The volatile composition and aroma profile of ‘Unique’, ‘Triumph’ and ‘Anatoki’ feijoa fruits (*Acca sellowiana*) during ripening

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A thesis submitted in fulfilment of the requirements for the degree of Master of Science in Food Science, The University of Auckland, New Zealand, February 2020.

Abstract

Feijoa (*Acca sellowiana*) is a climacteric fruit which is widely planted in the Mediterranean region, Tunisia, USA, Australia and New Zealand. Although the functional and biochemical properties of feijoa fruits have been well-recognised, the volatile composition and aroma profile are less studied. This study focused on the analysis of the free and bound volatiles, as well as identification of aroma active compounds of feijoa fruits. Factors include fruit ripening stages and cultivar difference could influence the synthesise of fruit volatiles. Therefore, feijoa fruits from different cultivars and ripening stages were selected to give a deeper insight of aroma profile of feijoa fruits.

Feijoa fruits from the 'Unique', 'Triumph' and 'Anatoki' cultivar were collected at four ripening stages (four weeks before ripening, two weeks before ripening, ripe and overripe). Their volatile was analysed by headspace solid phase microextraction (HS-SPME), gas chromatography mass spectrometry (GC-MS) and olfactory tests (GCO).

A total of 164 free volatiles with 60 terpenes, 52 esters, 20 alcohols, 9 ketones, 6 aldehydes, 2 hydrocarbons and 15 unknowns were identified. Terpene was the dominant group in the unripe fruits, whereas ester was the dominant class in the ripe and overripe fruits. During ripening, the concentration of free terpenes, alcohols and ketones decreased while that of ester largely increased. A total of 26 aroma active compounds were identified. At unripe stages, terpene dominated the free aroma and released a 'herbal and grassy' odour. At ripe and overripe stages, esters dominated the free aroma profile and gave a 'fruity, sweet and floral' aroma. The key aroma active compounds were found to be 'sweet, floral and fruity' ethyl butanoate and 'fruity, feijoa-like' methyl benzoate and ethyl benzoate, 'floral and fresh' linalool, 'fruity and green' α-cubebene and 'grass and woody' caryophyllene.

A total of 84 bound volatiles with 28 terpenes, 19 alcohols, 9 aldehydes, 8 ketones, 3 esters, 1 hydrocarbon and 16 unknowns were identified and 52 of them co-existed as free and bound volatiles. Bound alcohol, terpene and ketone were the main groups in the 4 weeks before ripening fruits and aldehyde was the dominant class in the 2 weeks before ripening fruits. In the ripe and overripe fruits, the major groups varied among the three cultivars. Comparable with the free terpene and ester, the bound terpene decreased while the bound ester increased. Both the number and concentration of bound volatiles were much lower than those of the free volatiles.

The importance of bound volatiles to its whole volatile composition in feijoa fruits could be limited due to their lower number and insignificant concentrations.

To sum up, the present thesis is the first study that focused on the analysis of free and bound volatile compounds in the whole feijoa fruit at different ripening levels. The study of feijoa volatile composition and aroma profile of feijoa fruits at different ripening stages could bring substantial insight into this fruit and give deeper understanding into fruit ripening and fruit aroma.

Acknowledgement

Firstly, I would like to sincerely express my gratitude and appreciation to my supervisor, Professor Siew-Young Quek, for providing me the opportunity to take part in this study and giving me continuous support, invaluable guidance and great encouragement throughout my Master year. She is a warm-hearted and enthusiastic supervisor who shared her bountiful knowledge and personal experience with me, as well as listening and giving advices whenever I needed. Thanks for all her precious time and suggestions. I felt deeply grateful to have her as my supervisor for the Master project.

Much appreciation goes to Yaoyao Peng who always offered suggestions, assistance and encouragement to me as my senior in the Master research. She is a hardworking, optimistic and passionate person who inspired me to attain better achievement. I am extremely thankful for her support during the whole year.

I am very appreciated to another senior, Daisy Zhang, whom I worked closely with for this project. I felt so thankful for her generous assistance inside and outside of the laboratory. She is also a hardworking pattern who gave me many motivation and support.

I also acknowledgement my seniors and fellows in the University of Auckland, Jingya Cui, He Lyu, Xuan Dong, Xiao Chen, Boyang Xu, Yongchao Zhu, Geoff Ang Xun, Pipat Tangjaidee for their support, suggestions and encouragement during my Master year.

Finally, I would like to express my deepest gratitude to my family members, partner and friends for supporting me study overseas and loving me.

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Abbreviations

Alcohol dehydrogenase	ADH
Alcohol acyltransferase	AAT
Analysis of variance	ANOVA
Anatoki 4 weeks before ripening sample	A4
Anatoki 2 weeks before ripening sample	A2
Anatoki ripen sample	AR
Anatoki overripen sample	AO
Carotenoid cleavage dioxygenases	CCDs
Diethyl ether-pentene	DP
Dimethylallyl diphosphate	DMAPP
Farnesyl pyrophosphate	FPP
Farnesyl pyrophosphate synthase	FPPS
Flavinadenine dinucleotide	FAD
Gas chromatography	GC
Gas chromatography–mass spectrometry	GC – MS
Gas Chromatography—Olfactometry	GC-O
Geranyl pyrophosphate	GPP
Geranyl pyrophosphate synthase	GPPS
Headspace – Solid-phase Microextraction	HS – SPME
Isopentenyl diphosphate	IPP
Lipoxygenase	LOX
Linear retention index	LRI

Methylerythritol phosphate	MEP
Mevalonic acid	MVA
Nicotinamide adenine dinucleotide	NAD
New Zealand	NZ
Odor activity values	OAVs
Principle component analysis	PCA
Retention index	RI
Solid phase extraction	SPE
Solid-phase Microextraction	SPME
Terpene synthases	TPSs
Triumph 4 weeks before ripening sample	T4
Triumph 2 weeks before ripening sample	T2
Triumph Ripen sample	TR
Triumph overripen sample	TO
Unique 4 weeks before ripening sample	U4
Unique 2 weeks before ripening sample	U2
Unique ripen sample	UR
Unique overripen sample	UO
4 weeks before ripening stage	4WBR
2 weeks before ripening stage	2WBR

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Chapter One: Introduction

1.1 Overview

1.1.1 Background

Feijoa (*Acca sellowiana*), also known as pineapple guava, was originally found in high altitude South American countries. In New Zealand, there are more than 800 tonnes of planting area of feijoa trees (Zhu, 2018) and more than 20 feijoa cultivars (Mosbah *et al*., 2018). Feijoa is regarded as a healthy fruit due to its wide range of nutritional compositions including vitamins, minerals, dietary fibres and phenolics (flavonoids). These bioactive components largely contribute to its antioxidant, anticancer, anti-inflammatory, antidiabetic, anti-microorganisms and probiotic properties (Mokhtari et al., 2018; Monforte et al., 2014). Feijoa plant could also be considered as a potential ingredient in functional foods with pleasant aroma and flavour (Sun-Waterhouse, 2011). The aroma profile of feijoa juice and essential oils extracted from feijoa peel has been studied by Peng et al. (2019). However, literature about volatile profile of feijoa during ripening was very limited and outdated, more study is needed to fill the gap.

Volatile compounds in fruits are very important because they affect the aroma quality and sensorial acceptance. Volatiles not only affect consumer acceptance but also reflect ripeness, rancid and safety to some extent (El Hadi *et al*., 2013). Based on the forms of volatiles, compounds could be classified as free volatiles and glycosidically bound volatiles (Baldwin, 2010). Bound volatiles of feijoas have not been studied yet. Besides, there are many factors that could influence the synthesise of fruit volatiles, which include fruits maturation and ripening stages, cultivars difference, pre- and post-harvest conditions, storage atmosphere and other chemical applications such as gaseous or calcium treatments. This study paid attention to the influence of ripening stages and cultivar difference of volatiles composition in feijoa fruits.

Characterisation of volatiles is a whole process including sample preparation, solvent selection, free volatile isolation and extraction, bound volatile precursors extraction, bound volatile hydrolysis, volatiles separation and identification, volatiles quantification and final statistical analysis. Gas chromatography–mass spectrometry (GC-MS) and gas chromatography-olfactory (GC-O) are frequently used to identify volatiles/aromas.

Study of free and bound volatiles during ripening provides a relative comprehensive volatile composition and aroma profile of feijoa fruits, which could bring substantial insight into this fruit and be helpful in future feijoa product development. Our group has studied the volatiles in

feijoa juice (Peng, Bishop, 2019) and in the essential oils extracted from feijoa peels (Peng *et al*, 2019). However, the volatile compounds in the whole fruits from different cultivars and different ripening stages were not studied. Therefore, this project focused on the characterisation of free and bound volatiles in three feijoa during ripening.

1.1.2 Significance

Previous studies paid more attention to the chemical and biological properties of feijoa fruits (Mokhtari et al., 2018; Monforte et al., 2014). As an important component in feijoa fruits study, volatile composition was less explored. There are some literatures studied the volatiles of feijoa but most of them are outdated (Shaw et al., 1990; Binder and Flath, 1989; Shaw et al., 1989; Shaw et al.,1983; Hardy and Michael, 1970). The most recent volatile studies were on feijoa juice (Peng et al., 2019) and the oils extracted from feijoa peel (Peng et al., 2019) rather than the whole fruit. Whereas whole fruits without further treatment are normally commercialised and consumed. Therefore, it was worthwhile to study the volatile profile of whole feijoa fruits. Nonetheless, there is no paper explored the volatile change of feijoa fruits during ripening. This project studied volatile composition of three feijoa cultivars during ripening to give a better understanding on feijoa volatiles. Moreover, based on the volatile profile, the aromatic volatile compounds in feijoa fruits were identified to build an aroma profile of feijoa fruits.

Glycosidically bound volatile is also an important aspect in fruit volatile study. However, for feijoa fruits, there was no paper discussed about bound volatile. Considering of this gap, this project also explored the change of bound volatiles of feijoa fruits from different cultivars during ripening. This knowledge is important to finalize aroma profile of feijoa and might be utilized in the future feijoa products.

Volatile composition and aroma profile of feijoa is very important for commercial utilization of feijoa such as feijoa flavours and fragrances development. Except academic value, this study could help growers and consumers to select desired feijoa fruits with proper ripening stage and cultivar.

1.1.3 Objectives

The overall objective of this project was to characterize the volatiles composition in three cultivars of feijoas (Unique, Triumph and Anatoki) during four ripening (four weeks before ripening, two weeks before ripening, ripe and overripe stages). This was divided into several specific objectives, as below,

- Measurement of physical characteristics of feijoa fruits
- Analysis of free volatiles in three feijoa cultivars with four ripening stages
- Analysis of bound volatiles in three feijoa cultivars with four ripening stages
- Analysis aromatic free volatiles in three feijoa cultivars with four ripening stages

1.1.4 Scope

The whole thesis was divided into four chapters, the first chapter was an introduction of feijoa fruits, fruits volatiles, procedure and techniques used in volatiles analysis. The second chapter was to describe the materials and methods used in this project. The third chapter gave a result presentation and a detailed discussion, and the last chapter was going to have some general discussion, conclusion, limitations and future works. The following is the scope of the thesis:

- Chapter One *Introduction*. This chapter contains an overview and literature review of this projects. General background of feijoa fruits, fruits volatiles, procedure and techniques used in volatiles analysis are included in this chapter.

- Chapter Two *Materials and Methods*. This chapter lists all the materials and equipment used in this project and gives a detailed method from fruit collection, volatile extraction, volatile detection and quantification to statistical analysis.

- Chapter Three *Results and Discussion*. This chapter illustrates all the results of feijoa fruits and volatile analysis and gives discussions of the results.

- Chapter Four *Conclusion, limitations and Future Works*. This chapter gives a conclusion, limitation and future development of this projects.

1.2 Literature review

1.2.1 Feijoa (*Acca sellowiana*)

Feijoa (*Acca sellowiana*), also known as pineapple guava, is a climacteric fruit and part of the Myrtaceae family (Landrum, 1986). This woody shrub plant was originally found in high altitude South American countries such as Brazil, Argentina, Uruguay and Paraguay (Ramírez & Kallarackal, 2017) and now widely planted in some tropical and subtropical climate countries such as the Mediterranean region (France, Italy, Tirkey), Tunisia, The USA, Australia and Nez Zealand (Giuseppe and Corrado, 2004, Beyhan, et al., 2011; Nguyên & Savage, 2013; Tuncel & Yılmaz, 2015). In New Zealand, there are more than 800 tonnes of planting area of feijoa trees (Zhu, 2018) and more than 20 feijoa cultivars (Mosbah et al., 2018). Because of its ornamental function and edible fruits, some Asian countries such as China (Zhu, 2018) and Japan (Aoyama, et al., 2018) start cultivating feijoas as well in recent years.

The feijoa tree looks comparable to olive trees but with a smaller shape (Mosbah et al., 2018). The common ripening season for feijoa is autumn, from March to June in the southern hemisphere and October to December in the northern hemisphere (Thorp & Bieleski, 2002). The feijoa cultivars in New Zealand include early season cultivars (‘Gemini’, ‘Unique’, ‘Pounamu’, ‘Kakariki’, ‘Kaiteri’ and ‘Anatoki’), mid-season cultivars (‘Apollo’, Kakapo’, ‘Mamoth’ and ‘Den's Choice’), and late season cultivars (‘Wiki Tu’, ‘Opal Star’ and ‘Triumph’) (NZ Feijoa Growers Association, n.d.). New cultivars are under development to have better climate tolerance, longer ripening season and to solve self-incompatibility problems (Bell et al., 2018).

Many parts of feijoa plants can be consumed by consumers. The flowers of feijoa are hermaphroditic with desirable taste, they are always used in salads or as dish decoration (De Souza, et al, 2016). The fruits of feijoa are with a green skin and a soft white pulp. The flesh near the seeds is sweet and juicy while those close to the skin is a little gritty. There are many ways to consume feijoa fruits. They can be served entirely as fresh fruit, or pressed into feijoa juice, or consumed as flavor or aroma enhancer in desserts, candy, yogurt, smoothie, muffin, jams, syrups, puree, ice cream, wine and chocolate (Mokhtari et al., 2018). Feijoa plant could also considered as a potential ingredient in functional foods with pleasant flavour and aroma (Sun-Waterhouse, 2011).

1.2.1.1 Composition of feijoa fruit

Feijoa is regarded as a healthy fruit due to its wide range of nutritional compositions. Table 1 lists the general nutritional composition of whole feijoa fruits.

Table 1. Nutritional composition of feijoa fruits.

Nutrients	unit	Value per 100g
proximate		
Water	g	83.28
Carbohydrate, by	g	15.21
Protein	g	0.71
Total Sugars	g	8.2
Total Dietary fibre	g	6.4
Total Fats	g	0.42
Energy kcal 61 148 26	kcal	61
Vitamins		
Total Vitamin C (ascorbic acid)	mg	32.9
Total Vitamin E (α-tocopherol)	mg	0.16
Total Vitamin A	IU	6
Total Vitamin K (phylloquinone)	µg	3.5
Total Folate	µg	23
Niacin	mg	0.295
Pantothenic acid	mg	0.233
Vitamin B6	mg	0.067
Riboflavin	mg	0.018
Minerals		
Potassium, K	mg	172
Phosphorus, P	mg	19
Calcium, Ca	mg	17
Magnesium, Mg	mg	9
Sodium, Na	mg	3
Iron, Fe	mg	0.14
Magnesium, Mn	mg	0.084
Zinc, Zn	mg	0.06
Copper, Cu	mg	0.036
Fats		
Polyunsaturated Fatty acids	g	0.136
Saturated Fatty acids	g	0.104
Monounsaturated Fatty acids	g	0.056

Databases obtained from USDA food composition, June 30st, 2018.

Feijoa has been reported to contain vitamins, minerals, dietary fibres and phenolics (flavonoids), these bioactive components contribute largely to its antioxidant, anticancer, anti-inflammatory, antidiabetic, anti-microorganisms and probiotic properties (Mokhtari et al., 2018; Monforte et al., 2014). For antioxidant property, radical scavenging capacities have been found in the extracts of feijoa fruits (Amarante, et al., 2017; Peng et al., 2019). Phenols such as flavones might be responsible for this function by reacting with protein-kinase C pathway to achieve free radical inhibition (Peng et al., 2019). Moreover, the superoxide anion formation in human neutrophils significantly decreased by drinking mucilage and sugars enriched feijoa juice (Monforte et al., 2014).

Furthermore, phenols such as ellagitannins and syringic acid in feijoa fruits can decrease microbial activity and inhibit the grow rate of some microorganisms such as bacteria and fungus (Amarante et al., 2017). Other health benefits of feijoa fruits such as nephroprotection (Aoyama et al., 2018), analgesia (Karami et al., 2014), carrier of probiotics(Amarante et al., 2017), and improving disaccharidase activity and tyrosinase inhibition (Mosbah et al., 2018) have also been reported.

1.2.1.2 Volatiles profile of feijoa

The volatiles (especially aroma) profile is one of the most significant characteristics of feijoa and worthwhile being studied. However, compared with compositional and biological research, the studies of feijoa aroma is very limited and outdated. Table 2 listed the previous studies on feijoa volatiles.

Table 2: References summary of aroma profiles in feijoa

Sample	Methods		Results		Reference
	Isolation	Identification	Major Class	Important Compounds	
Feijoa Juice (Unique, Apollo, Wiki Tu, Opal Star (NZ)	SPME	GC-MS (DB-WAX and 5MS column) GCO-MS	Esters Terpenes	Ethyl butanoate (apple-like); Ethyl pentanoate (fruity); Ethyl hexanoate (apple-like); Methyl benzoate (feijoa-like); Ethyl benzoate (fruity); Linalool (floral); 3-Octanone (mushroom); Myrcene (metallic); α -Cubebene (herbal); Humulene (herbal)	Peng et al. (2019)
Essential Oils from feijoa peel (Unique, Apollo, Wiki Tu, Opal Star (NZ)	SPME	GC-MS (DB-5MS column) GCO-MS	35 Terpenes 40 Esters 40 Alcohols	Methyl benzoate (Feijoa-like); Ethyl benzoate (fruit, fresh); Ethyl hexanoate (apple); α -terpineol (minty, fresh); Linalool (floral); α -Cubebene (herbal, sweet) and germacrene D (floral, sweet, herbal) β-Myrcene (metallic)	Peng et al. (2019)
Feijoa leaves (Tunisia)	HS-SPME	GC-MS (HP-5MS column)	19 Terpenes	limonene (36.2%); β-caryophyllene (27.8%); Aromadendrene (12.5%); α-copaene (6.6%); α-humulene (1.9%); Alloaromadendrene (2.7%); δ-cadinene (1.0%)	Mosbah et al. (2018)
Essential Oil from Feijoa Peel (France)	30°-35°C vacuum steam distillation	GC-MS (HP1 column)	29 Sesquiterpene (64.8%), 11 esters (9.1%)	β-caryophyllene (12%); ledene (9.6%); α-humulene (6.3%); β-Elemene (4.9%); δ-cadinene (4.8%); 3-octanone (1.1%); methyl benzoate (2.9%); α-Cubebene (1.4%); Allo-aromadendrene (1.4%); β-selinene (1.8%); cadina-1,4-diene (1.1%)	Fernandez et al. (2004)
Feijoa Fruit Flesh (NZ)	high vacuum steam distillation with liquid-liquid extraction	GC-MS (Carbowax 20M WCOT and OV-101 column)	Esters	(Z)-Hex-3enal (2.2%); (E)-Hex-2enal (1.7%); (E)-B-Ocimene (1.3%); (Z)-Hex-3en-l-ol (2.2 %); methyl benzoate (81.9%)	Shaw et al. (1990)
pineapple guava fruit (Hawaii)	vacuum distillation with solvent extraction	GC-MS (DB-1 column and DB-WAX column)	45 Sesquiterpenes 20 Alcohols	germacrene D (7.7 μg/g), Bicyclogermacrene (6.71μg/g), methyl benzoate (5.08 μg/g)), β-caryophyllene (4.91μg/g), (Z)-3-hexenyl benzoate (3.1 μg/g), linalool (2.67 μg/g), humulene (2.6 μg/g), and 3-octanone (2.33 μg/g)	Binder and Flath (1989)
Feijoa Skin Oil (NZ)	vacuum steam distillation with solvent extraction	GC-MS (Carbowax 20 M WCOT column)	Alcohols Esters	(Z)-3-Hexen-l-ol (20%), linalool (18.8); methyl benzoate (14.5%), 3-Octanone (5.8%) germacrene D (6.1%) β-caryophyllene (3.4%)	Shaw et al. (1989)
Intact Feijoa Fruit (NZ)	Headspace	GC-MS (Carbowax 20 M WCOT column)	Esters Terpenes	ethyl butanoate (29.6); trans -β-ocimene (4.7%); cis hex-3-enyl butanoate (8.5%); methyl benzoate (39.2%) ethyl benzoate (10.6%); cis hex-3-enyl hexanoate (2.5%)	Shaw et al. (1983)
Feijoa Fruit (Australia)	distillation	GC-MS	Esters	Methyl benzoate; Ethyl benzoate Ethyl acetate Ethyl butanoate Hexenal	Hardy & Michael (1970)

%: Relative abundance of each compound of class

Volatiles in essential oils extracted from feijoa peel were studied in several papers. The latest paper studied by Peng et al. (2019) identified 160 compounds. Essential oils from different cultivars had varied number of volatile compounds. These compounds were divided into 10 compositional groups, and esters (40 compounds) and terpenes (35 compounds) were identified as the dominant volatile classes. Furthermore, 24 compounds were identified by gas chromatograph-olfactory-mass spectrometry (GC-O-MS) as aroma active volatiles and contributed to the aroma profile of feijoa essential oils. Important aroma active compounds such as ethyl benzoate and *(E)*-geraniol gave 'fruity' odour; *α*-cubebene, germacrene D and cis-3-hexenyl hexanoate had 'sweet' odour and *(E)-β*-ocimene, *(4E,6Z)*-allo-ocimene, *α*-terpineol, *δ*-cadinene together with *α*-cubebene, germacrene D contributed to the 'herbal and grass' aroma of feijoa essential oils.

The study by Fernandez et al. (2004) also reported volatiles in essential oil extracted from feijoa peel. A total of 67 volatiles were detected which included 29 sesquiterpene hydrocarbons (64.8%), 14 oxygenated sesquiterpenes (17.8%), 11 esters (9.1%), 2 monoterpene hydrocarbons (0.5%) and 2 oxygenated monoterpenes (1%). Among them, *β*-caryophyllene (12%), ledene (9.6%), *α*-humulene (6.3%), *β*-elemene (4.9%) and *δ*-cadinene (4.8%) occupied for a large share. Another paper studied by Shaw et al. (1989) found a total of 34 volatiles in essential oils from feijoa peel. Linalool, methyl benzoate and cis-3-hexen-l-ol were the major compounds and together accounted for 53% of total compounds. However, for the volatiles' isolation and extraction part, Peng et al. (2019) used Solid Phase Microextraction (SPME) whereas Fernandez et al. (2004) and Shaw et al. (1989) used vacuum steam distillation in the temperature between 30°C-35°C and subsequent liquid-liquid extraction.

Aroma volatiles in feijoa juice was also studied by Peng, et al. (2019). A total of 63 volatiles were detected and 25 of them were identified as aromatic compounds. For aromatic volatiles, esters (11 compounds) with 'fruity and sweet' note and terpenes (10 compounds) with 'herbal and green' odour were the major groups. Methyl benzoate with 'feijoa-like' odour, (3Z)-3-hexenyl acetate with 'banana-like' odour and ethyl butanoate with 'apple-like' odour were the compounds with higher aroma intensities.

Instead of feijoa peel oils and juice, volatiles in feijoa leaves was also explored. A total of 31 compounds were found by Mosbah et al. (2018) using SPME combined with GC-MS. Among

them, sesquiterpene hydrocarbons (18 compounds) and monoterpenes (12 compounds) including Limonene (36.2%), β-caryophyllene (27.8%), Aromadendrene (12.5%), and α-Copaene (6.6%) were identified as dominant constituents in feijoa leaves.

Shaw et al. (1990) found a total of 15 volatiles in feijoa flesh by using high vacuum steam distillation and liquid-liquid extraction. Methyl benzoate was the most dominant compound and occupied for 82 % of the total volatiles. Shaw et al., (1983) studied the volatile constituents in feijoa intact fruit by using headspace combined with GC-MS and sniffing port. A total of 11 volatiles were identified with 93% esters, 5% acyclic monoterpenes and 2% aliphatic ketones. Besides, GC sniffing trials showed ethyl benzoate (10.6%), methyl benzoate (39.2%) and ethyl butanoate (29.6) were the significant aromas in intact feijoa fruit.

The same dealing with the fruit samples, referencing to Hardy & Michael (1970), 16 volatiles were found in feijoas by using vacuum steam distillation combined with GC-MS. Among these compounds, methyl benzoate and ethyl benzoate accounted for more than 90% of total concentration. Other components such as ethyl butanoate, ethyl acetate, ethyl cinnamate, ethyl p-anisate, 2-undecanone, 3-octanone, 2-heptanone, hexenyl acetate, 2-hexenal, 2-nonanone, and methyl p-anisate also been found had a strong ‘floral’ aroma and contributed to the aroma profile of feijoa to some extents.

Based on the studies of volatiles content in feijoa fruits, Shaw et al. (1983) and Shaw & Franich (1990) also did some researches on the changes of feijoa aroma compounds during ripening stages. Specifically, the fruit was stored at room temperature for around two weeks after natural abscission and named over ripen fruits. Comparisons were done between ripe and overripe fruits. According to their results, trans-β-ocimene, cis hex-3-enyl butanoate and ethyl butanoate decreased; Methyl benzoate increased first 7 days then kept constant; ethyl benzoate increased and dominant after 12 days at 17$^{\cdot}$C (Shaw et al., 1983). The comparable result has also been found by haw, Allen et al. (1990). They considered the sweeter and sickly aroma was formed because of the increased concentration of ethyl benzoate.

1.2.2 Overview of fruit volatiles

Volatile compounds are very important because they affect aroma quality and sensorial acceptance of the fruits. The aroma and appearance of the fruits can stimulate human appetite and build emotional links with consumer's past experiences with fruits (Siegmund, 2015). In addition, they not only affect consumer acceptance but also reflect ripeness, rancid and safety to some extent (El Hadi & Tao, 2013).

Food volatiles usually have low concentration, high polarity, varied boiling point and low chemical stability (Wampler, 2001). The relationship between their sensory acceptance by human and the quantitative composition varied in different aroma compounds depending on their aroma threshold (Obenland et al., 2012). The aromatic functions of volatiles need to be analysed under the help of gas chromatography-olfactometry (GC-O) and sensory evaluations.

Fruits volatiles can be divided into different groups by several classification methods. Based on compounds' functional groups, fruit volatiles could be separated into esters, alcohols, ketones, lactones, carboxylic acids, aldehydes, terpenes and terpenoids (Defilippi et al., 2009). Based on the forms of volatiles, compounds could be classified as free volatiles and bound volatiles (Baldwin, 2010). In addition, aromatically active volatiles and non-aroma active volatiles is also used to define fruit volatiles. fruits aroma is built on aroma active compounds (Beaulieu & Baldwin, 2002). Concentration and odour threshold of aromatic volatiles influence aroma intensities (Nuzzi et al., 2008).

Esters play the most important role in the aroma profiles for many fruits which generate 'floral, fruity and sweet' odour (El Hadi et al., 2013). Terpenes and terpenoids are common volatile compounds in fruits, spices and herbs (Breitmaier, 2006). Terpenes tend to impart 'herb, woody and citrus' notes (Nitsch et al. 2010) and terpenoids such as linalool, nerol and citronellol tend to impart 'weak lemon, floral' aromas in fruits (Lu & Huang, 2012). Aldehydes are also a popular aromatic compounds group in food because of their low threshold (Siegmund, 2015). Hexanal with 'green and grassy' note is a very popular C 6 aromatic aldehyde and exist in many fruits (Pino, 2014; Wen et al., 2014; Yang, 2019).

1.2.2.1 Volatiles precursors and their biosynthetic pathways

Fruit volatiles can be synthesized both in intact fruits and disrupted tissues (Defilippi et al., 2009). Fatty acids, amino acids, terpenoids and carotenoids are the main precursors of fruits volatiles (Buttery and Ling, 1993b). After various enzymatic reactions such as methylation, hydroxylation and acetylation, these precursors can be transformed into volatile compounds. The amount, composition and availability of related enzymes and precursors substrates greatly affect the biosynthesis of fruits aroma volatiles (Song & Bangerth, 2003).

Fatty Acids Pathway

Two main enzyme modifications, *β*-oxidation and lipoxygenase (LOX) reactions, utilize lipids to synthesize aroma compound. *β*-Oxidation is believed to be the main metabolism of volatiles formation in intact fruits, whereas the LOX pathway is considered to be the dominant volatiles pathway in disrupted fruits (Demole, 1985). However, with the change of fruits' maturity, in ripe fruits, the cell wall and membranes tend to be more permeable, which results in higher activity of LOX metabolism even in intact fruits (Defilippi et al., 2009).

β-oxidation pathway

β-oxidation is the primary metabolism for the formation of alcohol in fruits (El et al., 2013). As shown in figure 1, the pathway starts with losing C2 units from fatty acids to form fatty acid acyl CoAs derivatives. Then the derivatives are transformed to short-chain acyl CoAs by removing 2 carbons during every *β*-oxidation cycle under the help of free CoA, nicotinamide adenine dinucleotide (NAD) and flavinadenine dinucleotide (FAD). Followed by some reduction reactions, the acyl CoAs is reduced to aroma volatile aldehydes.

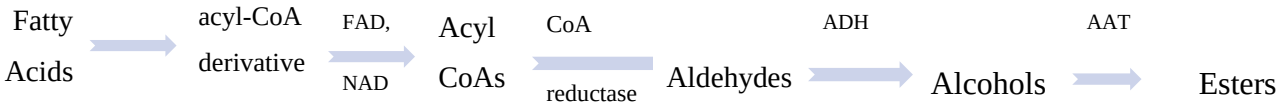

Figure 1.1: *β*-oxidation pathway of fruit volatiles generation

The aldehydes can be further reduced to alcohols by alcohol dehydrogenase (ADH). When the activity of alcohol acyltransferase (AAT) is high, alcohol can act as substrates to generate esters (Bartley et al., 1985). Besides aldehydes, alcohols and esters, lactones can be also synthesized by *β*-oxidation.

Lipoxygenase (LOX) pathway

LOX pathway is a frequent metabolic way of polyunsaturated acids to from aroma compounds include C6 and C9 aldehydes and alcohols (Liu et al., 2010). Hexanal, cis-3-Hexenal and trans-2-hexanal are formed from linolenic acid and linoleic acid (Feussner & Wasternack, 2002). LOX metabolism generally occurs in disrupted tissues because of higher substrates and enzymes availability as well in intact ripe fruits because of a lower membrane permeable (Siegmund, 2015).

As shown in figure 2, the pathway starts with the oxygenation of polyenoic fatty acids. To be exact, catalysed by LOX, polyunsaturated fatty acids at carbon 9 and carbon 13 positions are oxidized, producing 9-hydroperoxy and 13-hydroperoxy derivatives. The following reactions are metabolized by allene oxide synthase and hydroperoxide (HPL) to generate volatile compounds (El Hadi et al., 2013).

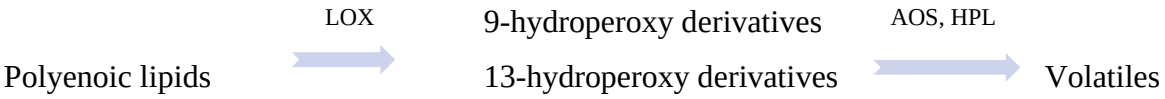

Figure 1.2: LOX pathway of fruit volatiles generation

Amino Acids metabolism

Amino acids are also major precursors of volatile compounds and can synthesize a wide range of aroma volatiles compromising alcohols, acids and esters (Baldwin et al., 2002). Alanine, leucine, aspartic acid isoleucine, valine and phenylalanine have been discovered as volatiles precursors (El Hadi et al., 2013). Ethyl ester compounds in strawberry have been reported as the final product of amino acid metabolism and AAT esterification (Beekwilder et al., 2004; Pérez et al., 2002).

The pathway starts with the transamination and deamination of the amino acids to α-keto acid. Then the carboxyl group in α-keto acid is removed by decarboxylation, followed by an array of oxidation, reduction or esterification, generating volatiles (Reineccius, 2005).

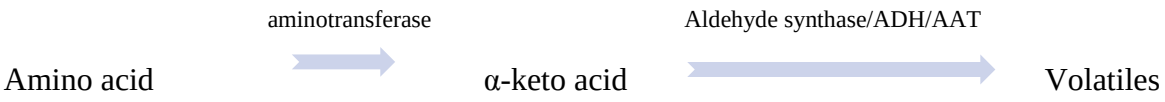

Figure 1.3: Amino acid metabolism of fruit volatiles generation

Terpenoids metabolism

Terpenoids are comprised of diterpenes (C20), homoterpenes (C16 and C11), sesquiterpenes (C15), monoterpenes (C10) and hemiterpenes (C5). Despite some terpenoids are generated by acylation, dehydrogenation and hydroxylation of primary terpenoids skeletons, most of these terpenes are catalysed by terpene synthases (TPSs) family (Breitmaier, 2006).

There are two pathways of terpenoids independently based on different intracellular locations, although both of them share the same terpenes precursors dimethylallyl diphosphate (DMAPP, C5) and its allylic isomer isopentenyl diphosphate (IPP) (Siegmund, 2015). One pathway called methylerythritol phosphate (MEP) (or MVA-independent) occurs in cell plastids. IPP and DMAPP are formed from MEP intermediate, which is generated by the initial precursors glyceraldehydes 3-phosphate and pyruvate. This pathway leads to the formation of most monoterpenes (El Hadi et al., 2013). From Figure 4, one molecule of DMAPP with one IPP is catalysed by geranyl pyrophosphate synthase (GPPS) and generate geranyl pyrophosphate (GPP, C10). Finally, C10 GPP is catalysed by TPSs and form monoterpenes (Tholl, 2006).

Figure 1.4: MEP pathway of fruit volatiles generation

The other terpenoids pathway called mevalonic acid (MVA) pathway occurs in cytosol. IPP is formed under the condensation of acetyl CoA (Mahmoud & Croteau, 2002). From Figure 5, geranyl pyrophosphate (FPP, C15) is generated from enzymatic reaction which is triggered by farnesyl pyrophosphate synthase (FPPS) and two molecules of IPP with one molecule of DMAPP. Comparable with the MEP pathway, under the assistance of TPSs, sesquiterpenes are formed.

Figure 1.5: MVA pathway of fruit volatiles generation

Carotenoids metabolism

Carotenoids can be the precursors of some important linear C8, C13, C18 and cyclic C5 isoprene volatiles compounds such as 6-methyl-5-hepte-2-ol, β-ionone (Lewinsohn et al., 2005). There are three steps to form carotenoids volatiles. The first stage is the generation of apocarotenoids. This oxidative cleavage reaction is catalysed by carotenoid cleavage dioxygenases (CCDs) family (El Hadi et al., 2013). Although CCDs exist throughout the whole ripening progress, this reaction only occurs on over ripe fruits (Simkin et al., 2004). The second step is the transformation of apocarotenoids to polar volatiles precursors which is followed by the last step of an acid-catalysed reaction to change the aroma precursors to the final volatile products (Winterhalter & Rouseff, 2002).

1.2.2.2 Factors influencing volatiles generation

Fruit volatiles are generated by complex mechanism and having varied compositions depending on many factors. These factors include fruits maturation and ripening stages, genetic makeup (or cultivars difference), fruits type (climacteric and non-climacteric fruits), pre- and post-harvest conditions, storage atmosphere and other chemical applications such as gaseous or calcium treatments.

Maturity and ripening stages

Fruit ripening is a process that starts with the latter stages of growth (fruit formation) until the early of fruit senescence (which refers to the death of tissues of plants) to achieve palatable food quality (Fellman et al., 2000). During this process, fruit characterizations including texture, flavor, odor, taste, and appearance (color) will change. These changes mainly derivate from physical and biochemical changes in fruits (Dotto et al., 2006). For example, with the aging of the fruits, the content of chlorophyll will decrease, which results in the change of pigment generation, followed by the change in fruit color. Moreover, as the ripening develops, the breakdown of the polymeric structure of amino acids, fatty acids and carbohydrates will happen. The loss of pectin softer the firmness of fruits. Moreover, the generations of low molecular weight volatiles develop more aroma active compounds (Siegmund, 2015).

During the ripening of fruits, ethylene plays an important role. As a phytohormone, ethylene is in charge of modulating a series of maturation process (Osorio et al., 2013). With the help of 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase and ACC synthase, substrates S-adenosylmethionine and ACC react, forming ethylene (Bleecker & Kende, 2000). Then the produced ethylene will bind to ethylene receptor in cell membrane, which will lead to a single transduction cascade. The transduction cascade will activate the ethylene response factors and activate the ethylene responsive genes expressions. Finally, the expressed ethylene genes will result in the fruit maturation and senescence (Bapat et al., 2010). The ethylene effects on fruit maturity are obvious on climacteric fruits such as banana, apple. Because ethylene formation can active autocatalysis in climacteric fruits, active autocatalysis can promote the production of ethylene, as a consequence, rising the rate of ripening and maturation (Siegmund, 2015).

Cultivar differences

Aroma volatiles in grapes from several different genotypes was explored by (Yang et al., 2011). According to their results, different cultivars had different dominant volatiles class and content. In *Vitis. vinif*era grapes, terpenes were the dominant aroma volatile class whereas in *labrusca* grapes and in grapes' cultivars of *vinifera* and *amurensis* grapes, esters were the major class.

Different genes in different fruits and cultivars could lead to the variations in the content and availability of volatiles precursors substrates and related enzymes (Canosa et al., 2011). For example, fruits or cultivars with lower ACC tend to have a lower number of volatiles. Fruits and cultivars with lower AAT content tend to have less ester compounds (Marsili & ProQuest, 2012).

1.2.3.2.3 Harvest effects

According to Auldridge et al., (2006), abundant rainfall before harvest lowered the intensity of aroma volatiles in tomatoes. Factors such as sunlight, humidity, fertilizers, soil condition can largely affect the growth of fruits, subsequently influencing the formation of aroma volatiles in fruits (Fellman et al., 2000). Furthermore, factors including temperature, storage atmosphere, chemical usage after fruit harvest will significantly influence the aroma of fruits. Particularly, low temperature (below 5℃) can easily cause chilling injury of fruits, which will lower the volatile content especially esters and lactones (Zhang et al., 2011). Low temperature may also decrease the activity of ADH and causes the loss of linalool, hexanol, tran-2-hexenal in tomatoes (De León-Sánchez et al., 2009). Moreover, although low oxygen content can maintain the quality and extend the shelf life of fruits, it may also induce anaerobic respiration and cause some adverse odour effects (Bapat et al., 2010).

1.2.3 Glycosidically bound volatiles

1.2.3.1 Free and glycosidically bound volatiles

Generally, from the origins of aroma volatiles, compounds can be divided into two groups, namely primary and secondary compounds. Primary aroma volatiles are generated from normal ripening process of plants by some catabolic and anabolic mechanisms, whereas the secondary aroma volatiles are formed from non-volatile precursors in disrupted tissues by enzymatically catalysed reactions and autoxidative reactions (Christensen et al., 2007). From the form of compounds, volatiles could be divided into free and glocosidically-bound volatiles,

Free volatiles are the primary compounds present that can be detected by methods such as gas chromatography-mass spectrometry (GC-MS) and gas chromatography-olfactometry (GC-O), directly after extraction (e.g. solvent extraction, Headspace Solid phase microextraction (HS-SPME). Bound volatiles are secondary compounds presented as flavorless glycoconjugates in non-volatile forms (stahl-Biskup et al. 1993, Sarry and Gunata, 2004). The volatile compound (which is called aglycone in bound volatiles) is bound to sugar moieties and lose its aromatic functions. However, volatiles can escape and contribute to aroma once they are released from the bound form. Glycosidic bound volatiles was firstly found in rose (Francis & Allcock, 1969) and volatile precursors was found in grape for the first time (Williams et al., 1982).

1.2.3.2 General background of glycosidically bound volatiles

Glycosidically bound volatiles have low reactivity and no odour activity in fruits, O-*β*- D -glucosides and O-diglycosides are the common glycosides of bound volatile compounds, and triglycosides have also be detected as aroma glycosides (Sarry & Günata, 2004). For example, the content of vanillin present in vanilla beans is relatively low, however, the glucovanillin (*β*-D-glucoside of vanillin) concentration is quite high in fresh vanilla plants. By enzymatic processing, free form vanillin can be released from its bound form (Pérez Silva et al., 2011).

In fruits bound volatiles, the sugar moieties could be *α*-*L*-rhamnopyranose, *β*-D-glucopyranose, *β*- D -apiofuranose, *α*-L-arabinopyranose, *α*-L-arabinofuranose, and *β*- D -xylopyranose. The aglycones part are usually phenols, terpenoids, norisoprenoids, alcohols and phenyl propane derivatives (Sarry and Günata, 2004, Stahl-Biskup et al., 1993).

The formation of glycosidic bound volatiles may come from a defensive mechanism to protect the cell membrane form lipophilic compounds destroying (Stahl-Biskup et al., 1993, Lucker et al., 2001). There is also a translocation theory to explain the forming of bound volatiles. To be exact, similar with the sugar movement in plants from leaves to other organs such as roots. glycosylated lipophilic volatile compounds can be regarded as sugar moieties and easily pass through the cell membrane to reach other organs in the plants (Bowles et al., 2006). Furthermore, Dietz et al. (2000) and Samuels et al. (2002) found glycosides in the apoplast of the plant cell along with their corresponding glycosidases. Apoplast is one of transport pathways in the plant cell.

1.2.3.3 Hydrolysis of glycosidically bound volatiles

The hydrolysis of bound volatile can achieve the transformation of odourless volatiles to odour active, thus enhancing sensory characteristics of fruits (Hjelmeland & Ebeler, 2015). Flavour volatiles such as C13-norisoprenoids, monoterpenes, hydroxy esters, fatty alcohols and benzenic compounds can be released from glycosidic precursors after enzymatic or acid hydrolysis (Lu, et al., 2009a; Gunata et al., 1985; Rodríguez-Bencomo et al., 2011). The hydrolysis of bound volatile has been used in many industries for aroma/flavour intensification, differentiation, diversification and modification (Liand others 2013). Wine and juice production are good examples of food processing to get desirable flavor and aroma. Fan and others (2012), Daenenand others (2008) and Li and others (2013) explored the hydrolysis of glycosidic bound volatiles in orange juice, fruit beer and mango wine respectively. The hydrolysis of different aglycones is not the same. For example, the volatile aroma of monoterpene glycosides can be directly produced after hydrolysis. Whereas for norisoprenoid glycosides, acid catalyzed rearrangements and other chemical reactions may be needed after hydrolysis to produce volatile active compounds. The procedures of releasing volatile aroma vary with the types of aglycones (Sefton et al. 2011, Winterhalter and Skouroumounis, 1997).

1.2.3.4 Changes of glycosidically bound volatiles during ripening

The content of glycosically bound volatiles is not constant during the ripening stages of the fruits. They are regarded as an important potential backup of flavor compounds. Gunata et al. (1985) found that both free and glycosidic bound terpenols volatile increased with maturity in grapes, the free ones decreased in the over-ripe stage while the bound ones continued to increase. Furthermore, Garcia et al., (2013) found the bound volatile content in kiwifruits tended to increase as the fruit ripened from under-ripe to ripe, and then declined in over-ripe fruit. According to Reineccius (2016), the concentration of bound volatile is as ten times bigger as that of free volatiles. During maturation, storage, processing or under the assistance of heat, acids and enzymes, the odorous volatile aglycones can be released to contribute to the aroma of the fruits.

1.2.5 Free and bound volatiles in varies fruits

At present, there is no reference on the bound volatiles of feijoa fruits. However, there are a few papers on bound volatiles in other fruits such as cherry, grapes, mango, lemon, kiwifruits, citrus has been studied. A few recently typical references have been concluded in Table 3 and the following paragraphs.

Table 3: References summary of aroma active volatiles in varies fruits

Fruits	Free volatiles	Bound volatiles	References
Rubus corchorifolius L. f. fruit during ripening	Hexanal - green; 2-Hexenal - grass, herbaceous; 1-Hexanol - flower, green, grass; 2-Heptanone - cinnamon, sweet; Myrcene - green; Ethyl Hexanoate - green, apple; D- Limonene - fruity, lemon; linalool - citrus, floral, sweet,	Benzaldehyde - caramel, fruity; terpinolene - floral, sweet, sour; linalool - spices, clove, honey; *α*-terpineol - anise, mint; geraniol - rose, geranium	Yang (2019)
Air-dried raisins from three seedless grape cultivars	Pentanal - pungent, strong acid; Nonanal - citrus, aldehyde; Ethyl hexanoate - apple, sweet, *p*-Cymene - citrus, carrot; trans-2-Hexenal - green, bitter,	Linalool - floral, citrus; 1-octen-3-ol - mushroom, grass, rubbery; 2-Hexanal - green, grass	Wang et al. (2015)
Mulberry	Ethyl butanoate - banana, pineapple, strawberry; cis-3-Hexenal - green, leaf; trans-2-Hexenal - apple, green; trans-2-Hexen-1-ol - green; 1-Octen-3-ol - mushroom; Linalool - flower, lavender	Hexanal - grass, green; 3-Methyl-1-butanol - malt, burnt; Octanal - fat, soap, lemon, green; Nonanal - fat, citrus, green; trans -2-Nonenal - cucumber, fat; Eugenol - clove, honey	Chen et al. (2015)

Table 3 continued

Cherry (*Prunus avium L.*)	hexanal - grassy, green; trans-2-hexenal - green, fruity, fresh; Methyl benzoate - sweet; trans-2-Nonenal - cucumber, green; linalool - citrusy, floral, sweet; benzyl alcohol and benzaldehyde	Octanal - fruity, green, lemon; Nonanal - citrusy, green; Decanal - sweet, citrusy, green; Eugenol - clove, honey, spice; Geraniol - floral, rose; Benzyl alcohol	Wen et al., (2014)
Juice and Peel of Eureka Lemon	*α*-Thujene - sour, flower; *α*-Pinene - sweet, fruit, grass; *β*-Myrcene - orange; D-Limonene - lemon; Ocimene- fruit; Terpinene - lemon, flower; Alloaromadendrene - lemon; Farnesene - lemon, flower; *β*-Gurjunene - mint; *γ*-Selinene - lemon;	Nerol – flower, sweet; Linalool oxide - sweet, fruit; *α*-Terpineol - lemon; Vanillin - mint, lemon, flower, sweet; Benzoic acid; Benzyl alcohol	Zhong et al. (2014)
Kiwifruit (*Actinidia eriantha*)	-	cis-3-Hexen-1-ol - grass; Isoamyl alcohol - alcoholic; Benzyl alcohol - floral; 1-Octen-3-ol - mushroom; Linalool - lavender; Hexanal - grass; trans-2-Hexenal - green	Garcia et al., (2012)

Bound volatiles in Satsuma mandarin fruit at four different ripening stages has been studied by Gao et al. in 2018. A total of 56 bound volatile compounds were identified. Monoterpenes (monoterpene hydrocarbons and oxygenated monoterpenes) were the most abundant chemical class in bound volatiles. Regarding the volatiles change with ripening stages, the total number of bound volatiles increased to peak before decreasing during ripening. The trends of monoterpene hydrocarbons, aldehydes and phenylpropanoids were comparable to the total bound volatiles, whereas oxygenated monoterpenes increased during ripening.

Three mulberry cultivars were studied for its free and bound volatiles by Chen et al. in 2015. A total of 55 free volatile compounds and 57 bound volatiles were identified. Aldehydes were the predominant free volatiles group and C 6 alcohols were the most abundant aromatic alcohols. For the bound volatiles part, the major class was the alcohols (2590 to 4025 μg/L), which ranged from 72% of the total bound volatiles. Compared with free volatiles, bound volatiles were found in lower amounts in all the three cultivars.

In addition, free and bound volatiles in five red grape cultivars has been explored (Canosa et al., 2011) . From the results, 35 free volatile and 61 glycosidically bound compounds were identified and quantified by GC-MS. For the five cultivars, Pedral shows the highest glycosidically bound compounds content. Besides, C6 - compounds were identified in lower concentrations in the bound fraction than that in the free fraction.

Yellow-fleshed clingstone nectarines with three ripening stages were studied (Aubert et al., 2003). A total of 54 bound volatiles were identified. Among those and terpenes (30) were the most abundant. As for the ripening stages, the concentration of C13 norisoprenoids and monoterpenols were greatly increased with the maturity process.

Lalel et al. (2003) studied glycosidically bound volatiles in ‘Kensington Pride’ mango pulp and skin from different ripening stages. A total of 92 aglycones and 85 aglycones were found in pulp and skin, respectively. Aromatic amino acid metabolites were the most abundant constituents in skin while terpenes were the most abundant and increased with ripening in pulp. At green and half ripen stages, bound volatiles generated by aromatic amino acid, lipid and carotenoid metabolism were higher in the skin than in the pulp. Bound acids volatiles were also higher in the skin at ripe stages.

Glycosidically bound volatiles in mango were also explored (Sakho et al., 1997). Acid and enzymatic hydrolysis were both used to release the bound precursors. According to the results, cis-Hexen-3-ol, hexanol, hexanoic acid, benzyl alcohol, linalool oxides, furaneol, R-terpineol, carvacrol, vanillin, benzoic acid, myristic and stearic acids were identified as aglycons in bound volatiles. Glucose, arabinose and rhamnose were detected as sugar moieties. Among them, glucose was the most important sugar moiety and occupied the most significant share.

1.2.6 Analysis of volatile compounds

Volatile compounds in foods, especially fruits, are very important. Good practice methods and scientific analysis are necessary to give a clear and precise aroma profile of the aimed food sample. A few challenges including sample complexity, sample composition, compound stability, low threshold of the constituents and low concentration of the aromatic compounds need to be considered when preforming aroma profile analysis. The whole process from sample preparation, solvent selection, free volatile isolation and extraction, bound volatile precursors extraction, bound volatile hydrolysis, volatiles separation and identification, volatiles quantification to final statistical analysis are essential for volatile profile analysis.

1.2.6.1 Sample preparation

Sample selection is the first step of volatile analysis. Due to the varied growth conditions, environmental situations, different ripening stages, different cultivars, the results can vary significantly. For example, for feijoas, the 'Unique' and 'Apollo' cultivars tend to have higher firmness than others (East et al., 2009); Ripe feijoas usually have softer and smoother skin and bigger size compared with the unripe ones (Schotsmans et al., 2011). Parameters such as colour, texture, total soluble solids content can indicate the maturity of the fruits (Fellman et al., 2000).

After sample selection, sample transportation and storage should also be controlled. Samples should be stored in cold environment (lower than – 20·C) immediately after picking until before using to avoid compound losses, microbiological deterioration, and some enzymatic reactions (Millard reaction). Besides, organic solvent and water should have high purity and proper operation environment and facilities are necessary to avoid further contamination (Reineccius, 2006b).

1.2.6.2 Isolation and extraction of Volatiles

Because of the variety of the sample properties, different isolation and extraction methods are employed for different aims. The isolation and extraction should be selected according to sample types, concentration of volatile compound and distribution in the food sample, boiling point and volatility of the interested compounds and the type of analysis (quantitative and/or qualitative) (Bemelmans, 1981). In general, two types of extraction and isolation methods, solid phase microextraction (SPME) techniques and solvent base extractions, were mainly used in recent studies.

Headspace SPME

Headspace SPME is largely selected in aroma extraction nowadays (Parker, 2014). This method is fast, easy, sensitive and can be directly connected with GC-MS analysis (Wardencki et al., 2004). By using a fiber coated with adsorbents, after dynamic headspace, the free volatiles are absorbed in the fiber and can be disported when transferred to GC. Free and bound volatile extraction in citrus fruits (Ren, 2015), cherry (Wen et al., 2014), lemon (Zhong et al., 2014), Satsuma mandarin fruits (Gao, 2018) and air-dried raisins from seedless grapes (Wang et al., 2015) were detected by using SPME technology (listed in Table 4). However, this method also has its disadvantages. A big amount of water could be extracted from liquid samples, although absorbent trap has been used to eliminate this problem, a new problem of air contaminations raised (Sugisawa, 1981). Besides, poor reproducibility of the results is also a drawback of HS-SPME (Teranishi and Kint, 1993).

Supercritical fluid extraction (SFE)

Due to the high temperature of steam distillation, SFE is a gentle method and widely used in food samples such as tea seed oils (Rajaei et al., 2005), black cumin (Pourmortazavi et al., 2005) and coriander seed (Grosso et al., 2008; Hamm et al., 2005). A supercritical fluid is between liquid and gas when temperature and pressure above its critical point. This fluid can effuse through solids like a gas, and dissolve food matrix like a liquid. It is suitable as a substitute for organic solvents in a range of industrial and laboratory processes (Brunner, 2005). Suitable pressure and temperature are used to assist the extraction. However, specialized equipment is needed (Mohamed & Mansoori, 2002).

Liquid extraction

Another method using organic solvent is also quite popular in aroma profile analysis as aromatic volatiles can dissolve in organic solvents successfully (Reineccius, 2002). Ethers, hydrocarbons, chlorinated hydrocarbons are common solvents for volatiles extraction (Tholl et al., 2006). Hydrocarbons such as diethyl ether and pentane are common solvents which are cheap and highly efficient. They are widely used when ethanol and water are not wanted such as in alcoholic beverages (Reineccius, 2005). Large amount of ethanol may interfere with other aromatic compounds. Therefore, hydrocarbon solvents can efficiently solve this problem.

Besides, for the separation of funnel extractions, Chlorinated solvents are commonly used (Sugisawa, 1981, Teranishi and Kint, 1993).

Vacuum steam distillation

Steam distillation was also a popular extraction method. However, the high temperature used for distillation might destroy the protein and some micronutrients in the food samples (Teranishi and Kint, 1993). Therefore, distillation under vacuum is commonly used to protect the samples by using a lower temperature, which has been widely applied in the extraction of volatiles from Spices and grains (Sugisawa, 1981). However, generation of artefacts may be a potential problem connected with vacuum steam distillation (Masango, 2005).

Solvent-assisted flavour evaporation (SAFE)

High vacuum transfer (HVT) is the initial technology of solvent-assisted flavour evaporation (SAFE). Volatiles are extracted by mixing the sample with 2-3 volumes of organic solvent such as dichloromethane (DCM), pentene, diethyl ether. After filtration or centrifugation, the food sample is removed from the solvent containing volatiles. Thereafter volatiles extraction is achieved by transferring volatiles from high temperature area to a low temperature area. Based on this mechanism, SAFE can achieve better separation of high boiling point polar compounds (Engel et al., 1999). Dichloromethane and diethyl ether are popular extraction solvents used in SAFE.

Solid-phase extraction (SPE)

SPE is used for the liquid sample containing small number of free volatiles. It can be used to extract or concentrate bound volatiles precursors (glycoside) very efficiently (Williams et al., 1995). Resins or cartridges are used to achieve SPE. A sorbent material within glass or plastic cartridge is pre-treated with a solvent. Liquid sample is firstly loaded onto the sorbent and desired compounds will be retained on the sorbent. Later the sorbent is washed with some solvents to remove undesired compounds and eluted with suitable solvent to collect wanted compounds. Examples and cartridge selection can be seen in Table 4.

Table 4: References summary of Free and bound volatiles extraction methods

Fruits	NaCl addition	Cartridge selection	Solvent to remove free volatile	Bound extraction solvent	Enzyme	Enzyme hydrolysis	aromatic compound detection	Reference
Rubus corchorifoliu s L. f. fruit	10ml juice with 2.00g NaCl	LC column (400 *40 mm) packed with Amberlite XAD-2 Resin	diethyl ether-pentane (1:1 v/v)	methanol	Aspergillus Niger pectinase (1.0 U/mg)	37℃ for 48h	OAVs	Yang (2019)
Satsuma mandarin	1.00g sample powder in 5ml NaCl solution	SPE LC-18 resins	dichloromethane	methanol	Rapidase AR2000	37℃ for 48h	-	Gao (2018)
Citrus fruits	10ml juice with 3.60g NaCl	50*1 cm Amberlite XAD-2 column	pentane: diethyl ether (1:1, v/v)	methanol	β-glucosidase (40 mg, 7.7 units/mg)	40℃ for 48 h	-	Ren (2015)
Air-dried raisins	-	Cleanert PED-SEP column	dichloromethane	methanol	Rapidase AR2000	40℃ for 16h	OAVs	Wang et al. (2015)
Mulberry	5ml juice with 1.00g NaCl	Clearnert PEP column (200mg, 6ML)	dichloromethane	methanol	AR 2000	40℃ for 16h	OAVs	Chen et al. (2015)
Eureka Lemon	10ml juice with 3.60g NaCl	50 * 1 cm column filled with XAD-2 resin	diethyl ether / pentane (1:1)	methanol	β-glucosidase	40℃ for 48 h	GCO	Zhong et al. (2014)

Table 4 continued

Cherry	5ml juice with 1.00g NaCl	Cleanert PEP-SPE resins	dichloromethane	methanol	Rapidase AR2000	40℃ for 16h	OAVs	Wen et al. (2014)
Kiwifruit ('Hayward' and 'Hort16A')	-	50 ml Amberlite XAD-2 column	pentane: diethyl ether (1:1, v/v)	methanol	Rapidase AR2000	37℃ for 48h	GC-O	Garcia et al. (2013)
Kiwifruit (*Actinidia eriantha*)	-	50 ml Amberlite XAD-2 column	pentane: diethyl ether (1:1, v/v)	methanol	Rapidase AR2000	37℃ for 48h	OAVs	Garcia et al. (2012)
Blackberry	-	Bakerbond SPE C18 disposable extraction cartridge	dichloromethane	methanol	Macer8 FJ enzyme solution	45℃ for 12h	-	Du et al., (2010)
Tomato	-	500mg C18 Sep-Pack cartridge	pentane	methanol	AR 2000 solution (100μl, 2.5%, W/V)	40℃ for 48h	-	Ortiz-Serrano and Gil (2009)
Mango	-	50 × 1 cm i.d. column packed with 20 mL of Amberlite XAD-2	pentane/dichloromethane mixture (2:1, v/v)	methanol	5mg/ml of almond *β*-glucosidase	40℃ for 16h	-	Lalel et al., (2003)

1.2.6.3 Hydrolysis of bound volatiles

Acid hydrolysis

As discussed in the glycosidically bound volatile part (Section 1.2.3.3), the bound volatiles can be separated by acid or enzyme hydrolysis. Acid hydrolysis liberates different aglycones at different acidity. Therefore, it is important to operate all the samples at the same pH. Williams et al. (1982) used acid hydrolysis on the study of grape volatile profiles. According to their results, at pH 3, the liberated volatiles was mainly -terpineol and linalool. At pH 10, other monoterpenes were released during the maturation of wine (Maicas and Mateo, 2005). However, the whole grape aroma profile is mainly neryl derivatives, geranyl and linalyl. This indicates that acid hydrolysis is insufficient to detect the intact aroma profile of the foods and it is not easy to find a suitable pH that can describe the whole profile as much as possible.

Enzymatic hydrolysis

Compared with acid hydrolysis, enzymatic hydrolysis is easier and more representative (as shown in Table 4). Although the number of volatiles released by enzymatic hydrolysis depends on enzymes specificity and the nature of sugar moiety, enzymatic hydrolysis can give aroma profiles closer to the original food samples (Williams, 1993). Enzymes used in bound volatiles hydrolysis can come from microbial or plant. Rapidase AR200 (a commercial enzyme with β-glucosidase, α-arabinofuranosidase, α-rhamnosidase, and β-apiosidase), β-glucosidase, pectinase and hemicellulase are all the enzymes that commonly used in the liberation of bound volatiles (Maicas and Mateo, 2005). There are some disadvantages of enzyme hydrolysis in industrial purpose. According to Maicas and Mateo (2005), enzymatic hydrolysis can also release some undesirable volatiles such as vinyl phenols in wine production, which will influence the attractive aroma of wine. Sefton and Williams (1991) also reported that some oxidation artefacts were generated by using fungal glycosidases hydrolysis.

1.2.6.4 Identification and quantification of volatile compounds

Gas chromatography-mass spectrometry (GC-MS) is an important system to analyze volatiles. GC is a highly sensitive method to separate different volatile compounds and MS is a method used for compound identification and quantification. For electron impact (EI) mode, fragmentation patterns of the compound could be identified to generate spectra and later related to their chemical structures (Mussinan, 1993).

Volatilization of samples in injector (a hot inlet port), separation of sample mixture in column and detection compounds by the detector are the three main parts of GC. A carrier gas such as helium, nitrogen, hydrogen is important to transfer sample volatiles from injector through the column into the detector. The efficiency of GC depends on the stationary phase (column) and a mobile phase (carrier gas) (Kitson et al., 1996). Two types of stationary columns are common in scientific research. Polar column such as DB-WAX is likely to give a better separation of polar volatile compounds, whereas non-polar column such as DB-5MS tends to give a better separation on non-polar compounds (Siegmund, 2015).

The MS principle is to measure the mass-to-charge ratio of gas phase ions (m/z). The electrons breakdown the molecules, causing the molecule to fragment. Next a relative abundance of each ionic species is recorded by the computer system (Parker, 2014). A chromatogram with the number of peaks and the retention time (RT) of each peak is then generated.

Constant GC conditions can give a constant retention time, however, different isolation abilities from different procedures will give different RT. Therefore, using retention time to identify compounds is not accurate. The Kovats index, also known as linear retention index (LRI), is an index that connects retention times with an alkane series (Mussinan, 1993) and can be calculated using the following equation (Siegmund, 2014).

$$LRI = 100 \times (T - T_n) / (T_{n+1} - T_n) + n$$

This equation shows the calculation of LRI, where T represents the retention time of target compound, T_n represents the RT of the alkane compound detected before target compound, and T_{n+1} is the RT of the alkane compound detected after the target compound.

Positive identification of target compounds can be conducted using chemical standards. While for those compounds without available standards, identification shall be conduct by comparing the LRI and mass spectra with the records in database/library or references. NIST/EPA/NIH Mass Spectral Library, Wiley Registry 10th Edition/NIST 2012 Mass Spectral Library are the common online libraries that can be used for compounds identification (Siegmund, 2014). Besides, for the compounds which are present in a trace amount, selective detectors are recommended for better results (Mussinan, 1993).

Regarding quantification methods, rough concentration can be calculated in percentage based on peak area ratios. However, because different compounds have different sensitivity to the detectors, this method is not accurate (Reineccius, 2006). Using standard (including internal standard and/or external standards) increases accuracy in the quantification of the compounds. The internal standards should be stable and not affecting sample peaks (Buttery, 1993). On the other hand, external standards can be used to generate standard curves. The concentration of the target volatiles can be calculated according to the equation conducted from the standard calibration curves.

A food sample could have more than hundreds of compounds but only a small portion of them contribute the aroma (Buttery, 1993). Volatile compounds from lemon such as caryophyllene, farnesene and alloaromadedrene can be detected by GC-MS but not by olfactory tests (Zhong et al., 2014). Only 10 compounds have been found as aromatic in fresh tomato (Buttery et al., 1987). This is because of the high thresholds or/and low quantity of the compounds. Gas chromatography-olfactory (GC-O) is a technique that enables human to physically detect the aromatic volatiles. After the separation, two parts of the compound will go to the GC and MS detector, and the rest will flow to a humidified nosepiece. The aroma

can be assessed by trained individuals, aroma description and intensity are recorded by the candidates.

Odor Active Value (OAV) is also a value that indicates the importance of a specific volatile compound to the odour of foods. The OAVs represent the ratio between the concentration of target aroma compound to its odour threshold in water or air (Erten & Cadwallader, 2017).

OAVs = Aroma compounds' concentration / Odour threshold

When OAV is over 1, the compounds can be defined as aroma active compound and contribute to the fruit aroma profile. However, high OVA value does not mean high intensity of volatile compounds, the sensorial intensity is not proportional to compounds' OAV. Some low OAV volatiles can contribute greater odour to the final aroma profile of food (Grosch, 2001).

Chapter Two:
Materials and Methods

The overview methods of feijoa volatiles characterization was shown in Figure 6. The whole structure is comprised of eight parts, which are fruits collection, texture analysis, fruits storage, free volatiles preparation, bound volatiles preparation, compounds identification, compounds quantification and data analysis.

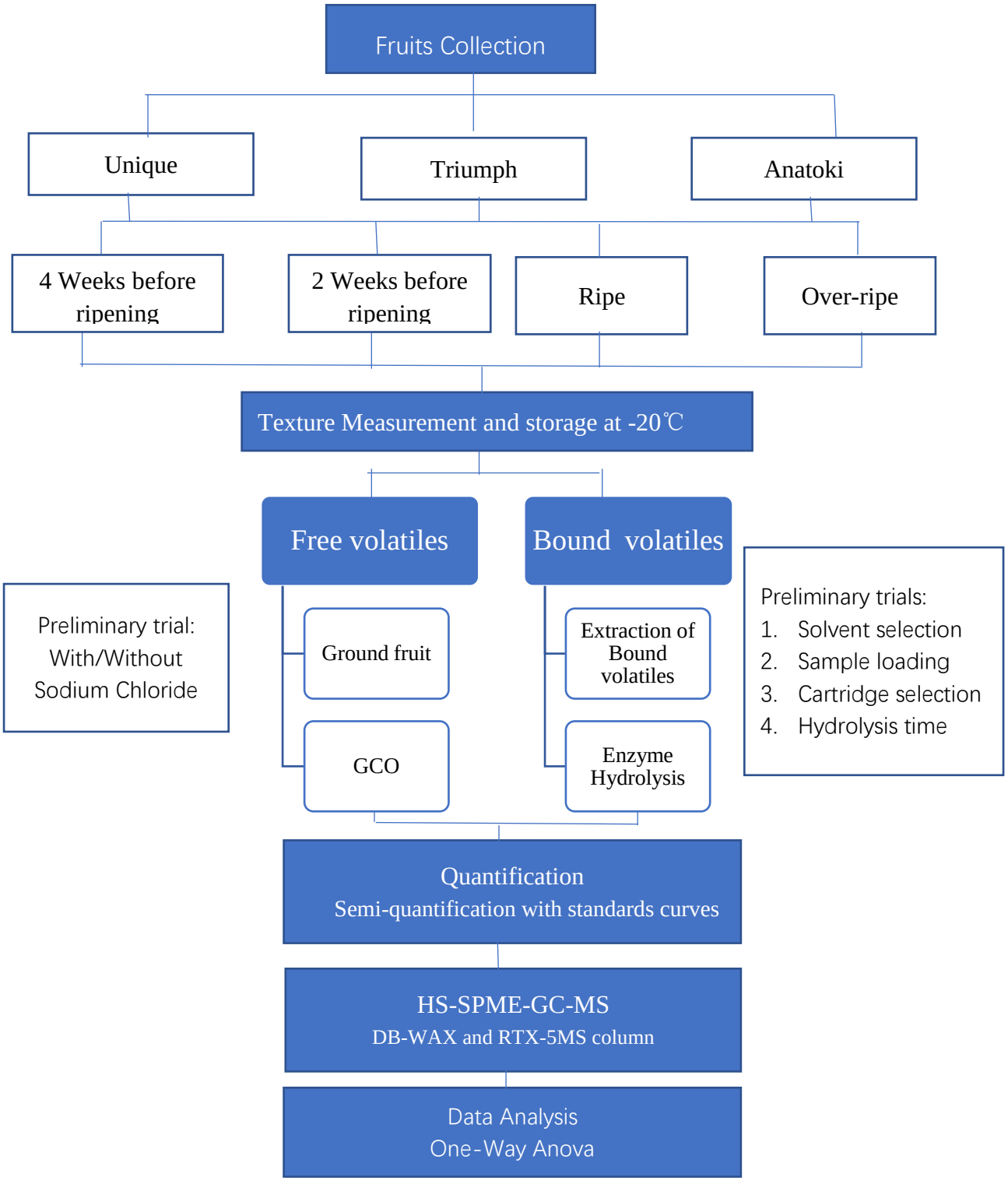

Figure 2.1: Method structure of feijoa volatiles characterization

2.1 Fruit materials

There Feijoa cultivars (Unique, Triumph and Triumph) were harvested during March to June 2019 from the Southern Bell Orchard in Matamata, Waikato, New Zealand (Figure 7). Fruits from four ripening stages of each cultivar were included, they are the Unique cultivar fruits - four weeks before ripening (U4), two weeks before ripening (U2), ripened (UR) and over-ripened (UO), the Triumph cultivar fruits - four weeks before ripening (T4), two weeks before ripening (T2), ripened (TR) and over-ripened (TO), the Anatoki cultivar fruits - four weeks before ripening (A4), two weeks before ripening (A2), ripened (AR) and over-ripened fruits (AO). 4 weeks, 2 weeks and ripe fruits were collected under the suggestion form the orchard farmers. Overripe fruits were obtained by keeping ripe fruits at 25℃ (room temperature) for two weeks. The firmness of fruits were measured immediately using a TA.XT. plus Texture Analyzer FTA (Stable Micro Systems Ltd., Goldaming, England). Fruits were stored at -20℃ prior to experimental procedures.

4 weeks before ripening 2 weeks before ripening ripe over-ripe

Triumph

Unique

Anatoki

Figure 2.2: Photos of the three feijoa cultivars during ripening

2.2 Standards and solvents

All chemical standards and solvents used were of analytical grade or higher. Diethyl ether was from Loba Chemie (Mumbai, India); DCM and pentane were obtained from Thermo Fisher Scientific (Auckland, NZ). Ethanol, methanol, ethyl acetate, anhydrous sodium sulphate (Na_2SO_4), anhydrous citric acid ($C_6H_8O_7$), anhydrous sodium phosphate (Na_2HPO_4) and sodium hydroxide (NaOH) were obtained from ECP Labchem Ltd. (Auckland, NZ).

Reference standards methyl butanoate, ethyl acetate, ethyl pentanoate, D-limonene, hexanal, methyl hexanoate, ethyl butanoate, hexyl acetate, 2-heptanol, 2-nonanone, cis-3-hexenyl butyrate, caryophyllene, methyl benzoate, ethyl benzoate, ethyl isobutyrate, myrcene, trans-β-ocimene/cis-*β*-ocimene, ethyl hexanoate, 2-heptanone, 3-octanone, cis-3-hexenyl acetate, 3-octanol, ethyl octanoate, linalool, 2-undecanone, humulene, farnesene, cis-3-hexenyl benzoate, pentanal, 1-hexanol, cis-3-hexen-1-ol, 2-methyl-3-heptanone and C7-C40 saturated alkane standard mixture were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3 Analysis of free volatiles in feijoa

2.3.1 Sample preparation for free volatiles analysis

A mortar from Taiping Asian Supermarket (Auckland, NZ) was used to ground whole fruits (including flesh and peel) into feijoa paste. The mortar was sitting on ice while grounding the fruits to minimize the oxidation of the fruit. Two grams of each sample (12 samples in total: U4, U2, UR, UO, T4, T2, TR, TO, A4, A2, AR, AO) was placed into SPME amber vials (75.5mm × 22.5mm, 20ml Precision Thread Headspace Vials, Supelco, PA, USA) and 10µl

of 2-Methyl-3-hetanone was added as an internal standard in each vail. All vials were sealed with lids fitted with a rubber septum (Agilent Technologies Inc., Santa Clara, CA, USA). All samples were prepared in triplicates (except bound volatiles in AO and TO were in duplicates because running out of samples), and along with unprepared pastes were kept in a freezer at -20°C before experiments.

2.3.2 Free volatiles analysis

2.3.2.1 HS-SPME

After sample preparation, the vials were taken to the HS-SPME machine for volatiles extraction. The HS-SPME system contained an autosampler (CTC PAL automated sample injector; CTC Analytics, Zwingen, Switzerland) which was controlled by a CombiPAL software (Cycle Composer Version 1.5.4; CTC Analytics AG, Zwingen Switzerland). The vials were incubated in a shaking container at 400 rpm for 25 minutes at 40°C . Volatiles were absorbed by a SPME fibre (Divinylbenzene, Carboxen and Polydimethylsiloxane; Agilent Technologies Inc., Santa Clara, CA, USA) in the headspace of the vials at 40°C for 20 minutes.

Salt addition

As mentioned previously in section 1.2.6.2, some researchers added NaCl (usually 20%) with samples aiming to improve the volatiles' vaporization (Reineccius, 2005). Therefore, in order to find out whether salt addition would enhance the volatile extraction, the addition of 0.4 grams NaCl in 2 grams of fruit paste was studied.

2.3.2.2 GC-MS

The HS-SPME was directly connected with a GC-MS system (Shimadzu GC-MS-QP2010 Plus; Kyoto, Japan). When the fiber adsorption completed, GC desorption started. Two columns were used for the separation of volatiles, which were a 29.46 m × 0.025mmm with 0.25 μm film thickness DB-WAX column (Agilent Technologies Inc., Santa Clara, CA,

USA) and a 23.58 m ×0.025mmm with 0.25 μm film thickness RTX-5MS column (Restek Corporation, Bellefonte, PA, USA). BD-WAX was a polar column and RTX-5MS was a non-polar column. Columns (stationary phase) with different polarity have different elution range and ability (Kitson et al., 1996). Therefore, a relatively comprehensive aroma profile can be achieved by conducting two columns with different polarity (Marsili & ProQuest, 2012). Helium with purity over 99.99% was used as the mobile phase with a flow rate of 2.10 mL per minute in the DB-WAX column. The GC-MS system was operated with a splitless injection mode. A 1.00-minute sampling time with 250°C injector temperature was employed. The temperature of the GC desorption chamber was not constant all the time. Sample was held at 40°C for 1 minute at the beginning, followed by temperature increasing with a rate of 5°C/minute until reaching 250°C . Finally, sample was kept at 250°C for 12 minutes. The MS was operated at 70 eV to produce an electron-impact mass spectrum with a scanning range between 40 to 500 m/z. The temperature of the ion source was 200°C and that of MS interface was 250°C.

Helium with purity over 99.99% was used as a mobile phase with a flow rate of 1.21 mL per minute in the RTX-5MS column. The GC-MS system was operated with a splitless injection mode. A 1.00-minute sampling time with 280°C injector temperature was employed. The starting temperature was 35°C inside the chamber. When a sample was injected in GC-MS, the temperature immediately increased with a rate of 5°C /minute until reaching 180°C, then holding at 180°C for 2 minutes. Later temperature was ramped with a rate of 10/minute to 230°C and continuously increased with the rate of 20°C per minute until reaching 280°C . Finally, the sample was kept at 280°C for 5 minutes. The MS was operated at 71.4 eV to produce an electron-impact mass spectrum with a scanning range between 41 to 500 m/z. The temperature of the ion source was 200°C and that of MS interface was 250°C

2.4 Aroma active compounds identification

HS-SPME-GC-O-MS system was used to detect aroma active volatiles among all the free volatiles. An olfactory port (Phaser OP275, ODP; ATAS/GL Sciences, Tokyo, Japan) connected to GC-MS was used in this system. Specifically, in the last part of the GC column, the effluent was divided into GC, MS and olfactory ports by a 4-port splitter (SilFlow, Trajan, Australia) with a flow rate of 3 mL/minute. The olfactory port was set at 220°C. To keep a satisfied humidity for human nasal cavity, a humidified air was provided with a flow rate of 20 mL/minute.

Four panellists (two females and two males) were involved in this GC-O section, they were all food science students around 23 years old with relevant sensory experience. A practice session was carried out before the formal experiments. Each panellist was asked to trial and familiar with the olfactory port by sniffing one feijoa sample. Retention time shown on the MS spectrum, odour description and odour intensity (from 0-10) were recorded while the panellists were sniffing via the olfactory port. A discussion was held to standardize the odour description and odour intensity when all panellists finished the sniffing section. The final GC-O experiment was operated by the four trained panellists for all the 12 samples (U4, U2, UR, UO, A4, A2, AR, AO and T4, T2, TR, TO).

2.5 Analysis of bound volatiles in feijoa

2.5.1 Fruits preparation for bound volatiles

Same as the sample preparation for free volatiles in section 2.3.1, for all three cultivars with four ripening stages, whole feijoas were ground into feijoa paste in a mortar sitting on ice. Then a two-step separation was employed to separate paste cake and clear juice. Firstly, a centrifugal machine is used to separate pomace and juice. The supernatant juice was saved for the following treatment. Secondly, a vacuum filtration was used to further separate the rest of the solids with juice. Finally, the clear feijoa juice was labelled and kept at -20°C until next step.

2.5.2 Trials on bound volatile extraction and analysis

Solvent selection trial to eliminate free volatiles

An essential step in bound volatile analysis was to get rid of the free volatiles in the samples. As mentioned in literature review (Table 4), several solvents can be used to remove free compounds in fruits. Among them, diethyl ether-pentane mixture (DP) at a ratio of 1:1 (v/v) was the most popular and the most widely used in fruits including lemon, kiwifruits and citrus fruits (Garcia et al., 2013; Ren, 2015; Zhong et al., 2014). The mixture of pentane and DCM (2:1, v/v) was also employed in mango, banana and grape berries (Aurore et al., 2011; Lalel et al., 2003; Torchio et al., 2016). The employment of pentane alone has also been seen in the study on tomato (Ortiz-Serrano & Gil, 2009). Therefore, these three most widely reported solvents were concluded as trials in this research.

Two grams of paste samples were mixed with 20 mL of each trial solvent (diethyl ether: pentane 1:1, DCM: pentane 1:2 and pentane) in a 50 ml centrifuge tube. Free volatile removal was conducted using a magnetic stirrer at a rate of 400 rpm per hour at room temperature. The supernatant was collected and dried to 2 mL under a gentle stream of nitrogen. The concentrated solvent was then filtered with syringe into a 2 mL screw top GC vial (Agilent

Technologies Inc., Santa Clara, CA, USA) and injected into GC-MS machine (7683B Series Injector, 7890A GC System, 5975 Inert XL MSD; Agilent Technologies Inc., Santa Clara, CA, USA) for identification and quantification. The detailed procedure is the same with free volatile analysis in Section 2.3.2.

Cartridge selection trial

According to literatures (Table 4), two different cartridges were used in trials. Amberlite XAD-2 was widely used in various fruits such as mango, kiwifruit, citrus fruits (Garcia et al., 2012; Garcia et al., 2013; Lalel et al., 2003; Ren, 2015) while C 18 cartridge was also a popular cartridge for bound volatiles extraction in fruits (Gao, 2018; Ortiz-Serrano & Gil, 2009). Therefore, a 500 mg 6 Strata C18-E cartridge (55μm, 70A, Teflon® Tubes; Phenomenex, Torrance, CA, USA) and a precleaned Amberlite XAD-2 cartridge (ORBO^{TM} 1500 Small PUF/Amberlite®/XAD®-2/PUF cartridge; Agilent Technologies Inc., Santa Clara, CA, USA) were tested in this experiment.

Following to Garcia et al. (2013)'s method, the cartridges were pre-treated with 12 mL of methanol and 12 of mL Milli-Q water in turn. Then, 20 mL of clear feijoa juice (obtained from bound juice preparation, section 2.5.1.) was loaded to the cartridge, and was eluted by 12 mL of milli-Q water followed by 12 mL of diethyl ether: pentane (1: 2, v/v) to remove water-soluble sugars (Ren, 2015) and eliminate any free volatiles. These two fractions were discarded. Bound volatile was collected by eluting with 12 mL of methanol into a 20 mL SPME vial and was then completely dried under nitrogen blow at room temperature and stored at -20°C until further experiments. The collected bound volatile samples further went through the same enzyme hydrolysis and SPME-GC-MS analysis.

Before the following enzyme hydrolysis procedure, a buffer made of 4.2 g citric acid with 2.84g sodium phosphate was prepared in a 100 mL volumetric flask. The pH was adjusted by 4 M NaOH to around 5. A volume of 5 mL buffer, 125 mg Rapidase Revelation Aroma (Oenobrands SAS; Montpellier, France), together with a magnetic stirrer were added to a

SPME vial containing the extracted bound volatile samples. The enzyme hydrolysis was carried on at 40℃ for 24 hours on a magnetic hot plate. After the hydrolysis, the temperature was increased to 60℃ for 15 minutes to inactivate the enzyme. Finally, the hydrolysed samples were subjected to the HS-SPME-GC-MS analysis, with the employment of a DB-WAX column. The detailed procedure is the same with free volatile analysis in Section 2.3.2.

Sample type and loading volume selection trial

Based on the cartridge selection trial experience, some samples (especially ripe and overripe juice samples) were too viscous to pass through the resins inside the cartridges. A dilution was made to trial the best loading sample. Three samples were chosen to trial in this section, which were pure clear feijoa juice prepared from section 2.5.1, homogenized sample containing 20 mL of pure juice and 20 mL of water, and supernatant from a fine mixture of 45 grams of feijoa paste vortexed 5 minutes with 45 mL of water. Furthermore, sample loading volume was also a part to maximize cartridge capacity. A volume of 100 ml sample (50 ml juice and 50 ml water) was tested to check the influence of higher loading volume on spectrum result.

Trial 5: Enzyme hydrolysis time trial

From literature review (Table 4), different enzyme incubation time, from 12 hours to 48 hours (Chen et al., 2015; Du et al., 2010; García-Carpintero et al., 2012; Ren, 2015; Wang et al., 2017; Wen et al., 2014), has been used. In this trial, six individual time points were selected, they were 6, 16, 24 and 48 hours.

2.5.3 Characterization of feijoa bound volatiles

Based on the above trials, the final methods for the characterization of feijoa bound volatile was determined. Briefly, a mixture of 20 mL of prepared juice with 20 mL of deionized water was used as samples. Activated Amberlite XAD-2 cartridge by 12 mL of methanol and 12 mL of Milli-Q water was used to extract bound precursors. A total of 40 ml samples was loaded on the cartridge and washed with 12 mL of water and 12 mL of diethyl ether: pentane mixture (1:2, v/v) to remove water-soluble sugars and free volatiles. Extraction of bound volatiles was achieved by eluting with 12 mL of methanol. Thereafter, a gentle stream of nitrogen was used to completely remove methanol and dry the bound volatiles precursor at room temperature. The dried bound volatile vails were stored in -4°C freezer until enzyme hydrolysis.

Enzyme hydrolysis of bound volatiles was conducted using 125 mg of Rapidase Revelation Aroma (Oenobrands SAS; Montpellier, France in 5 mL of r the Phosphate-citric acid buffer (pH 5), at 40℃ for 24 hours on a magnetic hot plate. After enzyme inactivation, 10 µL of Internal standard, 2-methyl-3-heptanone at a concentration of 9.75×10^{-3} mg/mL was added. Finally, the samples were analysed by HS-SPME-GC-MS system as in Section 2.3.2.

2.6 Identification of volatiles in feijoas

A MassHunter Workstation software (Agilent Technologies Inc., Santa Clara, CA, USA) was used to analyse the MS spectrums. Online NIST08 GC-MS Library on B.07.00 Qualitative Analysis software as well as the Unknown Analysis software was used to identify each compound. Available authentic standards (as mentioned in section 2.1.2) were used for positive confirmation. LRI was calculated using the equation in Literature Review (section 1.2.4.7). The LRI was further compared with records in the NIST Chemistry Webbook (http://webbook.nist.gov/chemistry/).

2.7 Quantification of volatiles in feijoas

The concentration of the compounds with standards was determined using standard curves which were plotted according to concentration ratios of the standard compounds to internal standard (ISTD) versus the ratio of peak area (TIC peak area of standard / TIC peak of ISTD). Semi-quantification was employed for those identified compounds without standards, as relative concentration to one of the standard compounds in each chemical group (ethyl butanoate for esters, linalool for alcohols, 3-octanone for ketones, caryophyllene for terpenes, hexanal for aldehydes).

2.8 Statistical analysis

Duplication was employed for all finalized experiments in this thesis and the results were represented as mean ± standard deviation. Windows software SPSS version 25.0 was used for statistical analysis (SPSS IBM Crop., Armonk, NY, USA). Significant differences were determined by Duncan's multiple range test at $p < 0.05$ in a one-way analysis of variance (ANOVA). Principle Component Analysis (PCA) (SIMCA Version X 14.0, Umetrics, Umea, Sweden) was also used to analyze free aroma active compounds.

Chapter Three:
Results and Discussion

3.1 Physical characteristics of feijoa fruits

Physical parameters such as color, firmness, size, shape are important indicators of fruit quality, and could change during fruit ripening and vary among different fruit cultivars (Song & Forney, 2008). As presented in Table 5, three different feijoa cultivars (Unique, Triumph and Anatoki) from different ripening stages, 4 weeks before ripening (4WBR) U4, T4, A4, 2 weeks before ripening (2WBR) U2, T2, A2, ripened UR, TR, AR and over-ripened UO, TO, AO exhibited different physical properties.

For the size of fruits, fruits were getting bigger from 4WBR to R and stayed stable to OR (Figure 7 in section 2.1). Thus, fruit size could be one of the parameters to evaluate the maturation of feijoas. Among the three cultivars, the late season Triumph cultivar always developed the biggest fruits with the largest fruit length and width, followed by early season Anatoki and early season Unique for all ripening stages.

As feijoas became ripe, the color tended to be more yellow and the skin became smoother. For unripe fruits (4WBR and 2WBR), the color was darker and greener. While as ripening progressed, the color tended to be less dark green and became a little yellow. The change of color with maturity is linked to the concentration change in carotenoids and chlorophyll, flavonoids, and betalains (Solovchenko et al., 2019). Chlorophyll with ‘green’ pigments is likely to degrade to colourless chlorophyll catabolites during ripening, which might explain the loss of dark green colour in more ripened fruits. Besides, ‘yellow’ colour carotenoids tend to accumulate from desaturation of colourless, condensed-isoprenoid precursors, which could build yellow colour in ripened fruits (Solovchenko et al., 2019).

Table 5: Physical properties of 'Unique, Triumph and Anatoki' feijoas at four ripening stages

Samples	Size	physical description	Force (N)
U4	6 x 4	Hard and required higher force to puncture, little dark green skin with smooth surface.	61.62 ± 0.35e
U2	7 x 5	Hard and required higher force to puncture, little dark green skin with smooth surface.	52.00 ± 1.27d
UR	7.9 x 6.4	Soft and required little force to puncture, yellow green skin with smooth surface.	17.91 ± 1.22b
UO	7.9 x 5.5	Very soft and required little force to puncture, yellow green and grey speckles skin with smooth surface.	14.79 ± 0.89a
T4	9 x 4.6	Very hard and required higher force to puncture, little dark green skin with wrinkled surface.	97.95 ± 0.88g
T2	9.5 x 4.6	Hard and required higher force to puncture, little dark green skin with unsmooth surface.	55.28 ± 0.68h
TR	10 x 6.5	Soft and required little force to puncture, yellow green skin with smooth surface.	38.12 ± 0.28c
TO	10 x 6.8	Very soft and required little force to puncture, yellow green and grey speckles skin with smooth surface.	14.70 ± 0.86a
A4	6 x 4.6	The hardest and required the most force to puncture, little dark green skin with wrinkled surface.	101.87 ± 0.52i
A2	7.9 x 5	Very hard and required higher force to puncture, green skin with unsmooth surface.	86.33 ± 0.35f
AR	8 x 6	Soft and required little force to puncture, yellow green skin with smooth surface.	20.24 ± 0.48b
AO	8 x 6.2	Very soft and required little force to puncture, yellow green and grey speckles skin with smooth surface.	17.81 ± 0.17b

a, b, c, d, e, f, g, h, i present statistically significant differences among cultivars and ripening stages by multiple comparisons using Duncan's test ($P < 0.05$), different letters indicate significant difference among samples;
Size is presented as Length cm x width cm.

Firmness is one of the most important attributes for consumer to evaluate the maturity and quality of fruits (Jha et al., 2010; Sirisomboon et al., 2008). Results illustrate the firmness of feijoas was significantly affected by ripening stages and cultivars (Figure 8). Decreased firmness was seen at more ripe stages and significant differences ($P < 0.05$) existed among samples from 4WBR, 2WBR, ripened and over-ripened for all the three cultivars. Additionally, for the cultivar differences, Anatoki (A4 101.87N and A2 86.33N) was the firmest cultivar at 4WBR and 2WBR while Triumph (38.12N) become the firmest cultivar at R. For OR samples, Unique (14.75N) and Triumph (14.70N) were comparable ($P > 0.05$) yet Anatoki (17.81N) was a little firmer than these two.

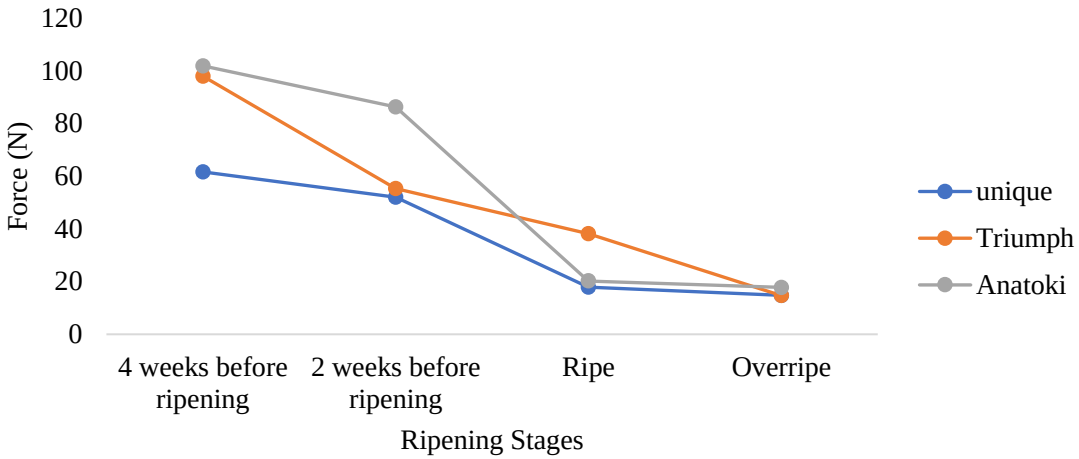

Figure 3.1: Firmness of feijoas in three cultivars at four ripening stages (the standard deviation (error bars) were included in this figure, but they are too small to be seen)

The change of firmness during ripening were also indicated by other fruits. For apple fruits, there was a significant difference in firmness of star apple from unripe to ripe fruits. Therefore, the firmness of star apple was believed to be a reliable maturity index to indicate the ripening stages of this fruit (Williams & Benkeblia, 2018). Textural properties of mango in different cultivars during ripening was also explored. From the results, the firmness of mango experienced a dramatic decrease during ripening and there were significant differences among each cultivar (Jha et al., 2013). Besides, there was an obvious decrease in strawberry firmness from unripe large green fruit to ripe fruit for both two cultivars, despite

firmness change of Fragaria ananassa cultivar was lower than that of Fragaria chiloensis cultivar (Figueroa et al., 2010). Moreover, firmness of apricot fruits (Bureau et al., 2009) and plum fruit (Usenik et al., 2008) decreased from unripe to ripe fruits as well.

The firmness changes of fruits during ripening maybe due to the structural changes of cell wall pectin polymers (Salunkhe & Kadam, 1995). Specifically, as a result of concentration and availability change in related enzyme such as pectin methylesterase (PME), polygalacturonase (PG), endoglucanase (EGase), α-arabinofuranosidase (αFase) and β-galactosidase (β Gal), plant wall cell polysaccharides such as hemi cellulosic cell wall fraction and pectin change to some extent, which further leads to the loss of structural and compositional functions of fruit cell wall (Figueroa et al., 2010).

3.2 Free volatiles in feijoas

Feijoa has a strong and very attractive aroma. As mentioned in section 1.2.1.2, the complete feijoa volatile composition and aroma profile has not been analysed at present. Therefore, it is meaningful to explore the free and bound volatiles in feijoas to give an overall profile. The following parts mainly focused on free volatiles in feijoas.

Salt addition trial

As mentioned by previous paper, addition of salt could promote the release of volatiles (Kelebek & Selli, 2011). This effect was trialled by adding NaCl in sample in the SPME vials. However, there was no significant difference between the chromatograph obtained from samples with or without salt addition (Figure 9). Therefore, salt was not added in the final free volatile sample preparation.

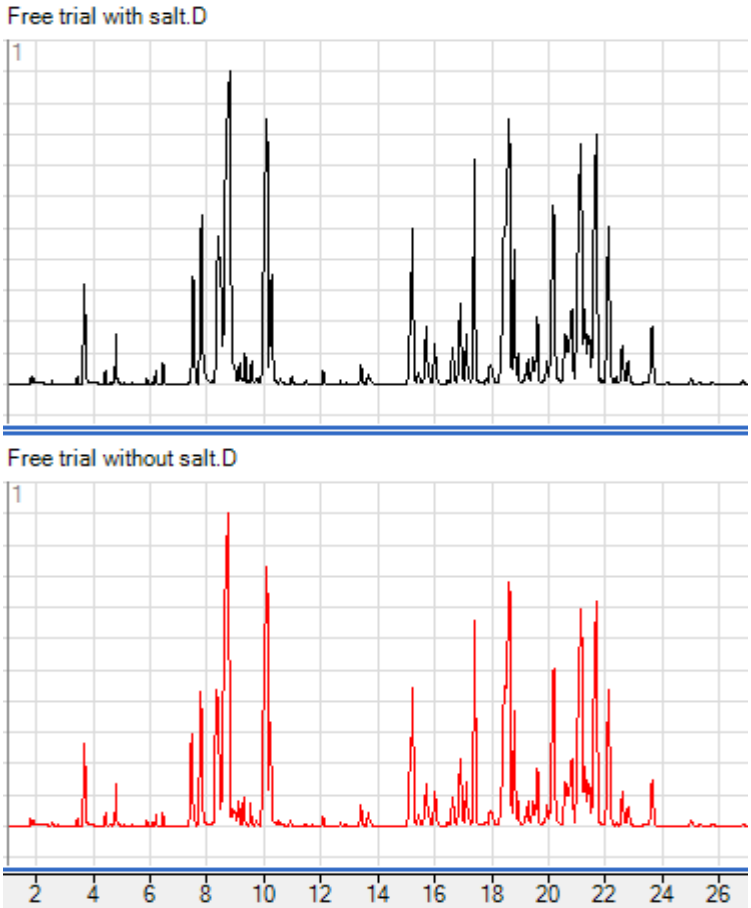

Figure 3.2: Salt addition trial in feijoa free volatile analysis

3.2.1 Free volatiles identified in feijoa fruits during ripening

A total of 164 free volatiles were detected in three cultivars at four ripening stages by HS-SPME-GC-MS (Table 6), among which 69 free volatiles were detected for the first time. Specifically, a total of 129 aroma volatiles were found in all three cultivars and the total number of free volatiles in Unique, Triumph and Anatoki was 147, 151 and 155 respectively.

Table 7 illustrates relative concentration of some odourless (not detected in GCO) free volatiles in the three feijoa cultivars during four ripening stages. These volatiles were selected either by their higher concentration (more than 1.5 mg/kg) or special absent and present in some cultivar and ripening stages (all aroma active compounds were discussed in detail in section 3.3).

Table 6: Compounds description of free volatiles in three feijoa cultivars at four ripening stages

NO.	Compounds	RIa (RIb)		Formula	CAS	Class	ID
		Wax	5MS				
1	Methyl acetate	804 (810)	-	$C_3H_6O_2$	79-20-9	C	MS, RI
2	2-Propenal	819 (828)	-	C_3H_4O	107-02-8	B	MS, RI
3	Ethyl Acetate	863 (900)	603 (613)	$C_4H_8O_2$	141-78-6	C	Std, MS, RI
4	Ethyl propanoate	939 (946)	713 (714)	$C_5H_{10}O_2$	105-37-3	C	MS, RI
5	Ethyl isobutyrate	950 (960)	758 (757)	$C_6H_{12}O_2$	97-62-1	C	Std, MS, RI
6	Pentanal	961 (968)	-	$C_5H_{10}O$	110-62-3	B	MS, RI
7	Methyl butanoate	968 (976)	722 (721)	$C_5H_{10}O_2$	623-42-7	C	Std, MS, RI
8	α-Pinene	1008 (1015)	-	$C_{10}H_{16}$	80-56-8	F	MS, RI
9	α-Thujene	1012 (1017)	931 (924)	$C_{10}H_{16}$	2867-5-2	F	MS, RI
10	Ethyl butanoate	1023 (1026)	801 (802)	$C_6H_{12}O_2$	105-54-4	C	Std, MS, RI
11	3-Hexanone	1037 (1046)	798 (801)	$C_6H_{12}O$	589-38-8	E	MS, RI
12	Ethyl 2-methylbutanoate	1039 (1041)	-	$C_7H_{14}O_2$	7452-79-1	C	MS, RI
13	Butyl acetate	1057 (1049)	-	$C_6H_{12}O_2$	123-86-4	C	MS, RI
14	Hexanal	1065 (1067)	804 (802)	$C_6H_{12}O$	66-25-1	B	Std, MS, RI
15	Isobutyl isobutyrate	1075 (1085)	915 (914)	$C_8H_{16}O_2$	97-85-8	C	MS, RI
16	Unknown	-	1663 (0)	$C_{15}H_{22}$	67517-14-0	G	-
17	L-β-pinene	1081 (NA)	984 (0)	$C_{10}H_{16}$	18172-67-3	F	MS, RI
18	Ethyl carbonate	1088 (1099)	789 (747)	$C_5H_{10}O_3$	105-58-8	C	MS, RI
19	Sabinene	1094 (1103)	-	$C_{10}H_{16}$	3387-41-5	F	MS, RI
20	Ethylbenzene	1102 (NA)	877 (879)	C_8H_{10}	100-41-4	D	MS, RI
21	Isoamyl acetate	1105 (1112)	877 (876)	$C_7H_{14}O_2$	123-92-2	C	MS, RI
22	Ethyl pentanoate	1118 (1128)	903 (902)	$C_7H_{14}O_2$	539-82-2	C	Std, MS, RI
23	Myrcene	1140 (1157)	994 (993)	$C_{10}H_{16}$	123-35-3	F	Std, MS, RI

Table 6 continued

24	Ethyl 2-butenoate	1147 (1148)	851 (844)	$C_6H_{10}O_2$	10544-63-5	C	MS, RI
25	Isobutyl 2-methylbutanoate	1164 (1179)	1004 (1002)	$C_9H_{18}O_2$	2445-67-2	C	MS, RI
26	2-Heptanone	1169 (1178)	902 (894)	$C_7H_{14}O$	110-43-0	E	Std, MS, RI
27	Methyl hexanoate	1170 (1176)	925 (927)	$C_7H_{14}O_2$	106-70-7	C	Std, MS, RI
28	D-Limonene	1177 (NA)	1035 (NA)	$C_{10}H_{16}$	5989-27-5	F	Std, MS, RI
29	2-Thujene	1178 (1133)	979 (NA)	$C_{10}H_{16}$	28634-89-1	F	MS, RI
30	2-Metylbutyl Isobutyrate	1183 (1194)	1017 (1018)	$C_9H_{18}O_2$	2445-69-4	C	MS, RI
31	α-Phellandrene	1185 (1162)	1011 (1010)	$C_{10}H_{16}$	99-83-2	F	MS, RI
32	3-Hexanol	1194 (1190)	798 (797)	$C_6H_{14}O$	623-37-0	A	MS, RI
33	β-Phellandrene	1196 (1195)	1043 (1045)	$C_{10}H_{16}$	555-10-2	F	MS, RI
34	2-Hexenal	1205 (1193)	856 (863)	$C_6H_{10}O$	505-57-7	B	MS, RI
35	2-Methylpentyl isobutyrate	1208 (NA)	-	$C_{10}H_{20}O_2$	84254-82-0	C	MS, RI
36	2-Ethylbutyl isobutyrate	1210 (NA)	-	$C_{10}H_{20}O_2$	74398-54-2	C	MS, RI
37	β-trans-Ocimene	1217 (1224)	1040 (1047)	$C_{10}H_{16}$	3779-61-1	F	MS, RI
38	Ethyl hexanoate	1221 (1220)	999 (999)	$C_8H_{16}O_2$	123-66-0	C	Std, MS, RI
39	γ-Terpinene	1227 (1224)	1064 (1064)	$C_{10}H_{16}$	99-85-4	F	MS, RI
40	β-cis-Ocimene	1234 (1234)	-	$C_{10}H_{16}$	3338-55-4	F	MS, RI
41	β-(E)-Ocimene	-	1050 (1044)	$C_{10}H_{16}$	3779-61-1	F	MS, RI
42	3-Octanone	1248 (1240)	991 (988)	$C_8H_{16}O$	106-68-3	E	Std, MS, RI
43	2-Heptyl acetate	1251 (1266)	1042 (1034)	$C_9H_{18}O_2$	5921-82-4	C	MS, RI
44	Isoamyl butanoate	1253 (1256)	1057 (1058)	$C_9H_{18}O_2$	106-27-4	C	MS, RI
45	o-Cymene	1254 (1268)	1035 (1050)	$C_{10}H_{14}$	527-84-4	F	MS, RI
46	Hexyl acetate	1261 (1274)	1013 (1011)	$C_8H_{16}O_2$	142-92-7	C	Std, MS, RI
47	α-Terpinene	1263 (1193)	1038 (1014)	$C_{10}H_{16}$	99-86-5	F	MS, RI
48	2-Methylbutyl 2-Methylbutanoate	1271 (1274)	1104 (1102)	$C_{10}H_{20}O_2$	2445-78-5	C	MS, RI

Table 6 continued

49	Acetyl methyl carbinol	1275 (1286)	714 (713)	$C_4H_8O_2$	513-86-0	E	MS, RI
50	2-Ethylbutyl butyrate	1283 (NA)	1108 (1093)	$C_{10}H_{20}O_2$	5129-48-6	C	MS, RI
51	Ethyl 3-hexenoate	1288 (1290)	-	$C_8H_{14}O_2$	2396-83-0	C	MS, RI
52	3-Heptanol	1292 (1290)	-	$C_7H_{16}O$	589-82-2	A	MS, RI
53	Unknown	-	1648(NA)	-	-	G	MS, RI
54	2-Methylpentyl isovalerate	1304 (NA)	1157 (NA)	$C_{11}H_{22}O_2$	1000236-38-7	C	MS, RI
55	cis-3-Hexenyl Acetate	1307 (1315)	1008 (1009)	$C_8H_{14}O_2$	3681-71-8	C	Std, MS, RI
56	2-Heptanol	1307 (1318)	-		543-49-7	A	Std, MS, RI
57	2-Heptenal, (Z)-	1313 (1319)	-	$C_7H_{12}O$	57266-86-1	B	MS, RI
58	Ethyl heptanoate	1324 (1317)	-	$C_9H_{18}O_2$	106-30-9	C	MS, RI
59	Methyl heptenone	1326 (1323)	-	$C_8H_{14}O$	110-93-0	E	MS, RI
60	3-Octyl acetate	1329 (1344)	1122 (1124)	$C_{10}H_{20}O_2$	4864-61-3	C	MS, RI
61	Ethyl 2-hexenoate	1334 (1329)	-	$C_8H_{14}O_2$	1552-67-6	C	MS, RI
62	3-Nonanone	1349 (1339)	-	$C_9H_{18}O$	925-78-0	E	MS, RI
63	1-Hexanol	1329 (1339)	-	$C_6H_{14}O$	111-27-3	A	MS, RI
64	(4E,6Z)-allo-Ocimene	1360 (NA)	1131 (1131)	$C_{10}H_{16}$	7216-56-0	F	MS, RI
65	cis-3-Hexenyl propionate	1375 (1375)	1073 (1074)	$C_9H_{16}O_2$	33467-74-2	C	MS, RI
66	3-Hexenol	1379 (1384)	-	$C_6H_{12}O$	544-12-7	A	MS, RI
67	2-Nonanone	1382 (1387)	1093 (1096)	$C_9H_{18}O$	821-55-6	E	Std, MS, RI
68	3-Octanol	1392 (1393)	998 (996)	$C_8H_{18}O$	589-98-0	A	Std, MS, RI
69	1-Methylhexyl butanoate	1396 (1401)	-	$C_{11}H_{22}O_2$	39026-94-3	C	MS, RI
70	Hexyl butanoate	1410 (1407)	-	$C_{10}H_{20}O_2$	2639-63-6	C	MS, RI
71	Unknown	1419(NA)	-	-	-	G	
72	3-Cyclohexenyl acetate	1422 (NA)	1209 (1010)	$C_8H_{12}O_2$	10437-78-2	C	MS, RI
73	Ethyl octanoate	1429 (1435)	1197 (1196)	$C_{10}H_{20}O_2$	106-32-1	C	Std, MS, RI

Table 6 continued

74	Cosmene	1435 (NA)		$C_{10}H_{14}$	460-01-5	F	MS, RI
75	cis-3-Hexenyl Butyrate	1456 (1451)	1187 (1186)	$C_{10}H_{18}O_2$	16491-36-4	C	Std, MS, RI
76	α-Cubebene	1458 (1455)	1363 (1360)	$C_{15}H_{24}$	17699-14-8	F	MS, RI
77	Elixene	1477 (1514)	1350 (1492)	$C_{15}H_{24}$	3242-8-8	F	MS, RI
78	Ylangene	1489 (1482)	1388 (1391)	$C_{15}H_{24}$	14912-44-8	F	MS, RI
79	Copaene	1489 (1499)	1392 (1393)	$C_{15}H_{24}$	3856-25-5	F	MS, RI
80	α-Bourbonene	1508 (1514)	-	$C_{15}H_{24}$	-	F	MS, RI
81	Benzaldehyde	1508 (1508)	986 (975)	C_7H_6O	100-52-7	B	MS, RI
82	β-Bourbonene	1515 (1515)	-	$C_{15}H_{24}$	5208-59-3	F	MS, RI
83	α-Gurjunene	1528 (1530)	1430 (1425)	$C_{15}H_{24}$	489-40-7	F	MS, RI
84	β-Cubebene	1536 (1535)	1403 (1392)	$C_{15}H_{24}$	13744-15-5	F	MS, RI
85	Linalool	1548 (1547)	1104 (1105)	$C_{10}H_{18}O$	78-70-6	A	Std, MS, RI
86	Aristol-9-ene	1566 (1582)	-	$C_{15}H_{24}$	6831-16-9	F	MS, RI
87	Ylangene	1571 (1482)	-	$C_{15}H_{24}$	14912-44-8	F	MS, RI
88	γ-Gurjunene	1574 (1676)	-	$C_{15}H_{24}$	22567-17-5	F	MS, RI
89	β-Elemen	1589 (1586)	1406 (1403)	$C_{15}H_{24}$	515-13-9	F	MS, RI
90	2-Undecanone	1601 (1599)	1295 (1294)	$C_{11}H_{22}O$	112-12-9	E	Std, MS, RI
91	Caryophyllene	1602 (1599)	1446 (1444)	$C_{15}H_{24}$	87-44-5	F	Std, MS, RI
92	4-Terpinenol	1605 (1601)	1187 (1180)	$C_{15}H_{24}$	562-74-3	A	MS, RI
93	Aromandendrene	1610 (1635)	-	$C_{15}H_{24}$	489-39-4	F	MS, RI
94	Methyl benzoate	1616 (1615)	1110 (1103)	$C_8H_8O_2$	93-58-3	C	Std, MS, RI
95	unknown	1625(NA)	-	-	-	G	-
96	Isoledene	1638 (NA)	1411 (NA)	$C_{15}H_{24}$	95910-36-4	F	MS, RI
97	Ethyl caprate	1644 (1643)	1396 (1397)	$C_{12}H_{24}O_2$	110-38-3	C	MS, RI
98	Alloaromadendren	1644 (1646)	1463 (1461)	$C_{15}H_{24}$	25246-27-9	F	MS, RI
99	cis-3-Hexenyl Hexanoate	1656 (1648)	1382 (1376)	$C_{12}H_{22}O_2$	31501-11-8	C	MS, RI

Table 6 continued

100	Ethyl benzoate	1657 (1650)	1183 (1173)	$C_9H_{10}O_2$	93-89-0	C	Std, MS, RI
101	Humulene	1670 (1671)	1478 (1477)	$C_{15}H_{24}$	6753-98-6	F	Std, MS, RI
102	γ-Muurolene	1690 (1681)	1490 (1449)	$C_{15}H_{24}$	30021-74-0	F	MS, RI
103	Methyl geraniate	1691 (1678)	-	$C_{11}H_{18}O_2$	2349-14-6	F	MS, RI
104	α-Terpineol	1697 (1692)	1199 (1200)	$C_{10}H_{18}O$	98-55-5	A	MS, RI
105	Varidiflorene	1700 (1698)	1518 (1495)	$C_{15}H_{24}$	21747-46-6	F	MS, RI
106	Germacrene D	1715 (1709)	1471 (1474)	$C_{15}H_{24}$	23986-74-5	F	MS, RI
107	β-Selinene	1721 (1719)	-	$C_{15}H_{24}$	17066-67-0	F	MS, RI
108	α-Muurolene	1727 (1727)	-	$C_{15}H_{24}$	10208-80-7	F	MS, RI
109	γ-Elemene	1741 (NA)	1339 (1339)	$C_{15}H_{24}$	339154-91-5	F	MS, RI
110	α-Muurolene	1746 (NA)	-	$C_{15}H_{24}$	31983-22-9	F	MS, RI
111	Farnesene	1750 (1754)	1531 (1511)	$C_{15}H_{24}$	502-61-4	F	Std, MS, RI
112	δ-Cadinene	1759 (1758)	1540 (1534)	$C_{15}H_{24}$	483-76-1	F	MS, RI
113	unknown	1770(NA)	-	-	-	G	-
114	Cadine-1,4-diene	1781 (1797)	1550 (1546)	$C_{15}H_{24}$	16728-99-7	F	MS, RI
115	Isobutyl benzoate	1786 (NA)	-	$C_{11}H_{14}O_2$	120-50-3	C	MS, RI
116	α-Amorphene	1791 (1751)	1496 (1490)	$C_{15}H_{24}$	483-75-0	F	MS, RI
117	2-Tridecanone	1816 (1814)	-	$C_{13}H_{26}O$	593-08-8	E	MS, RI
118	L-Calamenene	1829 (1832)	1542 (1546)	$C_{15}H_{22}$	483-77-2	F	MS, RI
119	Ethylphenyl propanoate	1881 (1880)	-	$C_{11}H_{14}O_2$	2021-28-5	C	MS, RI
120	α-Calacorene	1911 (1916)	1562 (NA)	$C_{15}H_{20}$	1000293-02-3	F	MS, RI
121	Isoamyl benzoate	1912 (1928)	-	$C_{12}H_{16}O_2$	94-46-2	C	MS, RI
122	Palustrol	1934 (1934)	1592 (1567)	$C_{15}H_{26}O$	95975-84-1	A	MS, RI
123	Caryophyllene oxide	1986 (1988)	-	$C_{15}H_{24}O$	1139-30-6	F	MS, RI
124	heptyl benzoate	2032 (2217)	1597 (NA)	$C_{14}H_{20}O_2$	7155/12/6	C	MS, RI
125	Cubenol	2058 (2063)	1640 (1642)	$C_{15}H_{26}O$	21284-22-0	A	MS, RI
126	Veridiflorol	2070 (NA)	-	$C_{15}H_{26}O$	1000122-17-3	A	MS, RI

Table 6 continued

127	Hexyl benzoate	2072 (2076)	-	$C_{13}H_{18}O_2$	6789-88-4	C	MS, RI
128	(-)-Globulol	2079 (NA)	-	$C_{15}H_{26}O$	489-41-8	A	MS, RI
129	Methyl p-anisate	2083 (2085)	-	$C_9H_{10}O_3$	121-98-2	C	MS, RI
130	Ledol	2085 (2062)	-	$C_{15}H_{26}O$	577-27-5	A	MS, RI
131	Globulol	2086 (2083)	-	$C_{15}H_{26}O$	51371-47-2	A	MS, RI
132	Rosifoliol	2108 (NA)	-	$C_{15}H_{26}O$	63891-61-2	A	MS, RI
133	cis-3-Hexenyl Benzoate	2117 (2118)	1582 (1571)	$C_{13}H_{16}O_2$	25152-85-6	C	Std, MS, RI
134	Ethyl cinnamate	2121 (2127)	-	$C_{11}H_{12}O_2$	103-36-6	C	MS, RI
135	(-)-Spathulenol	2122 (NA)	1602 (1619)	$C_{15}H_{24}O$	77171-55-2	A	MS, RI
136	α-Cadinol	2222 (2221)	-	$C_{15}H_{26}O$	481-34-5	A	MS, RI
137	Cadinene	1658 (NA)	-	$C_{15}H_{24}$	523-47-7	F	MS, RI
138	cis-3-Hexenyl-α-methylbutyrate		1234 (1234)	$C_{11}H_{20}O_2$	53398-85-9	C	MS, RI
139	1-Propylpentyl butyrate		1248 (NA)	$C_{12}H_{24}O_2$	20286-46-8	C	MS, RI
140	(3Z)-3-Octenyl acetate		1280 (NA)	$C_{10}H_{18}O_2$	69668-83-3	C	MS, RI
141	(Z)-3-Hexenyl pentanoate		1286 (1270)	$C_{11}H_{20}O_2$	35852-46-1	C	MS, RI
142	γ-Pyronene	-	921 (NA)	$C_{10}H_{16}$	4724-89-4	F	MS, RI
143	α-Guaiene	-	1371 (1409)	$C_{10}H_{16}$	3691-12-1	F	MS, RI
144	Clovene	-	1380 (1396)	$C_{15}H_{24}$	469-92-1	F	MS, RI
145	γ-Gurjunene	-	1450 (1472)	$C_{15}H_{24}$	22567-17-5	F	MS, RI
146	Aromadendr-1-ene	-	1415 (1440)	$C_{15}H_{24}$	1000152-25-6	F	MS, RI
147	β-Gurjurene	-	1432(NA)	$C_{15}H_{24}$	17334-55-3	F	MS, RI
148	Aromadendrene	-	1485 (1485)	$C_{15}H_{24}$	109119-91-7	F	MS, RI
149	γ-Cadinene	-	1532 (1534)	$C_{15}H_{24}$	39029-41-9	F	MS, RI
150	α-Cadinene	-	1554 (1541)	$C_{15}H_{24}$	24406-05-1	F	MS, RI
151	Guaia-10(14),11-diene	-	1619 (NA)	$C_{15}H_{24}$	1000159-39-3	F	MS, RI
152	unknown	-	1478 (NA)	-	-	F	MS, RI
153	β-Selinenol	-	1650 (1645)	$C_{15}H_{26}O$	473-15-4	A	MS, RI

Table 6 continued

154	δ-Cadinol	-	1678 (1644)	$C_{15}H_{26}O$	36564-42-8	A	MS, RI
155	Cadalene	-	1698 (1688)	$C_{15}H_{18}$	483-78-3	D	MS, RI
156	Unknown	-	1036 (NA)	-	-	G	-
157	Unknown	-	1191 (NA)	-	-	G	-
158	Unknown	-	1340 (NA)	-	-	G	-
159	Unknown	-	1527 (NA)	-	-	G	-
160	Unknown	-	1582 (NA)	-	-	G	-
161	Unknown	-	1589 (NA)	-	-	G	-
162	Unknown	-	1640 (NA)	-	-	G	-
163	Unknown	-	1653 (NA)	-	-	G	-
164	Unknown	-	1664 (NA)	-	-	G	-

ID: identification of compounds, RI- calculated retention index confirmed by NIST webbook database; MS- confirmed by mass spectrum; Std- confirmed by reference standards; NA: cannot find in references;

RIa: retention index calculated from equation; RIb: retention index from NIST Chemical webbook Database;

Compound group: A- Alcohol; B- Aldehyde; C- Ester; E- Ketone; F-Terpene; G-Unknown

The order of the compounds was arranged by RI calculated from DB-WAX column.

Table 7: Relative concentration of 26 typical odourless free volatiles in the three feijoa cultivars at four ripening stages

No.	Compound	Free aroma volatile relative concentration ($10^{\wedge 2}$ug/kg)											
		U4	U2	UR	UO	T4	T2	TR	TO	A4	A2	AR	AO
11	3-Hexanone	0.52 ± 0.01d	0.18 ± 0.02a	0.21 ± 0.02ab	ND	0.34 ± 0.03c	0.16 ± 0.00a	ND	ND	0.51 ± 0.02d	0.25 ± 0.02b	ND	ND
21	Isoamyl acetate	ND	ND	ND	4.42 ± 0.05a	ND	ND	ND	16.74 ± 0.34c	ND	ND	ND	8.61 ± 0.37b
26	2-Heptanone	ND	ND	1.76 ± 0.13b	0.24 ± 0.02a	ND	0.23 ± 0.01a	ND	0.21 ± 0.01a	ND	ND	0.42 ± 0.02a	2.46 ± 0.09c
28	D-Limonene	7.58 ± 0.12a	17.68 ± 0.07ab	17.55 ± 0.03ab	17.45 ± 0.02ab	18.34 ± 0.21ab	7.32 ± 0.03a	7.21 ± 0.01a	18.00 ± 0.09ab	290.33± 23.23e	188.98± 11.94d	46.55 ± 3.19c	38.16 ± 4.01bc
30	2-Metylbutyl Isobutyrate	12.73 ± 1.12d	5.08 ± 0.08b	1.98 ± 0.11a	0.73 ± 0.05a	17.88 ± 0.87e	5.39 ± 0.44b	1.58 ± 0.08a	6.14 ± 0.52bc	7.09 ± 0.17c	6.60 ± 0.36bc	1.92 ± 0.17a	0.39 ± 0.03a
33	β-Phellandrene	ND	ND	ND	ND	0.27 ± 0.02a	ND	ND	ND	96.60 ± 0.75c	96.76 ± 8.27c	20.21 ± 0.59b	19.73 ± 1.62b
34	2-Hexenal	ND	ND	2.49 ± 0.11de	1.54 ± 0.08bc	8.32 ± 0.21f	ND	0.68 ± 0.03a	2.90 ± 0.08e	2.28 ± 0.20cde	1.95 ± 0.19bcd	1.22 ± 0.49ab	ND
36	2-Ethylbutyl isobutyrate	11.04 ± 0.35bc	ND	ND	ND	ND	7.81 ± 0.64ab	ND	8.15 ± 0.33abc	25.82 ± 2.27d	11.81 ± 0.68c	6.05 ± 0.44a	ND
45	o-Cymene	ND	ND	2.49 ± 0.00a	ND	2.46 ± 0.01a	ND	ND	ND	19.55 ± 0.90b	21.72 ± 2.36b	4.72 ± 0.07a	3.62 ± 0.34a
52	3-Heptanol	0.02 ± 0.00a	0.01 ± 0.00a	ND	ND	0.02 ± 0.02a	ND	ND	ND	ND	ND	ND	ND
57	2-Heptenal, (Z)-	0.30 ± 0.02c	0.38 ± 0.02d	0.31 ± 0.01c	ND	ND	ND	ND	ND	0.14 ± 0.00a	0.22 ± 0.02b	ND	ND
62	3-Nonanone	0.03 ± 0.00b	0.01 ± 0.00a	ND	ND	0.04 ± 0.00b	0.01 ± 0.00a	ND	ND	ND	ND	ND	ND
77	Elixene	10.57 ± 1.36d	3.81 ± 0.37b	2.30 ± 0.12ab	1.97 ± 0.13a	7.99 ± 0.27c	2.29 ± 0.14ab	0.73 ± 0.02a	1.46 ± 0.11a	15.10 ± 0.68e	13.50 ± 0.84e	2.11 ± 0.08ab	1.81 ± 0.03
89	β-Elemen	26.27 ± 1.40j	8.64 ± 0.88d	6.00 ± 0.57bc	4.79 ± 0.65c	10.67 ± 0.76de	5.10 ± 0.74bc	1.65 ± 0.13a	3.38 ± 0.07ab	14.33 ± 0.02f	12.60 ± 1.06ef	3.31 ± 0.54ab	3.29 ± 0.31ab
93	Aromandendrene	15.00 ± 0.90e	8.11 ± 0.05d	ND	ND	4.89 ± 0.30c	3.62 ± 0.38bc	0.50 ± 0.04a	1.80 ± 0.19ab	25.85 ± 1.19g	17.76 ± 1.38f	ND	2.00 ± 0.07ab
98	Alloaromadendren	6.90 ± 0.49b	5.43 ± 0.56b	2.33 ± 0.14a	2.01 ± 0.17a	5.39 ± 0.15b	3.11 ± 0.20a	0.84 ± 0.06a	1.59 ± 0.02a	21.98 ± 1.04c	21.44 ± 1.86c	2.90 ± 0.28a	1.82 ± 0.27a
99	cis-3-Hexenyl Hexanoate	ND	ND	10.86 ± 0.57b	3.69 ± 0.30a	ND	ND	ND	4.72 ± 0.60a	ND	ND	17.24 ± 1.02c	5.58 ± 0.27a

Table 7 continued

103	Methyl geraniate	6.80 ± 0.40ab	5.96 ± 0.30a	23.69 ± 0.13e	10.50 ± 0.56d	7.95 ± 0.10bc	6.66 ± 0.41a	5.64 ± 0.04a	8.36 ± 0.02c	8.33 ± 0.10c	10.00 ± 0.94d	8.11 ± 0.10c	5.55 ± 0.25a
105	Varidiflorene	9.97 ± 1.88c	8.59 ± 0.41bc	8.76 ± 0.62bc	3.56 ± 0.05a	11.44 ± 1.39c	5.32 ± 0.56ab	1.58 ± 0.03a	2.69 ± 0.05a	25.72 ± 2.16d	30.43 ± 3.42e	3.44 ± 0.54a	2.69 ± 0.08a
111	Farnesene	67.25 ± 2.47c	ND	18.22 ± 0.27b	17.09 ± 1.02b	18.04 ± 0.06b	5.77 ± 0.80a	ND	16.06 ± 2.07b	22.13 ± 1.01b	101.22 ± 4.53d	21.57 ± 1.06b	19.76 ± 1.42b
120	α-Calacorene	0.62 ± 0.06cde	0.72 ± 0.03e	0.47 ± 0.03bc	2.52 ± 0.04g	0.69 ± 0.07de	0.53 ± 0.03bcd	0.29 ± 0.03a	0.39 ± 0.01ab	1.36 ± 0.05f	1.27 ± 0.12f	0.44 ± 0.07abc	2.46 ± 0.02g
124	Heptyl benzoate	ND	ND	9.38 ± 0.47d	10.90 ± 0.28e	ND	ND	ND	6.28 ± 0.04c	ND	ND	2.08 ± 0.33a	4.80 ± 0.47b
126	Veridiflorol	0.39 ± 0.03a	0.30 ± 0.01a	ND	ND	0.19 ± 0.15a	0.25 ± 0.01a	ND	ND	0.23 ± 0.02a	0.20 ± 0.01a	ND	0.28 ± 0.03a
132	Rosifoliol	ND	ND	27.21 ± 2.25c	5.23 ± 0.27b	0.17 ± 0.01a	0.11 ± 0.00a	ND	ND	ND	ND	ND	ND
136	α-Cadinol	0.76 ± 0.01b	0.46 ± 0.24ab	ND	ND	0.33 ± 0.01a	ND	0.11 ± 0.01a	0.16 ± 0.01a	1.58 ± 0.07c	ND	ND	ND
148	Aromadendrene	8.93 ± 0.60c	3.87 ± 0.35a	3.04 ± 0.06a	2.92 ± 0.19a	5.36 ± 0.05b	3.22 ± 0.13a	2.49 ± 0.00a	2.59 ± 0.02a	10.51 ± 0.00d	14.56 ± 1.34e	2.87 ± 0.00a	2.73 ± 0.13a

Volatiles concentration: present as mean ± standard deviation; ND: not detected; Compounds' number (No.) is consistent with that in Table 6.

a, b, c, d, e, f, g, h, i present statistically significant differences among cultivars and ripening stages by multiple comparisons using Duncan's test ($P < 0.05$), different letters indicate significant difference among samples;

These compounds were selected either by their higher concentration (more than 1.5 mg/kg) or special absent/present in some ripening stages (except all aroma active free compounds, which was discussed in detail in section 3.3).

The change of free volatiles in feijoas is shown in Figure 10. The progressions of all three cultivars were comparable, which decreased firstly then increased. Out of Unique, Triumph and Anatoki, Anatoki had the highest free volatile content for all the ripening stages. The volatile concentrations of Anatoki decreased firstly from 141.77 mg/kg (A4) to 137.88 mg/kg (A2) and 78.25 mg/kg (AR), followed by increasing to 125.63 mg/kg (AO). Triumph had the lowest free volatiles content throughout the whole growing process. The concentration of Triumph free volatiles started from 64.31 mg/kg in T4, followed by continuous declining to 32.73 mg/kg (T2) and 21.03 mg/kg (TR) before increasing to 79.84 mg/kg at over-ripe stage. The total free volatiles content of Unique was in between of Triumph and Anatoki with decreasing from 76.1 mg/kg (U4) to 42.4 mg/kg (U2) and increasing to 63.04 mg/kg (UR) and 100.28 mg/kg (UO).

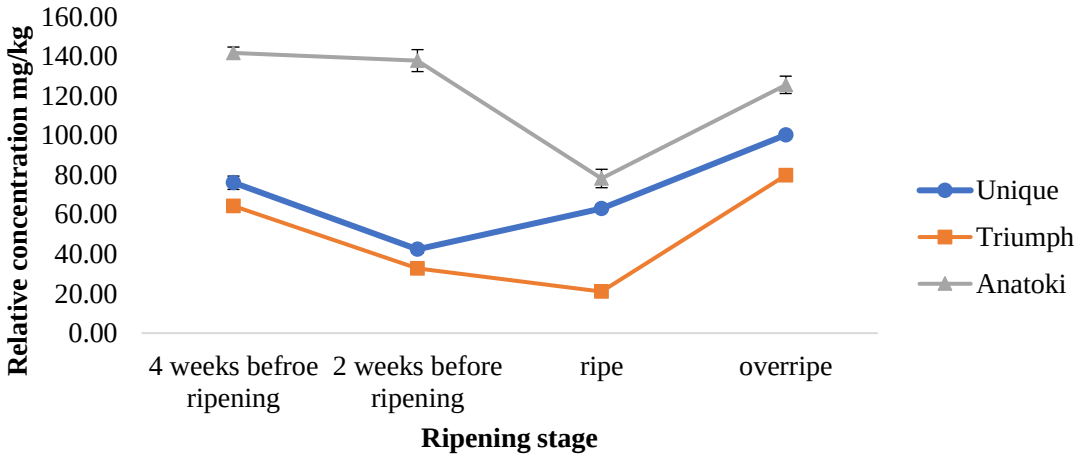

Figure 3.3: Total content of free volatiles in the three feijoa cultivars during four ripening stages

3.2.2 Changes in the composition of free volatile compounds during ripening

Free volatile compounds were classified into several groups, except 15 unknown compounds, free volatiles in feijoa was comprised of 60 terpenes, 52 esters, 20 alcohols, 9 ketones, 6 aldehydes and 2 hydrocarbons. The number of compounds in different groups for different cultivars was slightly different. Specifically, the number of alcohols in Anatoki was the highest (18), while that of ester compounds in Unique was the highest (50). The number of terpenes in Triumph was the lowest (52) yet in Anatoki feijoa was the highest (60).

Figure 11 illustrates the compositional changes during ripening for the three feijoa cultivars. Overall, the concentrations of terpenes, alcohols and ketones decreased during ripening while that of esters largely increased from approximate 7mg/kg to 80mg/kg. The levels of aldehydes fluctuated and that of hydrocarbons kept constant across ripening.

Terpenes and esters were most abundant volatile compounds in feijoa with significant higher than other compounds ($p < 0.05$). At 4 weeks before ripening stage (4WBR) and 2 weeks before ripening stage (2WBR), terpenes occupied the biggest share, while esters were the biggest portion when the fruits became ripe and overripe (Figure 11). Anatoki feijoas had the most terpene compounds at 4WBR and 2WBR, while Triumph fruits had the most terpenes at ripe stage (R) and over-ripe stage (OR). For esters, at unripe stages (4WBR and 2WBR), Triumph had the highest esters compared to Unique and Anatoki. Whereas at R and OR, Anatoki had highest esters compared with Unique and Triumph.

Except some slight increase from U4 to U2, the concentration of alcohols declined during fruit ripening for all three cultivars. Triumph always had the most alcohol than the Unique and Anatoki fruits at the same ripening stages. Similar to alcohols, there was also a decrease of ketones in fruits from unripe to ripe. However, the concentrations of aldehydes and hydrocarbons were too small for detection for some samples.

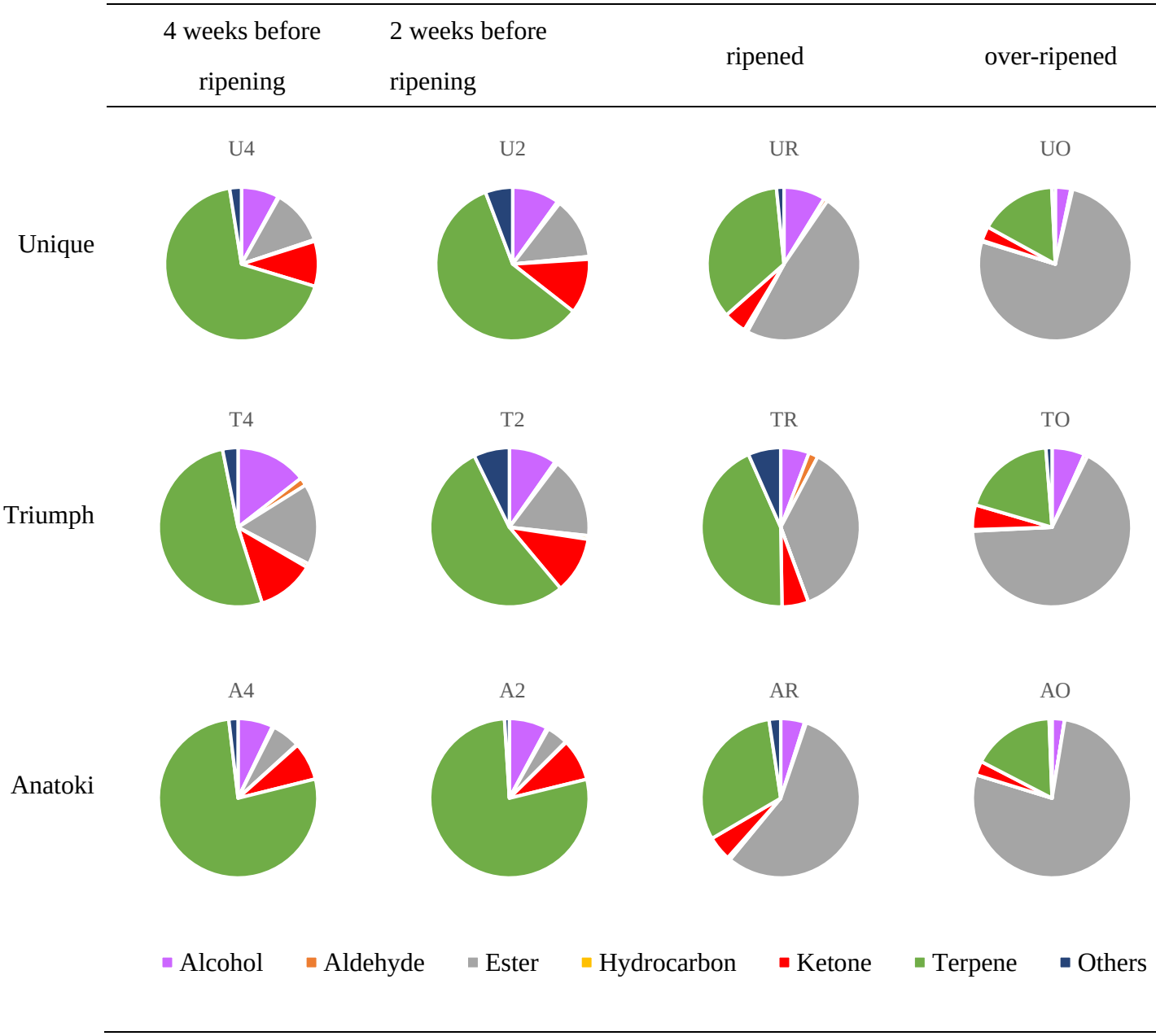

Figure 3.4: Compositional changes of free volatiles in three feijoa cultivars during ripening

3.2.2.1 Esters

As mentioned previously, a total of 52 esters were found as the dominant group in the feijoas at ripe and overripe. Among them, the number of free esters in Unique, Anatoki and Triumph were 50, 49 and 47 respectively. However, although the number of esters was the abundant in Unique cultivar, the relative concentration of esters was highest in Anatoki, followed by Unique and Triumph. The change in ester concentration during ripening stages across three cultivars can be seen in Figure 12.

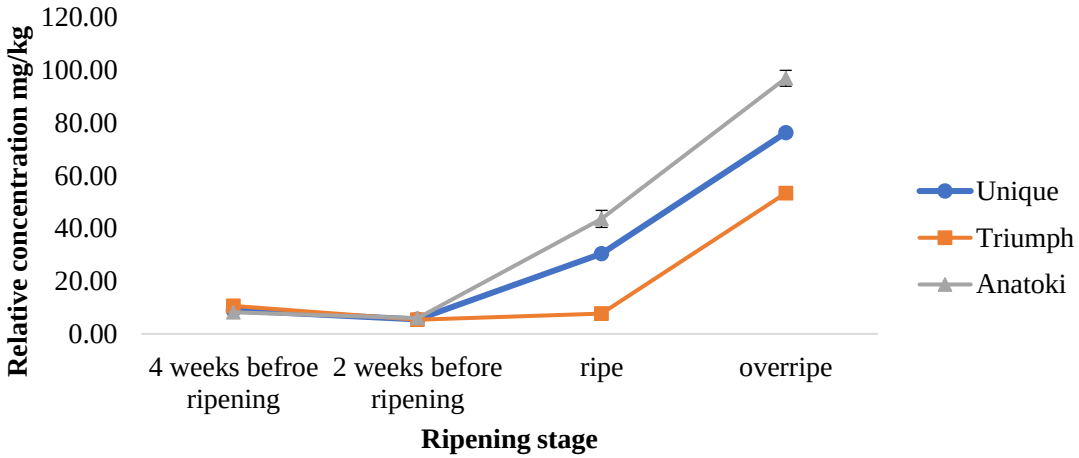

Figure 3.5: Total content of free esters in the three feijoa cultivars during four ripening stages

From Figure 12, all three cultivars showed the same overall trend on the change of the ester content from 4WBR to OR. Take the Anatoki cultivar as an example, the relative concentration of free esters slightly decreased from 8.17 mg/kg (A4) to 5.94 mg/kg (A2) then dramatically increased to 43.63 mg/kg (AR) and 96.98 mg/kg (AO). It was noteworthy that the ester level at the ripe stage for Triumph was significantly lower than the other two cultivars ($P < 0.05$).

Among all ester compounds, there were 12 compounds with concentrations more than 1.5 mg/kg (Table 7). The change of individual esters during ripening varied. Isoamyl acetate only existed in overripe fruits for all three cultivars and the concentration of 2-metylbutyl isobutyrate and 2-ethylbutyl isobutyrate were highest in 4WBR fruits. The levels of heptyl

benzoate increased from ripe to overripe stages in all three cultivars whereas the concentration of cis-3-hexenyl hexanoate decreased from ripe to overripe.

Esters have been frequently reported as dominant fruit volatiles. Acetate esters such as hexyl acetate, butyl acetate and 2-muthylbuty acetate were the important volatiles in apples (El Hadi et al., 2013), which were also found in feijoas. Butyl acetate, methyl hexanoate, ethyl hexanoate, ethyl butanoate and methyl butanoate were the key esters in strawberry (Du et al., 2011), which were also abundant in feijoas. Besides, ethyl esters in pear is abundant (Lu et al., 2012). Same with pears, ethyl ester such as ethyl benzoate was one of the most important esters in feijoas. What is more, comparable with ripe feijoas, banana is also a fruit with many aromatic ester volatiles and the concentration of acetate esters and butanoate esters increased with maturation. (Jayanty, Song, Rubinstein, Chong, & Beaudry, 2002; Jorge A. Pino & Febles, 2013).

With regards to the changes of esters during fruit ripening, according to Pérez and Sanz (2008), the level of esters varies in different fruits and at different ripening stages because of substrate quantity and enzyme activity. In feijoas, ester content also varied with different ripening stages and cultivars. From Table 7, the content of cis-3-hexenyl benzoate increased in fruits form unripe to ripe stages, however, the concentration of 3-octyl acetate was higher in unripe fruits. Some esters such as isomayl benzoate and isobutyl benzoate were only formed in overripe feijoas. Compared with apple fruits, same with feijoas, the changes of apple ester concentration during ripening were not constant for every different ester as well. In apple, butyl acetate and hexyl acetate increased with ripening progressed yet ethyl butanoate, methyl acetate and ethyl acetate fluctuate during maturation progresses (Echeverrıa et al., 2004).

Ester are formed by alcohol esterification and fatty acid metabolism. Carboxylic acid metabolism β-oxidation and lipoxygenase (LOX) pathway are two major pathways via which esters are synthesized form fatty acids (Siegmund, 2015). In feijoas, saturated esters such as

2-ethylbutyl isobutyrate, 2-metylbutyl isobutyrate and cis-3-hexyl ester could be formed in fatty acids metabolisms (Siegmund, 2015). In β-oxidation pathway (flow chart can be seen in Figure 1 in section1.2.2.1), by removing two carbons from lipids, fatty acid acyl-CoA derivatives were generated. Acyl CoAs are formed by losing two carbons with the assistance of flavinadenine dinucleotide (FAD), nicotinamide adenine dinucleotide (NAD) and free CoA. Acyl CoAs are reduced to aldehydes when CoA reductases are available. Moreover, aldehydes can be further reduced to alcohol by alcohol dehydrogenase (ADH) and alcohol could be transformed to corresponding esters by alcohol acyltransferase (AAT). LOX pathway is responsible for the generation of C6 and C9 aldehydes, which can be utilized to form C6 alcohols and finally transformed into ester form (flow chart can be seen in Figure 2 in section1.2.2.1). During the growth of fruits, there are many alcohols generated (El Hadi et al., 2013). However, ester content in fruits increases with the decreased alcohol content due to the esterification of alcohols (Siegmund, 2015).

Amino acid metabolism is also a way to form methyl-branched esters (Siegmund, 2015). In feijoas, compounds including methyl benzoate, ethyl benzoate, ethyl acetate, methyl butanoate are generated through this pathway. Amino acids firstly generate corresponding α-keto acid by deamination and transamination, and alcohols, aldehydes and esters can be further formed after decarboxylation, oxidation, reduction and esterification (flow chart can be seen in Figure 3 in section1.2.2.1). According to Gonda et al. (2010), amino acid metabolism is positively associated with maturation. That means the generation of these esters would increase with ripening progressed, which is consistent with the results in this thesis.

Ester formation pathways are related to enzyme activity and substrate availability. According to Siegmund (2015), ester concentration in fruits and vegetables is down-regulated by esterase. As ripening progresses, esterase activity increases (Lamikanra & Watson, 2003). This could explain the reduction of some esters such as 3-octyl acetate and 2-methylpentyl isovalerate during ripening. Moreover, the activity of ADH, AAT and LOX varies in different

fruit and cultivar, ripening stages and growth conditions. AAT is active in both flesh and peel in Fuji apple but in Granny Smith apples AAT only works on peel (El Hadi et al., 2013). Moreover, AAT in Fuji works on a wider range of alcohols whereas AAT in Granny Smith apples only focus on hexanol and cis-3-hexenol (Holland et al., 2005). According to Echeverrıa et al. (2004), saying the availability of ester precursors is more crucial than enzymes for some ester such as acetate esters. By analysing Fuji apples, they found that the content of acetate ester such as hexyl, butyl and 2-methylbutyl acetate esters and their precursors such as 1-hexanol, 1-butanol and 2-methyl-1-butanol increase with ripening progressed. However, the activity of AAT fluctuated in flesh and decreased in peel during ripening.

3.2.2.2 Terpenes

Terpene compounds are a dominant group in 4WBR and 2WBR feijoas. Monoterpenes (ten-carbon) and sesquiterpenes (fifteen-carbon) were the most common terpene compounds. In total, 60 terpenes were identified in three feijoa cultivars with 60 in Anatoki, 52 in Unique and 51 in Triumph.

The content of terpenes generally decreased with ripening progressed (Figure 13). Anatoki had the highest terpene content at all ripening stages with the concentrations of 109.19 mg/kg in A4, 107.33 mg/kg in A2, 24.31 mg/kg in AR and 21.1 mg/kg in AO. On the contrary, the Triumph cultivar contained the lowest terpenes across four ripening stages and the levels of terpenes in the Unique cultivar was between Anatoki and Triumph.

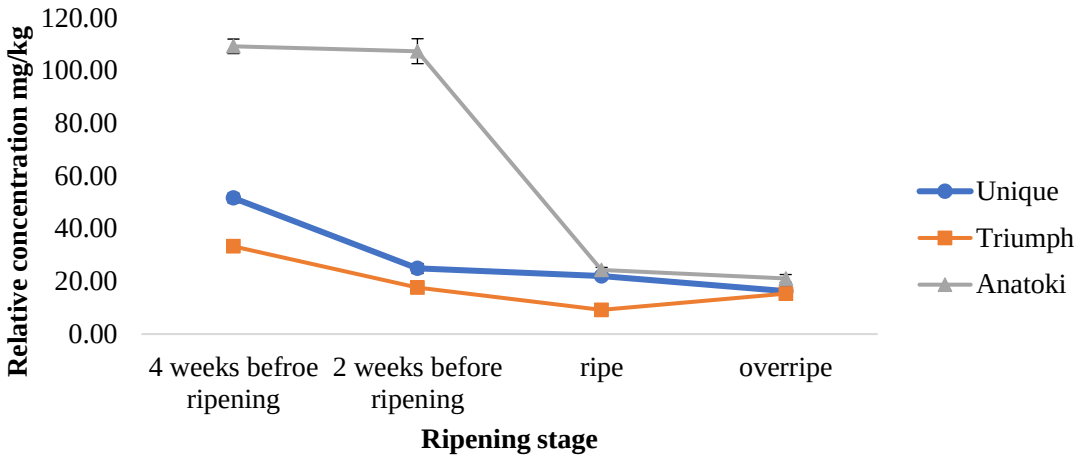

Figure 3.6: Total content of free terpenes in the three feijoa cultivars at four ripening stages

Among all free terpene compounds, there were 23 compounds with concentrations more than 1.5 mg/kg (Table 7). Despite the change of terpenes during ripening varied in individual compounds, the concentration of most free terpene compounds decreased with ripening progressed. For the total content of terpene, there was a significant drop when fruits changed from 4WBR to overripe fruits. For the 23 potent terpenes, except myrcene and *β*-(E)-ocimene, the concentrations of others were higher in unripe fruits than in ripe fruits. For myrcene, the concentration increased as ripening progressed and for *β*-(E)-ocimene, the progression fluctuated during ripening (Table 7).

Referencing to other papers, 35 terpenes were found in essential oil of feijoa peel. High concentration compounds such as humulene, *α*-cubebene, *β*-caryophyllene, germacrene D, *δ*-cadinene, *β*-elemene (Peng et al., 2019) were also found in this thesis. In papaya, terpenes declined significantly during maturation (Fuggate et al., 2010) and in blackcurrant, the relative concentration of monoterpenes and sesquiterpenes decreased as ripening progressed (Del Castillo & Dobson, 2002), which are both comparable with the results found in this thesis.

The formation of terpenes is mainly from carbohydrate pathway (El Hadi et al., 2013). Monoterpenes including D-limonene, *β*-phellandrene, *α*-terpinene and *β*-(E)-ocimene are

formed in plastids by methylerythritol phosphate (MEP) pathway. geranyl pyrophosphate (GPP) synthase catalysed enzymatic reaction of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) and form GPP. Under the assistance of terpene synthases (TPSs), monoterpense are generated from GPP (flow chart can be seen in Figure 4 in section 1.2.2.1). Sesquiterpenes including farnesene and caryophyllene are formed through mevalonic acid (MVA) pathway in cytosol (flow chart can be seen in Figure 5 in section 1.2.2.1). farnesyl pyrophosphate (FPP) synthases catalysed enzymatic reaction of IPP and DMAPP and form FPP. Lastly, with TPSs, sesquiterpenes are generated from FPP (Siegmund, 2015).

Comparable with ester synthesis mechanism, the generation of terpenes largely depends on the activity of terpene synthases. The change of Terpene synthases during mango ripening was explored by Pandit et al. (2010). They found that the content of terpene synthases decreased during ripening. This finding could be used to explain the dropped terpene trend in feijoas during ripening. However, some TPSs of monoterpense and sesquiterpenes specifically expressed at some ripening stages in fruits (Tholl, 2006). This can explain irregular trends of some terpene compounds such as β-(E)-ocimene and (4E,6Z)-allo-ocimene in feijoas.

3.2.2.3 Alcohols

Alcohol was the third biggest group after ester and terpenes in feijoas. A total of 20 alcohol volatile compounds were found. The number of alcohols in the Anatoki, Triumph and Unique was 18, 17 and 16 respectively

Despite of some fluctuations, the content of alcohol decreased during ripening (Figure 14). To be specific, the total content of alcohols in Anatoki increased a little from A4 (10.09 mg/kg) to A2 (10.74 mg/kg) and continuously dropped from A2 to AO (3.17 mg/kg). Contrast with Anatoki, the total concentration of alcohols in Triumph had a continuous decrease from T4 (9.29 mg/kg) to TR (1.22 mg/kg) and an increase from TR to TO (5.30

mg/kg). Different with Anatoki and Triumph, the change of alcohol concentration in Unique was the least with a gentle increase and decreases (highest in U4 6.02 mg/kg and lowest in UO 3.27mg/kg).

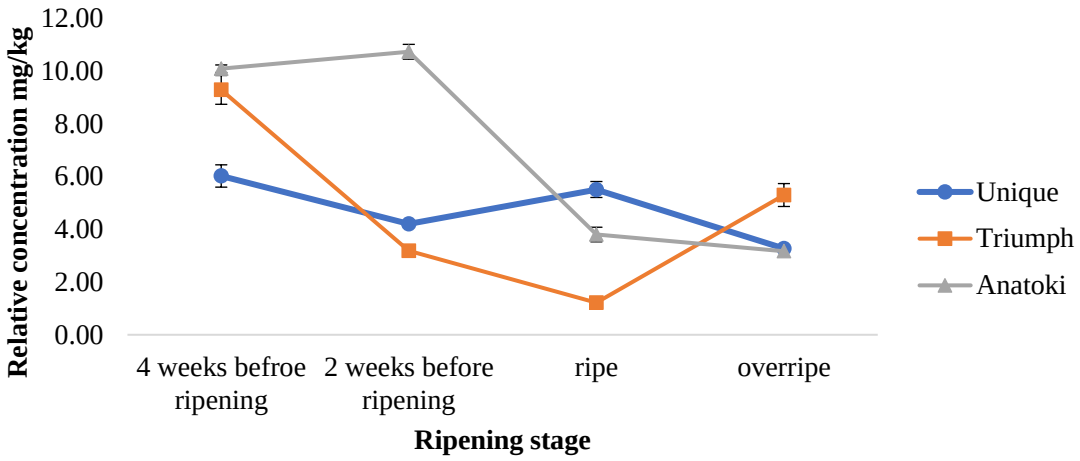

Figure 3.7: Total content of free alcohols in the three feijoa cultivars at four ripening stages

There were 5 alcohol compounds in feijoas with the concentration over 1.5 mg/kg (Table 7). Particularly, Linalool was the dominant alcohol in feijoas which presented in all the 12 samples. 3-heptanol was only presented in U4, U2 and T4 samples. For the progressions of individual alcohol volatiles during ripening, the concentration of 3-Hexanol, (-)-spathulenol and ledol increased. However, the concentration of 3-hexanol, 1-hexanol, cubenol, α-cadinol, α-terpineol δ-Cadinol, β-selinenol and linalool decreased in unripe to ripe fruits.

Alcohols in feijoa has also been detected in other papers. In essential oils extracted from feijoa peel, 19 alcohols were detected (Peng et al., 2019). Among them, 3-ocatnaol, rosifoliol, (-)-spathulenol, α-cadinol, α-terpineol and linalool were also found in this study. Moreover, 3-hexanol, 2-heptanol, 3-hexenol, 3-octanol, 4-terpinenol, palustrol, globulol, ledol, α-terpineol (-)-spathulenol and linalool were also detected in feijoa flesh (Binder & Flath, 1989) which were also detected in this study. Alcohol was also abundant in papaya. Typical alcohol hexanol, linalool, benzyl alcohol was found in papaya with a decrease trend during ripening (Fuggate et al., 2010; Liu et al., 2019). In feijoas, the concentration of alcohol hexanol,

linalool, benzyl alcohol decreased as well. In melons, some short-chain alcohols including 3-hexenol, heptanol, linalool were also detected. Furthermore, the concentration of alcohols in melon were dependent with cultivars and ripening stages (Yahyaoui et al., 2002). In strawberry, cis-3 hexanol, cis-2-hexanol and linalool were found with a decreasing progression during ripening (Ménager et al., 2004; Samykanno et al., 2013), which was comparable with that in feijoas.

Alcohol are mainly formed from fatty acid and amino acid metabolism (Siegmund, 2015). Six-carbon alcohols such as 3-hexanol is formed from fatty acid metabolism. As mentioned in literature review and section 3.3.2.1, alcohols are formed from aldehyde enzymatic reaction catalysed by ADH in both β-oxidation and LOX pathways. Aliphatic, methyl-branched aromatic alcohols including ledol and cubenol are likely to be generated from amino acid pathway, which is related with α-keto acids generation (El Hadi et al., 2013).

The concentration of alcohols during ripening is associated with related substrates and enzymes. In papaya, the decreased alcohol content was related to the availability of increased ADH concentration and increased AATs concentration (Fuggate et al., 2010). The increased AATs could explain the decline of some alcohols in current study. More alcohols are transformed into esters with a high activity of AATs in feijoas. The activity of LOXs varied in fruits and ripening stages. According to Griffiths, Barry, Alpuche-Solis, and Grierson (1999), LOXs can be divided into three groups which are Tomlox A, B and C. Tomlox A declines during ripening while Tomlox B and Tomlox C increases with maturation. They are differentially regulated therefore lead to a complex regulation system in tomato during ripening. This could explain the fluctuation of 3-octanol in feijoas. Moreover, the increased concentration of ledol and cubenol could be explained by the increased concentration of amino acids in ripe fruits, which observed in tomatoes (Sorrequieta et al., 2010). Higher substrates concentration in ripe fruits could result in the increase of alcohols that are generated form amino acids metabolism.

3.2.2.4 Aldehydes

A total of 6 aldehydes were found in feijoas, namely 2-propenal, pentanal, 2-hexenal, 2-heptenal, benzaldehyde and hexanal. Among them, 2-pentenal was absent in the Triumph cultivars.

The change of aldehyde varied with cultivars and compounds. For 4WBR fruits, T4 had the highest aldehyde content with 1.07 mg/kg, followed by A4 (0.49 mg/kg) and U4 (0.19 mg/kg). At 2WBR, T2 decreased dramatically to 0.19 mg/kg. While with a slight increase, A2 and U2 increased to 0.52 mg/kg and 0.24 mg/kg respectively. For ripe fruits, aldehyde in UO peaked at 0.53 mg/kg and in TR and AR were 0.40 mg/kg and 0.28 mg/kg respectively. Finally, for overripe fruits, TO had the highest aldehyde content (0.59 mg/kg) flowed by UO (0.38 mg/kg) and AO (0.06 mg/kg) (Figure 15).

For hexanal, its progression differed between cultivars. In the Unique, hexanal increased first from U4 (0.16 mg/kg) to UR (0.24 mg/kg) and declined from UR to UO (0.19 mg/kg). Whereas for the Triumph, the opposing trend was observed whereby an increased was followed with a decrease. Moreover, the concentration of 2-heptenal was highest in 2WBR, but for 2-hexenal, the concentration was highest in 4 WBR.

The change of aldehyde during ripening also varied in different plants. In avocados, the concentration of hexanal and 2-hexenal significant decreased in amount during ripening (Obenland et al., 2012). On the contrary, in climacteric pear, the content of aldehydes increased with ripening progressed (G. Li et al., 2014). The different trends of aldehyde could be explained by substrate and enzyme differentiation in various fruits and different ripening stages.

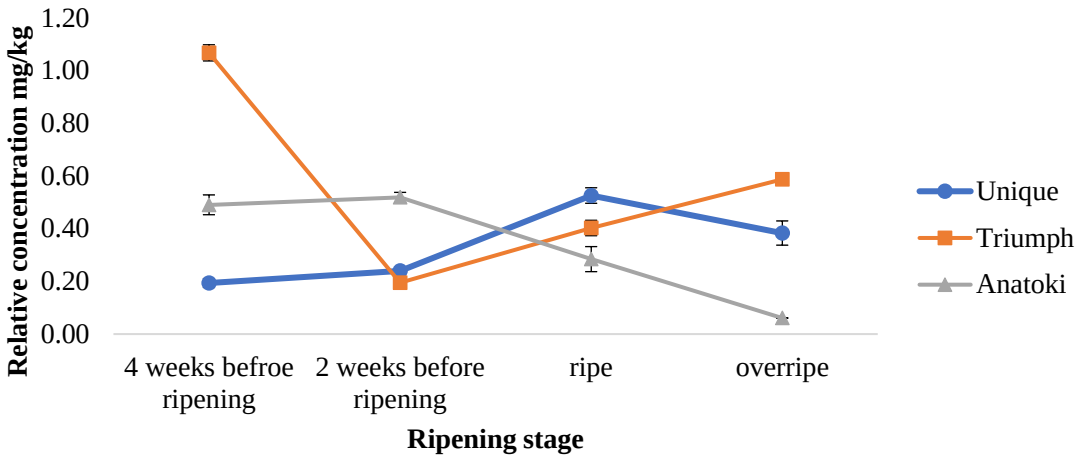

Figure 3.8: Total content of free aldehydes in the three feijoa cultivars at four ripening stages

The formation of aldehydes is mainly through fatty acids metabolism. As mentioned previously, aldehydes are synthesized before alcohols and esters. Saturated and unsaturated six-carbon and nine-carbon aldehydes including hexenal and 2-hexenal are derivate from linolenic acids as primary oxidation products by LOX pathway (El Hadi et al., 2013; Siegmund, 2015). The variation of aldehydes in fruits is related to the fatty acids' availability and enzymes (LOXs and CoA reductase, ADHs and AAT) activity. In mango fruits, fatty acids content accumulated during ripening (Lalel, Singh, & Tan, 2003), which could explain the increasing content of aldehydes in feijoas. The decreased content of aldehyde in Unique and Anatoki could be related to the higher concentration of esters. More aldehydes could be transformed to alcohols and subsequent esters from unripe to ripe stages in feijoas.

3.2.2.5 Ketones

A total of 9 ketones were found in feijoas. Both Unique and Triumph feijoas contained all these 9 ketones were detected in Unique and Triumph but methyl heptanone and 3-nonanone were not detected in Anatoki. Anatoki had the highest ketones throughout the ripening progress (Figure 16). The level of ketone in Unique was in between of Triumph and Anatoki. The concentrations of ketones in Triumph were lowest except TO sample.

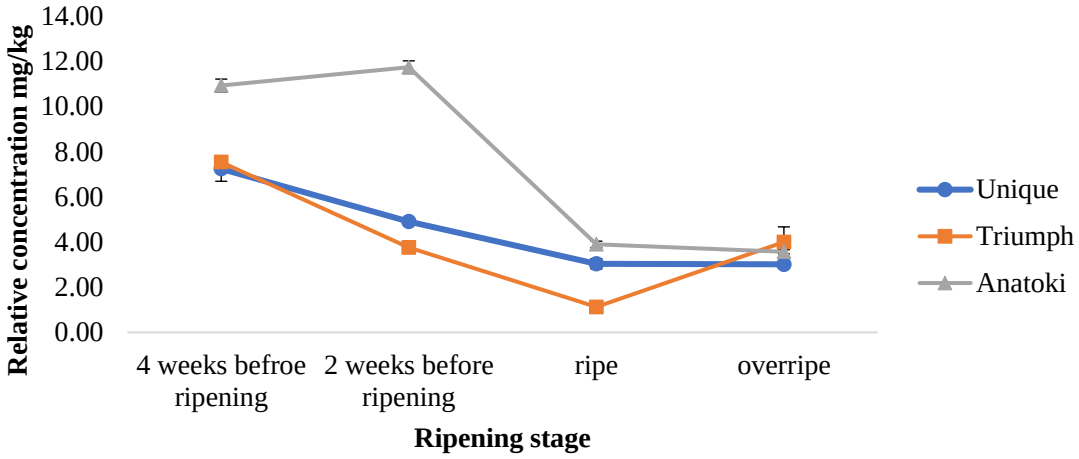

Figure 3.9: Total content of free ketones in the three feijoa cultivars at four ripening stages

Despite of some fluctuations, the total content of ketones in these three cultivars declined. However, the changes of individual ketones had different patterns. Specifically, the concentration of 3-hexanone and 3-octanone dropped from unripe to ripe stages. While the trends of 2-nonanone varied with cultivars. The concentration of 2-nonanone were the highest in the Anatoki fruits at 4WBR and 2WBR but highest in the Unique cultivar at ripe and overripe stages.

Ketones are synthesized through fatty acid and amino acid pathway (Siegmund, 2015). The formation of ketones depends on substrates and enzymes and varied in different fruits (Sanz et al.,1996). In raspberry fruits (Koeduka et al., 2011) and tomatoes (Simkin et al., 2004), the ketone content was upregulated as ripening progressed. In feijoas, the content of 2-nonanone increased in ripe fruits as well.

3.2.2.6 Hydrocarbons

Compared with esters, terpenes, alcohols, aldehydes and ketones, very little hydrocarbons were present in feijoas. There were only two compounds ethylbenzene and cadalene found in current project. The concentrations of ethylbenzene varied in different cultivars during ripening the concentrations of cadalene were higher in unripe feijoas Ethylbenzene was found in olive fruits (Scarpati et al.,1993) whereas cadalene was found in essential oils extracted from feijoa peel (Peng et al., 2019) and grapes (Yang et al., 2011).

In summary, the change of free volatiles during ripening can benefit people understand more characteristics about feijoas. Distinct changes of terpenes and esters free volatiles may be used as a parameter to judge feijoa fs maturation and growth conditions. Furthermore, by understanding the volatiles formation mechanisms, genetic modification could be applied on feijoas to boost the generation of more desirable free volatiles in feijoas.

3.3 Aroma active compounds in feijoa fruits

Although many free volatiles were detected, not all of them contributed to the sensorial aroma profile of feijoa. Aroma active compounds were identified by HS-SPME-GCO-MS by four trained panellists in this study. Compounds approved by at least two panellists were selected as aromatically active compounds in feijoas. The feijoa aroma profile is built upon the intensity of these compounds.

3.3.1 Aroma active compounds detected in feijoa fruits during ripening

A total of 26 aroma active compounds were identified over 164 free volatiles, with 2 alcohols, 1 aldehyde, 7 esters, 2 ketones, 12 terpenes and 2 unknown volatile compounds. Odour description, intensity and relative concentration of aroma active compounds are listed in Table 8 and Table 9. According to Kamadia et al., (2006) and Peng et al., (2019), the concentration of aroma compounds should have positive correlations with their odour intensity. In this research, compound's odorant intensity was proportional to its relative concentration.

Consistent with the free volatiles results, aromatic esters and terpenes were also the predominant groups among all classes. At unripe stages, terpenes dominated free aromas and released 'herbal, grassy' odour. While for ripe fruits, esters dominated free aromas and gave 'fruity, sweet, floral' aroma. According to Peng et al., (2019) who explored the aroma active compounds in feijoa juice, a total of 25 aroma active compounds were identified. Esters and terpenes were the main groups and contributed to the 'green, grass, herbal and fruity' odour of feijoa juice as well. Moreover, terpenes and esters were the main aromatic classes in essential oils extracted from feijoa peel as well (Peng et al., 2019).

Table 8: Intensity and description of aromatic free volatile in the three feijoa cultivars during four ripening stages

No.	Compound Name	RIa (RIb)		CAS	C	ID	Odor Description	Average Intensity											
		RI Wax	RI 5MS					U4	U2	UR	UO	T4	T2	TR	TO	A4	A2	AR	AO
1	Ethyl Acetate	863 (900)	603 (613)	141-78-6	C	Std, MS, RI	sweet, floral	ND	ND	0.5	2	ND	ND	1	2	ND	ND	0.25	2
2	Methyl butanoate	968 (976)	722 (721)	623-42-7	C	Std, MS, RI	fruity (apple), sweet	ND	0.5	4	2.25	ND	ND	ND	2	ND	ND	2	ND
3	Ethyl butanoate	1023 (1026)	801 (802)	105-54-4	C	Std, MS, RI	sweet, floral, fruity	2.25	2	4.25	5.75	0.75	2.25	4	6	ND	ND	6.25	7
4	Hexanal	1065 (1067)	804 (802)	66-25-1	B	Std, MS, RI	green, fruity(lemon)	2	2.5	3.75	2.5	4	2.75	4.25	4.5	4	3.75	3	ND
5	unknown1	1085(NA)			G	-	unpleasant (Gasoline, rubber, metal)	3	2	ND	ND	3.75	3	ND	ND	4	2	ND	ND
6	Myrcene	1140 (1157)	994 (993)	123-35-3	F	Std, MS, RI	unpleasant(metallic)	2	2	5.5	4.25	2.75	1	1.25	3	4.5	4	2.75	2
7	Ethyl hexanoate	1221 (1220)	999 (999)	123-66-0	C	Std, MS, RI	sweet, floral, fruity	ND	ND	4	4.5	ND	ND	2	4.25	ND	ND	5.25	4.75
8	β-(E)-Ocimene	1219(1224)	1039(1039)	3779-61-1	F	MS, RI	Herbal, sweet	2	1.75	4.25	4	2.25	2	1.75	4.5	3.5	3.75	4.75	4.25
9	3-Octanone	1248 (1240)	991 (988)	106-68-3	E	Std, MS, RI	Herbal, mint	6.25	6	4.5	4.75	6	4.75	2.5	5	6.5	6	3.75	3
10	unknown2	1292(NA)			G	-	Mushroom, mould	6.25	6	5.75	5.5	5.75	5	5.5	6.25	5	6	5.75	5.25
11	cis-3-Hexenyl Acetate	1307 (1315)	1008 (1009)	3681-71-8	C	Std, MS, RI	sweet, fruity(banana)	ND	3.5	6.25	5.75			2.75	4.5	ND	ND	5.75	3.75
12	(4E,6Z)-allo-Ocimene	1360 (NA)	1131 (1131)	7216-56-0	F	MS, RI	herb, unpleasant (metal, plastic)	5.75	5.25	5.25	3.75	4.75	2.75	ND	2.5	4.75	4.25	2.25	2
13	2-Nonanone	1382 (1387)	1093 (1096)	821-55-6	E	Std, MS, RI	Fruity, green	2	ND	3	ND	2.25	1	0.25	0.75	3.25	2.75	2	1
14	α-Cubebene	1458 (1455)	1363 (1360)	17699-14-8	F	MS, RI	Fruity, sweet, green	3.75	2.75	2.25	ND	2.5	2	ND	1.25	4	3.25	ND	ND
15	Linalool	1548 (1547)	1104 (1105)	78-70-6	A	Std, MS, RI	Flora, fresh	6.75	6.25	4.5	4.5	6.25	4.5	3.75	5.75	6.75	7	5	5.25
16	Caryophyllene	1602 (1599)	1446 (1444)	87-44-5	F	Std, MS, RI	grass, woody	5	2.5	1.75	1.5	5	2.5	ND	3	5.25	4	2.75	2
17	Methyl benzoate	1616 (1615)	1110 (1103)	93-58-3	C	Std, MS, RI	fruity, feijoa-like	ND	ND	7	7.5	2	2	2.75	6.25	ND	ND	7.25	6
18	Ethyl benzoate	1657 (1650)	1183 (1173)	93-89-0	C	Std, MS, RI	fruity, feijoa-like	ND	ND	5.75	7.5	ND	ND	1.25	4.75	ND	ND	5	7.75
19	Humulene	1670 (1671)	1478 (1477)	6753-98-6	F	Std, MS, RI	sweet, fresh, herbal	3	2	1.25	0.75	3.25	2.25	1.25	2	2.75	2	ND	ND
20	γ-Muurolene	1690 (1681)	1490 (1449)	30021-74-0	F	MS, RI	Fruity, sweet	3	2	1.75	ND	1.75	1	ND	ND	2.75	2.5	ND	ND
21	α-Terpineol	1697 (1692)	1199 (1200)	98-55-5	A	MS, RI	minty, woody	ND	ND	ND	ND	ND	ND	ND	ND	2.75	2.5	2	0.75

Table 8 continued

22	Germacrene D	1715 (1709)	1471 (1474)	23986-74-5	F	MS, RI	sweet, floral, green	2.75	2	1	ND	2.25	2	1	1	3.75	2.25	1.25	ND
23	β-Selinene	1721 (1719)		17066-67-0	F	MS, RI	green, woody	4.75	3	1.75	ND	2.75	2	ND	ND	3	2.75	1	ND
24	γ-Elemene	1741 (NA)	1339 (1339)	339154-91-5	F	MS, RI	herbal, grass	4	ND	2	1.5	4.75	3.75	2	2	2.75	2.25	1	0.75
25	δ-Cadinene	1759 (1758)	1540 (1534)	483-76-1	F	MS, RI	Fresh, herbal	3	2	ND	ND	2.75	2	ND	ND	2.75	2.25	1	1.25
26	L-Calamenene	1829 (1832)	1542 (1546)	483-77-2	F	MS, RI	Herbal, sour, fruity	4.5	2.5	1	ND	4.5	3.25	2	2	3	3.5	1	ND

The order of the compounds was arranged by RI calculated from DB-WAX column.

ID: identification of compounds, RI- calculated retention index confirmed by NIST webbook database; MS- confirmed by mass spectrum; Std- confirmed by reference standards; NA: cannot find in references; ND: not detected;

RIa: retention index calculated from equation; RI^b: retention index from NIST Chemical webbook Database;

Compound group: A- Alcohol; B- Aldehyde; C- Ester; D- Hydrocarbon; E- Ketone; F-Terpene; G- Unknown

The intensity scale was from 0 to 10. The average intensity was calculated by the sum of intensity divided by the number of panellists

Table 9: Relative concentration of aromatic free volatile in three feijoa cultivars at four ripening stages

No.	Compound	Free aroma volatile relative concentration (10^2ug/kg)											
		U4	U2	UR	UO	T4	T2	TR	TO	A4	A2	AR	AO
1	Ethyl Acetate	0.56 ± 0.00a	0.52 ± 0.05a	19.55 ± 0.53ab	291.40± 10.30e	2.36 ± 0.12a	3.42 ± 0.30ab	26.96 ± 1.02bc	256.95 ± 1.21d	1.37 ± 0.12a	0.95 ± 0.07a	45.26 ± 4.48c	587.24±23.02f
2	Methyl butanoate	ND	0.26 ± 0.05a	31.49 ± 0.12c	6.62 ± 0.23b	ND	0.24 ± 0.03a	0.12 ± 0.01a	3.33 ± 0.15ab	ND	ND	40.34 ± 3.57d	2.59 ± 0.04ab
3	Ethyl butanoate	0.18 ± 0.01a	0.17 ± 0.01a	33.27 ± 0.27c	95.03 ± 1.09e	0.17 ± 0.01a	0.52 ± 0.01a	9.29 ± 0.21b	57.84 ± 0.32d	0.16 ± 0.00a	0.09 ± 0.01a	89.14 ± 6.99e	106.17 ± 2.34f
4	Hexanal	1.57 ± 0.15b	1.97 ± 0.03bcd	2.42 ± 0.17cd	1.92 ± 0.51bc	2.35 ± 0.10cd	1.91 ± 0.13bc	2.58 ± 0.21d	2.42 ± 0.06cd	2.39 ± 0.18cd	2.41 ± 0.05cd	1.50 ± 0.02b	0.31 ± 0.02a
5	unknown1	2.50 ± 0.07a	2.46 ± 0.02a	ND	ND	2.45 ± 0.01a	2.43± 0.01a	ND	ND	2.49 ± 0.00a	ND	ND	ND
6	Myrcene	15.75 ± 0.20a	15.67 ± 0.02a	18.11±0.18abc	17.01 ±0.31ab	16.55 ±0.17ab	15.70 ± 0.06a	15.79 ± 0.01a	18.88± 0.0abc	41.23 ± 3.40e	31.91 ± 0.21d	21.87 ± 1.20c	19.95 ±1.82bc
7	Ethyl hexanoate	0.30 ± 0.05a	0.34 ± 0.03a	10.21 ± 0.16c	19.88 ± 0.18d	0.20 ± 0.00a	0.36 ± 0.02a	3.85 ± 0.01b	18.09 ± 0.35d	0.16 ± 0.00a	ND	22.71 ± 2.36e	22.94 ± 0.71e
8	β-(E)-Ocimene	2.16 ± 0.15b	1.53 ± 0.17ab	8.55 ± 0.23g	5.66 ± 0.20de	4.73 ± 0.28d	1.54 ± 0.04ab	2.05 ± 0.01b	6.53 ± 0.43ef	0.68 ± 0.01a	3.21 ± 0.15c	11.18 ± 0.83h	6.64 ± 0.38f
9	3-Octanone	59.39 ± 4.30f	42.20 ± 1.50e	23.05 ± 2.04b	25.51 ± 0.16bc	69.52 ± 0.37g	33.57±2.55cbe	9.82 ± 0.01a	36.37 ± 6.61de	99.16 ± 3.06h	107.22 ±2.44h	35.68 ±1.10de	29.94± 1.03bcd
10	unknown2	2.42 ± 0.00b	2.42 ± 0.00b	2.41 ± 0.00a	2.41 ± 0.00a	2.41 ± 0.01a	2.41 ± 0.00a	2.41 ± 0.00a	2.41 ± 0.00a	2.42 ± 0.01b	2.42 ± 0.00c	2.41 ± 0.01a	2.42 ± 0.00b
11	cis-3-Hexenyl Acetate	ND	3.26 ± 0.03a	19.37 ± 0.27b	15.73 ± 0.29b	ND	ND	4.54 ± 0.16a	26.91 ± 1.35c	ND	ND	38.35 ± 3.25d	7.92 ± 0.26a
12	(4E,6Z)-allo-Ocimene	1.52 ± 0.05h	0.90 ± 0.06ef	0.66 ± 0.05cd	0.49 ± 0.01bc	4.78 ± 0.10hi	0.69 ± 0.00cde	0.05 ± 0.00a	1.14 ± 0.01g	1.01 ± 0.16fg	0.81 ± 0.08def	0.62 ±0.03bcd	0.45 ± 0.03b
13	2-Nonanone	1.18 ± 0.06fg	0.89 ± 0.02de	1.81 ± 0.12h	0.78 ± 0.02cd	1.30 ± 0.03g	0.80 ± 0.04cd	0.12 ± 0.01a	0.51 ± 0.04b	2.25 ± 0.04i	1.97 ± 0.01h	1.05 ± 0.10ef	0.67 ± 0.04bc
14	α-Cubebene	25.14 ± 2.07e	7.91 ± 1.10c	3.82 ± 0.35ab	4.42 ± 0.02ab	12.81 ± 0.19d	6.11 ± 0.42bc	1.86 ± 0.12a	3.26 ± 0.07ab	33.47 ± 0.10f	27.79 ± 2.15e	2.88 ± 0.24ab	3.53 ± 0.53ab
15	Linalool	41.53 ± 3.47d	24.68 ± 1.03c	16.30 ± 0.77b	15.27 ± 0.01b	74.18 ± 5.67e	16.53 ± 0.09b	5.00 ± 0.10a	44.62 ± 3.83d	68.47 ± 0.99e	71.77 ± 1.76e	20.83 ± 1.94bc	21.05 ± 0.00bc

Table 9 continued

16	Caryoph-yllene	48.82 ± 4.24d	20.21 ± 1.61bc	9.55 ± 0.59a	7.81 ± 0.50a	26.02 ± 2.69c	13.07 ± 0.91ab	3.50 ± 0.19a	6.32 ± 0.10a	72.99 ± 3.34e	71.83 ± 7.58e	7.93 ± 0.23a	6.90 ± 0.46a
17	Methyl benzoate	ND	ND	86.79 ± 2.04c	122.57 ±7.25d	3.62 ± 0.30a	3.98 ± 0.29a	8.90 ± 0.04a	39.83 ± 0.85b	ND	ND	88.62 ± 7.39c	51.39 ± 2.09b
18	Ethyl benzoate	ND	2.59 ± 0.02a	6.94 ± 0.16b	113.56 ±3.21e	2.71 ± 0.05a	2.48 ± 0.04a	3.67 ± 0.05ab	33.32 ± 0.20d	ND	2.87 ± 0.10a	11.18 ± 0.54c	114.25 ±1.62e
19	Humulene	43.45 ± 0.05e	32.81 ± 4.08d	17.16 ±0.97bc	14.73 ±1.45bc	40.79 ± 3.27e	19.21 ± 1.78c	5.44 ± 0.05a	10.08 ±0.66ab	108.98 ± 2.13f	132.82 ±4.65g	12.85 ±0.90bc	17.52 ±1.15bc
20	γ-Muurolene	13.59 ± 0.53e	6.36 ± 0.49d	2.78 ± 0.07bc	1.89 ± 0.17ab	7.80 ± 0.59d	3.64 ± 0.28c	1.07 ± 0.02a	1.75 ± 0.12ab	16.02 ± 0.54f	16.83 ± 1.17f	2.19 ± 0.12abc	2.20 ± 0.07abc
21	α-Terpineol	ND	ND	ND	ND	ND	ND	ND	ND	3.67 ± 0.03b	4.52 ± 0.18c	1.92 ± 0.14a	1.69 ± 0.54a
22	GermacreneD	12.60 ± 0.37f	9.39 ± 0.24e	5.36 ± 0.40bc	4.78 ± 0.34b	7.17 ± 0.50d	6.54 ± 0.14cd	2.47 ± 0.17a	3.88 ± 0.34ab	20.31 ± 0.16h	17.23 ± 1.18g	6.33 ± 0.61cd	4.66 ± 0.01b
23	β-Selinene	31.70 ± 3.74c	4.11 ± 0.25ab	1.07 ± 0.11a	0.262 ± 0.00a	3.09 ± 0.23ab	1.43 ± 0.96a	0.23 ± 0.01a	0.57 ± 0.00a	6.11 ± 0.44b	6.09 ± 0.31b	0.70 ± 0.02a	ND
24	β-Elemene	8.49 ± 0.24b	4.50 ± 4.27ab	5.83 ± 0.13ab	5.18 ± 0.47ab	8.65 ± 0.58b	5.64 ± 0.41ab	2.00 ± 0.10a	3.94 ± 0.27a	21.76 ± 0.73b	18.04 ± 0.84b	5.75 ± 0.22ab	5.00 ± 0.16ab
25	δ-Cadinene	28.33 ± 1.76f	10.56 ± 1.05d	5.74 ± 0.27bc	3.89 ± 0.27ab	13.69 ± 1.38e	6.99 ± 0.46c	2.54 ± 0.09a	3.73 ± 0.02ab	26.94 ± 0.51f	28.58 ± 1.36f	4.66 ± 0.13abc	3.78 ± 0.44ab
26	L-Calam-enene	5.52 ± 0.37d	5.78 ± 0.39d	2.82 ± 0.41ab	2.31 ± 0.23ab	5.02 ± 0.47cd	3.77 ± 0.21bc	1.38 ± 0.09a	2.36 ± 0.04ab	8.91 ± 0.32e	13.40 ± 1.37f	2.71 ± 0.10ab	2.14 ± 0.17ab

These compounds were selected from free volatile which were aromatically active.

Volatiles concentration: present as mean ± standard deviation; ND: not detected; Compounds' number (No.) is consistent with that in Table 8.

a, b, c, d, e, f, g, h, i present statistically significant differences among cultivars and ripening stages by multiple comparisons using Duncan's test ($P < 0.05$), different letters indicate significant difference among sample

3.3.2 Compositional changes of aromatic free compounds

The total of 26 aroma active compounds were divided into 5 groups (expect the unknown compounds). The following paragraphs will discuss these compounds based on different classes.

3.3.2.1 Esters

A total of 7 esters were found as aroma active compounds in intact feijoas which were ‘sweet, floral’ ethyl acetate, ‘apple fruity, sweet’ methyl butanoate, ‘sweet, floral, fruity’ ethyl butanoate and ethyl hexanoate, ‘sweet, banana’ cis-3-hehenyl acetate, ‘fruity, feijoa-like’ methyl benzoate and ethyl benzoate. Similar to the relative concentration proregression during ripening, the intensity of esters was higher in ripe and overripe fruits. To be exact, all aromatic ester compounds were not detected in A4 and A2 samples. ‘Sweet, floral’ ethyl acetate, ethyl hexanoate and ‘fruity, feijoa-like’ ethyl benzoate was absent in 4WBR and 2WBR fruits in Unique and Triumph cultivars. ‘Apple and sweet’ methyl butanoate was not detected in U4, T4, T2 and TR. ‘Banana sweet’ cis-3-hexenyl acetate was no detected in U4, T4 and T2. ‘Feijoa-like’ methyl benzoate was not found in U4 and U2 samples. There were cultivar differences in feijoas, the absent compound might be an indicator to distinguish different cultivars.

All previous studies on feijoa volatiles (including feijoa fruit, feijoa peel, feijoa oils) (Fernandez et al., (2004); Hardy and Michael (1970); Peng et al.,(2019); Peng et al. (2019); Saj et al., (2008); Shaw et al., (1989); Shaw et al., (1990); Shaw et al., (1983)), have reported that esters such as methyl benzoate, ethyl benzoate, ethyl butanoate were the major compounds which contributed to the aroma of feijoas. In this study, for ripe and overripe fruits, the intensity of ethyl benzoate was the highest, followed by methyl benzoate and ethyl butanoate, which was consistent with the references. Besides, except ethyl acetate, all other aromatic esters were detected in feijoa juice reported by (Peng et al., 2019). Odour descriptions such as ‘banana-like’ cis-3-hexenyl butanoate, ‘fruity’ methyl butanoate and

ethyl hexanoate were the same with current study. Other than feijoas, aroma active esters were also found as the key contributor in ripe strawberry (Du et al., 2011). 'Sweet and fruit' notes methyl butanoate, ethyl butanoate, methyl hexanoate and ethyl butanoate were decided as the major aromatic esters in strawberry (Du et al., 2011).. In mango, 'Sweet, apple fruity' ethyl butanoate and 'fruity' note methyl benzoate were found as one of the most important compounds (Bonneau et al., 2016). In passion fruits, cis-3-henxyl butanoate was found with a 'floral and orange' note (Janzantti et al., 2012).

3.3.2.2 Terpenes

A total of 12 terpenes were identified as aroma active compounds, namely 'herbal and sweet' *β-(E)*-ocimene, 'fruity and green' *α*-cubebene, 'grass and woody' caryophyllene, 'fresh and herbal' humulene, 'fruity and sweet' *γ*-muurolene, 'sweet, floral and green' germacrene D, 'green and woody' *β*-selinene, 'herbal and grass' *β*-elemene, 'fresh and herbal' *δ*-cadinene, 'herbal, sour and fruity' L-calamenene, 'metallic unpleasant' *β*-myrcene and 'herbal, metallic and plastic' (4E,6Z)-allo-ocimene. Opposite to esters, aromatic terpenes were abundant in unripe feijoas. Specifically, most aroma active terpenes were sniffed with the highest intensity in 4WBR fruits. 'Fruity and green' *α*-cubebene was absent in UO, TR, AR and AO samples. 'Sweet and fruity' *γ*-Muurolene germacrene D and 'green, woody' *β*-Selinene were not detected in overripe fruits. *δ*-cadinene was absent in UR, UO, TR and TO samples and 'herbal' L-calamenene was absent in UO and AO samples. However, contrast with most terpenes, intensity of *β*-myrcene and *β*-(E)-ocimene were stronger in ripe and overripe fruits. Furthermore, among these aromatic terpenes, *β*-selinene, *β*-elemene, L-calamenene, *α*-cubebene, (4E,6Z)-allo-ocimene were the terpenes with higher intensity.

Comparable results can be seen from papers reported by Peng et al. (2019) , which studied aroma active volatiles in feijoa essential oils and feijoa juice. According to their results, terpenes was one of the most important classes in feijoa. Myrcene, *β*-(E)-ocimene, *α*-cubebene, caryophyllene, humulene, *δ*-Cadinene, *β*-selinene were all identified in their papers. Caryophyllene *δ*-cadinene and humulene with higher concentrations were also

detected in feijoa leaves (Monforte et al., 2014). Caryophyllene, humulene, *β*-selinene and *α*-cubebene were found in feijoa essential oils as well (Fernandez et al., 2004).

Except feijoa fruits, aromatic terpenes were found in other fruits. In mango, by calculating OAV of volatiles, 'Woody, resinous, musty, balsamic and ethereal' myrcene, 'citrus, herbal and sweet' *β*-(E)-ocimene, 'woody and green' caryophyllene were detected (Bonneau et al., 2016). In passion fruits, 'sweet and citrus' myrcene, 'Citrus, peppermint, sweet and sharp' germacrene D were detected by GCO (Janzantti et al., 2012). In strawberry guava, 'sweet-balsamic' note myrcene, 'floral and herbal' note *β*-(E)-ocimene, 'woody and spicy' humulene, *δ*-cadinene and caryophyllene were identified (Pino et al., 2001).

3.3.2.3 Alcohols

According to Peng et al. (2019); Zhang et al. (2011), alcohol volatiles are likely to give 'floral, sweet, fruity, green and fresh' odour in fruits. Moreover, referring to Defilippi et al. (2009); El Hadi et al. (2013); Peng et al. (2019); Siegmund (2015), six carbon alcohols pass a 'green, herbal' note in various fruits. In this study, 'floral, fresh' linalool and 'minty, woody' *α*-terpineol were two aromatic alcohols identified in feijoas.

Aroma importance of linalool in feijoas was quite significant across all ripening stages. The intensity of linalool decreased during ripening. Both relative concentration and sensorial intensity were strongest in 4WBR fruits. Many references approved that linalool is an essential volatile in feijoa. 'Floral' linalool was identified in feijoa juice and essential oils extracted from feijoa peels (Peng et al., 2019; Shaw et al., 1989). Not only feijoa juice and oils, intact fruits and feijoa leaves were also experimented to have linalool as aroma volatiles (Binder & Flath, 1989; Mosbah et al., 2018).

Linalool is also very common and important aromatic volatile in many other fruits. In raspberry, linalool and *α*-terpineol were both identified as important aroma volatiles (Koeduka et al., 2011). In strawberries, linalool was identified with 'citrus, fruity and floral'

note. The intensity and relative concentration of linalool in strawberries were also significant different among different cultivars (Jetti et al., 2007; Schieberle & Hofmann, 1997). Linalool was also found in strawberry guava with 'floral' note (Pino et al., 2001) and 'lemon, citrus, sour' note in passion fruits (Janzantti et al., 2012). Moreover, linalool is treated as terpenoid in some papers because it is synthesized the same pathway with terpenes by MEP or MEP metabolisms (Defilippi et al., 2009). Besides, linalool also has functions to resist oxidant, inflammatory and microbial hazards (Babahmad et al., 2018).

α-Terpineol in feijoa was also reported in other feijoa papers. Essential oils extracted from feijoa peel had α-terpineol with a 'minty, fresh' note (Fernandez et al., 2004; Peng et al., 2019). Feijoa leaves and fruits were also detected with *α*-terpineol content (Binder & Flath, 1989; Poodi et al., 2018). In mandarin juice, *α*-terpineol and linalool were also identified (Pérez-López et al., 2006). In freshly quizzed citrus juice, *α*-terpineol was identified with a 'citrus' odour (Dharmawan et al., 2007).

3.3.2.4 Aldehydes

Hexanal with 'green, fruity' note was identified in feijoas. According to literatures, comparable with six carbon aromatic alcohols, six carbon aldehydes usually pass 'green' note in fruits (Defilippi et al., 2009; Siegmund, 2015; Zhang et al., 2011). The change of intensity across ripening varied a lot. Precisely, in Unique cultivar, the intensity score of UR was highest, followed by UO, U2 and U4. In Triumph, TO had the strongest hexanal odour, followed by TR, T4 and T2. In Anatoki feijoa, A4 had the highest hexanal intensity, followed by A2 and AR (Table 8). There was no hexanal odour detected in AO because of the very low hexanal concentration (OAV < 1).

Hexanal in feijoa was detected several times in other papers. Referencing to Shaw et al. (1990); Binder and Flath (1989) and Fernandez et al. (2004), hexanal has been found in feijoa flesh and feijoa oils. Because of the low odour thresholds of aldehydes (hexanal), it is an important aromatic compound in fruits (El Hadi et al., 2013). Hexanal with 'fresh and green'

note was detected strawberries with relative high intensities (Du et al., 2011). In strawberry guava, ‘fatty green’ note hexanal was identified (Jorge A. Pino et al., 2001). ‘green, grass and herbal’ hexanal and ‘sweet, citrus and green’ hexanal was found in cashew apple juice (Garruti et al., 2003) and passion fruits (Janzantti et al., 2012) respectively. Furthermore, hexanal with ‘herbal, green and grass’ aroma was identified in banana as well (Jordan et al., 2001).

3.3.2.4 Ketones

Aromatic ketones could be a contributor to ‘green, fruity and sweet’ notes in many fruits (Jorge A Pino, 2014). Ketones such as ‘herbal and minty’ 3-octanone and ‘fruity and green’ 2-nonanone were identified in this study. ‘Herbal and minty’ 3-octanone was easily identified by panellists with relative high intensity for all samples. As for the intensity change during ripening, unripe fruits (4WBR and 2WBR) had higher sensorial intensities than ripe and overripe fruits. For ‘fruity and green’ 2-nonanone, the intensity change varied with different cultivars. In Unique feijoa, 2-nonanone was not detected in U2 and UO samples. The 2-nonanone odour was strongest in T4 sample in the Triumph and highest in A4 in the Anatoki cultivar (Table 8).

These two compounds were found in feijoa many times. ‘Mushroom-like’ 3-octanone was detected in essential oils from feijoa peels (Fernandez et al., 2004; Peng et al., 2019; Shaw et al., 1989) and in intact feijoas (Binder & Flath, 1989; Hardy & Michael, 1970). ‘Fruity and green’ 2-nonanone was found only in essential oils extracted from feijoa peels (Hardy & Michael, 1970; Peng et al., 2019).

Aromatic ketones were also found in other fruits. 3-Octanone was found in wild blueberry (Lugemwa et al., 1989), guava (Bashir & Abu-Goukh, 2003; Idstein & Schreier, 1985) and grape and dried vine fruit (Zhang et al., 2017). In mushroom, ‘buttery and mushroom-like’ 3-octanone was identified (Cho, et al., 2008; Costa et al., 2015). The odour description of 3-octanone (‘mushroom-like’ note) in other papers was little different with ‘herbal and minty’

note in this study. This might be explained by different aroma profile and concentrations. Although same compounds were detected in various fruits, the odour description were not the constant. This is because of the synergistic effects of the whole aroma system. Different volatiles might influence each other and emit different odour (Janzantti et al., 2012). Moreover, the concentration of aromatic compounds could also influence odour description to some extent (Marsili & ProQuest, 2012).

For 2-nonanone, 'fruity and floral' note was detected in strawberry guava (Jorge A. Pino et al., 2001). In papaya, the odour description of 2-nonanone was 'fruity and soapy' (Balbontin et al., 2007). Moreover, according to Vaughn (1993), except aromatic property, other function of 2-nonanone is to inhibit postharvest decay fungi in strawberry and raspberry samples.

3.3.3 Principle component analysis (PCA) of aromatic compounds in feijoa fruits

Principle component analysis (PCA) is a clear way to represent the correlations and differentiations among three feijoas across four ripening stages. Aroma intensity and the three cultivars with four ripening stages was evaluated in Figure 17. Aroma active compounds and their intensity listed in Table 8 were data source of this PCA plot.

As shown in the PCA plot, TR, T2 and U2 samples were far away from aroma active volatiles, which indicates that these three samples were less aromatic compared with other samples. This could be explained by their low total aroma intensities and relative concentrations. TR was the least aromatic sample with 39.25 total aroma intensity and 115.63 mg/kg concentration, followed by T2 (51.75 intensity, 152.99 mg/kg) and U2 (56.5 intensity, 201.49 mg/kg).

Ripe and unripe samples were separated by PC1, ripe and overripe samples were more correlated to compounds on the left, which suggested that all ripe and overripe fruits were dominated by ‘fruity, sweet, floral’ aromas. Moreover, UO could be more aromatic than other samples due to its closer relation to important aromatic compounds ‘feijoa-like’ methyl benzoate, ‘fruity and feijoa-like’ ethyl benzoate and ‘sweet, floral, fruity’ ethyl butanoate. AO and TR were separated by PC2 from TO, UO, AR, UR, indicating that they were closer to unripe samples with less ‘fruity and sweet’ aromas.

All 2WBR and 4WBR samples were located on the right side together with aromatic ‘green, herbal and fresh’ terpenes and ‘herbal, minty and green’ ketones, which illustrates that unripe samples had greener and more herbal odour. Among these unripe samples, A4, U4, T4 were closer to most right-side compounds, indicating 4WBR samples were the samples with the most ‘green and herbal’ aromas. U2 and T2 were on the right down side were relative far away from right up side compounds, suggesting that they were still dominated by ‘green, herbal’ aroma yet with much weaker odour

The ‘metallic’ myrcene and ‘mould and mushroom-like’ unknown 2 were locating in the middle of the PCA plot and far away from all samples, illustrating that none of these samples were characterized by these two unpleasant odours.

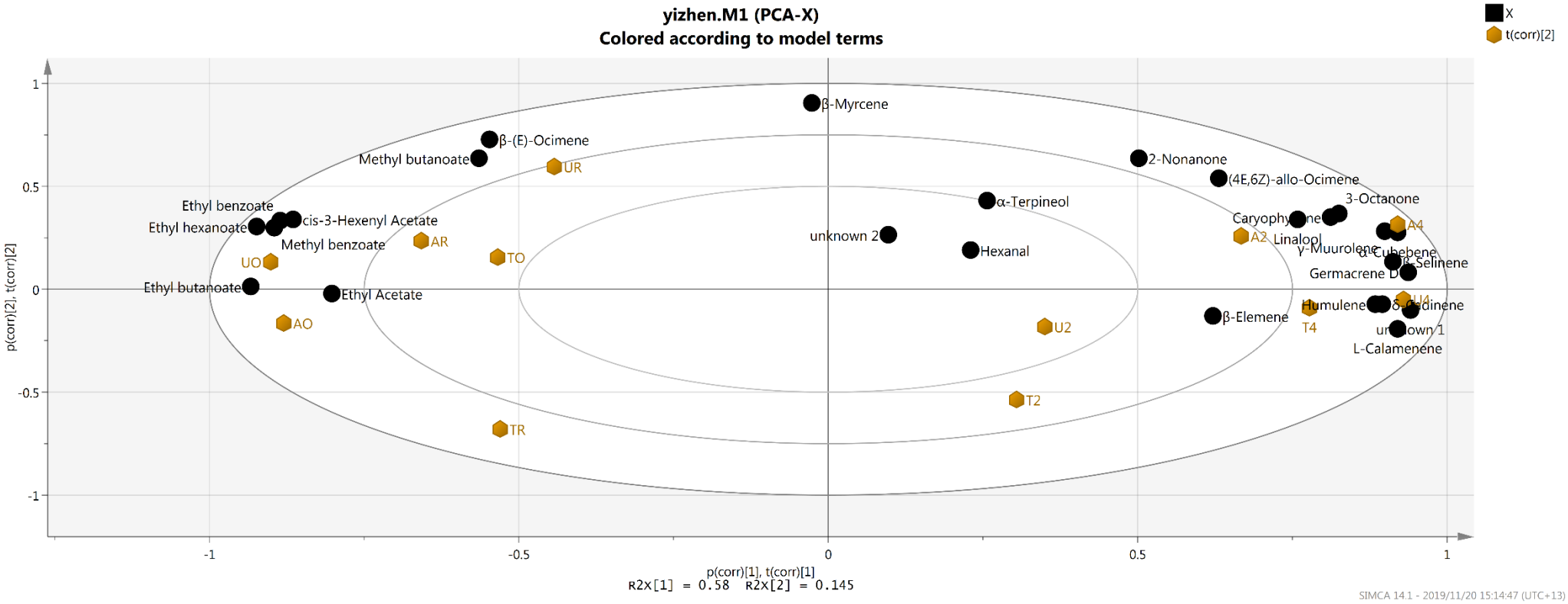

Figure 3.10: PCA analysis of aroma active volatiles in three feijoa cultivars at four ripening stages.

3.3.4 Aroma changes during ripening

The aromatically active volatiles were divided into six attributes based on their odour descriptions, which were sweet, floral, fruity, green, herbal and unpleasant. Compounds with their aroma groups were listed in Table 11. After classifying all the aroma active volatiles, aroma profile of Unique, Triumph and Triumph feijoas during ripening was built and presented in Figure 18.

Table 11: Aroma attributes classification

Flavour note	Compounds
Sweet	Ethyl Acetate; Methyl butanoate; Ethyl butanoate; Ethyl hexanoate; cis-3-Hexenyl Acetate; *β-cis*-Ocimene; α-Cubebene; Humulene; *γ*-Muurolene; Germacrene D
Floral	Linalool; Ethyl Acetate; Ethyl butanoate; Ethyl hexanoate; Germacrene D
Fruity	Hexanal; Methyl butanoate; Ethyl butanoate; Ethyl hexanoate; cis-3-Hexenyl Acetate; Methyl benzoate; Ethyl benzoate; *α*-Cubebene; 2-Nonanone; *γ*-Muurolene
Green	Hexanal; *β*-Elemene; *β*-Caryophyllene; cis-3-Hexenyl Acetate; 3-Octanone; 2-Nonanone;
Herbal	*δ*-Cadinene; *L*-Calamenene; *α*-Cubebene; Germacrene D; *α*-Terpineol; *β*-Selinene Methyl benzoate; Ethyl benzoate; 3-Octanone; (4E,6Z)-allo-Ocimene; *β*-Elemene; *δ*-Cadinene; *β*-(cis)-Ocimene; *β*-Caryophyllene; Humulene; *L*-Calamenene; *α*-Terpineol; *β*-Selinene
Unpleasant	unknown 1; unknown 2; *β*-Myrcene; *(4E,6Z)*-allo-Ocimene

Changes of aroma during ripening in these three cultivars were not the same (Figure 18). In Unique and Triumph feijoa, 4WBR fruits and R fruits were more aromatic. In the Triumph cultivar, the T4 and TO had relative high aroma intensity than the TR sample. However, all 2WBR feijoas were positioned in the aroma transition status with relative lower aromatic

intensity. According to Zhu et al. (2018) and Ménager et al., (2004), unripen bananas and strawberries had fewer aromatic compounds compared with ripe fruits.

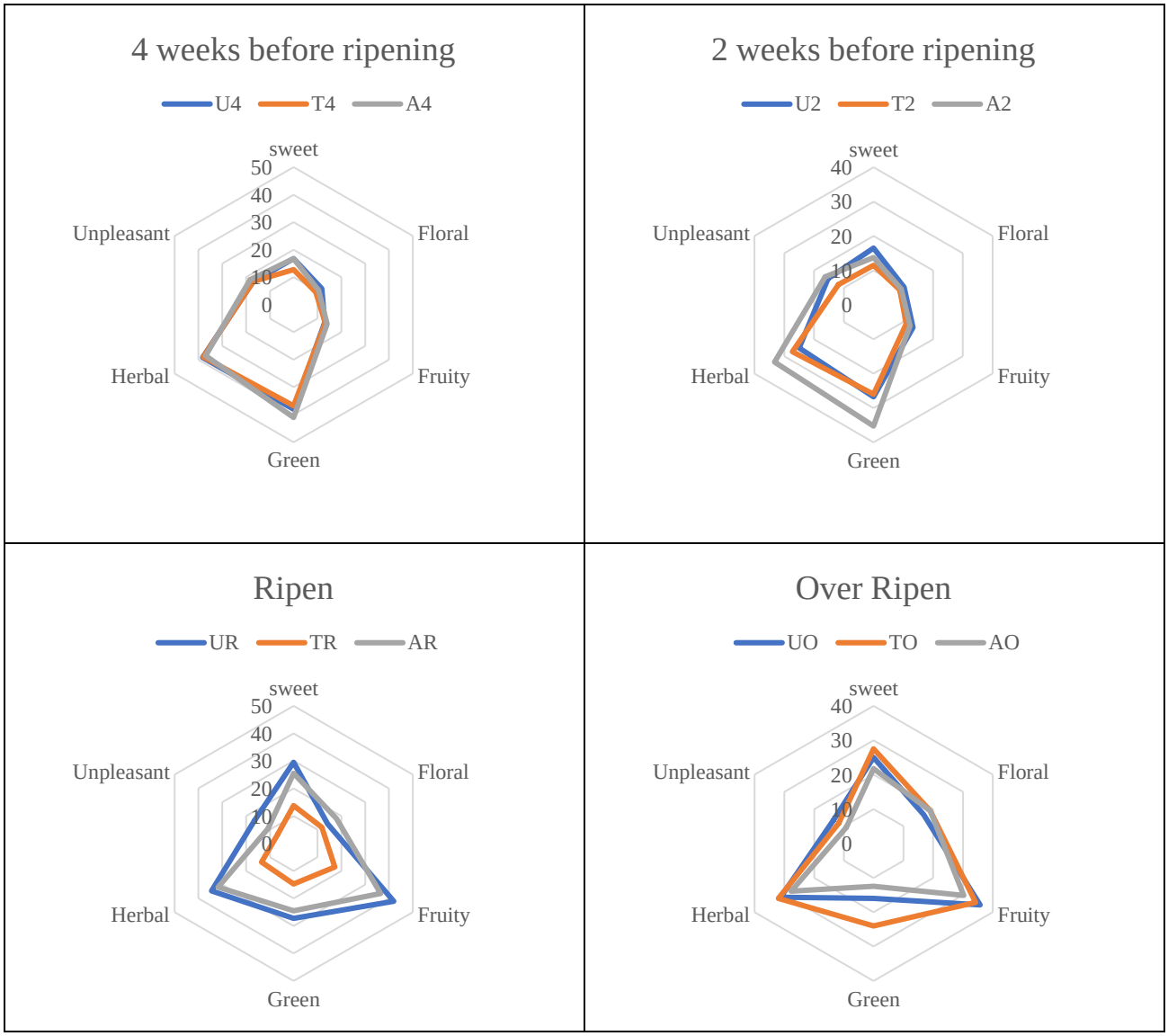

Figure 3.11: Aroma profiles of three feijoa cultivars during ripening

For 4WBR fruits, the intensities of herbal and green attributes were significant (Figure 18). Other odour description including ‘sweet, floral, fruity and unpleasant’ were quite weak. At 2WBR, the ‘sweet’ odour was developed but the ‘fruity, floral and unpleasant’ aromas were weakened. The ‘green and herbal’ aromas still occupied the key position despite a little decrease compared with the 4WBR fruits. Comparable results can be seen in the guava fruits,

where ‘grassy and green’ attributes were more important in unripe fruits (Sinuco, Steinhaus, Schieberle, & Osorio, 2010). As ripening progressed, the ‘herbal and green’ odour degraded a lot whereas the ‘fruity and sweet’ aroma became stronger in the ripe feijoas.

Class transition was the reason for aroma changes from ‘herbal, green’ in unripe fruits to ‘sweet, fruity’ in ripe fruits. The concentration of aromatic terpenes decreased during ripening but the level of esters increased (Table 9). The generation of esters in ripe fruits could greatly contribute to their sweet and fruity aroma as reported in apple (Williams & Benkeblia, 2018), banana (Jayanty et al., 2002), mango (Pandit et al., 2010) and strawberry (Du et al., 2011).

There was cultivars difference in feijoa aroma profiles. The Triumph cultivar was less aromatic at 4WBR and 2WBR. However, the TO had comparable ‘sweet, floral, fruity, unpleasant and herbal’ aroma intensities and higher ‘green’ aroma intensities compared with the UO and AO samples. Unique and Anatoki cultivars had similar aroma profiles throughout all feijoa ripening stages. Based on these results, the Triumph cultivar may not be a good choice for possible aroma applications due to its lower aromatic property.

3.4 Glycosidically bound volatiles in feijoa fruits

Odourless glocosidically bound volatiles are important flavor compounds which act as a reserve of aroma in many fruits (Sarry & Günata, 2004). It is meaningful to analyze the glycosidically bound volatiles in feijoas to build a comprehensive understands on the volatile profile of specific fruit. As mentioned in Chapter one literature review, no information was reported about bound volatiles in feijoas. The following results show the identification and quantification of bound volatiles in the Unique, Triumph and Anatoki feijoas during ripening.

3.4.1 Trials for bound volatile extraction and analysis

Solvent Selection

To remove the free volatiles in samples, the efficiency of three solvent mixtures were compared. One was Diethyl ether and pentane mixture (1/1; V/V), one was DCM and pentane mixture (1/2; v/v) and the last one was pentane. From Figure 19, diethyl ether and pentane mixture showed higher peak area but pentane gave more peaks. As a result of this, modifications were conducted by increasing the percentage of pentane. Hence, a mixture of 1 volume diethyl ether and 2 volume pentane was selected to remove free volatiles in sample.

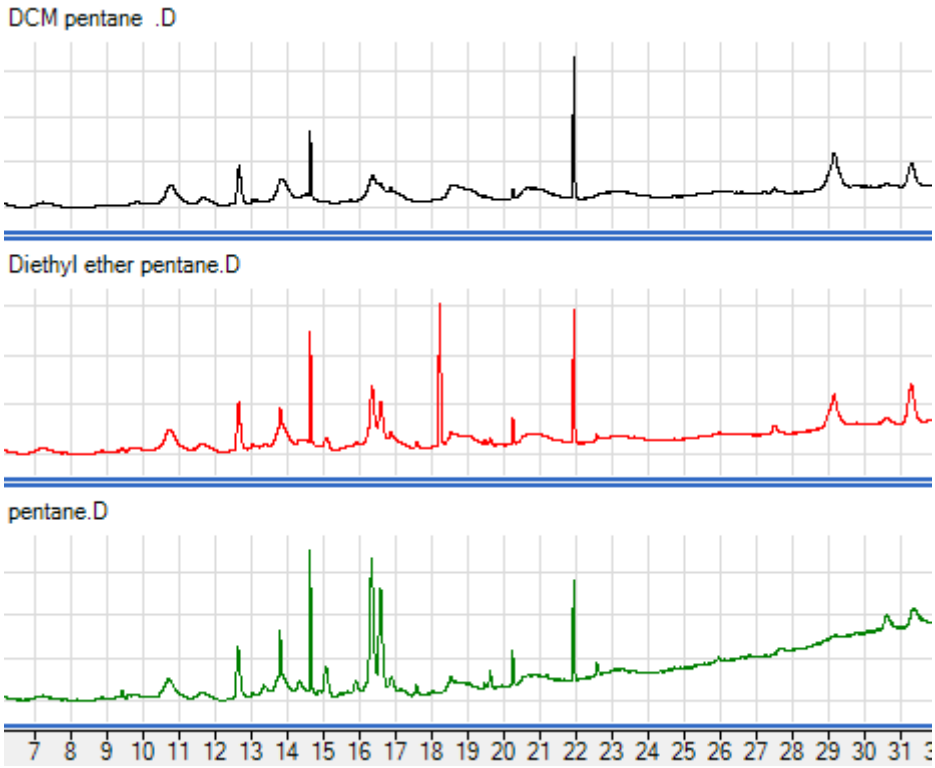

Figure 3.12: Solvent selection trial in bound volatile analysis

Cartridge selection

From Table 4 in section 1.2.6, two cartridges Amberlite XAD-2 and Strata C18-E were tested in this section. From Figure 20, Amberlite XAD-2 gave a higher area height and a better separation of peaks. Hence, Amberlite XAD-2 was selected to extract bound volatiles.

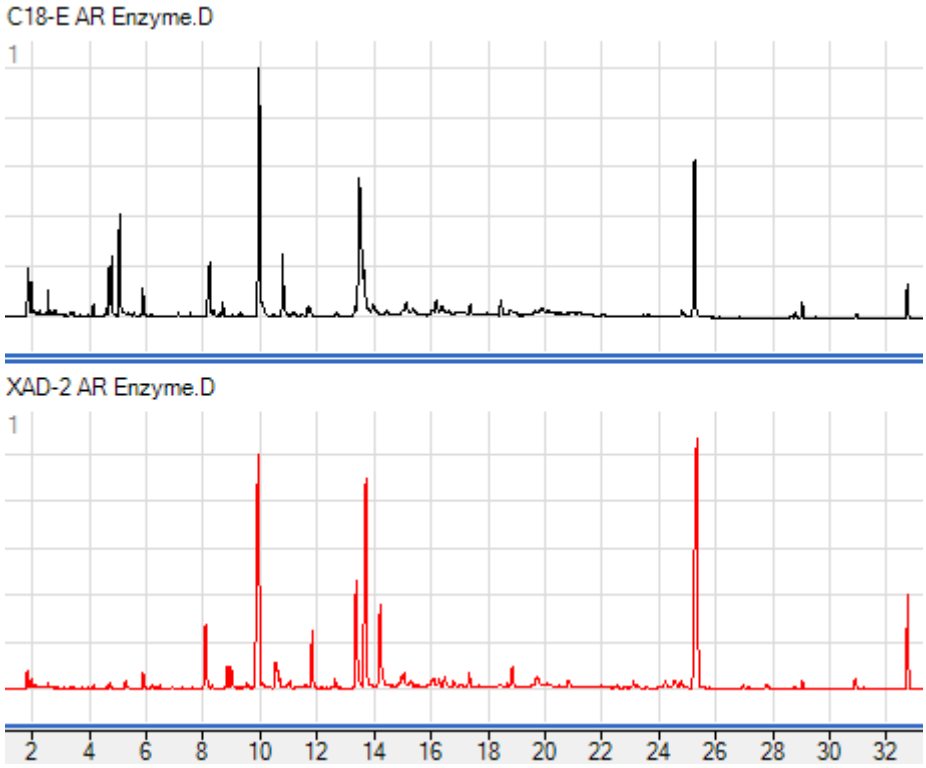

Figure 20: Cartridges selection trial in bound volatile analysis

Sample type and loading volume selection

Sampler concentration and volume selection was trialled together. From Figure 21, 40 mL of homogenized sample mixed with 20 mL clear juice 20mL and 20 mL water gave more peaks and better peak separation than 20 mL pure juice and supernatant from a fine mixture of 45 grams of feijoa paste vortexed 5 minutes with 45 mL of water. Hence homogenized sample with 20 mL clear juice and 20 mL water was selected. For the volume selection, two trialled volumes (20 ml juice with 20 ml water versus 50 ml juice with 50 ml water) illustrated comparable peak separation and total peak area (Figure 21). Considering of time consumption, 40 mL solution made with 20 mL clear juice and 20 mL water was chosen as the final loading sample.

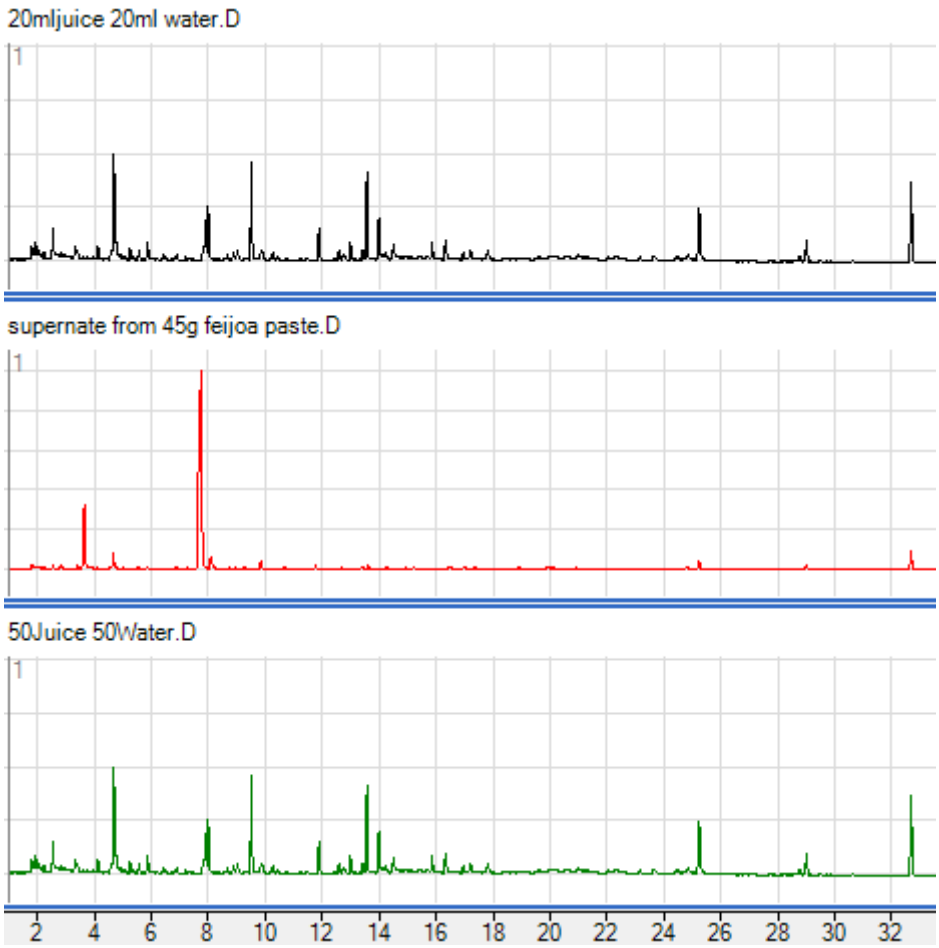

Figure 3.13: Sample selection trial in bound volatile analysis

Enzyme hydrolysis time selection

As mentioned in section 2.5.2, six different enzyme hydrolysis times (6 hours, 16 hours, 24 hours and 48 hours) were studied. The final hydrolysis time was set to 24 hours due to better separation of peaks and higher peak area (Figure 22).

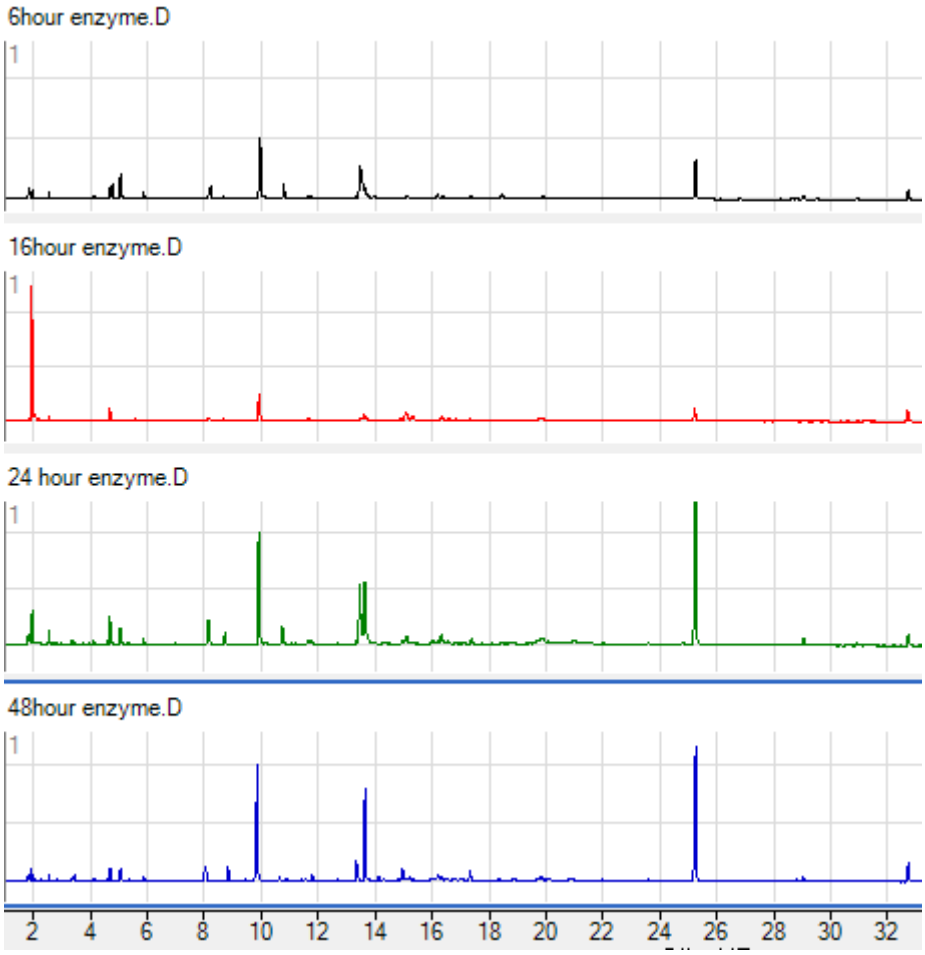

Figure 3.14: Enzyme hydrolysis time selection trial in bound volatile analysis

3.4.2 Bound volatiles identified in feijoa fruits during ripening

Many studies reported that the quantification of glycosidically bound volatiles was achieved by measuring hydrolytically liberated aglycones (volatiles) (Sarry & Günata, 2004). Therefore, volatile analysis by HS-SPME-GC-MS was used to analyze the bound volatiles in feijoas. A total of 84 bound volatile compounds were identified, with 19 alcohols, 9 aldehydes, 3 esters, 1 hydrocarbon, 8 ketones, 28 terpenes and 16 unknowns (Table 12). Comparable with cherry (Wen et al., 2014), nectarines (Aubert, Ambid, Baumes, & Günata, 2003), mulberry (Chen et al., 2015) and kiwifruits (Garcia et al., 2012), glycosidically bound alcohols and terpenes were the top two abundant classes in feijoas.

The number of bound volatiles (84) was almost half of that of free volatiles (164) in feijoas (Table 12 and Table 6). Similar results were reported in lemon juice, with 25 free volatiles versus 4 bound volatiles (Zhong et al., 2014). Nonetheless, out of 84 bound volatiles, 52 co-existed as free volatiles. It was noteworthy that although the total number of bound was less, the number of bound aldehydes (8) was higher than the number of free aldehydes (6) and the number of alcohols (20 free and 19 bound) and ketones (9 free and 8 bound) were comparable in bound and free forms.

Based on the results of free and bound volatiles in many fruits such as mango (Lalel et al., 2003), nectarines (Aubert et al., 2003), blackberry (Du et al., 2010), kiwifruits (Garcia et al., 2012; Valdizon Garcia, 2013), strawberry (Ubeda et al., 2012), cherry (Wen et al., 2014), grapes (Hjelmeland & Ebeler, 2015), the concentrations of bound volatiles are usually two to eight-fold greater than that of the free counterparts (Sarry & Günata, 2004). However, from Table 7 and Table 13, the concentrations of glycosidically bound volatiles in the feijoa fruits were much lower than that of their free volatiles (around 10^{-1} ug/kg of bound volatiles versus 10^{3} ug/kg of free volatiles). The importance of bound volatiles to its whole volatile composition in feijoa fruits could be limited due to their lower number and insignificant concentrations.

Table 12: Intensity and Description of Bound Volatiles in three feijoa cultivars at four ripening stages

No.	Compound Name	CAS	RIa (RIb)		Formula	Class	ID	Odor Description	Reference
			WAX	5MS					
1	Ethyl Acetate	141-78-6	863 (900)	-	$C_4H_8O_2$	C	MS, RI	ethereal fruity sweet green	C, D
2	Pentanal	110-62-3	961 (968)	693 (701)	$C_5H_{10}O$	B	Std, MS, RI	Acrid, pungent	A
3	1R-α-Pinene*	7785-70-8	1012 (1017)	939 (922)	$C_{10}H_{16}$	F	MS, RI	Mint	C
4	3-Hexanone	589-38-8	1037 (1046)	798 (801)	$C_6H_{12}O$	E	MS, RI	Grape, wine-like	A
5	2-Hexanone	591-78-6	1063 (1069)	-	$C_6H_{12}O$	E	MS, RI	Fruity, buttery	C
6	Hexanal	66-25-1	1065 (1067)	804 (802)	$C_6H_{12}O$	B	Std, MS, RI	Apple, green	A, D
7	3-Carene*	13466-78-9	1120 (1135)	1017 (1002)	$C_{10}H_{16}$	F	MS, RI	Citrus, herbal	C
8	3-Heptanone	106-35-4	1138 (1131)	-	$C_7H_{14}O$	E	MS, RI	Fruity, green	A
9	α-Phellandrene	99-83-2	1140 (1150)	1011 (1010)	$C_{10}H_{16}$	F	MS, RI	citrus, herbal, green	C
10	β-Myrcene	123-35-3	1140 (1157)	-	$C_{10}H_{16}$	F	MS, RI	Metallic, spicy, peppery	B, C, D
11	2-Ethylhexanal	123-05-7	1169(NA)	957 (955)	$C_8H_{16}O$	B	MS, RI	-	-
12	2-Heptanone	110-43-0	1169 (1178)	902 (894)	$C_7H_{14}O$	E	Std, MS, RI	Banana, cinnamon	A
13	D-Limonene	5989-27-5	1177(NA)	1035(NA)	$C_{10}H_{16}$	F	MS, RI	Lemon-like, pleasant	A, D
14	β-Phellandrene	555-10-2	1189 (1200)	1043 (1045)	$C_{10}H_{16}$	F	MS, RI	minty	C
15	3-Hexanol	623-37-0	1194 (1190)	798 (801)	$C_6H_{14}O$	A	Std, MS, RI	Alcoholic, medicinal	A
16	2-Methyl-1-butanol	137-32-6	1198 (1206)	-	$C_5H_{12}O$	A	MS, RI	Fruity, roasted	A
17	Isoamyl alcohol	123-51-3	1200 (1209)	735 (730)	$C_5H_{12}O$	A	Std, MS, RI	Banana, fruity	C
18	(E)-2-Hexenal	6728-26-3	1205(1204)	-	$C_6H_{10}O$	B	MS, RI	Fruity, grassy	A, D
19	2-Hexanol	626-93-7	1216 (1217)	-	$C_6H_{14}O$	A	MS, RI	Cauliflower, fruity	C
20	3-Octanone	106-68-3	1248 (1240)	991 (988)	$C_8H_{16}O$	E	Std, MS, RI	Mushroom-like, herbal, mint	B, D
21	3-Methyl-5-heptanone	541-85-5	1249(NA)		$C_8H_{16}O$	E	MS, RI	Herbal, sweet	C
22	Unknown*	123-38-6	1270 (784)	900 (474)	C_3H_6O	B	MS, RI	-	-
23	Octanal	124-13-0	1272 (1287)	1007 (1011)	$C_8H_{16}O$	B	MS, RI	Citrus, honey-like	A
24	Unknown*	-	1280(NA)	-		G	MS, RI	-	-

Table 12 continued

25	3-Heptanol	589-82-2	1288 (1290)	-	$C_7H_{16}O$	A	MS, RI	Herbal, pungent	A
26	2,3-Octanedione	585-25-1	1308 (1325)	-	$C_8H_{14}O_2$	E	MS, RI	Herbal, earthy	C
27	2-Heptanol	543-49-7	1318 (1322)	907 (903)	$C_7H_{16}O$	A	Std, MS, RI	Lemon-like, herbal	A
28	1-Hexanol	111-27-3	1329 (1339)	-	$C_6H_{14}O$	A	Std, MS, RI	Herbal, woody	A
29	3-Hexen-1-ol	544-12-7	1379 (1384)		$C_6H_{12}O$	A	MS, RI	green, leafy	C
30	Nonanal	124-19-6	1382 (1390)	1108 (1107)	$C_9H_{18}O$	B	MS, RI	Orange rose	A
31	3-Octanol	589-98-0	1392 (1393)	998 (996)	$C_8H_{18}O$	A	Std, MS, RI	Herbal, sweet	A
32	(E)-2-Octenal	2548-87-0	1422 (1424)		$C_8H_{14}O$	B	MS, RI	Cucumber, fresh	C
33	Benzaldehyde	100-52-7	1508 (1508)	986 (975)	C_7H_6O	B	MS, RI	Bitter, almond	A
34	Linalool	78-70-6	1548 (1547)	1104 (1105)	$C_{10}H_{18}O$	A	Std, MS, RI	Floral, fresh	B, D
35	Caryophyllene	87-44-5	1602 (1599)	1446 (1444)	$C_{15}H_{24}$	F	Std, MS, RI	Grassy, woody	A, D
36	Methyl benzoate	93-58-3	1616 (1615)	1110 (1103)	$C_8H_8O_2$	C	Std, MS, RI	Feijoa-like, herbal	B, D
37	Aromadendrene	109119-91-7	1644 (1646)	1485 (1485)	$C_{15}H_{24}$	F	MS, RI	-	-
38	Ethyl benzoate	93-89-0	1657 (1650)	1183 (1173)	$C_9H_{10}O_2$	C	Std, MS, RI	Fresh, fruity	B
39	Humulene	6753-98-6	1670 (1671)	1478 (1477)	$C_{15}H_{24}$	F	Std, MS, RI	Sweet, woody	D
40	Terpineol	98-55-5	1697 (1692)	1199 (1200)	$C_{10}H_{18}O$	A	Std, MS, RI	Citrus woody floral	B, C
41	α-Muurolene	10208-80-7	1727 (1727)	-	$C_{15}H_{24}$	F	MS, RI	Woody	C
42	β-Cadinene	523-47-7	1753(NA)	-	$C_{15}H_{24}$	F	MS, RI	-	-
43	δ-Cadinene	483-76-1	1754 (1758)	1540 (1534)	$C_{15}H_{24}$	F	MS, RI	Fresh, herbal	B, D
44	Cadine-1,4-diene	16728-99-7	1781 (1797)	1550 (1546)	$C_{15}H_{24}$	F	MS, RI	-	-
45	L-Calamenene	483-77-2	1829 (1832)	1542 (1546)	$C_{15}H_{22}$	F	MS, RI	Herbal, sour	D
46	Benzyl Alcohol	100-51-6	1872 (1877)	-	C_7H_8O	A	MS, RI	Floral, rose	C
47	unknown	-	1887 (NA)	-	-	G	MS, RI	-	-
48	unknown	-	1903 (1906)	-	-	G	MS, RI	-	-
49	α-Calacorene	1000293-02-3	1911 (1916)	1561(NA)	$C_{15}H_{20}$	F	MS, RI	-	-
50	o-Cresol	95-48-7	1988 (2000)	-	C_7H_8O	A	MS, RI	Musty, plastic	C
51	Cubenol	21284-22-0	2058 (2063)	-	$C_{15}H_{26}O$	A	MS, RI	Spicy, herbal	C

Table 12 continued

52	Veridiflorol	1000122-17-3	2084(NA)	-	$C_{15}H_{26}O$	A	MS, RI	Herbal, green, fruity	C
53	Ledol	577-27-5	2085 (2062)	-	$C_{15}H_{26}O$	A	MS, RI	-	-
54	(-)-Spathulenol	77171-55-2	2122(NA)	-	$C_{15}H_{24}O$	A	MS, RI	Honey-like	C
55	α-Cadinol	481-34-5	2222 (2221)	-	$C_{15}H_{26}O$	A	MS, RI	Herbal, woody	C
56	unknown	-	1700(NA)	-	-	G	MS, RI	-	-
57	unknown	-	2105(NA)	-	-	G	MS, RI	-	-
58	α-Cubebene	17699-14-8	-	1363 (1360)	$C_{15}H_{24}$	F	MS, RI	Herbal, sweet	A, D
59	Copaene	3856-25-5	-	1392 (1393)	$C_{15}H_{24}$	F	MS, RI	Woody, spicy	C, D
60	β-Elemen	515-13-9	-	1406 (1403)	$C_{15}H_{24}$	F	MS, RI	Herbal, grassy	D
61	α-Gurjunene	489-40-7	-	1430 (1425)	$C_{15}H_{24}$	F	MS, RI	Woody	C
62	Alloaromadendren	25246-27-9	-	1463 (1461)	$C_{15}H_{24}$	F	MS, RI	Woody	C
63	Germacrene D	23986-74-5	-	1471 (1474)	$C_{15}H_{24}$	F	MS, RI	Floral, herbal, sweet	B
64	γ-Muurolene	30021-74-0	-	1449(NA)	$C_{15}H_{24}$	F	MS, RI	Fruity, sweet	D
65	α-Amorphene	483-75-0	-	1496 (1490)	$C_{15}H_{24}$	F	MS, RI	-	-
66	Viridiflorene*	21747-46-6	-	1518 (1495)	$C_{15}H_{24}$	F	MS, RI	-	-
67	γ-Cadinene	39029-41-9	-	1532 (1534)	$C_{15}H_{24}$	F	MS, RI	Herbal, woody	C
68	α-Cadinene	24406-05-1	-	1554 (1541)	$C_{15}H_{24}$	F	MS, RI	Woody	C
69	γ-Gurjunene	22567-17-5	-	1493 (1472)	$C_{15}H_{24}$	F	MS, RI	Musty	C
70	Guaia-10(14),11-diene	1000159-39-3	-	1619(NA)	$C_{15}H_{24}$	F	MS, RI	-	-
71	2-Ethylhexanol	104-76-7	-	1035 (1035)	$C_{8}H_{18}O$	A	MS, RI	Floral, sweet	A
72	δ-Cadinol	36564-42-8	-	1678 (1644)	$C_{15}H_{26}O$	A	MS, RI	Herbal	C
73	β-Ionone	14901-07-6	-	1482 (1488)	$C_{13}H_{20}O$	E	MS, RI	Fruity, violet-like	A
74	Unknown*	-	-	1001(NA)		G	MS, RI	-	-
75	Unknown*	-	-	1260 (1245)		G	MS, RI	-	-
76	Unknown*	-	-	1463(NA)		G	MS, RI	-	-
77	Unknown*	-	-	1603(NA)		G	MS, RI	-	-
78	Unknown*	-	-	1615(NA)		G	MS, RI	-	-

Table 12 continued

79	Unknown*	-	-	1630(NA)		G	MS, RI	-	-
80	Unknown*	-	-	1653(NA)		G	MS, RI	-	-
81	Unknown*	-	-	1665(NA)		G	MS, RI	-	-
82	Unknown*	-	-	1679(NA)		G	MS, RI	-	-
83	Cadalene	-	-	1698 (1688)	$C_{15}H_{18}$	D	MS, RI	-	-
84	Unknown*	-	-	1720(NA)		G	MS, RI	-	-

The number of compounds No. is consistent with that in Table 12; The order of the compounds was arranged by RI calculated from DB-WAX column.

ID: identification of compounds, RI- calculated retention index confirmed by NIST webbook database; MS- confirmed by mass spectrum; Std- confirmed by reference standards; NA: cannot find in references;

'*' Means compounds that are not present in free form;

RIa: retention index calculated from equation; RIb: retention index from NIST Chemical webbook Database;

Compound group: A- Alcohol; B- Aldehyde; C- Ester; D- Hydrocarbon; E- Ketone; F-Terpene; G- Unknown

Odour description references: A means from Burdock and Fenaroli (2009), B means from Peng *et al.* (2019), C means from The Good Scents Company (2019), D means from aromatic free volatiles detected by HS-SPME-GC-O-MS in Unique, Triumph or Anatoki feijoa cultivars.

Table 13: Relative concentration of bound volatiles in three feijoa cultivars at four ripening stages

No.	Compound	Bound aroma volatile relative concentration (ug/kg)											
		U4	U2	UR	UO	T4	T2	TR	TO	A4	A2	AR	AO
1	Ethyl Acetate	ND	ND	ND	ND	ND	ND	ND	1.40 ± 0.00a	ND	ND	ND	ND
2	Pentanal	0.37 ± 0.02ab	1.83 ± 0.17d	0.69 ± 0.01abc	1.08 ± 0.11c	0.53 ± 0.03abc	1.13 ± 0.03c	9.53 ± 0.59e	0.74 ± 0.02abc	0.17 ± 0.01a	0.87 ± 0.08bc	0.53 ± 0.06abc	1.72 ± 0.01d
3	1R-α-Pinene	0.16 ± 0.00abc	0.23 ± 0.03de	0.20 ± 0.02cd	0.26 ± 0.00de	0.12 ± 0.00a	0.15 ± 0.01abc	0.14 ± 0.01ab	ND	0.17 ± 0.00bc	0.25 ± 0.04de	0.16 ± 0.00abc	0.27 ± 0.00e
4	3-Hexanone	0.26 ± 0.01e	0.13 ± 0.01c	0.17 ± 0.01d	0.10 ± 0.01b	0.15 ± 0.01d	0.03 ± 0.00a	0.05 ± 0.00a	0.04 ± 0.00a	0.17 ± 0.01d	0.41 ± 0.00f	0.13 ± 0.00c	0.09 ± 0.01b
5	2-Hexanone	ND	ND	ND	ND	ND	ND	ND	ND	0.22 ± 0.07c	0.01 ± 0.00a	0.35 ± 0.03d	0.12 ± 0.00b
6	Hexanal	0.51 ± 0.00abc	0.93 ± 0.08e	0.57 ± 0.03bcd	0.69 ± 0.10cd	0.46 ± 0.03ab	0.93 ± 0.03e	1.39 ± 0.08f	0.68 ± 0.07cd	0.33 ± 0.01a	2.12 ± 0.08g	0.52 ± 0.01abc	0.71 ± 0.04d
7	3-Carene	0.18 ± 0.00a	0.21 ± 0.02ab	0.32 ± 0.01d	0.19 ± 0.01ab	0.20 ± 0.00ab	0.22 ± 0.01bc	0.21 ± 0.01ab	0.19 ± 0.01a	0.19 ± 0.00ab	0.25 ± 0.01c	0.20 ± 0.01ab	0.20 ± 0.00ab
8	3-Heptanone	0.05 ± 0.00c	0.13 ± 0.02e	0.03 ± 0.00b	ND	0.02 ± 0.00ab	0.01 ± 0.00a	0.09 ± 0.00d	0.01 ± 0.00ab	ND	ND	ND	ND
9	α-Phellandrene	ND	0.26 ± 0.00e	ND	ND	0.23 ± 0.00c	0.07 ± 0.00a	ND	ND	0.06 ± 0.00a	0.18 ± 0.01b	0.24 ± 0.00d	0.25 ± 0.01d
10	β-Myrcene	ND	0.01 ± 0.00ab	ND	0.03 ± 0.01bc	0.05 ± 0.02c	ND	ND	ND	ND	ND	ND	ND
11	2-Ethylhexanal	2.85 ± 0.26ab	6.98 ± 4.19bc	4.86 ± 0.06abc	2.04 ± 0.13a	1.56 ± 0.07a	3.66 ± 0.05abc	3.06 ± 0.21ab	2.33 ± 0.21a	2.07 ± 0.04a	5.54 ± 0.93abc	7.51 ± 0.31c	2.30 ± 0.10a
12	2-Heptanone	1.52 ± 0.15c	1.48 ± 0.13c	9.59 ± 0.25e	13.54 ± 0.78f	0.58 ± 0.10ab	0.80 ± 0.01abc	0.93 ± 0.03bc	1.41 ± 0.10bc	ND	1.54 ± 0.01c	3.54 ± 0.08d	3.60 ± 0.25d
13	D-Limonene	0.24 ± 0.00c	0.37 ± 0.00e	0.30 ± 0.02a	0.26 ± 0.02cd	0.12 ± 0.00b	0.26 ± 0.02cd	0.29 ± 0.05cd	0.09 ± 0.01ab	0.11 ± 0.01b	0.14 ± 0.00b	0.04 ± 0.00a	0.11 ± 0.00b
14	β-Phellandrene	0.23 ± 0.00b	ND	ND	ND	0.25 ± 0.03b	0.23 ± 0.03b	0.27 ± 0.01b	0.24 ± 0.00b	0.09 ± 0.00a	0.34 ± 0.02c	0.32 ± 0.03c	0.08 ± 0.01a
15	3-Hexanol	1.22 ± 0.04ab	2.24 ± 1.27b	0.53 ± 0.03a	0.49 ± 0.04a	0.97 ± 0.01a	0.20 ± 0.01a	0.31 ± 0.05a	0.17 ± 0.01a	0.42 ± 0.01a	0.50 ± 0.04a	0.33 ± 0.02a	0.18 ± 0.01a
16	2-Methyl-1-butanol	0.10 ± 0.01b	ND	ND	ND	0.07 ± 0.01ab	ND	ND	ND	0.45 ± 0.07c	ND	ND	ND
17	Isoamyl alcohol	ND	ND	ND	0.12 ± 0.01a	ND	ND	ND	ND	ND	ND	ND	0.16 ± 0.01b
18	(E)-2-Hexenal	0.13 ± 0.00bc	ND	0.07 ± 0.01ab	ND	0.11 ± 0.00ab	0.16 ± 0.01bc	1.19 ± 0.07e	0.38 ± 0.09d	0.09 ± 0.00ab	0.22 ± 0.02c	0.09 ± 0.01ab	ND
19	2-Hexanol	0.01 ± 0.00a	ND	ND	0.07 ± 0.01a	ND	ND	ND	ND	0.37 ± 0.13b	1.04 ± 0.07c	0.43 ± 0.01b	0.12 ± 0.01a
20	3-Octanone	5.87 ± 0.67e	5.47 ± 0.16e	3.38 ± 0.12cd	2.42 ± 0.04b	3.45 ± 0.12cd	1.13 ± 0.05a	1.42 ± 0.01a	0.96 ± 0.08a	4.07 ± 0.10d	5.15 ± 0.38e	3.24 ± 0.20c	0.96 ± 0.09a

Table 13 continued

21	3-Methyl-5-heptanone	0.13 ± 0.00a	1.88 ± 1.60b	ND	ND	ND	ND	ND	0.15 ± 0.01a	ND	ND	ND	ND
22	Unknow 1	ND	0.66 ± 0.00f	0.07 ± 0.01b	0.21 ± 0.03c	ND	0.47 ± 0.04e	ND	0.11 ± 0.00b	0.10 ± 0.01b	0.26 ± 0.02d	0.06 ± 0.00ab	0.42 ± 0.02e
23	Octanal	0.15 ± 0.02a	1.98 ± 0.02f	0.75 ± 0.04c	2.00 ± 0.12f	0.45 ± 0.06b	0.85 ± 0.09cd	0.50 ± 0.05b	0.47 ± 0.02b	0.20 ± 0.00a	2.38 ± 0.02g	1.21 ± 0.07e	1.00 ± 0.02d
24	Unknown 2	0.14 ± 0.01ab	0.60 ± 0.03ab	ND	ND	0.09 ± 0.00ab	ND	1.51 ± 1.49b	ND	0.05 ± 0.00ab	ND	0.05 ± 0.00ab	ND
25	3-Heptanol	0.05 ± 0.00c	0.19 ± 0.02e	0.02 ± 0.00b	ND	0.07 ± 0.00d	ND	0.22 ± 0.00f	0.01 ± 0.00ab	ND	ND	ND	ND
26	2,3-Octanedi-one	0.49 ± 0.00f	ND	0.20 ± 0.02d	0.18 ± 0.01cd	0.15 ± 0.01bc	ND	0.11 ± 0.01b	ND	0.33 ± 0.02e	0.64 ± 0.02g	0.12 ± 0.01b	0.11 ± 0.01b
27	2-Heptanol	0.93 ± 0.07a	2.70 ± 0.15c	9.30 ± 1.07e	4.31 ± 0.06d	0.71 ± 0.06a	1.09 ± 0.05a	1.39 ± 0.13ab	1.16 ± 0.03a	0.83 ± 0.06a	0.80 ± 0.03a	2.45 ± 0.41bc	1.46 ± 0.19ab
28	1-Hexanol	ND	0.26 ± 0.02b	ND	ND	0.16 ± 0.00a	0.31 ± 0.03bc	0.35 ± 0.03c	ND	0.12 ± 0.00a	0.43 ± 0.04d	ND	ND
29	3-Hexen-1-ol	0.10 ± 0.00a	0.22 ± 0.02b	0.38 ± 0.01c	ND	0.10 ± 0.01a	0.20 ± 0.01b	0.19 ± 0.00b	0.51 ± 0.00b	0.08 ± 0.00a	0.38 ± 0.06c	0.46 ± 0.04d	ND
30	Nonanal	0.80 ± 0.07a	3.65 ± 0.00e	1.33 ± 0.10b	1.64 ± 0.04b	0.86 ± 0.03a	2.28 ± 0.26c	3.04 ± 0.17d	0.49 ± 0.04a	0.58 ± 0.04a	3.54 ± 0.39e	3.88 ± 0.13e	0.82 ± 0.02a
31	3-Octanol	2.68 ± 0.12d	2.74 ± 0.18d	1.37 ± 0.08b	0.61 ± 0.03a	1.96 ± 0.03c	0.51 ± 0.04a	0.66 ± 0.09a	0.37 ± 0.03a	1.88 ± 0.14bc	3.00 ± 0.54d	1.31 ± 0.02b	0.39 ± 0.05a
32	(E)-2-Octenal	ND	ND	ND	ND	0.47 ± 0.03b	ND	ND	ND	0.30 ± 0.01a	0.31 ± 0.02a	ND	ND
33	Benzaldehyde	0.26 ± 0.01ab	2.00 ± 0.30e	0.56 ± 0.01bcd	0.48 ± 0.02abc	0.20 ± 0.01a	0.46 ± 0.00abc	0.84 ± 0.07d	2.01 ± 0.02e	0.22 ± 0.00a	0.25 ± 0.02a	0.68 ± 0.03cd	0.50 ± 0.03abc
34	Linalool	0.54 ± 0.00a	0.72 ± 0.10bc	0.70 ± 0.01abc	0.60 ± 0.00ab	0.68 ± 0.04abc	0.64 ± 0.01ab	0.71 ± 0.03bc	0.69 ± 0.01abc	0.81 ± 0.01c	1.25 ± 0.11d	0.71 ± 0.00bc	0.55 ± 0.01a
35	Caryophyllene	0.39 ± 0.03c	0.36 ± 0.01c	0.58 ± 0.03e	0.25 ± 0.02ab	0.28 ± 0.02b	0.19 ± 0.04a	0.29 ± 0.00b	0.26 ± 0.01ab	0.29 ± 0.01b	0.49 ± 0.02d	0.28 ± 0.03b	0.24 ± 0.00ab
36	Methyl benzoate	ND	ND	0.37 ± 0.03b	ND	ND	ND	1.80 ± 0.14c	0.10 ± 0.01a	ND	ND	1.75 ± 0.01c	0.04 ± 0.00a
37	Aromaden-drene	0.19 ± 0.01d	0.40 ± 0.00f	ND	ND	ND	ND	0.03 ± 0.00b	0.02 ± 0.00a	0.05 ± 0.00c	0.36 ± 0.01e	ND	ND
38	Ethyl benzoate	ND	ND	ND	2.29 ± 0.04c	ND	ND	1.92 ± 0.31b	1.42 ± 0.01a	ND	ND	ND	1.43 ± 0.00a
39	Humulene	0.22 ± 0.01d	0.24 ± 0.01d	0.41 ± 0.04f	0.13 ± 0.01c	0.04 ± 0.01a	0.10 ± 0.01bc	0.06 ± 0.01ab	0.08 ± 0.00abc	0.13 ± 0.01c	0.32 ± 0.03e	0.24 ± 0.00d	0.06 ± 0.00ab
40	Terpineol	ND	ND	ND	ND	ND	ND	ND	ND	0.07 ± 0.00b	0.34 ± 0.00c	0.04 ± 0.00a	ND
41	α-Muurolene	ND	ND	ND	ND	0.15 ± 0.01b	ND	0.07 ± 0.00a	ND	ND	ND	ND	ND
42	β-Cadinene	1.56 ± 0.13c	ND	ND	ND	ND	ND	ND	ND	0.65 ± 0.00b	0.41 ± 0.01a	ND	ND

Table 13 continued

43	δ-Cadinene	0.39 ± 0.02bc	1.31 ± 0.20e	0.33 ± 0.05abc	0.18 ± 0.01a	0.82 ± 0.00d	0.22 ± 0.01ab	0.31 ± 0.01abc	0.15 ± 0.03a	0.29 ± 0.01abc	0.46 ± 0.01c	0.17 ± 0.00a	0.26 ± 0.01abc
44	Cadine-1,4-diene	0.24 ± 0.00 c	0.29 ± 0.01d	ND	ND	0.11 ± 0.01b	0.07 ± 0.01a	0.37 ± 0.02e	0.24 ± 0.00c	0.23 ± 0.00c	0.29 ± 0.01d	ND	ND
45	L-Calamenene	0.69 ± 0.01g	0.49 ± 0.02ef	0.56 ± 0.01f	0.29 ± 0.05c	0.40 ± 0.04d	0.26 ± 0.00bc	0.42 ± 0.05de	0.18 ± 0.02ab	0.38 ± 0.00d	0.66 ± 0.04g	0.16 ± 0.01a	0.24 ± 0.01abc
46	Benzyl Alcohol	ND	ND	ND	ND	ND	0.04 ± 0.00a	0.67 ± 0.15c	0.27 ± 0.02b	ND	ND	ND	ND
47	Unknown 3	0.38 ± 0.07b	2.87 ± 0.01d	0.39 ± 0.03b	ND	0.31 ± 0.03b	ND	1.91 ± 0.26c	ND	ND	ND	0.17 ± 0.01ab	0.22 ± 0.01ab
48	Unknown 4	ND	ND	ND	ND	ND	ND	4.18 ± 0.17c	2.23 ± 0.23b	ND	ND	ND	0.30 ± 0.00a
49	α-Calacorene	0.24 ± 0.01cd	0.32 ± 0.01g	0.32 ± 0.00g	0.23 ± 0.00b	0.22 ± 0.00b	0.27 ± 0.00e	0.07 ± 0.00a	0.23 ± 0.00b	ND	0.29 ± 0.01f	0.25 ± 0.00d	0.23 ± 0.00b
50	o-Cresol	ND	ND	ND	ND	ND	ND	0.21 ± 0.01a	0.25 ± 0.00b	ND	ND	ND	ND
51	Cubenol	ND	0.08 ± 0.00b	ND	ND	0.04 ± 0.01a	0.04 ± 0.00a	0.08 ± 0.00b	ND	ND	ND	ND	ND
52	Veridiflorol	ND	ND	ND	0.12 ± 0.01a	ND	0.02 ± 0.00b	0.70 ± 0.14a	0.08 ± 0.01a	0.09 ± 0.00a	0.09 ± 0.01a	0.03 ± 0.00a	ND
53	Ledol	1.46 ± 0.03d	2.02 ± 0.22e	0.18 ± 0.02ab	ND	0.23 ± 0.01b	0.24 ± 0.02b	0.54 ± 0.03c	0.10 ± 0.00ab	ND	ND	ND	ND
54	(-)-Spathulenol	0.52 ± 0.02e	1.72 ± 0.01f	0.15 ± 0.01de	0.07 ± 0.01abc	0.12 ± 0.00bcd	0.09 ± 0.00bcd	0.53 ± 0.07e	0.05 ± 0.00ab	0.17 ± 0.01d	0.55 ± 0.04e	0.07 ± 0.01abc	ND
55	α-Cadinol	ND	ND	ND	ND	ND	0.03 ± 0.00a	0.07 ± 0.00b	0.83 ± 0.02c	ND	ND	ND	ND
56	unknown	0.59 ± 0.02a	0.10 ± 0.02b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
57	Unknown 5	ND	ND	0.63 ± 0.06c	ND	0.28 ± 0.02a	0.54 ± 0.05bc	ND	ND	0.44 ± 0.02b	1.22 ± 0.13d	0.52 ± 0.01bc	0.51 ± 0.07bc
58	α-Cubebene	0.23 ± 0.00b	0.27 ± 0.00f	0.26 ± 0.01d	0.22 ± 0.00a	0.22 ± 0.00a	0.27 ± 0.00e	0.24 ± 0.00c	0.22 ± 0.00a	0.22 ± 0.00a	0.28 ± 0.00f	0.24 ± 0.00c	0.22 ± 0.00a
59	Copaene	0.23 ± 0.00a	0.29 ± 0.00b	ND	ND	ND	ND	ND	0.23 ± 0.00a	0.23 ± 0.00a	0.29 ± 0.00b	ND	ND
60	β-Elemen	0.23 ± 0.00a	0.29 ± 0.00b	ND	ND	0.24 ± 0.01a	ND	ND	ND	ND	0.29 ± 0.01b	ND	ND
61	α-Gurjunene	0.23 ± 0.00a	0.29 ± 0.01c	0.27 ± 0.01b	0.22 ± 0.00a	0.22 ± 0.00a	0.27 ± 0.00b	ND	ND	0.23 ± 0.00a	0.29 ± 0.00c	0.26 ± 0.01b	0.22 ± 0.00a
62	Alloaromadendren	0.28 ± 0.01bcd	0.28 ± 0.01abcd	0.48 ± 0.03e	ND	0.23 ± 0.00a	0.32 ± 0.00cd	0.27 ± 0.01abc	0.24 ± 0.00ab	0.24 ± 0.01ab	0.32 ± 0.01d	0.32 ± 0.03d	ND
63	GermacreneD	0.22 ± 0.00a	0.35 ± 0.01c	0.26 ± 0.00b	ND	0.22 ± 0.00a	ND	ND	ND	0.22 ± 0.00a	ND	ND	ND
64	γ-Muurolene	ND	0.40 ± 0.03bc	0.43 ± 0.02c	ND	0.29 ± 0.07a	ND	ND	ND	0.24 ± 0.01a	0.33 ± 0.01ab	0.54 ± 0.05d	ND
65	α-Amorphene	0.22 ± 0.00a	0.43 ± 0.02d	ND	ND	0.26 ± 0.00b	0.39 ± 0.03c	0.28 ± 0.01b	ND	ND	ND	ND	ND
66	Viridiflorene	0.47 ± 0.01b	0.66 ± 0.03c	0.98 ± 0.07d	ND	ND	ND	ND	ND	0.33 ± 0.03a	0.42 ± 0.04b	0.32 ± 0.00a	ND

Table 13 continued

67	γ-Cadinene	0.23 ± 0.00a	0.28 ± 0.01ab	0.27 ± 0.01ab	ND	0.47 ± 0.07c	0.27 ± 0.00ab	ND	0.22 ± 0.00a	0.22 ± 0.00a	0.28 ± 0.01ab	0.30 ± 0.03b	ND
68	α-Cadinene	0.22 ± 0.00a	ND	ND	ND	ND	ND	ND	ND	ND	0.27 ± 0.00b	ND	ND
69	γ-Gurjunene	0.25 ± 0.00b	0.33 ± 0.01e	0.29 ± 0.01d	ND	0.23 ± 0.01a	0.27 ± 0.00c	0.25 ± 0.00b	ND	0.22 ± 0.00a	ND	ND	ND
70	Guaia-10(14),11-diene	0.34 ± 0.01c	ND	ND	ND	0.26 ± 0.02b	ND	0.28 ± 0.00b	0.24 ± 0.00a	0.25 ± 0.00a	0.34 ± 0.02c	0.28 ± 0.02b	ND
71	2-Ethylhexanol	ND	ND	ND	ND	ND	ND	0.08 ± 0.01a	ND	ND	ND	ND	ND
72	δ-Cadinol	ND	0.13 ± 0.00c	ND	ND	ND	ND	ND	0.06 ± 0.01b	0.03 ± 0.00a	0.04 ± 0.00b	ND	ND
73	β-Ionone	ND	ND	ND	0.11 ± 0.00a	0.46 ± 0.05b	ND	0.12 ± 0.01a	ND	ND	ND	ND	ND
74	Unknown 6	ND	0.27 ± 0.00ab	ND	0.23 ± 0.00a	ND	0.27 ± 0.00ab	ND	0.22 ± 0.00a	0.22 ± 0.00a	ND	ND	0.31 ± 0.07b
75	Unknown 7	0.25 ± 0.01ab	0.28 ± 0.00abc	0.30 ± 0.01c	0.25 ± 0.01ab	0.24 ± 0.00a	0.30 ± 0.02bc	0.25 ± 0.01ab	0.27 ± 0.00abc	0.26 ± 0.01abc	0.47 ± 0.03d	0.47 ± 0.02d	0.25 ± 0.02abc
76	Unknown 8	ND	0.41 ± 0.01a	ND	ND	0.23 ± 0.01b	ND	ND	ND	ND	ND	0.49 ± 0.03c	ND
77	Unknown 8	0.30 ± 0.00a	0.36 ± 0.01b	0.35 ± 0.00b	ND	ND	0.35 ± 0.00b	ND	ND	ND	0.35 ± 0.03b	ND	ND
78	Unknown 9	ND	0.42 ± 0.00c	0.36 ± 0.01b	ND	0.37 ± 0.04b	ND	0.31 ± 0.00a	ND	ND	0.29 ± 0.01a	ND	0.30 ± 0.01a
79	Unknown 10	ND	0.51 ± 0.00c	0.47 ± 0.04c	0.23 ± 0.00a	ND	ND	ND	ND	ND	0.29 ± 0.02c	0.61 ± 0.03d	0.24 ± 0.00ab
80	Unknown 11	0.24 ± 0.00a	0.28 ± 0.01c	0.27 ± 0.00b	ND	0.24 ± 0.01a	ND	ND	ND	ND	ND	ND	ND
81	Unknown 12	0.30 ± 0.01b	ND	0.44 ± 0.02c	0.23 ± 0.00a	ND	ND	0.26 ± 0.00a	ND	ND	ND	ND	ND
82	Unknown 13	0.30 ± 0.01b	ND	0.37 ± 0.02c	ND	ND	0.37 ± 0.02c	0.25 ± 0.00a	ND	0.23 ± 0.00a	0.29 ± 0.01b	ND	0.23 ± 0.00a
83	Cadalene	0.22 ± 0.00a	0.27 ± 0.00bc	0.28 ± 0.01c	ND	ND	ND	ND	ND	0.22 ± 0.00a	0.27 ± 0.00b	ND	ND
84	Unknown 14	ND	0.27 ± 0.00c	0.25 ± 0.00b	0.22 ± 0.00a	ND	ND	ND	ND	0.22 ± 0.00a	0.27 ± 0.00c	0.30 ± 0.01d	0.22 ± 0.00a

Volatiles concentration: present as mean ± standard deviation; ND: not detected

a, b, c, d, e, f, g, h, i present statistically significant differences among cultivars and ripening stages by multiple comparisons using Duncan's test ($P < 0.05$), different letters indicate significant difference among samples;

The order of the compounds was arranged by RI calculated from DB-WAX column.

The total contents of bound volatiles increased from 4WBR to 2WBR, following by continuously decreasing as ripening progressed for Unique and Anatoki feijoas (Figure 23). This pattern is also evident in mango, which reached the highest total concentration at half ripe stage (Lalel et al., 2003). Whereas for the Triumph fruits, the total content of bound volatiles peaked at ripe stage.

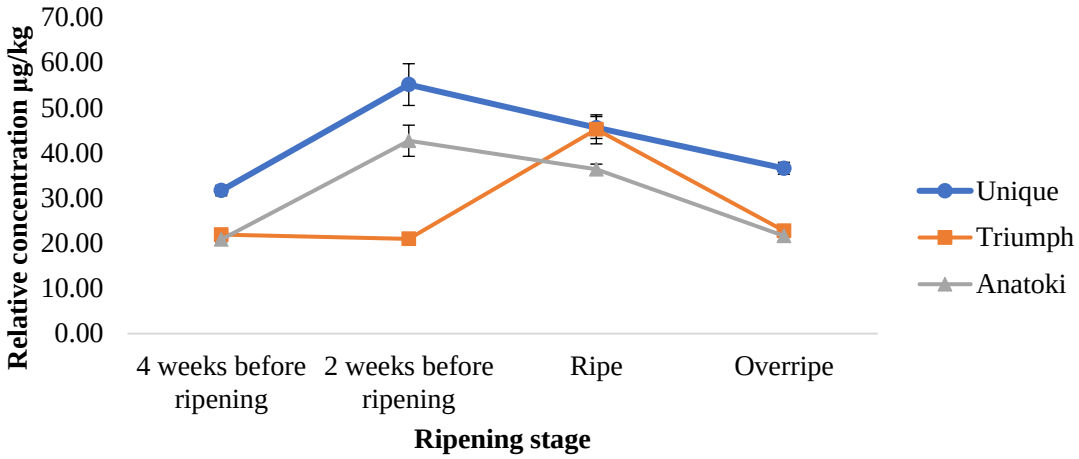

Figure 3.15: Total content of bound volatiles in three feijoa cultivars at four ripening stages

Enzymes play an important role in releasing of bound volatiles (Hjelmeland & Ebeler, 2015). *β*-glucosidases are a crucial enzymes in aroma released in many plants (Sarry & Günata, 2004). The activities of *β*-glucosidases have been found to increase as ripening progressed in melon (Fils-Lycaon & Buret, 1991) and grape berries (Sarry et al., 2004). However, the activity of some *β*-glucosidases is weakened by glucose and the extent of inhibition vary with different sources of enzyme (Günata & Vallier, 1999; Sarry et al., 2004). Moreover, hydrolysis of glycosidic aroma precursors depends on activities of enzymes and availabilities of substrates. Plant cells might compartment substrates from enzymes (Sarry & Günata, 2004). Therefore, the changes of bound volatiles during ripening is diverse and complex.

3.4.3 Changes in the composition of bound volatile compounds during ripening

Various trends of individual bound volatiles during ripening has been found (Table 13). Numerous gene expression of fruits, formation mechanisms, enzymes availability and substrates concentration lead to the differences among each compound (Wen et al., 2014). Likewise to feijoa fruits, nectarines (Aubert et al., 2003), cherry (Wen et al., 2014), mango (Lalel et al., 2003), mulberry (Chen et al., 2015), which had different progressions for individual bound volatiles.

Figure 24 illustrates compositional changes of each sample during maturation. The concentrations of terpenes were higher in unripe feijoas than ripen fruits, which was consistent with the progression of free terpenes. On the contrary, the concentration of esters was highest in ripe and overripe stages, which was also evident in mango (Lalel et al., 2003). Although the concentration of free esters in feijoa was much higher but the patterns were the same with that of bound esters. For bound alcohols and aldehydes, the concentrations firstly increased, peaking at 2WBR, followed by declining from ripe to overripe stages. The change of ketones varied among cultivars. For the Unique cultivar, the concentrations of ketones accumulated from unripe to ripe stages. For Triumph and Anatoki, the progressions fluctuated during maturation.

Different feijoa cultivars were discovered with inconsistent results on bound volatiles, and significant cultivar differences were also reported in many fruits, including kiwifruits (Garcia et al., 2013), citrus juice (Ren, 2015), mango (Ollé et al., 1998), tomato (Ortiz-Serrano & Gil, 2009). For all 4WBR fruits, the total concentrations of alcohol, terpene and ketone occupied the top three positions and for 2WBR fruits, aldehyde became the dominant class. In ripe feijoas, the dominant groups varied within the three cultivars. Aldehydes still shared the biggest portion in Triumph and Anatoki, while ketone and alcohol were the dominant groups

in Unique. At overripe stage, aldehyde was still the dominant class in Triumph and Anatoki whereas ketone was the top class in the Unique cultivar.

Figure 3.16: Compositional changes of feijoa bound volatiles in three cultivars during ripening

3.4.3.1 Ester

There were only three esters found in glycosidically bound volatiles in feijoa, which were ethyl acetate, methyl benzoate and ethyl benzoate. Dramatically lower concentrations of bound esters were found (maximum 1.92 ug/kg, Table 13) compared to those detected in their free forms (minimum 183 ug/kg, Table 7). Nevertheless, the concentrations accumulated as ripening progressed for both free and bound volatiles (Figure 12 and Figure 25). For many fruits, esters concentrations increase during ripening and account for the biggest portion in ripe fruits (Beaulieu & Baldwin, 2002).

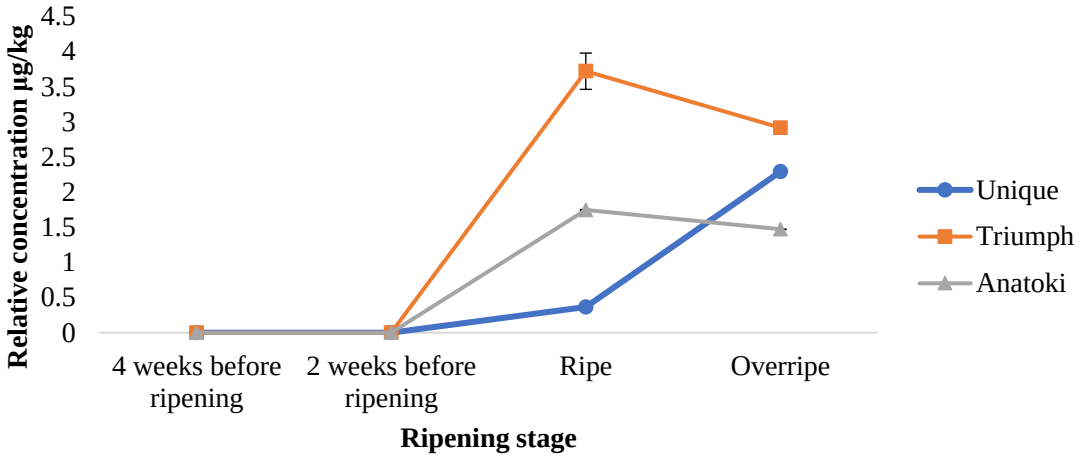

Figure 3.17: Total content of bound esters in three feijoa cultivars at four ripening stages

Their negligible concentrations as bound form might indicate that ester compounds are less likely to link to glycosides and to be aglycone molecules. They may be directly synthesized from fatty acid and amino acid pathways and released as volatiles. Moreover, In *Rubus corchorifolius* L. f. fruit, the content of bound esters were lower than that of free form because bound esters were already hydrolysed to free form (Yang, 2019). This might be another explanation for less esters in bound form in feijoas.

3.4.3.2 Terpenes

A total of 28 terpenes were found in feijoa bound volatiles (27 in the Unique cultivar, 25 in Triumph and Anatoki). Among them, only three compounds did not exist in free form (1R-*α*-pinene, 3-carene and viridiflorene). In general, the concentrations of terpenes in unripe stages (4WBR and 2WBR) were higher than that in ripe and overripe fruits (Figure 26). For the Unique and Anatoki feijoas, the relative concentrations firstly increased from 4WBR to 2WBR then continuous decreased from ripe to overripe stages. For Triumph, contrarily, the concentration decreased from T4 to T2 then slightly increased to TR, followed by declining to TO.

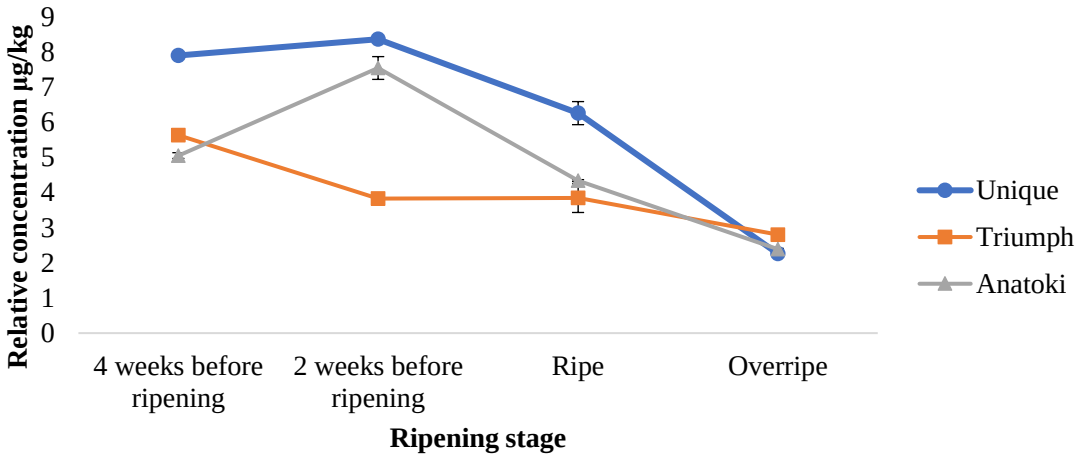

Figure 3.18: Total content of bound terpenes in three feijoa cultivars at four ripening stages

3-Carene was only detected in bound form. It is widely existed in bound from in mango (Lalel et al., 2003), citrus juice (Ren, 2015), oranges (Fan et al., 2009) and mandarins (Gao, 2018). Especially in mango, 3-carene with ‘herbal, citrus and sweet’ aroma was an important flavor contributor to mango (Lebrun, Plotto, Goodner, Ducamp, & Baldwin, 2008). 1R-*α*-pinene was also only detected in bound form. This compound was not popular in fruits but has been found in essential oils extracted from *Myrica* rubra leaves (Langhasova et al., 2014) and Chinese chestnut (Q. Li et al., 2016). Although, according to (Breitmaier, 2006), bound

terpenes widely existing in plants and were regarded as potential aroma precursors. The concentrations of bound terpenes in feijoa were too low to release aroma (OAVs <1).

Bound *β*-cadinene, *L*-calamenene and caryophyllene showed higher concentrations among terpenes. The change of *L*-calamenene during ripening varied for different cultivars. In Unique, the concentrations of *L*-calamenene decreased during ripening while in Triumph and Anatoki the concentrations increased firstly then decreased. The variation between cultivars also existed on bound caryophyllene. The content of caryophyllene increased from 4WBR to 2WBR and decreased to overripe stage in the Anatoki cultivar. The concentration of caryophyllene fluctuated during ripening in the Unique and Triumph cultivars. *β*-Cadinene only existed in U4, A4 and A2 and was completely absent in Triumph cultivars. Therefore, *β*-cadinene could become a chemical indicator of ripening stages and for cultivar identification.

3.4.3.3 Alcohols

Alcohol was one of the main groups of the bound volatiles in feijoas. Similarly, Alcohols was also the major class of bound volatiles in mulberry (Chen et al., 2015) and cherry (Wen et al., 2014). The number of alcohols found in this study was 19 in total, with 15 in Unique, 17 in Triumph and 14 in Anatoki. Moreover, the OAVs of bound alcohols were lower than 1, which means that bound alcohol could not contribute to feijoa aroma.

Alcohols in glycosidically bound form underwent different patterns, unlike bound alcohols, despite some fluctuation, the concentrations of free alcohols sharply decreased during ripening for all three cultivars. To be exact, the progression of bound alcohols in the Triumph cultivar fluctuated across ripening (Figure 27) and the concentrations of bound alcohols in Unique increased from 4WBR to 2WBR and decreased from ripe stage to overripe stage. Likewise to Unique, the concentration of Anatoki fruits also witnessed an increase followed by a decrease. The decreased relative concentrations of bound alcohols during ripening was also found in R. corchorifolius fruit juice (Yang, 2019) and mandarin (Gao, 2018).

The reduction of bound alcohols could indicate that the bound alcohols were hydrolysed to free form as ripening progressed after 2WBR. Afterwards the hydrolysed alcohols may be transformed into aldehydes and esters. In addition, the breakdown of some non-volatile precursors could generate alcohols such as linalool and terpineol which existed in both free and bound forms (Janzantti et al., 2012).

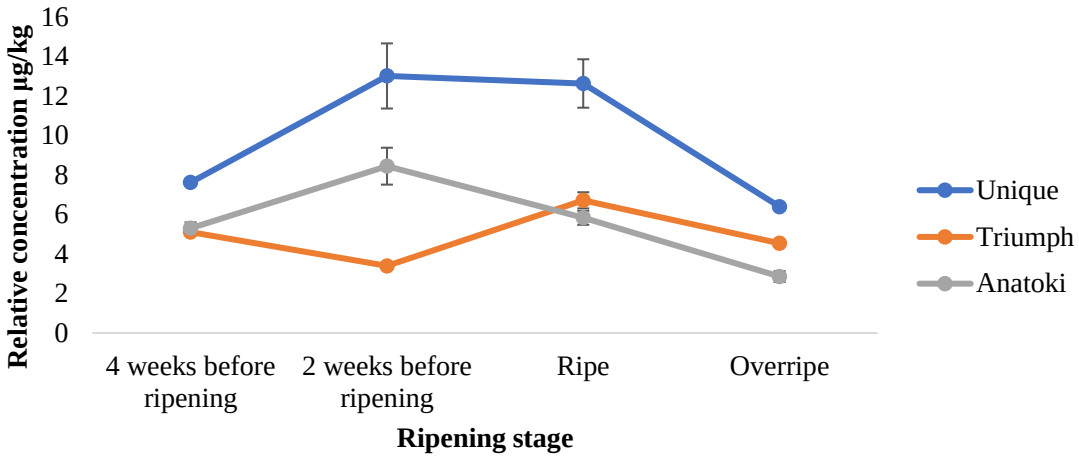

Figure 3.19: Total content of bound alcohols in three feijoa cultivars at four ripening stages

Moreover, 3 out of 19 alcohols were not present in free form, which were 2-hexanol, benzyl alcohol and 2-ethylhexanol. Impressively, all these three alcohols could be used as indicators of cultivar type. 2-Hexanol was absent in Triumph cultivar and only existed in U4 and UO samples in Unique cultivar. Benzyl alcohol only existed in T2, TR and TO and was not found in Unique and Anatoki feijoas. For 2-ethylhexanol, it was only detected in U2 and TR samples.

3.4.3.4 Aldehydes

A total of 9 aldehydes were identified in bound form in feijoas. Except (E)-2-octenal was absent in the Unique feijoas, all other aldehydes were identified in all three feijoa cultivars. Bound aldehyde was the dominant class in the 2 WBR, ripe and overripe fruits in the Triumph and Anatoki fruits.

The trends of bound alcohols and bound aldehyde were comparable (Figure 27 and Figure 28). Both classes experienced an increase followed by a decrease. Same progressions have also been found in mango (Lalel et al., 2003) and mandarins (Gao, 2018). Compared with the change of free aldehydes in feijoas, the change of bound aldehyde was also similar. During the stages of ripening, bound aldehydes were released into free form and free aldehydes were transformed into esters under the assistance of AAT (Siegmund, 2015).

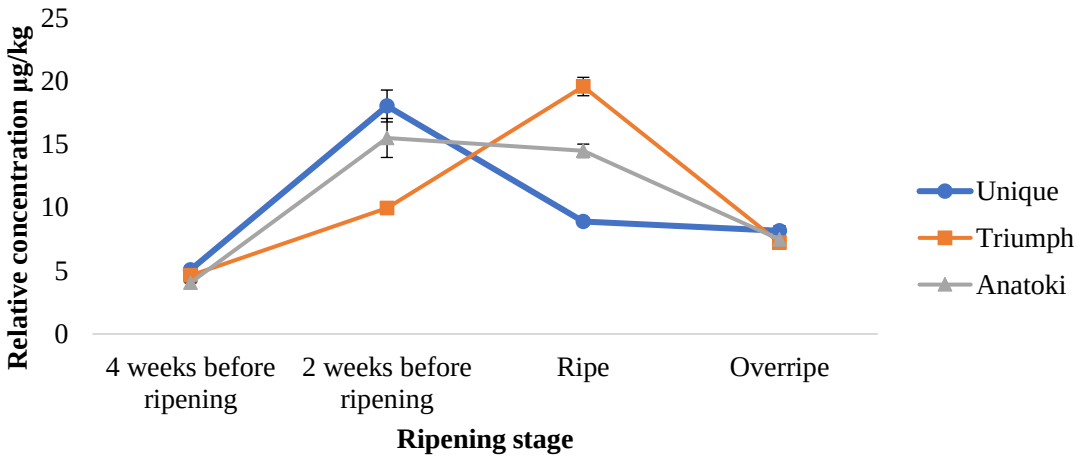

Figure 3.20: Total content of bound aldehydes in three feijoa cultivars at four ripening stages

Similar to a study on mulberry (Chen et al., 2015), bound aldehydes were found to have significant lower levels compared with these detected in their free form. Nevertheless, the number of bound aldehydes in feijoas was higher than that in free form. Octanal, 2-ethylhexanal, nonanal and *(E)*-2-octenal were only found in bound form.

(E)-2-Octenal only existed in unripe fruits (T4, A4 and A2). It has been found in cherry (Wen et al., 2014) and tomato (Birtic et al., 2009) as a potential aroma with 'fresh and cucumber' notes. The concentrations of bound 2-ethylhexanal were the highest throughout all ripening for all three cultivars. There was an increase followed by a decline in the relative concentrations of 2-ethylhexanal during ripening. Peach (Ortiz et al., 2010) and mulberry fruit (Zhang et al., 2018) have been tested to have 2-ethylhexanal as well. Though the concentrations of octanal and nonanal fluctuated during ripening, the peak concentration usually appeared in 2WBR fruits. Many other fruits includes grapes (Wang et al., 2015), mandarins (Gao, 2018), tomatoes (Ortiz-Serrano & Gil, 2009) and cherry (Wen et al., 2014) have been reported to have octanal and nonanal at the same time.

3.4.3.5 Ketones

Ketone was the dominant class of bound volatiles in the Unique cultivar at the ripe stages. A total of 8 ketones were found with 7 in the Unique and Triumph cultivars and 5 in the Anatoki fruits.

The change of free and bound ketones' concentration during ripening were different (free ketone content decreased during ripening, Figure 16 and Figure 29). The concentrations of bound ketones gradually rose during ripening (from U4 8.33 ug/kg to UO 16.33 ug/kg) in the Unique fruits. As mentioned previously, plant cells might compartment substrates from enzymes (Sarry & Günata, 2004). As ripening progressed, more enzymes are available for substrates because the cell wall and membranes tend to be more permeable during ripening (Defilippi et al., 2009). Whereas for Anatoki bound ketones, pattern changed (Figure 24). There was a mild increase from A4 (4.79 ug/kg) to A2 (7.76 ug/kg) followed by continuous decrease to AR (7.38 ug/kg) and to AO (4.88 ug/kg). As for the Triumph fruits, after a decrease (T4 4.81 ug/kg to T2 1.97 ug/kg), the concentration roughly kept constant around 2.7 ug/kg (TR). The decreased ketones concentrations might suggest that bound volatiles in Anatoki and Triumph might be released during ripening.

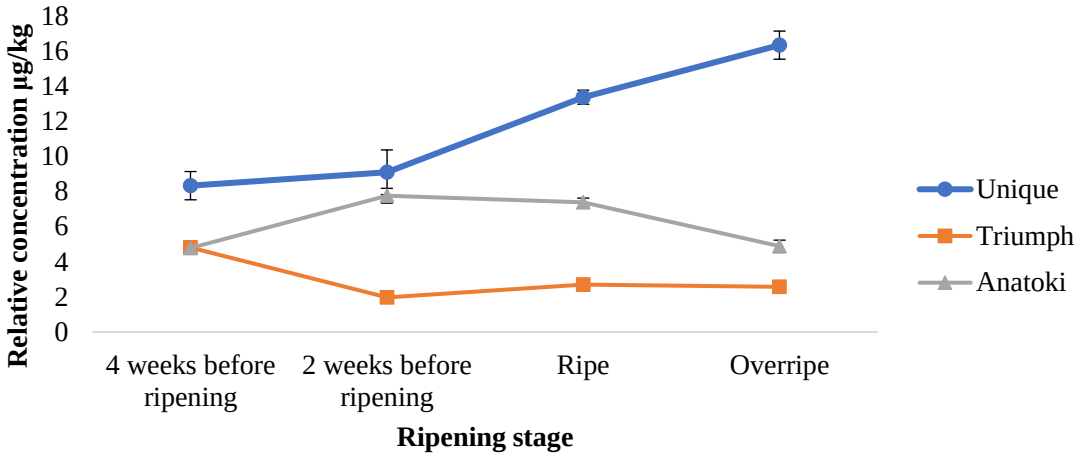

Figure 3.21: Total content of bound ketones in three feijoa cultivars at four ripening stages

Unlike bound ketones, a dramatic decrease was seen for feijoa free ketones (Figure 29), which suggested that the activities of ketones related enzymes may decrease during ripening. Additionally, methyl heptanone, 3-nonanone, 2-undecanone, 2-tridecanone 2-nonanone were only detected in free form, which means that these compounds are less likely to become glycosides by bonding to sugars.

The top two bound volatiles with the highest concentration throughout ripening were 2-heptanone and 3-octanone. Unfortunately, the OAVs of these two compounds were below 1 (threshold of 2-heptanone was 140 ug/L (Yang, 2019) and threshold of 3-octanone was 15-20 ug/L (Siegmund & Pöllinger-Zierler, 2006)), which means they were not potential aromatic compounds in feijoa bound volatiles. In addition, bound 2-hexanone was only detected in the Anatoki feijoas with fluctuated concentrations during ripening, which means that 2-hexanone could be a volatiles indicator of Anatoki cultivar. Bound 2-hexanone was also detected in essential oils extracted from orange (Jerkovic et al., 2007). Bound 3-heptanone only existed in the Unique and Triumph feijoas with very low concentrations and it has been found in gooseberry (Yilmaztekin, 2014) and grapes (Girard et al., 2002) as well. *β*-Ionone was a popular volatiles in many fruits like cherry (Wen et al., 2014), blackberry (Du et al., 2010) and grapes (Vilanova et al., 2012). In feijoas, bound *β*-ionone completely missed in Anatoki cultivar and only detected in UO, T4 and T2 samples.

In summary, bound volatiles which co-existed as free volatiles may be hydrolysed during ripening and released as free form. Volatiles which only present in free form may be directly synthesized from fatty acid, amino acid, carbohydrate pathway without glycosylation. Volatiles which only present in bound from might suggest that the potential of their auto-transformation into free form is low. Furthermore, bound volatiles that were specific to some cultivars and maturation stages could be used as indicator to distinguish different cultivars or ripening stages.

Chapter Four: Conclusion, limitation and Future Work

4.1 Conclusion

Similar with many fruits, the Unique, Triumph and Anatoki feijoa fruits showed significant physical changes during ripening. Fruits were getting bigger from 4WBR to R, the color tended to be more yellow and the skin became smoother as ripening progressed. Furthermore, firmness of fruits decreased during ripening and significant differences ($P < 0.05$) existed among different ripening stages and cultivars. Additionally, for the cultivar differences, Anatoki was the firmest cultivar at 4WBR and 2WBR while Triumph become the firmest cultivar at R. For OR samples, Unique and Triumph were comparable ($P > 0.05$) yet Anatoki was a little firmer than these two.

A total of 164 free volatiles were detected in three cultivars at four ripening stage through HS-SPME-GC-MS, with 60 terpenes, 52 esters, 20 alcohols, 9 ketones, 6 aldehydes, 2 hydrocarbons and 15 unknowns, among which 69 free volatiles were detected for the first time. Terpene was the dominant class in 4WBR and 2WBR fruits and ester was the major group in R and OR fruits. During ripening, the concentration of terpenes, alcohols and ketones decreased while that of ester largely increased. The levels of aldehydes fluctuated and that of hydrocarbons kept constant across ripening. Free volatiles in feijoas are synthesized from amino acid, fatty acid and carbohydrate metabolism. Relative substrates and related enzymes including lipoxygenase, alcohol dehydrogenase and alcohol acyltransferase are the factors influencing the content of volatiles (Siegmund, 2015). Besides, there were significant difference among some compounds in different ripening stages and cultivars, and these compounds could be used as chemical differentiators for feijoas at different ripening stages and cultivars.

A total of 26 aroma active compounds with 12 terpenes, 7 esters, 2 alcohols, 2 ketones, 1 aldehyde and 2 unknowns were identified by HS-SPME-GCO-MS by four trained panellists. Consistent with all free volatiles, at unripe stages, terpenes dominated free aromas and released more ‘herbal, grassy’ odour. While for ripe fruits, esters dominated free aromas and gave more ‘fruity, sweet, floral’ aroma. During ripening, the overall aroma of feijoas moved

from ‘green and herbal’ to ‘sweet and fruity’ aromas. There was cultivars difference in feijoa aroma profiles, the Triumph cultivar was less aromatic at 4WBR and 2WBR and the Unique and Anatoki cultivars had similar aroma profiles throughout all feijoa ripening stages. Therefore, the Triumph cultivar may not be a good choice for possible aroma applications due to its lower aromatic property.

A total of 84 bound volatile compounds were identified with 28 terpenes, 19 alcohols, 9 aldehydes, 8 ketones, 3 esters, 1 hydrocarbon, and 16 unknowns in all three cultivars and 52 co-existed as free and bound volatiles. It was noteworthy the concentration of glycosidically bound volatiles were much lower than that of free volatiles and the OAVs of most bound volatiles in feijoa were less than 1, which means that the hydrolysed bound volatiles may not contribute to the aroma of feijoa fruits. For all 4WBR fruits, bound alcohol, terpene and ketone were the dominant groups and for 2WBR fruits, aldehyde became the dominant class. In ripe and overripe fruits, the dominant groups varied within the three cultivars. During ripening, comparable with free volatiles, the concentrations of bound terpenes decreased, and the concentration of esters increased. For bound alcohols and aldehydes, the concentrations firstly increased, peaking at 2WBR, flowed by declining from R to OR. The change of ketones varied among cultivars. In brief, the importance of bound volatiles in feijoas could be limited due to their lower numbers and insignificant concentrations.

4.2 Limitations

Challenges occurred during fruit collection, fruit storage, volatile extraction and volatile identification and quantification were faced in this study. Firstly, the collected fruits should be representative and be maintained with the maximum effort after harvest. However, in realistic conditions, improper sample storage during delivery and prolonged refrigeration might influence the quality of fruits. Secondly for volatile extraction, free volatile is easily to release during sample preparation, which might cause the loss of free volatile in result. Extraction of bound volatile might bring in some contaminants and influence the volatile analysis.

Thirdly for volatile identification, the equipment for characterising the volatile composition were not of the latest edition, which could give less precise result. Fourthly, chemical standards were not available for every volatile, the semi-quantified concentration of some compounds maybe not accurate enough. Lastly, there was individual difference in olfactory detection. Besides, the aroma of feijoa fruits is perceived as a whole rather than each volatile compound, sensorial tests might be needed to evaluate the aromas of whole fruits.

4.3 Future works

Because of the time limitations, this study only focused on the characterisation and aroma profile of free and bound volatile in three feijoa cultivars during ripening. More development and improvement could be done in the future to contribute to the understanding of comprehensive aroma and volatile profile of feijoa fruits during ripening, which includes,

- Exploring the relevant metabolic pathway for synthesising individual volatile compound, especially for those with strong aromas or higher concentration. More understanding of the mechanism of their formation can help scientists have a deeper insight of the formation for volatiles.

- Developing better extraction procedures of aromatically active compounds, as they could be widely used in flavour and fragrance industries.

- Analysing of the concentration of related substrates and enzymes to explain the reasons for the change of volatiles during ripening and the variation among cultivars. This could give scientists more knowledge in this area and provide information to growers for cultivar selection.

- Conducting sensorial test to complete the aroma profile of feijoa fruits. Because consumer assess the fruit flavour as a whole rather than for each compound, a complete feijoa aroma profile could be build based on the evaluation of aromas from both whole fruits and individuals.

- Detecting of sugar moieties and related enzymes of some important bound volatiles to widely understand the synthesis of those bound volatiles.

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