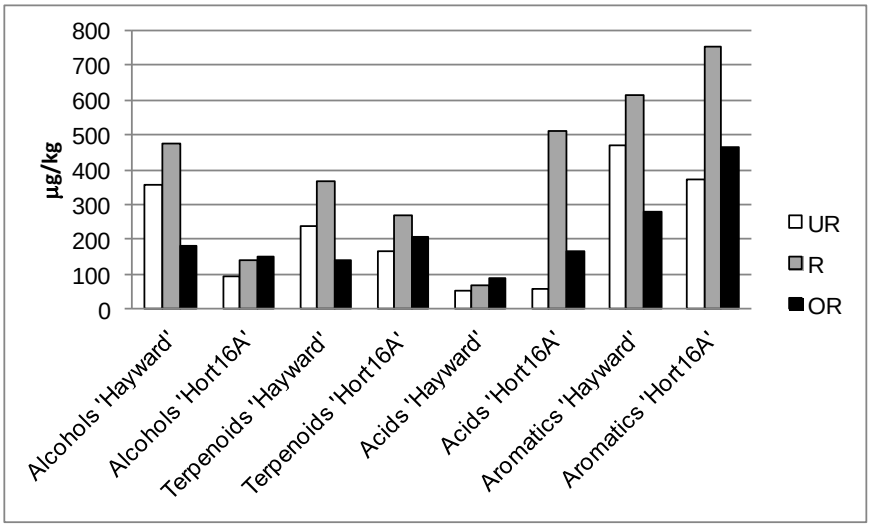

Fig. 5-1 Comparison of the bound volatile compositions of 'Hayward' and 'Hort16A' kiwifruit at different ripening stages (UR, under-ripe; R, ripe; OR, over-ripe).

In a previous report, the major components were (*E*)-2-hexenal and benzaldehyde (Young and Paterson, 1995). These authors reported several compounds not found in the present experiment, such as camphor, 4-methylbenzaldehyde, neral, geranial and iridomyrmecin. However, both the number of compounds and their concentrations were considerably higher in the present study, which may be due to the higher sensitivity of modern GC-MS equipment, or to differences in sample handling, or between years, orchards and storage conditions.

There were also some differences between the results of this analysis and those of the preliminary study (Table 2-2 and Fig 2-6), which may be explained by differences in the fruit (fruit stored for five months for the prelimnary study vs. fruit stored for less than a month for this analysis), the enzymes and the equipment used. This indicates that although good reproducibility was obtained in this analysis, changes in the source and handling of the fruit or in the laboratory conditions could produce different results. For example, 95 compounds were detected in ripe 'Hayward' in this analysis, while 62 compounds were detected in the preliminary study. 2,5-Dimethyl-4-hydroxy-3(2H)-furanone (DMHF) was the compound with the highest concentration in the solvent extract of bound volatiles of 'Hayward' in the preliminary study, while (*Z*)-3-hexen-1-ol was the compound with the highest concentration in the current analysis. Nevertheless, several major compounds (2-phenylethanol, benzyl alcohol, 3-hydroxy-β-damascone) were found in both this analysis and the preliminary study.

There were few compounds in the control samples (no enzyme added), mainly carboxylic acids including acetic, hexanoic, heptanoic, octanoic and octadecanoic acids. Small amounts of hexanal, (*E*)-2-hexenal, 4-vinylguaiacol, benzoic acid, DMHF and vanillin were also found. Their presence can be explained as the result of hydrolysis in the slightly acidic environment of the buffer (pH 5.0), because it is known that bound volatiles can be released

in such conditions (Williams et al., 1982b).

Alcohols included 2-butanol, (*Z*)-3-hexen-1-ol, isoamyl alcohol, 3-hexanol, hexanol, 3-octanol and 1-octen-3-ol among others (Table 5-1).

Green note compounds were an important component of the bound volatile profile of 'Hayward' kiwifruit. (*Z*)-3-Hexen-1-ol was the compound with the highest concentration. Other green note compounds found were hexanal, (*E*)-2-hexenal, hexanol and (*E*)-2-hexen-1-ol. Green note C6 compounds are among the most important odorants for 'Hayward' and their abundance in both the free and bound volatile portions indicates that lipoxygenase action on fatty acid precursors (linoleic and linolenic acid) is a vital part of the biosynthesis of kiwifruit aroma (Hatanaka, 1993).

Terpenoids were a major part of the bound volatile extracts (Table 5-2). Geraniol was the terpenoid with the highest concentration. Other terpenoids detected in the samples were prenol, linalool and its oxides, hotrienol, α-terpineol, isogeraniol, perilla alcohol and hydroxylinalool. Terpenoids arise from the mevalonic acid pathway or pyruvate/glyceraldehyde-3-phosphate pathway, and are commonly found as bound volatiles in many fruits, being particularly important for the varietal flavour of wines (Maicas and Mateo, 2005, Reineccius, 2006b).

The benzenoids with the highest concentrations, 2-phenylethanol and benzyl alcohol (Table 5-3), have sweet, floral aromas and are common in volatile extracts of flowers (Crowhurst et al., 2008).

Several norisoprenoids were identified, including 3-hydroxy-β-damascone, 3-oxo-α-ionol and 3-hydroxy-7,8-dihydro-β-ionol, among others (Table 5-2). Small amounts of β-damascenone, a powerful apple-like odorant with an odour threshold of only 0.002 μg/kg (http://leffingwell.com/odorthre.htm) were detected in ripe fruit.

DMHF was present at considerable levels (Table 5-4). This compound has been identified

as a key odorant for strawberry and pineapple, and is characterised by a strong caramel-like smell and a very low odour threshold (0.04 μg/kg) (Buechi et al., 1973, Siegmund and Leitner, 2010). DMHF has been reported as free and bound volatile in baby kiwifruit (*A. arguta*) (Yang et al., 2010, Garcia et al., 2011), but it has not been previously reported in 'Hayward' kiwifruit, either as free or bound volatile. It is surprising to find such considerable levels of glycosylated DMHF in 'Hayward' kiwifruit, as this fruit is known for its green, fresh, acidic flavour, in contrast with the sweet, candy-like baby kiwifruit, which indicates that the compound remains mainly in glycosylated form throughout ripening. Small amounts of the related compound 2,5-dimethyl-4-methoxy-3(2H)-furanone (DMMF) were also found in ripe and over-ripe fruit, but as with DMHF, this is the first report of their presence in 'Hayward' kiwifruit. DMMF also has a very low odour threshold (0.03 μg/kg) and a smell described as caramel, sweet, mildew (Siegmund and Leitner, 2010).

Novel compounds in 'Hayward' kiwifruit, tentatively identified by MS and RI, included hotrienol, 2-bornene, terpinyl formate, γ-hexalactone and massoia lactone.

The potential of glycosylated compounds to be not only a source of flavour compounds, but of off-odours, in particular in processed products where heat is involved, has been described in some reports. Young and Paterson (1995) hypothesised that the apple-like flavour of pasteurised kiwifruit juice is due to β-damascenone released by heating. On the other hand, the heat involved during the canning of pineapple releases glycosidically bound DMHF, giving it its characteristic flavour (Reineccius, 2006b). Interestingly, the possible presence of DMHF was described in a paper dealing with the effects of postharvest treatments on the sensory quality of kiwifruit, where the authors reported the high incidence of caramel/burnt sugar off-flavours in kiwifruit stored at 4 ºC for 4 weeks or 0 ºC for 12 weeks (MacRae et al., 1990). It is important to note that if this is the case, the glycosylated compound would be acting as a potential source of off-odours. Noticeably, DMHF was the

compound with the highest concentration in the solvent extract of the bound volatiles from 'Hayward' kiwifruit used for the preliminary study (Table 2-2 and Fig. 2-6), which had been stored at 0 ºC for five months.

5.3.2 Bound volatiles in 'Hort16A' kiwifruit

'Hort16A' has been described as having tropical, sweet, banana and blackcurrant flavours (Jaeger et al., 2003). The most important odorants have been identified as ethyl butanoate, acetaldehyde, hexanal, (*E*)-2-hexenal and dimethyl sulfide (Friel et al., 2007).

A total of 80 compounds were detected in the bound volatile extract of under-ripe 'Hort16A' kiwifruit, while 95 and 92 compounds were found in the bound volatile extracts of ripe and over-ripe fruit, respectively. Benzenoids and terpenoids were the most abundant compound classes, except in ripe fruit, where carboxylic acids were present in unusually high concentrations (Fig. 5-1).

Major compounds were 2-phenylethanol, benzyl alcohol, benzoic acid, (*Z*)-3-hexen-1-ol, and 3-oxo-α-ionol. In ripe fruit, linoleic and linolenic acid were also major compounds.

There were few compounds in control samples (no enzyme added), mainly carboxylic acids such as acetic, hexanoic, octanoic, nonanoic, stearic and linoleic acids. Small amounts of vanillin, 4-vinylguaiacol, (*E*)-2-hexenal and benzoic acid were also present. As mentioned above, they probably arise from hydrolysis in the slightly acidic conditions of the buffer.

Benzenoid compounds derive from phenylalanine and are synthesised via the shikimate pathway. 2-Phenylethanol and benzyl alcohol are floral compounds, and have been found as free and bound volatiles in several kiwifruit species (Crowhurst et al., 2008). Benzoic acid is an important precursor of benzoate esters (Moerkercke et al., 2009). All these compounds were found in the bound volatile extract of 'Hort16A', as well as in the other *Actinidia* fruit analysed.

Phenols and phenylpropanoids, which are also synthesised via the shikimate pathway, included vanillin, eugenol, 4-vinylguaiacol, (*E*)-isoeugenol, and coniferyl alcohol (Table 5-3). These compounds, except (*E*)-isoeugenol, were also found in the bound volatile extracts of all the other *Actinidia* species analysed.

A number of terpenoids were found in the bound volatile extracts of 'Hort16A', including linalool, geraniol, sesquicineole, α-terpineol, myrtenol, and cumin alcohol (Table 5-2). The levels of bound terpenoids in 'Hort16A' were slightly lower than in 'Hayward'.

Linoleic and linolenic acids are the precursors of C6 green note compounds, such as hexanal, (*Z*)-3-hexen-1-ol and (*E*)-2-hexen-1-ol, which were found as bound volatiles of 'Hort16A' kiwifruit. These compounds are synthesised via the lipoxygenase pathway, and involve the action of lipoxygenases, hydroperoxide lyases and alcohol dehydrogenases (Hatanaka, 1993). C6 green note compounds have been reported in the free volatile extracts of both 'Hayward' and 'Hort16A', but their levels are much higher in the former, reflecting its green aroma notes (Wang et al., 2011).

Apart from green note compounds, other alcohols found were 1-octen-3-ol, 1-octanol, 3-octanol, 2-butanol, 2-pentanol, butanol, and isoamyl alcohol (Table 5-1).

Norisoprenoids detected in the samples included 3-hydroxy-β-damascone, 3-oxo-α-ionol, and 3-hydroxy-7,8-dihydro-β-ionol. However, the latter could not be identified in ripe fruit samples (Table 5-2).

Novel compounds in 'Hort16A' kiwifruit (tentatively identified) included pinocarveol, sesquicineole, cumin alcohol, δ-terpineol, verbenol and syringaldehyde.

Table 5-1 Bound volatile compounds produced via lipid metabolism found in extracts of 'Hayward' and 'Hort16A' kiwifruit at three ripening stages (UR, under-ripe; R, ripe; OR, over-ripe). Amounts expressed in μg/kg.

Compound	CAS	RI [a]	'Hayward'			'Hort16A'			ID [b]
			UR	R	OR	UR	R	OR	
Alcohols									
2-Butanol	78-92-2	1043	17.6	29.7	12.2	3.8	4.1	3.7	MS, RI, Std
Isobutanol	78-83-1	1093	5.3	3.6	2.3	1.8	1.8	1.7	MS, RI, Std
3-Pentanol	584-02-1	1108	3.1	3.1	1.5	2.4	3.7	2.1	MS, RI, Std
2-Pentanol	6032-29-7	1119	7.5	7.4	3.7	5.8	9.1	5.3	MS, RI, Std
Butanol	71-36-3	1143	0.9	1.1	0.9	4.9	6.4	8.8	MS, RI, Std
1-Penten-3-ol	616-25-1	1159	0.9	1.1	0.4	-	-	0.3	MS, RI
3-Hexanol	623-37-0	1197	2.2	2.5	0.9	-	0.2	-	MS, RI
Isoamyl alcohol	123-51-3	1208	54.9	75.5	26.7	21.9	27.4	28.0	MS, RI, Std
Cyclopentanol	96-41-3	1302	0.5	0.7	-	-	-	-	MS, RI, Std
3-Methyl-1-pentanol	589-35-5	1328	-	0.4	-	-	-	-	MS, RI
3-Methylcyclopentanol	18729-48-1	1340	0.3	0.3	-	-	-	-	MS, RI
Hexanol	111-27-3	1354	14.0	20.8	8.4	7.4	11.6	24.5	MS, RI, Std
(*Z*)-3-Hexen-1-ol	928-96-1	1386	213.9	285.9	101.7	37.0	46.8	52.9	MS, RI, Std
3-Octanol	589-98-0	1395	6.9	8.9	2.8	0.3	0.5	0.3	MS, RI, Std
(*E*)-2-Hexen-1-ol	928-95-0	1408	12.0	12.0	12.3	2.1	-	3.5	MS, RI, Std
5-Methyl-1-hexanol	627-98-5	1417	-	-	-	-	0.5	0.4	MS
1-Octen-3-ol	3391-86-4	1453	4.9	7.3	2.0	3.6	5.6	4.3	MS, RI, Std
Heptanol	111-70-6	1457	0.5	0.8	0.3	-	-	0.2	MS, RI
6-Methyl-5-hepten-2-ol	1569-60-4	1465	1.0	1.1	0.4	0.3	0.4	0.4	MS, RI, Std
2-Ethylhexanol	104-76-7	1492	0.4	0.5	0.3	0.2	0.4	0.2	MS, RI, Std
1-Octanol	111-87-5	1559	-	0.4	-	0.2	0.3	0.3	MS, RI, Std
2,3-Butanediol	513-85-9	1580	-	-	-	-	-	0.5	MS, RI
Nonanol	28473-21-4	1662	-	-	-	-	0.2	0.2	MS, RI
3-Methyl-4-heptanol	1838-73-9	1706	0.8	1.3	0.3	-	-	-	MS, RI
1,2-Pentanediol	5343-92-0	1735	3.6	4.1	1.4	-	-	-	MS
4,5-Octanediol	22607-10-9	1748	2.1	3.6	0.8	0.5	0.8	0.6	MS
Decanol	112-30-1	1773	0.2	0.1	-	-	-	-	MS, RI
Dodecanol	112-53-8	1970	-	-	-	0.8	1.3	-	MS, RI
2-Nonenol	22104-79-6	2087	0.4	0.8	-	-	-	-	MS
1,3-Octanediol	23433-05-8	2134	2.6	3.6	0.9	-	18.1	13.5	MS
Carbonyl compounds									
Hexanal	66-25-1	1089	5.0	15.6	15.6	8.2	4.8	5.3	MS, RI, Std
(*E*)-2-Hexenal	6728-26-3	1221	4.5	3.6	12.4	0.7	-	-	MS, RI, Std

Table 5-1 (Continued)

4-Hydroxy-4-methylpentan-2-one	123-42-2	1363	1.3	2.0	1.7	0.6	1.0	0.9	MS, RI
4-Hydroxy-2-pentanone	4161-60-8	1460	-	-	0.2	-	0.4	-	MS
5-Hydroxy-4,6-dimethyl-6-hepten-3-one	62338-59-4	1488	0.7	0.9	0.3	-	0.3	0.3	MS
γ-Hexalactone	695-06-7	1703	0.2	-	-	-	-	-	MS, RI
Massoia lactone	54814-64-1	2683	6.0	-	-	-	-	-	MS
Acids									
Acetic acid	64-19-7	1468	-	-	4.8	10.6	12.1	11.2	MS, RI, Std
Isobutyric acid	79-31-2	1583	0.8	1.3	1.5	0.9	8.1	1.4	MS, RI
Butanoic acid	107-92-6	1645	1.7	3.2	1.5	-	0.7	1.6	MS, RI
Isovaleric acid	503-74-2	1683	-	-	1.3	0.5	9.7	2.0	MS, RI, Std
Pentanoic acid	109-52-4	1752	2.2	3.6	1.5	1.1	1.1	0.8	MS, RI
Hexanoic acid	142-62-1	1861	5.2	8.6	4.9	-	-	-	MS, RI, Std
Heptanoic acid	111-14-8	1966	-	-	3.7	1.4	3.0	2.5	MS, RI
(*E*)-2-Hexenoic acid	13419-69-7	1984	13.7	10.4	20.5	4.5	0.9	2.2	MS, RI
Octanoic acid	124-07-2	2072	4.1	6.3	3.1	3.2	4.9	4.5	MS, RI
Hexadecanoic acid	57-10-3	2915	16.2	20.4	18.4	8.0	59.2	22.5	MS, RI, Std
(*Z*)-9-Hexadecenoic acid	2091-29-4	2955	-	-	-	-	28.6	9.0	MS, RI
Octadecanoic acid	57-11-4	3162	9.1	13.9	10.8	3.9	18.4	8.8	MS, RI, Std
Linoleic acid	60-33-3	3303	-	-	4.6	7.4	199.3	45.5	MS, RI, Std
Linolenic acid	463-40-1	3444	-	-	11.6	13.6	162.8	51.1	MS, RI, Std

[a] RI, retention indices on Stabilwax column
[b] ID, identification method (Std = positive identification using authentic standard)

Table 5-2 Terpenoids and norisoprenoids found in the bound volatile extracts of 'Hayward' and 'Hort16A' kiwifruit at three ripening stages (UR, under-ripe; R, ripe; OR, over-ripe). Amounts expressed in μg/kg.

Compound	CAS	RI [a]	'Hayward'			'Hort16A'			ID [b]
			UR	R	OR	UR	R	OR	
Terpenoids									
Isoprenol	763-32-6	1250	5.5	7.4	2.6	5.3	7.7	6.3	MS, RI
Prenol	556-82-1	1323	16.6	23.2	7.6	18.4	28.3	23.2	MS, RI, Std
trans-Linalool oxide (furanoid)	5989-33-3	1441	1.5	1.9	0.9	0.2	0.5	0.4	MS, RI, Std
cis-Linalool oxide (furanoid)	34995-77-2	1470	6.0	7.7	2.9	-	-	-	MS, RI, Std
2-Bornene	464-17-5	1519	0.3	0.5	-	-	-	-	MS
Linalool	78-70-6	1551	0.7	0.6	0.7	1.4	0.6	1.2	MS, RI, Std
Dehydrolinalool	29957-43-5	1614	1.5	2.1	0.7	-	-	-	MS, RI
Pinocarveol	5947-36-4	1654	-	-	-	3.3	5.1	3.8	MS, RI
δ-Terpineol	7299-42-5	1672	-	-	-	0.4	0.3	0.3	MS, RI
Verbenol	473-67-6	1678	-	-	-	0.4	-	0.2	MS, RI
α-Terpineol	98-55-5	1697	1.5	2.0	0.8	7.7	12.5	10.2	MS, RI, Std
Verbenone	80-57-9	1702	-	-	-	0.8	-	-	MS, RI
cis-Linalool oxide (pyranoid)	41720-62-1	1739	1.3	1.7	0.9	-	-	-	MS, RI
trans-p-Menth-2-en-7-ol	19898-87-4	1755	-	-	-	3.6	6.0	4.9	MS
trans-Linalool oxide (pyranoid)	14049-11-7	1764	1.3	1.8	0.8	-	-	-	MS, RI
cis-p-Menth-2-en-7-ol	19898-86-3	1775	-	-	-	-	1.5	2.9	MS
Unknown terpenoid		1781	-	-	-	19.1	32.6	25.5	MS
Myrtenol	515-00-4	1792	-	-	-	22.0	39.9	21.0	MS, RI
Nerol	106-25-2	1801	1.8	2.1	0.6	-	-	-	MS, RI, Std
Isogeraniol	5944-20-7	1814	1.9	2.8	1.0	1.3	1.9	1.9	MS, RI, Std
Geraniol	106-24-1	1852	78.1	103.7	46.7	5.1	5.9	7.0	MS, RI, Std
Exo-2-hydroxycineole	66965-45-5	1861	-	-	-	8.8	12.5	9.8	MS, RI
Sesquicineole	90131-02-5	1865	-	-	-	26.4	45.2	34.9	MS
2,6-Dimethyl-3,7-octadiene-2,6-diol	13741-21-4	1951	14.4	17.1	9.3	0.7	1.1	0.9	MS, RI
6,7-Dihydro-7-hydroxylinalool	29210-77-3	1981	3.7	5.1	1.8	-	2.3	-	MS, RI
p-Menth-3-en-7-al	27841-22-1	1982	-	-	-	1.6	-	1.8	MS
p-Mentha-1,8-dien-7-ol	536-59-4	1992	12.8	18.7	4.9	2.6	3.1	2.9	MS, RI
p-Mentha-1(7),8(10)-dien-9-ol	29548-13-8	2036	3.8	5.3	2.2	1.0	0.9	1.0	MS
p-Mentha-1,4-dien-7-ol	22539-72-6	2059	-	-	-	9.8	15.9	12.6	MS, RI
4-(1-Methylethyl)-1,3-cyclohexadiene-1-methanol	1413-55-4	2095	-	-	-	1.4	2.5	1.7	MS
3-Caranol	4017-79-2	2219	0.9	1.5	-	-	-	-	MS

Table 5-2 (Continued)

(*Z*)-8-Hydroxylinalool	103619-06-3	2277	13.1	18.7	7.3	1.7	1.7	3.1	MS
(*E*)-8-Hydroxylinalool	64142-78-5	2315	16.6	24.5	8.1	17.5	31.8	20.8	MS, RI, Std
Epoxy-α-terpenyl acetate		2331	21.4	29.0	12.6	3.7	6.6	4.8	MS
Geranic acid	459-80-3	2352	5.3	2.7	1.1	-	-	-	MS, RI
Terpinyl formate	2153-26-6	2493	12.3	18.5	5.6	-	-	-	MS
(*Z*)-8-Hydroxygeraniol	26489-17-8	2603	-	42.3	13.4	-	-	4.5	MS
(*E*)-8-Hydroxygeraniol	26488-97-1	2637	18.1	25.3	7.2	-	4.5	-	MS
Norisoprenoids									
β-Damascenone	23726-93-4	1820	-	0.4	-	-	-	-	MS, RI, Std
1-(1a,2,3,5,6a,6b-Hexahydro-3,3,6a-trimethyloxireno [g]benzofuran-5-yl)-ethanone	80114-25-6	2451	4.2	6.9	2.3	-	7.8	4.4	MS
3-Hydroxy-β-damascone	102488-09-5	2550	64.3	94.5	22.6	17.0	36.2	19.8	MS, RI
3-Oxo-α-ionol	34318-21-3	2656	89.3	140.7	32.6	34.9	67.5	35.3	MS, RI
3-Hydroxy-7,8-dihydro-β-ionol	172705-13-4	2755	47.3	76.0	19.4	16.1	-	20.0	MS, RI
(3*E*)-4-(2-Hydroxy-2,6,6-trimethylcyclohexyl)-3-buten-2-one	55955-46-9	2812	21.6	26.1	14.1	11.0	15.7	11.6	MS

[a] RI, retention indices on Stabilwax column
[b] ID, identification method (Std = positive identification using authentic standard)

Table 5-3 Bound volatile compounds produced via aromatic acid metabolism found in extracts of 'Hayward' and 'Hort16A' kiwifruit at three ripening stages (UR, under-ripe; R, ripe; OR, over-ripe). Amounts expressed in μg/kg.

Compound	CAS	RI [a]	'Hayward'			'Hort16A'			ID [b]
			UR	R	OR	UR	R	OR	
Benzenoids									
Benzaldehyde	100-52-7	1526	1.5	1.7	0.8	0.6	1.2	0.8	MS, RI, Std
3-Ethylbenzaldehyde	34246-54-3	1709	tr.	0.6	0.3	-	0.4	-	MS
Benzyl alcohol	100-51-6	1881	79.1	111.7	45.5	52.8	97.9	77.7	MS, RI, Std
2-Phenylethanol	60-12-8	1915	199.6	236.5	121.5	162.5	291.0	191.2	MS, RI, Std
2,4-Dimethylphenethyl alcohol	6597-59-7	2023	-	-	-	2.3	1.1	1.3	MS
Cumin alcohol	536-60-7	2106	-	-	-	21.9	36.9	24.6	MS, RI, Std
o-Methoxybenzyl alcohol	612-16-8	2169	0.8	1.0	0.7	0.4	0.8	0.8	MS, RI
2,3-Dihydro-benzofuran	496-16-2	2409	16.0	32.3	11.1	2.3	6.9	13.5	MS, RI
Benzoic acid	65-85-0	2463	33.5	39.6	25.1	39.4	57.4	33.7	MS, RI, Std
Phenylacetic acid	103-82-2	2588	-	-	-	-	47.3	9.3	MS, RI
Indole-3-ethanol	526-55-6	2831	17.0	19.7	10.3	-	34.9	13.1	MS
Phenols and phenylpropanoids									
Methyl salicylate	119-36-8	1776	1.5	1.9	0.8	-	-	-	MS, RI, Std
Phenol	108-95-2	2015	5.0	8.0	0.9	2.6	2.5	1.6	MS, RI
Eugenol	97-53-0	2175	7.8	8.6	4.4	8.3	12.7	9.8	MS, RI, Std
4-Vinylguaiacol	7786-61-0	2206	8.0	17.0	7.9	2.7	5.3	4.4	MS, RI, Std
o-Acetyl-*p*-cresol	1470-52-2	2241	-	-	-	-	2.2	1.4	MS, RI
Cinnamyl alcohol	104-54-1	2295	1.3	0.8	-	-	-	-	MS, RI, Std
2-Allyl-4-methylphenol	6628-06-4	2302	-	-	-	-	4.1	2.7	MS
(*E*)-Isoeugenol	5932-68-3	2365	-	-	-	26.9	30.9	20.4	MS, RI, Std
1-(1a,2,3,5,6a,6b-Hexahydro-3,3,6a-trimethyloxireno [g]benzofuran-5-yl)-ethanone	80114-25-6	2451	4.2	6.9	2.3	-	7.8	4.4	MS
Vanillin	121-33-5	2594	11.6	19.9	7.7	13.7	46.2	23.8	MS, RI, Std
o-Hydroxyphenethyl alcohol	7768-28-7	2865	12.1	15.2	4.0	9.7	39.7	9.0	MS
4-Hydroxy-3,5-dimethoxy-benzaldehyde	134-96-3	2975	-	-	-	1.8	6.0	5.6	MS, RI
Homovanillic acid	306-08-1	3006	9.3	13.9	4.8	-	-	-	MS
p-*t*-Butylcatechol	98-29-3	3013	8.1	-	-	3.7	-	-	MS
p-Hydroxyphenethyl alcohol	501-94-0	3035	27.0	34.4	7.0	16.9	27.7	13.3	MS, RI, Std
3,5-Dimethoxy-4-hydroxycinnamaldehyde	87345-53-7	3082	-	-	-	6.6	-	-	MS
3-(4-Hydroxy-3-methoxyphenyl)-2-propenal	458-36-6	3239	-	-	18.8	-	-	-	MS
Coniferyl alcohol	458-35-5	3437	31.9	52.2	7.7	7.8	2.6	8.1	MS, RI, Std

[a] RI, retention indices on Stabilwax column
[b] ID, identification method (Std = positive identification using authentic standard)
tr., traces (below 0.1 μg/kg)

Table 5-4 Bound volatile compounds produced via carbohydrate metabolism (furans) found in extracts of 'Hayward' and 'Hort16A' kiwifruit at three ripening stages (UR, under-ripe; R, ripe; OR, over-ripe). Amounts expressed in μg/kg.

Compound	CAS	RI [a]	'Hayward'			'Hort16A'			ID [b]
			UR	R	OR	UR	R	OR	
2,5-Dimethyl-4-methoxy-3(2H)-furanone (DMMF)	4077-47-8	1594	-	0.3	1.0	-	-	-	MS, RI
2-Furanmethanol	98-00-0	1666	49.0	75.4	18.8	20.2	36.5	27.2	MS, RI, Std
5-Methyl-2-furanmethanol	3857-25-8	1727	0.5	1.8	-	-	-	-	MS, RI
5-Isopropyl-2,2-dimethyltetrahydrofuran	97265-00-4	2026	-	1.2	-	-	-	-	MS
2,5-Dimethyl-4-hydroxy-3(2H)-furanone (DMHF)	3658-77-3	2040	30.5	60.5	4.9	-	1.9	-	MS, RI, Std

[a] RI, retention indices on Stabilwax column
[b] ID, identification method (Std = positive identification using authentic standard)

Table 5-5 Miscellaneous compounds found in bound volatile extracts of 'Hayward' and 'Hort16A' kiwifruit at three ripening stages (UR, under-ripe; R, ripe; OR, over-ripe). Amounts expressed in μg/kg.

Compound	CAS	RI [a]	'Hayward'			'Hort16A'			ID [b]
			UR	R	OR	UR	R	OR	
Sulfur compounds									
2-(Methylthio)-ethanol	5271-38-5	1531	0.8	1.2	0.2	0.2	0.4	0.6	MS, RI, Std
3-(Methylthio)-1-propanol	505-10-2	1719	0.3	0.6	0.3	-	-	-	MS, RI, Std
Benzothiazole	95-16-9	1958	-	-	0.1	-	-	-	MS, RI
Alkanes									
Pentacosane	629-99-2	2498	-	-	-	-	59.2	-	MS, RI, Std
Heptacosane	593-49-7	2697	-	-	-	-	11.1	3.6	MS, RI, Std
Octacosane	630-02-4	2797	-	-	-	-	13.2	-	MS, RI, Std
Nonacosane	630-03-5	2898	-	-	-	-	11.2	-	MS, RI, Std
Triacontane	638-68-6	2997	-	-	-	-	10.0	-	MS, RI, Std
Others									
1-Acetyl-3-hydroxyadamantane	39917-38-9	2199	6.0	9.4	-	-	-	-	MS

[a] RI, retention indices on Stabilwax column
[b] ID, identification method (Std = positive identification using authentic standard)

5.3.3 Differences between the bound volatile profiles of 'Hayward' and 'Hort16A' kiwifruit

Of the total 138 compounds found in 'Hayward' and 'Hort16A' kiwifruit, 30 were present only in 'Hayward' and 32 only in 'Hort16A'. Some of the compounds found only in 'Hayward' kiwifruit included *trans*-linalool oxide, DMMF, hotrienol, methyl salicylate, nerol, 3-(methylthio)-1-propanol, (*E*)-cinnamyl alcohol and homovanillic acid. Compounds found exclusively in 'Hort16A' included pinocarveol, myrtenol, sesquicineole, cumin alcohol and (*E*)-isoeugenol.

The levels of bound alcohols in under-ripe and ripe 'Hayward' were more than twice those of 'Hort16A'. The concentrations of 2-butanol, isoamyl alcohol, (*Z*)-3-hexen-1-ol, 3-octanol and (*E*)-2-hexen-1-ol were considerably higher in the 'Hayward' cultivar. The content of norisoprenoids in ripe fruit was also around twice as high in 'Hayward' as in 'Hort16A'. 3-Hydroxy-β-damascone, 3-oxo-α-ionol and 3-hydroxy-7,8-dihydro-β-ionol had considerably higher concentrations in the 'Hayward' cultivar. The levels of terpenoids were similar in both cultivars, but their compositions differed.

The content of phenols and phenylpropanoids in under-ripe and ripe fruit of both cultivars were similar. However, 4-vinylguaiacol and coniferyl alcohol had considerably higher concentrations in the 'Hayward' cultivar. Furthermore, homovanillic acid and (*E*)-cinnamyl alcohol were only found in 'Hayward' samples. There were unusually high levels of linoleic, linolenic and hexadecanoic acids in ripe 'Hort16A' fruit, while the acid content in under-ripe and ripe fruit was similar in both cultivars. The levels of the main benzenoid compounds, benzyl alcohol and 2-phenylethanol, were slightly higher in 'Hayward' kiwifruit; but overall the content of benzenoids was slightly higher in 'Hort16A'. Furans in 'Hayward' were represented mainly by DMHF and furfuryl alcohol, while in 'Hort16A', DMHF was absent, except for a small amount in ripe fruit. The related compound DMMF was detected only in

samples of ‘Hayward’.

5.3.4 Changes in the bound volatile composition of ‘Hayward’ kiwifruit during ripening

All compound classes, except acids, experienced an increase in their concentrations at the ripe stage, and a subsequent decrease in the over-ripe stage. Changes were significant ($P < 0.05$) for most compound classes, except aldehydes and acids. The difference in benzenoid content at the ripe and over-ripe stages was significant ($P < 0.05$) (Table 5-6).

Individual compounds that experienced only a slight change during ripening include 1-octen-3-ol, linalool, (*E*)-2-hexen-1-ol, isobutanol and hexanal.

Changes in the bound volatile content are different to the reported changes in the free volatile content. Young and Paterson (1985) found that the levels of aroma compounds of kiwifruit increased significantly during a short ripening period, as confirmed by Bartley and Schwede and Wang et al. (Young and Paterson, 1985, Bartley and Schwede, 1989, Wang et al., 2011). According to Young and Paterson (1985) and Wan et al. (1999), the aroma of under-ripe kiwifruit had a green note, which changed to fruity with ripening; they attributed this change to the decrease of aldehydes responsible for the green aroma, and the increase of esters responsible for the fruity aroma (Young and Paterson, 1985, Wan et al., 1999). Similar results were obtained by Bartley and Schwede and Wang et al., who found that esters such as ethyl butanoate increased drastically, at the same time aldehydes like (*E*)-2-hexenal decreased (Bartley and Schwede, 1989, Wang et al., 2011).

5.3.5 Changes in the bound volatile composition of 'Hort16A' kiwifruit during ripening

As with 'Hayward', all compound classes, except alcohols, showed an increase in the ripe stage and a subsequent decrease in the over-ripe stage. For most compound classes, the difference in compound levels in ripe fruit compared to under-ripe or over-ripe fruit was significant ($P < 0.05$), but the difference in compound levels between under-ripe and over-ripe fruit was not ($P > 0.05$) (Table 5-6). This contrasts with the situation in 'Hayward', where compound levels in fruit at the three ripening stages showed significant differences ($P < 0.05$). Furthermore, the difference in alcohol and aldehyde levels between ripe and over-ripe fruit was not significant ($P > 0.05$).

The changes in both the reported free volatiles and the bound volatiles of 'Hort16A' are similar to those observed in 'Hayward', noticing that the bound volatile content decreases in over-ripe fruit, which is the opposite of what was reported for the free volatiles (Young and Paterson, 1985, Wang et al., 2011). As discussed previously, this may reflect changes in enzymatic activity, and may indicate that glycosidically bound compounds are being released at that stage.

Table 5-6 Levels (μg/kg) of bound volatile groups in 'Hayward' and 'Hort16A' kiwifruit at different ripening stages.

	Ripening stage			
'Hayward'	**UR**	**R**	**OR**	**P<0.05**
Total volatiles	1443.0 ± 168.8 b	2025.0 ± 66.8 c	827.0 ± 180.9 a	<0.001
Alcohols	356.2 ± 34.2 b	478.8 ± 14.9 c	179.8 ± 18.5 a	<0.001
Carbonyls	14.3 ± 4.1 a	11.6 ± 9.1 a	30.2 ± 10.9 a	0.075
Terpenoids	240.3 ± 26.8 b	363.7 ± 24.7 c	139.7 ± 34.0 a	<0.001
Norisoprenoids	226.7 ± 32.0 b	344.6 ± 16.2 c	91.0 ± 27.0 a	<0.001
Acids	53.0 ± 10.2 a	67.7 ± 9.2 a	88.2 ± 21.5 a	0.069
Aromatics	465.3 ± 54.0 b	608.0 ± 81.1 b	273.0 ± 85.0 a	0.004
Furans	80.0 ± 11.9 b	138.8 ± 8.5 c	24.3 ± 4.4 a	<0.001
'Hort16A'				
Total volatiles	795.0 ± 48.7 a	1939.0 ± 113.3 b	1110.0 ± 197.5 a	<0.001
Alcohols	93.5 ± 6.1 a	139.3 ± 8.8 b	151.4 ± 10.9 b	<0.001
Carbonyls	9.4 ± 2.3 a	6.6 ± 1.0 a	6.5 ± 1.6 a	0.136
Terpenoids	163.8 ± 12.6 a	270.8 ± 11.2 b	203.3 ± 30.1 a	0.002
Norisoprenoids	79.0 ± 6.9 a	127.2 ± 6.3 b	91.1 ± 20.2 a	0.009
Acids	52.6 ± 6.8 a	508.7 ± 173.7 b	162.2 ± 76.7 a	0.005
Aromatics	374.2 ± 21.6 a	743.8 ± 82.5 b	464.8 ± 60.3 a	<0.001
Furans	20.2 ± 0.3 a	37.1 ± 0.6 c	27.2 ± 3.1 b	<0.001

UR, under-ripe; R, ripe; OR, over-ripe. Values are mean ± standard deviation (n= 3). Means in the same row, followed by the same letter, are not significantly different ($P < 0.05$), as determined by Tukey's LSD test.

5.3.6 Results of GC-MS/O analysis

Eleven odour-active compounds were found in the bound volatile extract of ripe 'Hayward' kiwifruit, while 12 odour-active compounds were found in ripe 'Hort16A' (Table 5-7). The chromatograms showing the odour descriptors and intensities are shown in Fig. 5-2 and Fig. 5-3. Most odour-active compounds were found at moderate to low concentrations, with the notable exception of 2-phenylethanol, which was among the compounds with the highest concentrations and was also an important odorant for both cultivars.

Table 5-7 Aroma compounds detected by GC-MS/O in the bound volatile extracts of ripe *Actinidia deliciosa* 'Hayward' and *A. chinensis* 'Hort16A'.

Compound	CAS	RI [a]	Detection frequency [b]		Intensity [c]		Odour description
			'Hayward'	'Hort16A'	'Hayward'	'Hort16A'	
Sulcatol	1569-60-4	1466	5	-	weak	-	green, green beans
Isovaleric acid	503-74-2	1682	-	3	-	weak	sweat, rancid
Unknown		1694	2	3	weak	weak	plastic
β-Damascenone	23726-93-4	1826	5	5	middle	middle	apple juice, floral
2-Phenylethanol	60-12-8	1922	5	5	strong	strong	sweet, rose
DMHF	3658-77-3	2044	5	5	strong	middle	sweet, pineapple, caramel
Unknown		2120	2	-	weak	-	green
Eugenol	97-53-0	2183	5	5	middle	weak	spice, flower, sweet
4-Vinylguaiacol	7786-61-0	2215	5	3	weak	weak	spice, smoke
o-Acetyl-*p*-cresol	1470-52-2	2247	3	5	middle	weak	sweet, flower
Phenylacetic acid	103-82-2	2602	-	5	-	strong	sweet, honey
Vanillin	121-33-5	2609	5	5	strong	strong	vanilla
Syringaldehyde	134-96-3	2980	-	1	-	weak	berry, flower
Raspberry ketone	5471-51-2	3033	5	5	weak	middle	raspberry

[a] RI, retention indices on Stabilwax column
[b] Detection frequency in 5 GC-O runs
[c] Intensities are described relative to the compound perceived with the strongest smell, namely, DMHF for 'Hayward', and vanillin for 'Hort16A'

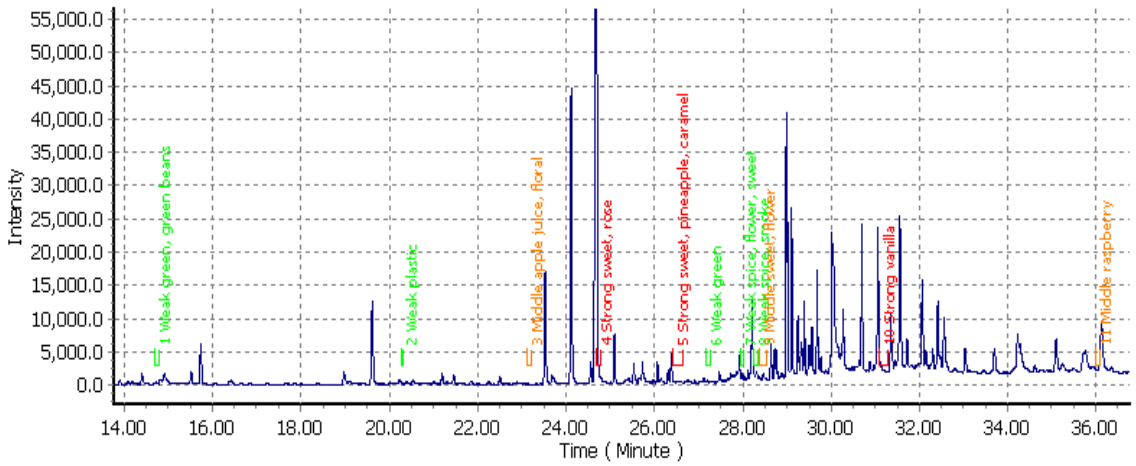

Fig. 5-2 Chromatogram of bound volatiles from ripe 'Hayward' kiwifruit showing the aromas detected and their intensities.

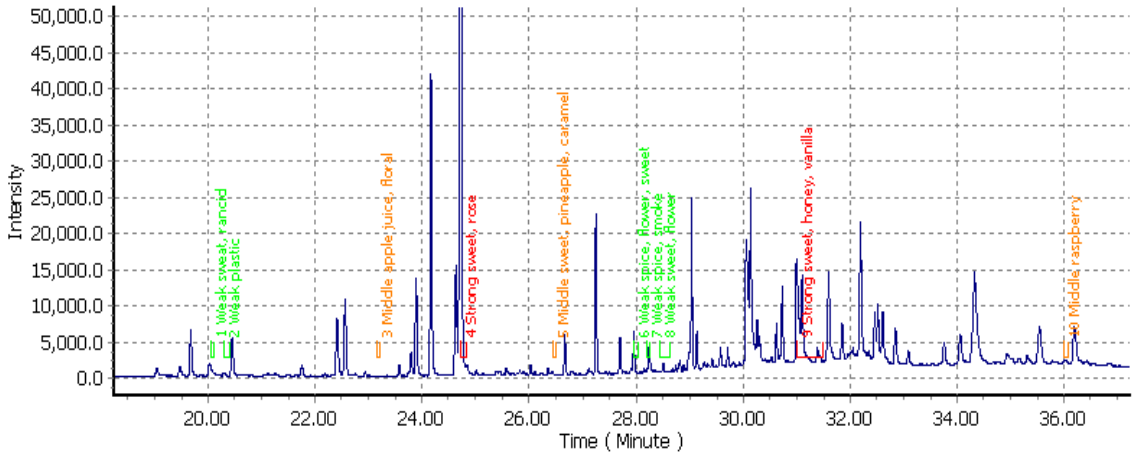

Fig. 5-3 Chromatogram of bound volatiles from ripe 'Hort16A' kiwifruit showing the aromas detected and their intensities.

In the bound volatile extract from 'Hayward', DMHF was the odorant perceived with the strongest intensity, and which lasted longer, the intensities of all other odour-active compounds were judged relative to this compound. DMHF had a caramel or pineapple-like smell. Vanillin and 2-phenylethanol were also perceived with strong intensities. β-Damascenone, eugenol, and *o*-acetyl-*p*-cresol, which had sweet and floral-type odours, were perceived with intermediate intensities. Among these compounds, β-damascenone contributed significantly to the aroma, although only traces of it were present. Other compounds of interest were sulcatol (6-methyl-5-hepten-2-ol) and raspberry ketone (4-(4-hydroxyphenyl)-2-butanone). Sulcatol had an aroma resembling green beans, while raspberry ketone, of which only traces were present and no GC-MS peak was observed, smelled of raspberries. Raspberry ketone was identified by comparison of odour and RI with an authentic standard. DMHF has not been identified in the free volatile portion of 'Hayward' kiwifruit, suggesting that it remains in bound form. This is the first report of sulcatol and *o*-acetyl-*p*-cresol as odorants in 'Hayward' kiwifruit.

In the bound volatile extract from 'Hort16A', three sweet-smelling compounds were perceived with strong intensities, namely, 2-phenylethanol, phenylacetic acid and vanillin.

Vanillin had the strongest odour intensity and the longest lasting smell; all other odour-active compounds were judged relative to it. Vanillin had the characteristic aroma of vanilla, while phenylacetic acid had a sweet smell similar to honey. 2-Phenylethanol had a sweet, rose-like aroma. β-Damascenone, eugenol and *o*-acetyl-*p*-cresol were also contributors to the aroma of the bound volatile extract from 'Hort16A'. In contrast to 'Hayward', no green-smelling compounds were contributors to the aroma of the bound extract from 'Hort16A'. On the other hand, the sweat-smelling isovaleric acid (3-methylbutanoic acid) was only found in 'Hort16A'. This is the first report of syringaldehyde (4-hydroxy-3,5-dimethoxy-benzaldehyde) as an odorant in 'Hort16A'.

For both 'Hayward' and 'Hort16A', the odour-active compounds found in the bound volatile extracts were different from those reported as contributors to the aroma of these fruits (Frank et al., 2007, Friel et al., 2007). This suggests that, for 'Hayward' and 'Hort16A', bound volatiles are not among the most significant contributors to the aroma. However, the presence of compounds such as DMHF, β-damascenone and isovaleric acid suggests that bound volatiles could be a source of off-odours for these kiwifruit cultivars.

5.3.7 Effect of ripening on β-glucosidase activity

Total free volatile levels are at their highest in over-ripe kiwifruit, which is mainly due to the increase in the content of esters (Wang et al., 2011). The opposite occured with bound volatiles, as over-ripe kiwifruit had a lower bound volatile content. The decrease in bound volatile content after the fruit reached the ripe stage may indicate that glycosylated compounds were hydrolysed and contributed to aroma production, and may also reflect the possibly increased activity of glycosidases or reduced activity of glycosyl transferases (Maicas and Mateo, 2005, Sarry and Günata, 2004).

Total β-glucosidase activity was measured during fruit ripening for both 'Hayward' and

'Hort16A', using *p*-nitrophenyl-β-D-glucopyranoside as the substrate.

The β-glucosidase specific activity of 'Hayward' was slightly higher than that of 'Hort16A' (Fig. 5-4). However, for both cultivars, the β-glucosidase activity did not undergo major changes during ripening. For 'Hort16A', β-glucosidase activity was highest in under-ripe fruit, and then decreased slightly in ripe and over-ripe fruit, while for 'Hayward', β-glucosidase activity kept fairly constant throughout ripening and only experienced a slight increase in the ripe stage. These results suggest that neither the β-glucosidase activity nor the content of glycosides is a limiting factor for flavour development in kiwifruit, although it is possible that other glycosidases and glycosyl transferases (which are responsible for glycoside formation) have an effect on the volatile content or that there is competition between these enzymes.

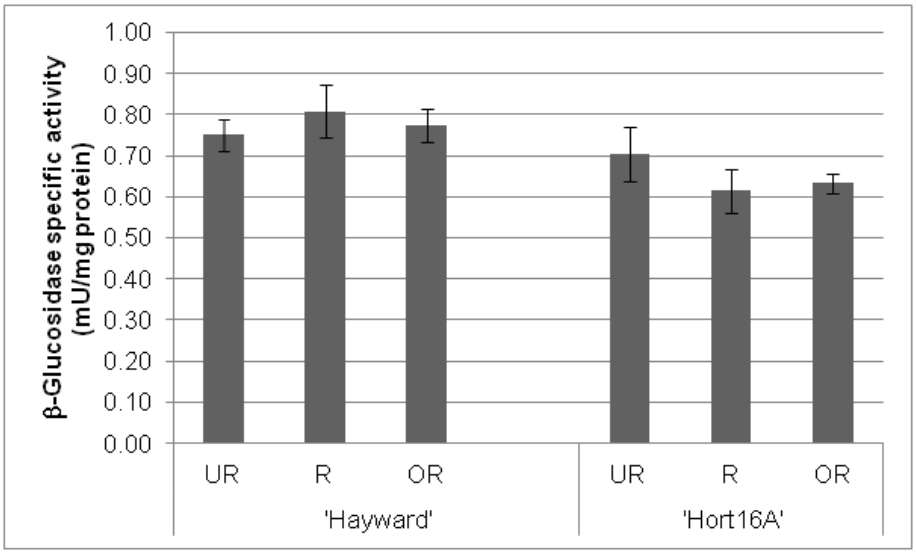

Fig. 5-4 β-Glucosidase specific activity (mU/mg protein) in 'Hayward' and 'Hort16A' kiwifruit at different ripening stages (UR, under-ripe; R, ripe; OR, over-ripe), expressed as mean values of three replicates with standard deviation bars.

5.4 Conclusion

The results obtained in this chapter provide insight into the changes occurring in two kiwifruit cultivars during ripening, and help to complement the knowledge on kiwifruit ripening and aroma production. It is known that kiwifruit ripening involves the increase in ethylene production, the softening of the flesh and the production of sugars and volatiles. It is also known that those volatiles tend to increase during ripening, in particular esters responsible for fruity notes, while green-smelling aldehydes tend to decrease. It has been observed that bound volatiles do not follow that trend, and instead tend to increase at the ripe stage, while decreasing in over-ripe fruit. In contrast with *A. arguta* and *A. eriantha*, terpenoids were a major component of the bound volatiles of 'Hayward' and 'Hort16A'. For both 'Hayward' and 'Hort16A', the odour-active compounds found in the bound volatile extracts were different from those reported as major contributors to the aroma of these fruits. This suggests that for these two kiwifruit cultivars, bound volatiles are probably not the most significant contributors to the aroma, but could be a possible source of off-odours.

Chapter 6

General discussion and conclusion

6.1 General discussion

Kiwifruit is an important crop for New Zealand, being its second major horticultural export. The 'Hayward' cultivar is the most common in the global market.

Kiwifruit flavour is the result of the presence of various volatile and non-volatile compounds. The free volatile portion of kiwifruit has been studied, with most reports having focused on the 'Hayward' cultivar. However, in fruits, aroma compounds are not only found in their free form, but also as glycosidically bound volatiles. The bound volatile composition of four kiwifruit species was studied: *Actinidia deliciosa* 'Hayward' (Green), *A. chinensis* 'Hort16A' (Gold), *A. arguta* (baby kiwifruit), and *A. eriantha*. The present study shows that glycoconjugates constitute a major portion of the aroma compounds of kiwifruit. As observed, the odorous aglycones may be released from the sugar moiety during ripening, or by enzymatic and acid treatments. Analysis of the liberated aglycones can show their potential contribution to the aroma.

This study provides new knowledge on kiwifruit aroma, by focusing on the bound volatile portion of the cultivars mentioned above. The information obtained complements the information already available on the free volatile portion, and provides a more inclusive picture of kiwifruit aroma, as in the past the bound volatile fraction of kiwifruit had been ignored, except for one publication more than 15 years old. In addition, the publication mentioned (Young and Paterson, 1995) reported only a small number of bound volatiles, and the predominant compounds identified were aldehydes (benzaldehyde and (*E*)-2-hexenal), which is unexpected considering that aldehydes cannot be directly bound to sugars. The composition and low concentration of the bound volatiles reported suggested that the low sensitivity of the equipment available at that time, as well as the handling of the sample, affected the results. This, coupled to the fact that the bound volatile composition of other fruits was being studied and publications were being produced, encouraged a deeper and

more careful investigation of the bound volatiles of kiwifruit.

Thus, the bound volatile composition of the three most important commercial kiwifruit types (Green, Gold, baby kiwifruit) was elucidated. A non-commercial, low flavour kiwifruit species (*A. eriantha*) was also analysed, producing surprising results.

To conduct this study, a preliminary analysis of the bound volatiles was performed to determine the best way to hydrolyse the glycosides and extract the bound volatiles. It was found that the use of the commercial enzyme preparation Rapidase AR2000 followed by solvent extraction produced good results, and it was thus selected as the methodology for this study. Solvent extraction has been previously used by many researchers to extract the released aglycones, because some important compounds (e.g.: DMHF, norisoprenoids) are difficult to extract with other techniques, and a more accurate quantification can be achieved with solvent extracts.

It has been reported that esters, aldehydes and alcohols are among the major contributors to the aroma of all the kiwifruit species analysed. Differences in the aroma of the fruits are thus due to variations in the concentrations of the compounds and to the presence of particular odorants. On the other hand, benzenoids, alcohols and terpenoids were the predominant compound classes in the bound volatile extracts; several norisoprenoids commonly found as bound volatiles, such as 3-hydroxylated and 3-oxo derivatives, were also detected. Shikimic acid derivatives predominated in the bound volatile extracts of all the kiwifruit species analysed.

A. arguta is an interesting case, because DMHF (2,5-dimethyl-4-hydroxy-3(2H)-furanone) has been reported as being one of the most important odorants in the free volatile fraction, and it was the compound with the highest concentration and strongest odour (determined by GC-MS/O) in the bound volatile fraction, suggesting that the glycoside may play a role as a potential source of aroma. However, it appears that most of this compound

remains in glycosylated form, as it has not been possible to determine its concentration in the free fraction, and the reported concentration of the related compound DMMF in the free fraction was almost 20 times lower than the concentration of glycosylated DMHF detected in this study. Because of the very low odour threshold of DMHF (0.04 μg/kg), even a low concentration would have an effect on the aroma of the fruit. DMHF-β-D-glucopyranoside was identified by LC-MS as the glycosidic precursor of DMHF, which agrees with previous studies done on pineapple and strawberry. For these fruits, the glycoside has been confirmed as a source of free DMHF, in particular in processed products. Analysis of the sugar portion of the hydrolysate from *A. arguta* revealed that the predominant sugar moiety was glucose.

The analysis of *A. eriantha* produced the most interesting and unexpected results of this study. This non-commercial kiwifruit is known for its bland, grassy flavour, which is a reflection of its low volatile content. The free volatile fraction of this fruit contains mainly butyl acetate and green note alcohols. Notwithstanding its poor flavour, *A. eriantha* is of interest to kiwifruit breeders because the fruit can be easily peeled by hand, so it could be used to generate new kiwifruit cultivars with convenient attributes. The bound volatile fraction of *A. eriantha* contained a number of compounds with green, floral and sweet notes, including several odorants such as 2-phenylethanol, sulcatol, (*E*)-isoeugenol, linalool and vanillin, among others. The presence of such a diversity of compounds in bound form is intriguing as the aroma of the fruit does not hint at their presence, suggesting that these compounds remain in glycosylated form during ripening. It is possible that a lack of gene expression or enzymatic activity is the reason these compounds remain as glycosides, although further research would be needed to clarify this point.

The free volatile composition of ‘Hayward’ and ‘Hort16A’ is qualitatively similar; however, ‘Hayward’ is richer in C6 aldehydes, responsible for its green aroma, while ‘Hort16A’ contains sulfur compounds responsible for its tropical notes. In a similar fashion,

many bound volatiles were found in both ‘Hayward’ and ‘Hort16A’, but at different concentrations, and the hydrolysates also had different smells. For ‘Hayward’, DMHF was an important component of the bound volatile extract, giving it a sweet, caramel note. Kiwifruit stored for long periods or at relatively high temperatures has been reported to have caramel-like off-flavour, suggesting the presence of DMHF in such fruit. It should be noted that DMHF has not been previously identified in ‘Hayward’. For ‘Hort16A’, sweet-smelling compounds including vanillin, phenylacetic acid and 2-phenylethanol were the strongest odorants in the hydrolysate. Glycosides of compounds with green notes were present in considerable amounts in both cultivars, but particularly in ‘Hayward’. (Z)-3-Hexen-1-ol was a major green note bound volatile in both ‘Hayward’ and *A. eriantha*. In contrast with *A. arguta* and *A. eriantha*, terpenoids were a major component of the bound volatile extracts of ‘Hayward’ and ‘Hort16A’. It is known that free volatiles tend to increase during ripening, in particular esters responsible for fruity notes, while green-smelling aldehydes tend to decrease. It has been observed that bound volatiles do not follow that trend, and instead tend to increase at the ripe stage, while decreasing in over-ripe fruit. Because the compounds identified as odour-active in the bound volatile extract are different from those having been reported as important odorants for these fruits, it is likely that bound volatiles are not among the major contributors to the aroma of either ‘Hayward’ or ‘Hort16A’. The ripening behaviour of the bound volatiles and the results of GC-MS/O analysis hint at their possible role as precursors of off-odours, and may reflect changes in the action of enzymes, in particular glycosidases and glycosyl transferases. However, it is noteworthy that β-glucosidase activity in both cultivars was fairly low and did not experience major changes during ripening.

GC-MS/O allowed the identification of odour-active compounds in the bound volatile extracts. It is notable that several odorants appeared as small peaks or did not appear in the chromatograms, indicating that the size of the GC peak does not necessarily correlate with

the contribution of a compound to the aroma. Hence, compounds with low odour thresholds can be important contributors to the aroma, even if present at low concentrations, as observed in this study. GC-MS/O, apart from being useful for the identification of the important odorants for each cultivar, revealed that some odorous compounds occur commonly as bound volatiles in kiwifruit. β-Damascenone, 2-phenylethanol, eugenol, 4-vinylguaiacol, vanillin, and raspberry ketone were found in all four kiwifruit species analysed. The presence of raspberry ketone is particularly interesting, because this compound is the key aroma compound of raspberries, and its release from glycosidic precursors could be of significance for the generation of novel kiwifruit cultivars with berry-like flavours.

6.2 Concluding remarks

6.2.1 Analysis methodology of bound volatiles

In this study, the release of kiwifruit bound volatiles by acid and enzymatic hydrolysis was investigated. In addition, an experiment comparing the volatile profiles of solvent extract vs. SPME was conducted.

The volatiles released by treatment with 1 M HCl (pH 0) included compounds not found in hydrolysates obtained at higher pH, or by enzymatic action, and their presence suggests that chemical changes in the released volatiles occur in strong acidic conditions. Hydrolysis of the glycosidic isolates with almond β-glucosidase and Rapidase AR2000 produced an aroma more resembling of the fruits. Rapidase AR2000 was chosen for the experiments, as this commercial preparation contains several glycosidase activities apart from β-glucosidase, which could be advantageous for the hydrolysis of diglycosides.

The effect of the extraction technique was evaluated by enzymatically hydrolysing the glycoconjugates and extracting the released volatiles with solvent (pentane:ether) and HS-SPME. More volatile compounds, in particular terpenoids and alcohols, were detected in the

SPME sample. However, important bound volatiles such as β-damascenone, 3-hydroxy-β-damascone, 3-oxo-α-ionol and vanillin were only found in the solvent extract. In addition, important odorants such as DMHF are known to be difficult to extract using SPME. Therefore, solvent extraction was chosen for the experiments.

6.2.2 Bound volatiles and glycosides of *Actinidia arguta*

The aroma of *A. arguta* has been described as banana, floral, fruit candy, grassy, green, melon and tropical. The odour-active compounds of *A. arguta* have been reported as ethyl butanoate, methyl benzoate, DMHF, 1-octen-3-one, (*E*)-2-hexenal, pentanal, and hexanal.

DMHF, benzyl alcohol, 3-hydroxy-β-damascone, hexanal, and (*Z*)-3-hexen-1-ol were the major compounds in the bound volatile extract from *A. arguta*.

GC-MS/O analysis revealed 11 odour-active compounds in the *A. arguta* hydrolysate. Sweet odorants predominated. DMHF was not only the compound with the highest concentration, but also the compound perceived with the strongest odour intensity. Vanillin (co-eluted with phenylacetic acid), 2-phenylethanol and raspberry ketone were also identified as important components for the aroma of the bound extract.

To elucidate the structure of the DMHF glycoside, the corresponding fraction was purified and analysed by LC-MS. On the basis of the mass spectra obtained, the glycoside was identified as DMHF-β-D-glucopyranoside.

Analysis of the sugar moieties after acid hydrolysis revealed that glucose was the most abundant sugar, with low amounts of arabinose, xylose and galactose also having been found.

6.2.3 Bound volatiles of *Actinidia eriantha*

Actinidia eriantha is a kiwifruit species which produces fruit with a bland flavour, but that can be easily peeled, and thus, has the genetic potential to generate new kiwifruit

cultivars with convenient attributes. The free volatile content of *A. eriantha* is lower than in other kiwifruit species, with butyl acetate being the major compound. The volatile fraction of this fruit also contains several C6 alcohols, known for their green notes, including (*E*)-2-hexenol, (*Z*)-3-hexenol and hexanol.

Compounds found with high concentrations in the bound volatile extract of *A. eriantha* included important aroma precursors such as linoleic and linolenic acids, as well as terpenoids and phenylpropanoids. Major compounds were 2-phenylethanol, furfuryl alcohol, (*Z*)-3-hexen-1-ol, coniferyl alcohol, isoamyl alcohol and linolenic acid. GC-O analysis revealed 15 odour-active compounds in the *A. eriantha* hydrolysate, including 2-phenylethanol, (*E*)-isoeugenol and vanillin, which were also among the compounds with the highest concentrations. The presence of several important odorants in *A. eriantha*, which is known for its bland flavour and low volatile content, was unexpected and suggests that there is a pool of potential odorants that remain as glycosides within the fruit cells, and are not released during ripening.

6.2.4 Changes in the bound volatiles of *Actinidia deliciosa* 'Hayward' and *A. chinensis* 'Hort16A' during ripening

The bound volatiles of 'Hayward' and 'Hort16A' kiwifruit at three ripening stages (under-ripe, ripe and over-ripe) were analysed.

The aroma of eating-ripe 'Hayward' kiwifruit has been described as grassy and sulfurous, with a melon and sweet candy flavour. The most important components of the aroma have been reported as (*E*)-2-hexenal, 1-penten-3-one, hexanal, ethyl-2-methylpropanoate, dimethyl trisulfide, (*E*)-3-hexenol and 1-octen-3-one. Among the bound volatiles of 'Hayward', (*Z*)-3-hexen-1-ol, 2-phenylethanol, 3-oxo-α-ionol, benzyl alcohol, geraniol and 3-hydroxy-β-damascone showed the highest concentrations. DMHF, vanillin and 2-phenylethanol were

identified as the strongest odorants among the 11 odour-active compounds detected by GC-O in the hydrolysate.

'Hort16A' has been described as having tropical, sweet, banana and blackcurrant flavours. The most important odorants have been reported as ethyl butanoate, acetaldehyde, hexanal, (*E*)-2-hexenal and dimethyl sulfide. Major compounds in the bound volatile extract of 'Hort16A'included 2-phenylethanol, benzyl alcohol, benzoic acid, (*Z*)-3-hexen-1-ol, and 3-oxo-α-ionol. 2-Phenylethanol, phenylacetic acid and vanillin were identified as the strongest odorants among the 12 odour-active compounds detected by GC-O in the hydrolysate.

There were differences between the bound volatile compositions of 'Hayward' and 'Hort16A'. The levels of alcohols and norisoprenoids were considerably higher in 'Hayward' kiwifruit. The bound volatile content of both kiwifruit cultivars changed with ripening. In general, compounds experienced an increase in their concentrations at the ripe stage, and a subsequent decrease in the over-ripe stage.

The β-glucosidase specific activity in 'Hayward' and 'Hort16A' did not experience considerable changes during ripening.

6.3 Future prospects

The results of this study complement the information about the volatile compositions of the commercially important kiwifruit cultivars, as well as a kiwifruit species with low flavour but with potential use in the breeding of new cultivars. The bound volatile compositions were elucidated, the changes in the bound volatile compositions and β-glucosidase activity during ripening were investigated, and the structural characteristics of the glycosides were explored. However, new questions arose, opening up potential areas of future research, including:

- Study of the balance between free and bound volatiles, in particular concerning their

relationship *in planta*. Questions about the circumstances under which bound volatiles become free and free volatiles become bound remain to be answered.

- Elucidation of the role of glycosidases and glycosyl transferases in the balance between free and bound volatiles, and aroma production in kiwifruit.
- Further study of the role of the DMHF glycoside as a source of aroma for *A. arguta*.
- Study of the ethylene generation in *A. eriantha*, and comparison with its volatile production, to help explain the poor aroma of this fruit.
- Experiments involving the feeding of labelled precursors to clarify the biosynthetic pathways of aroma compounds and the role of glycosides in *A. eriantha*.
- Elucidation of the role of DMHF as off-odour in 'Hayward' kiwifruit stored for long periods or at relatively high temperatures.
- Study of the free and bound volatiles of novel kiwifruit cultivars (e.g.: Gold3) and promising kiwifruit lines.
- Application of the methodology for the study of bound volatiles to other fruits, such as tamarillo and feijoa.

REFERENCES

ACREE, T. E. 1993. Bioassays for flavor. *In:* ACREE, T. E. & TERANISHI, R. (eds.) *Flavor science: sensible principles and techniques.* Washington D.C.: American Chemical Society.

ANANTHAKUMAR, A., VARIYAR, P. S. & SHARMA, A. 2006. Estimation of aroma glycosides of nutmeg and their changes during radiation processing. *Journal of Chromatography A,* 1108**,** 252-257.

ATKINSON, R. G., SHARMA, N. N., HALLET, I. C., JOHNSTON, S. L. & SCHRÖDER, R. 2009. *Actinidia eriantha*: A parental species for breeding kiwifruit with novel peelability and health attributes. *New Zealand Journal of Forestry Science,* 39**,** 207-216.

AUBERT, C., AMBID, C., BAUMES, R. & GÜNATA, Z. 2003. Investigation of bound aroma constituents of yellow-fleshed nectarines (*Prunus persica* L. Cv. Springbright). Changes in bound aroma profile during maturation. *Journal of Agricultural and Food Chemistry,* 51**,** 6280-6286.

BALL, R. D., MURRAY, S. H., YOUNG, H. & GILBERT, J. M. 1998. Statistical analysis relating analytical and consumer panel assessments of kiwifruit flavour compounds in a model juice base. *Food Quality and Preference,* 9**,** 255-266.

BARTLEY, J. P. & SCHWEDE, A. M. 1989. Production of volatile compounds in ripening kiwi fruit (*Actinidia chinensis*). *Journal of Agricultural and Food Chemistry,* 37**,** 1023-1025.

BEEVER, D. J. & HOPKIRK, G. 1990. Fruit development and fruit physiology. *In:* WARRINGTON, I. & WESTON, G. C. (eds.) *Kiwifruit: science and management.* Auckland: Ray Richards Publisher.

BELROSE INC. 2011. *World kiwifruit review*. Pullman, WA, USA: Belrose, Inc.

BEMELMANS, J. M. H. 1981. Isolation and concentration from the product phase. *In:* MAARSE, H. & BELZ, R. (eds.) *Isolation, separation and identification of volatile compounds in aroma research.* Berlin: Akademie-Verlag.

BITTEUR, S., GÜNATA, Z., BRILLOUET, J. M., BAYONOVE, C. & CORDONNIER, R. 1989. GC and HPLC of grape monoterpenyl glycosides. *Journal of the Science of Food and Agriculture,* 47**,** 341-352.

BLOM, H. 1983. Partial characterization of a thermostable anthocyanin-β-glycosidase from *Aspergillus niger*. *Food Chemistry,* 12**,** 197-204.

BUECHI, G., DEMOLE, E. & THOMAS, A. F. 1973. Syntheses of 2,5-dimethyl-4-hydroxy-2,3-dihydrofuran-3-one (furaneol), a flavor principle of pineapple and strawberry. *The Journal of Organic Chemistry,* 38**,** 123-125.

BURDON, J., LALLU, N., BILLING, D., BURMEISTER, D., YEARSLEY, C., WANG, M., GUNSON, A. & YOUNG, H. 2005. Carbon dioxide scrubbing systems alter the ripe fruit volatile profiles in controlled-atmosphere stored `Hayward' kiwifruit. *Postharvest Biology and Technology,* 35**,** 133-141.

BUREAU, S., RAZUNGLES, A., BAUMES, R. & BAYONOVE, C. 1996. Glycosylated flavor precursor extraction by microwaves from grape juice and grapes. *Journal of Food Science,* 61**,** 557-560.

BUTTERY, R. G. 1981. Vegetable and fruit flavors. *In:* TERANISHI, R., FLATH, R. A. & SUGISAWA, H. (eds.) *Flavor research: recent advances.* New York: Marcel Dekker.

BUTTERY, R. G. 1993. Quantitative and sensory aspects of flavor of tomato and other vegetables and fruits. *In:* ACREE, T. E. & TERANISHI, R. (eds.) *Flavor science: sensible principles and techniques.* Washington D.C.: American Chemical Society.

BUTTERY, R. G., TAKEOKA, G., TERANISHI, R. & LING, L. C. 1990. Tomato aroma components: identification of glycoside hydrolysis volatiles. *Journal of Agricultural and Food Chemistry,* 38**,** 2050-2053.

BUTTERY, R. G., TERANISHI, R. & LING, L. C. 1987. Fresh tomato aroma volatiles: a quantitative study. *Journal of Agricultural and Food Chemistry,* 35**,** 540-544.

CHAINTREAU, A. 2001. Simultaneous distillation-extraction: from birth to maturity - A review. *Flavour and Fragrance Journal,* 16**,** 136-148.

CHASSAGNE, D., BOULANGER, R. & CROUZET, J. 1999. Enzymatic hydrolysis of edible *Passiflora* fruit glycosides. *Food Chemistry,* 66**,** 281-288.

CHEN, K., XU, C., LOU, J., ZHANG, S. & ROSS, G. S. 1999. Lipoxygenase in relation to the ripening and softening of *Actinidia* fruit. *Zhi wu sheng li xue bao = Acta phytophysiologica Sinica / Zhongguo zhi wu sheng li xue hui zhu bian,* 25**,** 138-144.

CRAIG, J. T. 1988. A comparison of the headspace volatiles of kiwifruit wine with those of wine of *Vitis vinifera* variety Müller-Thurgau. *American Journal of Enology and Viticulture,* 39**,** 321-324.

CROWHURST, R., GLEAVE, A., MACRAE, E., AMPOMAH-DWAMENA, C., ATKINSON, R., BEUNING, L., BULLEY, S., CHAGNE, D., MARSH, K., MATICH, A., MONTEFIORI, M., NEWCOMB, R., SCHAFFER, R., USADEL, B., ALLAN, A., BOLDINGH, H., BOWEN, J., DAVY, M., ECKLOFF, R., FERGUSON, A. R., FRASER, L., GERA, E., HELLENS, R., JANSSEN, B., KLAGES, K., LO, K., MACDIARMID, R., NAIN, B., MCNEILAGE, M., RASSAM, M., RICHARDSON, A., RIKKERINK, E., ROSS, G., SCHRODER, R., SNOWDEN, K., SOULEYRE, E., TEMPLETON, M., WALTON, E., WANG, D., WANG, M., WANG, Y., WOOD, M., WU, R., YAUK, Y.-K. & LAING, W. 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 Genomics,* 9**,** 351.

DEFILIPPI, B. G., DANDEKAR, A. M. & KADER, A. A. 2004. Impact of suppression of ethylene action or biosynthesis on flavor metabolites in apple (*Malus domestica* Borkh) fruits. *Journal of Agricultural and Food Chemistry,* 52**,** 5694-5701.

DEFILIPPI, B. G., DANDEKAR, A. M. & KADER, A. A. 2005. Relationship of ethylene biosynthesis to volatile production, related enzymes, and precursor availability in apple peel and flesh tissues. *Journal of Agricultural and Food Chemistry,* 53**,** 3133-3141.

DU, X., FINN, C. E. & QIAN, M. C. 2010. Bound volatile precursors in genotypes in the pedigree of 'Marion' blackberry (*Rubus* Sp.). *Journal of Agricultural and Food Chemistry,* 58**,** 3694-3699.

DUTTAROY, A. K. & JØRGENSEN, A. 2004. Effects of kiwi fruit consumption on platelet aggregation and plasma lipids in healthy human volunteers. *Platelets,* 15**,** 287 - 292.

FERGUSON, A. R. 1984. Kiwifruit: A botanical review. *Horticultural Reviews,* 6**,** 1-64.

FERGUSON, A.R. 1999. New temperate fruits: *Actinidia chinensis* and *Actinidia deliciosa*. *In*: JANICK, J.(ed.) *Perspectives on new crops and new uses*. Alexandria, VA: ASHS Press.

FERGUSON, A. R. & STANLEY, R. 2003. Kiwifruit. *Encyclopedia of Food Sciences and Nutrition.* Oxford: Academic Press.

FISCHBÖCK, G., PFANNHOUSER, W. & KELLNER, R. 1988. GC-FTIR as a powerful tool for the characterization of flavor components in Kiwi. *Mikrochimica Acta,* 96**,** 249-257.

FLATH, R. A., SUGISAWA, H. & TERANISHI, R. 1981. Problems in flavor research. *In:* TERANISHI, R., FLATH, R. A. & SUGISAWA, H. (eds.) *Flavor research: recent advances.* New York-Basel: Marcel Dekker.

FORASTIERE, F., PISTELLI, R., SESTINI, P., FORTES, C., RENZONI, E., RUSCONI, F., DELL'ORCO, V., CICCONE, G. & BISANTI, L. 2000. Consumption of fresh fruit rich in vitamin C and wheezing symptoms in children. *Thorax,* 55**,** 283-288.

FORSS, D. A. 1981. Sensory characterization. *In:* TERANISHI, R., FLATH, R. A. & SUGISAWA, H. (eds.) *Flavor research: recent advances.* New York-Basel: Marcel Dekker.

FRANK, D., O'RIORDAN, P., VARELIS, P., ZABARAS, D., WATKINS, P., CECCATO, C. & WIJESUNDERA, C. 2007. Deconstruction and recreation of 'Hayward' volatile flavour using a trained sensory panel, olfactometry and a kiwifruit model matrix. *Acta Horticulturae (ISHS),* 753**,** 107-119.

FRIEL, E. N., LINFORTH, R. S. T. & TAYLOR, A. J. 2000. An empirical model to predict the headspace concentration of volatile compounds above solutions containing sucrose. *Food Chemistry,* 71**,** 309-317.

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. *Journal of Agricultural and Food Chemistry,* 55**,** 6664-6673.

GALLIARD, T. & MATTHEW, J. A. 1977. Lipoxygenase-mediated cleavage of fatty acids to carbonyl fragments in tomato fruits. *Phytochemistry,* 16**,** 339-343.

GARCIA, C. V., QUEK, S.-Y., STEVENSON, R. J. & WINZ, R. A. 2011. Characterization of the bound volatile extract from baby kiwi (*Actinidia arguta*). *Journal of Agricultural and Food Chemistry,* 59**,** 8358-8365.

GARCIA, C. V., QUEK, S.-Y., STEVENSON, R. J. & WINZ, R. A. 2012. Kiwifruit flavour: A review. *Trends in Food Science & Technology,* 24**,** 82-91.

GHOLAP, A. S., BANDYOPADHYAY, C. & NADKARNI, G. B. 1986. Aroma development in mango fruit. *Journal of Food Biochemistry,* 10**,** 217-229.

GILBERT, J. M., YOUNG, H., BALL, R. D. & MURRAY, S. H. 1996. Volatile flavor compounds affecting consumer acceptability of kiwifruit. *Journal of Sensory Studies,* 11**,** 247-259.

GIOVANNONI, J. J. 2004. Genetic regulation of fruit development and ripening. *Plant Cell,* 16**,** S170-180.

GÜNATA, Y. Z., BAYONOVE, C. L., BAUMES, R. L. & CORDONNIER, R. E. 1985a. The aroma of grapes I. Extraction and determination of free and glycosidically bound fractions of some grape aroma components. *Journal of Chromatography A,* 331**,** 83-90.

GÜNATA, Y. Z., BAYONOVE, C. L., BAUMES, R. L. & CORDONNIER, R. E. 1985b. The aroma of grapes. Localisation and evolution of free and bound fractions of some grape aroma components c.v. Muscat during first development and maturation. *Journal of the Science of Food and Agriculture,* 36**,** 857-862.

GÜNATA, Z., BITTEUR, S., BRILLOUET, J.-M., BAYONOVE, C. & CORDONNIER, R. 1988. Sequential enzymic hydrolysis of potentially aromatic glycosides from grape. *Carbohydrate Research,* 184**,** 139-149.

GÜNATA, Z., WIRTH JÉRÉMIE, L., GUO, W. & BAUMES RAYMOND, L. 2001. Norisoprenoid aglycon composition of leaves and grape berries from Muscat of Alexandria and Shiraz cultivars. *Carotenoid-derived aroma compounds.* American Chemical Society.

HARKER, F. R., CARR, B. T., LENJO, M., MACRAE, E. A., WISMER, W. V., MARSH, K. B., WILLIAMS, M., WHITE, A., LUND, C. M., WALKER, S. B., GUNSON, F. A. & PEREIRA, R. B. 2009. Consumer liking for kiwifruit flavour: A meta-analysis of five studies on fruit quality. *Food Quality and Preference,* 20**,** 30-41.

HATANAKA, A. 1993. The biogeneration of green odour by green leaves. *Phytochemistry,*

34**,** 1201-1218.
HUANG, C., ZHANG, Z., LI, G. & ZHOU, J. 1988. Isolation and identification of two new triterpenes from *Actinidia eriantha*. *Acta Botanica Yunnanica,* 10**,** 93-100.
HUANG, H. W. & FERGUSON, A. R. 2007. *Actinidia* in China: Natural diversity, phylogeographical evolution, interspecific gene flow and kiwifruit cultivar improvement. *Acta Horticulturae (ISHS),* 753**,** 31-40.
ISHIDA, T., ENOMOTO, H. & NISHIDA, R. 2008. New attractants for males of the Solanaceous fruit fly *Bactrocera latifrons*. *Journal of Chemical Ecology,* 34**,** 1532-1535.
JAEGER, S. R., ROSSITER, K. L., WISMER, W. V. & HARKER, F. R. 2003. Consumer-driven product development in the kiwifruit industry. *Food Quality and Preference,* 14**,** 187-198.
JO, Y. S., CHO, H. S., PARK, M. Y. & BANG, G. P. 2007. Selection of a sweet *Actinidia eriantha*, 'Bidan'. *Acta Horticulturae (ISHS),* 753**,** 253-258.
JOHNSON, J. H., GOULD, W. A., BADENHOP, A. F. & JOHNSON, R. M. 1968. Quantitative comparison of isoamylol, pentanol, and 3-hexen-1-ol in tomato juice. Varietal and harvest differences and processing effects. *Journal of Agricultural and Food Chemistry,* 16**,** 255-258.
JORDÁN, M. J., MARGARÍA, 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. *Journal of Agricultural and Food Chemistry,* 50**,** 5386-5390.
KOEDUKA, T., FRIDMAN, E., GANG, D. R., VASSÃO, D. G., JACKSON, B. L., KISH, C. M., ORLOVA, I., SPASSOVA, S. M., LEWIS, N. G., NOEL, J. P., BAIGA, T. J., DUDAREVA, N. & PICHERSKY, E. 2006. Eugenol and isoeugenol, characteristic aromatic constituents of spices, are biosynthesized via reduction of a coniferyl alcohol ester. *Proceedings of the National Academy of Sciences,* 103**,** 10128-10133.
KRAMMER, G. E., TAKEOKA, G. R. & BUTTERY, R. G. 1994. Isolation and identification of 2,5-dimethyl-4-hydroxy-3(2H)-furanone glucoside from tomatoes. *Journal of Agricultural and Food Chemistry,* 42**,** 1595-1597.
KRECK, M., PÜSCHEL, S., WÜST, M. & MOSANDL, A. 2002. Biogenetic studies in *Syringa vulgaris* L.: Synthesis and bioconversion of deuterium-labeled precursors into lilac aldehydes and lilac alcohols. *Journal of Agricultural and Food Chemistry,* 51**,** 463-469.
LALEL, H. J. D., SINGH, Z. & TAN, S. C. 2003. Glycosidically-bound aroma volatile compounds in the skin and pulp of `Kensington Pride' mango fruit at different stages of maturity. *Postharvest Biology and Technology,* 29**,** 205-218.
LAWLESS, H. T. & LEE, C. B. 1993. Common chemical sense in food flavor. *In:* ACREE, T. E. & TERANISHI, R. (eds.) *Flavor science: sensible principles and techniques.* Washington D.C. : American Chemical Society.
LIU, J. 2005. Oleanolic acid and ursolic acid: Research perspectives. *Journal of Ethnopharmacology,* 100**,** 92-94.
LÜCKER, J., BOUWMEESTER, H. J., SCHWAB, W., BLAAS, J., VAN DER PLAS, L. H. W. & VERHOEVEN, H. A. 2001. Expression of Clarkia S-linalool synthase in transgenic petunia plants results in the accumulation of S-linalyl-β-D-glucopyranoside. *Plant Journal,* 27**,** 315-324.
MAARSE, H. & BELZ, R. 1981. *Isolation, separation and identification of volatile compounds in aroma research,* Berlin, Akademie-Verlag.
MACLEOD, A. J. & SNYDER, C. H. 1985. Volatile components of two cultivars of mango from Florida. *Journal of Agricultural and Food Chemistry,* 33**,** 380-384.

MACRAE, E. A., STEC, M. G. H. & TRIGGS, C. M. 1990. Effects of postharvest treatment on the sensory qualities of kiwifruit harvested at different maturities. *Journal of the Science of Food and Agriculture,* 50**,** 533-546.

MAICAS, S. & MATEO, J. J. 2005. Hydrolysis of terpenyl glycosides in grape juice and other fruit juices: a review. *Applied Microbiology and Biotechnology,* 65**,** 322-335.

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 Quality and Preference,* 17**,** 376-386.

MATEO, J. J., GENTILINI, N., HUERTA, T., JIMENEZ, M. & DI STEFANO, R. 1997. Fractionation of glycoside precursors of aroma in grapes and wine. *Journal of Chromatography A,* 778**,** 219-224.

MATICH, A. J., YOUNG, H., ALLEN, J. M., WANG, M. Y., FIELDER, S., MCNEILAGE, M. A. & MACRAE, E. A. 2003. *Actinidia arguta*: volatile compounds in fruit and flowers. *Phytochemistry,* 63**,** 285-301.

MAYERL, F., NÄF, R. & THOMAS, A. F. 1989. 2,5-Dimethyl-4-hydroxy-3(2H)-furanone glucoside: Isolation from strawberries and synthesis. *Phytochemistry,* 28**,** 631-633.

MCDONALD, B. 1990. Precooling, storage and transport of kiwifruit. *In:* WARRINGTON, I. J. & WESTON, G. C. (eds.) *Kiwifruit: science and management.* Auckland: Ray Richard Publisher.

MOERKERCKE, A. V., SCHAUVINHOLD, I., PICHERSKY, E., HARING, M. A. & SCHUURINK, R. C. 2009. A plant thiolase involved in benzoic acid biosynthesis and volatile benzenoid production. *The Plant Journal,* 60**,** 292-302.

MOOKHERJEE, B. D., WILSON, R. A., TRENKLE, R. W., ZAMPINO, M. J. & SANDS, K. P. 1989. New dimensions in flavor research. *In:* TERANISHI, R., BUTTERY, R. G. & SHAHIDI, F. (eds.) *Flavor chemistry: trends and developments.* Washington, D.C.: American Chemical Society.

MOTOHASHI, N., SHIRATAKI, Y., KAWASE, M., TANI, S., SAKAGAMI, H., SATOH, K., KURIHARA, T., NAKASHIMA, H., MUCSI, I., VARGA, A. & MOLNÁR, J. 2002. Cancer prevention and therapy with kiwifruit in Chinese folklore medicine: a study of kiwifruit extracts. *Journal of Ethnopharmacology,* 81**,** 357-364.

MUSSINAN, C. J. 1993. Instrumental analysis in the flavor industry. *In:* ACREE, T. E. & TERANISHI, R. (eds.) *Flavour science: sensible principles and techniques.* Washington D.C.: American Chemical Society.

MYERS, M. J., ISSENBERG, P. & WICK, E. L. 1970. L-Leucine as a precursor of isoamyl alcohol and isoamyl acetate, volatile aroma constituents of banana fruit discs. *Phytochemistry,* 9**,** 1693-1700.

NASI, A., FERRANTI, P., AMATO, S. & CHIANESE, L. 2008. Identification of free and bound volatile compounds as typicalness and authenticity markers of non-aromatic grapes and wines through a combined use of mass spectrometric techniques. *Food Chemistry,* 110**,** 762-768.

NISHIYAMA, I. 2007. Fruits of the *Actinidia* genus. *Advances in Food and Nutrition Research,* 52**,** 294-324.

OGAWA, K., IJIMA, Y., GUO, W., WATANABE, N., USUI, T., DONG, S., TONG, Q. & SAKATA, K. 1997. Purification of a β-primeverosidase concerned with alcoholic aroma formation in tea leaves (Cv. Shuixian) to be processed to Oolong tea. *Journal of Agricultural and Food Chemistry,* 45**,** 877-882.

ORRUÑO, E., APENTEN, R. O. & ZABETAKIS, I. 2001. The role of β-glucosidase in the biosynthesis of 2,5-dimethyl-4-hydroxy-3(2H)-furanone in strawberry (Fragaria × ananassa cv. Elsanta). *Flavour and Fragrance Journal,* 16**,** 81-84.

PABST, A., BARRON, D., ETIEVANT, P. & SCHREIER, P. 1991. Studies on the enzymic

hydrolysis of bound aroma constituents from raspberry fruit pulp. *Journal of Agricultural and Food Chemistry,* 39**,** 173-175.
PATERSON, V. J., MACRAE, E. A. & YOUNG, H. 1991. Relationships between sensory properties and chemical composition of kiwifruit (*Actinidia deliciosa*). *Journal of the Science of Food and Agriculture,* 57**,** 235-251.
PATTERSON, K., BURDON, J. & LALLU, N. 2003. 'Hort16A' kiwifruit: Progress and issues with commercialisation. *Acta Horticulturae (ISHS),* 610**,** 267-273.
PERERA, C. O., YOUNG, H. & BEEVER, D. J. 1998. Kiwifruit. *In:* SHAW, P. E., CHAN, H. T. & NAGY, S. (eds.) *Tropical and subtropical fruits.* Auburndale: Agscience Inc.
PLANT & FOOD RESEARCH. 2010. *Fresh facts: New Zealand horticulture*. Auckland: Plant & Food Research.
POGORZELSKI, E. & WILKOWSKA, A. 2007. Flavour enhancement through the enzymatic hydrolysis of glycosidic aroma precursors in juices and wine beverages: a review. *Flavour and Fragrance Journal,* 22**,** 251-254.
REINECCIUS, G. 2002. Instrumental methods of analysis. *In:* TAYLOR, A. J. (ed.) *Food flavour technology.* Blackwell Publishing.
REINECCIUS, G. 2006a. Choosing the correct analytical technique in aroma analysis. *In:* ETIÉVANT, P. & VOILLEY, A. (eds.) *Flavour in food.* Woodhead Publishing.
REINECCIUS, G. 2006b. *Flavor chemistry and technology.* Boca Raton: Taylor and Francis.
ROSSITER, K. L., YOUNG, H., WALKER, S. B., MILLER, M. & DAWSON, D. M. 2000. The effects of sugars and acids on consumer acceptability of kiwifruit. *Journal of Sensory Studies,* 15**,** 241-250.
RUSH, E. C., PATEL, M., PLANK, L. D. & FERGUSON, L. R. 2002. Kiwifruit promotes laxation in the elderly. *Asia Pacific Journal of Clinical Nutrition,* 11**,** 164-168.
SAKHO, M., CHASSAGNE, D. & CROUZET, J. 1997. African mango glycosidically bound volatile compounds. *Journal of Agricultural and Food Chemistry,* 45**,** 883-888.
SALLES, C., JALLAGEAS, J.-C. & CROUZET, J. 1990. Chromatographic separation and partial identification of glycosidically bound volatile components of fruit. *Journal of Chromatography A,* 522**,** 255-265.
SAMPAIO, T. S. & NOGUEIRA, P. C. L. 2006. Volatile components of mangaba fruit (*Hancornia speciosa* Gomes) at three stages of maturity. *Food Chemistry,* 95**,** 606-610.
SARRY, J. E. & GÜNATA, Z. 2004. Plant and microbial glycoside hydrolases: volatile release from glycosidic aroma precursors. *Food Chemistry,* 87**,** 509-521.
SCHAEFER, J. 1981. Isolation and concentration from the vapour phase. *In:* MAARSE, H. & BELZ, R. (eds.) *Isolation, separation and identification of volatile compounds in aroma research.* Berlin: Akademie-Verlag.
SCHWAB, W., DAVIDOVICH-RIKANATI, R. & LEWINSOHN, E. 2008. Biosynthesis of plant-derived flavor compounds. *The Plant Journal,* 54**,** 712-732.
SCHWAB, W. & SCHREIER, P. 1988. Simultaneous enzyme catalysis extraction: a versatile technique for the study of flavor precursors. *Journal of Agricultural and Food Chemistry,* 36**,** 1238-1242.
SCHWAB, W. & SCHREIER, P. 1990. Vomifoliol 1-O-β-D-xylopyranosyl-6-O-β-D-glucopyranoside: A disaccharide glycoside from apple fruit. *Phytochemistry,* 29**,** 161-164.
SEFTON, M. A. & WILLIAMS, P. J. 1991. Generation of oxidation artifacts during the hydrolysis of norisoprenoid glycosides by fungal enzyme preparations. *Journal of Agricultural and Food Chemistry,* 39**,** 1994-1997.
SHIOTA, H. 1982. Kiwifruit. *Koryo,* 137**,** 59-64.
SHOSEYOV, O., BRAVDO, B. A., SIEGEL, D., GOLDMAN, A., COHEN, S., SHOSEYOV,

L. & IKAN, R. 1990. Immobilized endo-β-glucosidase enriches flavor of wine and passion fruit juice. *Journal of Agricultural and Food Chemistry,* 38**,** 1387-1390.

SIEGMUND, B. K. & LEITNER, E. 2010. Furaneol and mesifuran in strawberries - an analytical challenge. *In:* BLANK, I., WÜST, M. & YERETZIAN, C. (eds.) *Expression of Multidisciplinary Flavour Science.* Winterthur: ZHAW Züricher Hochschule für Angewandte Wissenschaften.

SOULEYRE, E. J. F., GÜENTER, C. S., WANG, M. Y., NEWCOMB, R. D., MARSH, K. B. 2011. Ester biosynthesis in kiwifruit-from genes to enzymes to pathways. *Acta Horticulturae (ISHS),* 913**,** 205-211.

STAHL-BISKUP, E., INTERT, F., HOLTHUIJZEN, J., STENGELE, M. & SCHULZ, G. 1993. Glycosidically bound volatiles - A review 1986-1991. *Flavour and Fragrance Journal,* 8**,** 61-80.

STANLEY, R., WEGRZYN, T. & SALEH, Z. 2007. Kiwifruit processed products. *Acta Horticulturae,* 753**,** 795-800.

STRAUSS, C. R., GOOLEY, P. R., WILSON, B. & WILLIAMS, P. J. 1987. Application of droplet countercurrent chromatography to the analysis of conjugated forms of terpenoids, phenols, and other constituents of grape juice. *Journal of Agricultural and Food Chemistry,* 35**,** 519-524.

SUGISAWA, H. 1981. Sample preparation: isolation and concentration. *In:* TERANISHI, R., FLATH, R. A. & SUGISAWA, H. (eds.) *Flavor research: recent advances.* New York-Basel: Marcel Dekker.

SUZUKI, M., MATSUMOTO, S., MIZOGUCHI, M., HIRATA, S., TAKAGI, K., HASHIMOTO, I., YAMANO, Y., ITO, M., FLEISCHMANN, P., WINTERHALTER, P., MORITA, T. & WATANABE, N. 2002. Identification of (3S, 9R)- and (3S, 9S)-Megastigma-6,7-dien-3,5,9-triol 9-O-β-D-glucopyranosides as damascenone progenitors in the flowers of *Rosa damascena* Mill. *Bioscience, Biotechnology, and Biochemistry,* 66**,** 2692-2697.

TAKEOKA, G. R., GUNTERT, M., FLATH, R. A., WURZ, R. E. & JENNINGS, W. 1986. Volatile constituents of kiwi fruit (*Actinidia chinensis* Planch.). *Journal of Agricultural and Food Chemistry,* 34**,** 576-578.

TAYLOR, R. B. 1996. Introduction to fruit processing. *In:* ARTHEY, D. & ASHURST, P. R. (eds.) *Fruit processing.* London: Blackie Academic & Professional.

TERANISHI, R. & KINT, S. 1993. Sample preparation. *In:* ACREE, T. E. & TERANISHI, R. (eds.) *Flavor science: sensible principles and techniques.* Washington D.C.: American Chemical Society.

WAN, X. M., STEVENSON, R. J., CHEN, X. D. & MELTON, L. D. 1999. Application of headspace solid-phase microextraction to volatile flavour profile development during storage and ripening of kiwifruit. *Food Research International,* 32**,** 175-183.

WANG, M. 2007. Distribution of important flavour precursors in *Actinidia*: long chain fatty acids. *Acta Horticulturae (ISHS),* 753**,** 427-432.

WANG, M. Y., MACRAE, E., WOHLERS, M. & MARSH, K. 2011. Changes in volatile production and sensory quality of kiwifruit during fruit maturation in *Actinidia deliciosa* 'Hayward' and *A. chinensis* 'Hort16A'. *Postharvest Biology and Technology,* 59**,** 16-24.

WANG, Y., WYLLIE, S. G. & LEACH, D. N. 1996. Chemical changes during the development and ripening of the fruit of *Cucumis melo* (Cv. Makdimon). *Journal of Agricultural and Food Chemistry,* 44**,** 210-216.

WEIN, M., LAVID, N., LUNKENBEIN, S., LEWINSOHN, E., SCHWAB, W. & KALDENHOFF, R. 2002. Isolation, cloning and expression of a multifunctional *O*-methyltransferase capable of forming 2,5-dimethyl-4-methoxy-3(2H)-furanone, one

of the key aroma compounds in strawberry fruits. *The Plant Journal,* 31**,** 755-765.
WERKHOFF, P., BRENNECKE, S., BRETSCHNEIDER, W. & BERTRAM, H. J. 2002. Modern methods for isolating and quantifying volatile flavor and fragrance compounds. *In:* MARSILI, R. (ed.) *Flavor, fragrance, and odor analysis.* New York-Basel: Marcel Dekker.
WILLIAMS, M. H., BOYD, L. M., MCNEILAGE, M. A., MACRAE, E. A., FERGUSON, A. R., BEATSON, R. A. & MARTIN, P. J. 2003. Development and comercialization of 'baby kiwi' (*Actinidia arguta* Planch). *Acta Horticulturae (ISHS),* 610**,** 81-86.
WILLIAMS, P. J. 1993. Hydrolytic flavor release in fruit and wines through hydrolysis of nonvolatile precursors. *In:* ACREE, T. E. & TERANISH, R. (eds.) *Flavor science: sensible principles and techniques.* Washington D.C.: American Chemical Society.
WILLIAMS, P. J., CYNKAR, W., FRANCIS, I. L., GRAY, J. D., ILAND, P. G. & COOMBE, B. G. 1995. Quantification of glycosides in grapes, juices, and wines through a determination of glycosyl glucose. *Journal of Agricultural and Food Chemistry,* 43**,** 121-128.
WILLIAMS, P. J., SEFTON, M. A. & WILSON, B. 1989. Nonvolatile conjugates of secondary metabolites as precursors of varietal grape flavor components. *In:* TERANISHI, R., BUTTERY, R. G. & SHAHIDI, F. (eds.) *Flavor chemistry: trends and developments.* Washington D.C.: American Chemical Society.
WILLIAMS, P. J., STRAUSS, C. R., WILSON, B. & MASSY-WESTROPP, R. A. 1982a. Studies on the hydrolysis of *Vitis vinifera* monoterpene precursor compounds and model monoterpene β-D glucosides rationalizing the monoterpene composition of grapes. *Journal of Agricultural and Food Chemistry,* 30**,** 1219-1223.
WILLIAMS, P. J., STRAUSS, C. R., WILSON, B. & MASSY-WESTROPP, R. A. 1982b. Use of C18 reversed-phase liquid chromatography for the isolation of monoterpene glycosides and nor-isoprenoid precursors from grape juice and wines. *Journal of Chromatography A,* 235**,** 471-480.
WINTERHALTER, P., SEFTON, M. A. & WILLIAMS, P. J. 1990. Two-dimensional GC-DCCC analysis of the glycoconjugates of monoterpenes, norisoprenoids, and shikimate-derived metabolites from Riesling wine. *Journal of Agricultural and Food Chemistry,* 38**,** 1041-1048.
WINTERHALTER, P. & SKOUROUMOUNIS, G. 1997. Glycoconjugated aroma compounds: Occurrence, role and biotechnological transformation. *In:* BERGER, R., BABEL, W., BLANCH, H., COONEY, C., ENFORS, S., ERIKSSON, K., FIECHTER, A., KLIBANOV, A., MATTIASSON, B., PRIMROSE, S., REHM, H., ROGERS, P., SAHM, H., SCHÜGERL, K., TSAO, G., VENKAT, K., VILLADSEN, J., VON STOCKAR, U. & WANDREY, C. (eds.) *Biotechnology of Aroma Compounds.* Springer Berlin / Heidelberg.
WU, P., KUO, M. C., HARTMAN, T. G., ROSEN, R. T. & HO, C. T. 1991. Free and glycosidically bound aroma compounds in pineapple (*Ananas comosus* L. Merr.). *Journal of Agricultural and Food Chemistry,* 39**,** 170-172.
WU, P., KUO, M. C., ZHANG, K. Q., HARTMAN, T. G., ROSEN, R. T. & HO, C. T. 1990. Analysis of glycosidically bound 2,5-dimethyl-4-hydroxy-3(2H)-furanone in pineapple. *Perfumer & Flavorist,* 15**,** 51-53.
WU, Y. J., XIE, M., ZHANG, Q. C., JIANG, G. H., ZHANG, H. Q., LONG, Q. J., HAN, W. J., CHEN, J. W. & SHONG, G. H. 2009. Characteristics of 'White': a new easy-peel cultivar of *Actinidia eriantha*. *New Zealand Journal of Crop and Horticultural Science,* 37**,** 369-373.
YANG, E., ZHAO, Y. & QIAN, M. C. 2010. Effect of edible coating on volatile compounds of hardy kiwifruit during storage. *Flavor and Health Benefits of Small Fruits.*

American Chemical Society.
YOUNG, H. & PATERSON, V. J. 1985. The effects of harvest maturity, ripeness and storage on kiwifruit aroma. *Journal of the Science of Food and Agriculture,* 36**,** 352-358.
YOUNG, H. & PATERSON, V. J. 1990. The flavour of exotic fruit. *In:* MORTON, I. D. & MACLEOD, A. J. (eds.) *Food flavours. Part C. The flavour of fruits.* Amsterdam: Elsevier.
YOUNG, H. & PATERSON, V. J. 1995. Characterisation of bound flavour components in kiwifruit. *Journal of the Science of Food and Agriculture,* 68**,** 257-260.
YOUNG, H., PATERSON, V. J. & BURNS, D. J. W. 1983. Volatile aroma constituents of kiwifruit. *Journal of the Science of Food and Agriculture,* 34**,** 81-85.
YOUNG, H., PERERA, C. O. & PATERSON, V. J. 1992. Identification of E-Hex-3-enal as an important contributor to the off-flavour aroma in kiwifruit juice. *Journal of the Science of Food and Agriculture,* 58**,** 519-522.
ZABETAKIS, I., GRAMSHAW, J. W. & ROBINSON, D. S. 1999. 2,5-Dimethyl-4-hydroxy-2H-furan-3-one and its derivatives: analysis, synthesis and biosynthesis—a review. *Food Chemistry,* 65**,** 139-151.
ZHANG, B., YIN, X.-R., LI, X., YANG, X.-L., FERGUSON, I. B. & CHEN, K.-S. 2009. Lipoxygenase gene expression in ripening kiwifruit in relation to ethylene and aroma production. *Journal of Agricultural and Food Chemistry,* 59**,** 2875-2881.

PUBLICATIONS AND PRESENTATIONS

Peer-reviewed papers

GARCIA, C. V., STEVENSON, R. J., ATKINSON, R. G., WINZ R. A. & QUEK, S. -Y. 2013. Changes in the bound aroma profiles of 'Hayward' and 'Hort16A' kiwifruit (*Actinidia* spp.) during ripening and GC-olfactometry analysis. *Food Chemistry*, 137, 45-54.

GARCIA, C. V., QUEK, S. -Y., STEVENSON, R. J. & WINZ R. A. 2012. Characterisation of bound volatile compounds of a low flavour kiwifruit species: *Actinidia eriantha*. *Food Chemistry*, 134, 655-661.

GARCIA, C. V., QUEK, S. -Y., STEVENSON, R. J. & WINZ R. A. 2012. Kiwifruit flavor: A review. *Trends in Food Science & Technology*, 24, 82-91.

GARCIA, C. V., QUEK, S. -Y., STEVENSON, R. J. & WINZ R. A. 2011. Characterization of the bound volatile extract from baby kiwi (*Actinidia arguta*). *Journal of Agricultural and Food Chemistry*, 59, 8358-8365.

Oral presentations

Analysis of glycosidically bound volatile compounds of several kiwifruit cultivars

Coralia V. Garcia

6th International Student Forum, 25-29 September 2010, Beijing, China

Poster presentations

Kiwifruit: hidden flavour compounds in a super fruit

Coralia V. Garcia, Siew-Young Quek, Robert A. Winz

International Conference and Exhibition on Nutraceuticals and Functional Foods, 14-17 November 2011, Sapporo, Japan

Bound aroma compounds of baby kiwifruit

Coralia V. Garcia, Siew-Young Quek, Robert A. Winz

44th Australian Institute of Food Science and Technology (AIFST) Convention, 10-13 July 2011, Sydney, Australia

Bound aroma compounds in Gold kiwifruit

Coralia V. Garcia, Siew-Young Quek, Robert A. Winz

The New Zealand Institute of Food Science and Technology (NZIFST) Annual Conference, 29 June-1 July 2011, Rotorua, New Zealand

Characterization of bound aroma precursors in *Actinidia eriantha*

Coralia V. Garcia, Siew-Young Quek, Robert A. Winz

10th Functional Foods Symposium, 16 November 2010, Auckland, New Zealand

Aroma compounds in kiwifruit determined by solvent extraction and SPME

Coralia V. Garcia, Siew-Young Quek, Robert A. Winz

15th World Congress of Food Science and Technology (IUFoST), 22-26 August 2010, Cape Town, South Africa

Kiwifruit bound volatile compounds isolated by solvent extraction and solid phase microextraction

Coralia V. Garcia, Siew-Young Quek, Ralph Stevenson, Laura Nicolau, Robert Winz, Mindy Wang

The New Zealand Institute of Food Science and Technology (NZIFST) Annual Conference, 23-25 June-2010, Auckland, New Zealand