

# Identification of flavour in fresh feijoa fruit

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mass spectrometry, D-limonene, cis-rose oxide

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## **Abstract**

The aim of this study was to extract and identify volatile flavour components from peel, flesh and seeds of feijoa fruit separately and further determine its flavour profile. Obtained information is aimed to be used to develop a new flavour mixture for the food industry. The volatile flavour fraction of feijoa fruit flesh, seeds and skin were isolated by ethanol extraction and steam distillation. Further an organoleptic evaluation was carried out to distinguish flavour differences within the obtained distillates to decrease the number of samples to be analysed by gas chromatography and gas chromatography-mass spectrometry. Gas chromatography analysis resulted in 13 identified flavour compounds of the 23 reference compounds used. Results showed gas chromatography-mass spectrometry to be most successful and this technique presented a great amount of data of which seven compounds are reported for the first time as flavour compounds of feijoa fruit; citronellyl acetate, cis-rose oxide, geraniol acetate, isoamyl acetate, ethyl octanoate, D-limonene and humulane-1,6-dien-3-ol. Further analysis is needed to evaluate the extent of unique findings of flavour compounds and to evaluate how they contribute to the flavour of feijoa fruit to be able to produce a flavour mixture for the food industry.

Key words: feijoa fruit, extraction, distillation, organoleptic evaluation, gas chromatography-mass spectrometry, D-limonene, cis-rose oxide.

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# Identification of flavour in fresh feijoa fruit

## 1. Introduction

*Feijoa sellowiana* Berg is a shrub native to South America and is a close relative of the guava (*Psidium guajava* L.) (Hardy & Michael, 1970). Because the feijoa plant can tolerate a wide range of climatic and soil conditions, it is grown either as an ornamental or exploited commercially in warm temperate areas of the world for its fruit which is consumed fresh or processed (Shaw, Allen & Yates, 1989; Hardy & Michael, 1970). Feijoa fruit is used to manufacture products such as juice, jam, ice-cream, chutney and fruit smoothies. The two major flavour sensations in most fruits are sweetness and sourness which mainly are due to sugars and non-volatile acids. Bitterness and astringency are not generally considered desirable flavour characteristics in fruits although if adequately masked by sugars, low levels of these attributes may well add body and depth of flavour. When producing a food product it is important to consider its flavour and aroma as both are important parameters that affect the acceptability of a product. It is also important to consider the presence of trace amounts of volatile components since they contribute a lot of character to fruits and their processed products (Williams, 1979).

### 1.1 *The flavour of feijoa fruit*

The flavour of feijoa fruit or pineapple guava (*F. sellowiana* Berg) is characterized by its high content of the flavour compounds methyl benzoate and ethyl benzoate. Hardy & Michael (1970) distilled volatiles from intact fruit and showed that methyl benzoate and ethyl benzoate constituted of 90% of the flavour and they provided much of the distinctive aroma which is characteristic of feijoa fruit. Both esters have a similar odour but the methyl ester is much stronger. Although benzoate esters have been detected in other fruits, the feijoa is the only fruit examined in which benzoate esters are the major aroma compounds (Hardy & Michael, 1970). Data presented from another analysis of intact feijoa fruit showed that esters, generally important in fruit for their flavour contribution, constituted approximately 93% of the aroma concentrate. Further preliminary sensory evaluation experiments suggested that

only methyl benzoate, ethyl benzoate and ethyl butyrate had feijoa-like aroma qualities. Ethyl butyrate was considered to be contributing significantly to the aroma of intact feijoa fruit (Shaw, Ellingham & Birch, 1983).

## ***1.2 What is a flavour?***

The four basic taste sensations are sweet, salty, sour and bitter (Seidman, 1979). The term aroma is used to describe the 'intangible conception' of odour and flavour. Aroma could also be referred to as a processed raw material used by the flavourist (Arctander, 1960).

Flavour is often explained as a combination of taste and aroma (Laing & Jinks, 1996). The flavour of food is caused by chemical substances present in the food and consists of a combination of taste, odour and other sensations. When considering flavour components it is usual to divide these into volatiles and non-volatiles (Williams, 1979). Taste is caused by stimulation of the gustatory cells of the buccal surface of the tongue by soluble substances, most of them non-volatile released from the food into the saliva. Flavour and aroma are said to be obtained mainly due to odorous volatile substances being released from the food into the air in the mouth and further carried to the olfactory epithelium in the nose (Laing, 1979; Williams, 1979). What is recognized as the response to an odour is dependent on the amounts of those volatile substances which are released into the air from food and reaching the olfactory sensors as well as the interaction with the perceiving organism (Laing, 1979).

## ***1.3 Extraction of volatile flavour components***

Flavour extraction can be demanding due to several factors including their presence in very low concentrations in food products and fruits. In foods the concentrations of the volatile components cover a wide range where the major volatile components vary from  $10^{-3}$  to  $10^{-1}$  mg/litre while those of the trace constituents may be as low as  $10^{-7}$  mg/litre. Even those constituents of really low concentrations might be important odour constituents since odour threshold values as low as  $10^{-9}$  mg/litre has been detected (Acree, 2005; Bebelmans, 1979). To identify flavour compounds in food products it normally requires initial isolation from the bulky constituents of the food combined with substantial concentration; for example using

distillation. It is important to minimize the distortion of the native composition, especially when studying the quality of flavours. Distillation techniques have the advantage to assure adequate isolation of compounds of higher boiling points and low concentrations but precautions have to be taken to ensure that volatile flavour compounds remain unchanged during their isolation or concentration since some volatiles are heat sensitive or reactive and so are easily lost. There is a risk for formation of new volatiles, artefacts, due to reactions between chemical constituents of a food matrix (Bebelmanns, 1979).

The use of gas-liquid chromatography and mass spectrometry enables detection of trace compounds of small concentrations as well it enables work with smaller amounts of food concentrates. Areas of the chromatogram or certain peaks can be detected by smell during analysis using GC-MS and its odour is described (Seidman, 1979). The relative amount of components in an odour concentrate prepared from the product is not the same as those in the vapour. Such differences of ratios may affect the odour (Bebelmanns, 1979).

Mass spectrometer analysis makes it possible to determine the chemical structure of the unknown compound and this can be verified by comparison with authentic compounds (Seidman, 1979).

### **1.3.1 Gas Chromatography/Mass Spectrometry**

A gas chromatograph (GC) separates components of a mixture while the mass spectrometry gives structural information of each component. If standards of known concentration are used together with the unknown compounds gas chromatography/mass spectrometry can also provide quantitative data.

Gas chromatography analysis of a mixture involves the injection of a dilute solution (1 $\mu$ l or less) into a head port of the gas chromatograph. The sample is evaporated and swept by a flow of inert gas into a capillary column. The capillary column is a tube with a normal length of 10-30 m and width of 0.1-0.5 mm. It is contained within a chamber ('oven') and the temperature can be varied according to the volatility of the analysed samples. A stationary

phase is usually coating the inside of the capillary column and the stationary phase is often a non-polar silicon based polymer.

As molecules of the sample are swept by the inert gas through the column, the molecules travel at different rates. The rate they are travelling is dependent on their boiling points and the degree of affinity for the stationary phase. The higher boiling point or the stronger affinity for the stationary phase the longer time it will take for the molecule to pass through the column. The length of time it takes for a compound to pass the column is called retention time. There is also a partition of the compound between the gas phase and the liquid phase; if the compound spends more time in the liquid phase it gets retained on the column and comes out later on.

When the components exit the gas chromatograph one by one it travels into the mass spectrometer. Molecules of the sample get bombarded by electrons; ions and fragments of the molecule are formed resulting in a mass spectrum. One mass spectrum is formed for every molecule (Solomons & Fryhle, 2006).

### ***1.3.2 Aim of project***

To extract and identify volatile flavour components from peel, flesh and seeds of feijoa fruit separately and further determine its flavour profile. This information is needed to be able to develop a nature identical feijoa flavour; a product that does not exist on the market.

## 2. Materials and methods

### 2.1 Extraction of flavour components

#### 2.1.1 Sample preparation

Approximately 4.7 kg of feijoa fruit of unknown variety was purchased from a Gordon's vegetable market in Prebbleton, Christchurch, New Zealand, and stored at room temperature (25°C) during 4 days until ripe and ready for use. Approximately 2 kg of ripe feijoa fruit were picked out and peeled to an approximate depth of 1 mm. Fruit flesh, seeds and peel were collected separately.



**Figure 1.** Feijoa skin, flesh and seed extracts

Fractions of flesh, seeds and peel were weighed and ethanol was added in a 2:1 ratio. The samples were further homogenized for 2 minutes using a Cascade Blender (Model No. CE071BR, China). The fruit-ethanol mixtures were poured into 1 l glass jars and flushed with nitrogen gas to avoid oxidation during storage. The sealed glass jars were placed in a fridge at 4°C for seven days to allow the flavour fractions to be extracted into the ethanol from the homogenised tissue.

#### 2.1.2 Distillation

A distillation method to extract and separate volatile flavour components of fresh feijoa fruit was developed. There is no previous work published where feijoa seeds have been extracted separated from the fruit flesh.

In the first trial extracts were poured directly into round glass flasks which were placed on a heating mantle and connected to a water-cooled condenser. When the required temperature (78°C) for extraction was reached the fruit puree extracts started to burn.

The method was modified by filtering the extracts to separate the fruit puree (the non-volatile food matrix) from the liquid fraction of ethanol using glass microfiber filters (11.0 cm, GF/C) and an electric vacuum pump. The filtered extracts were placed in a round bottomed flask on a heating mantle. The vapour passed through an 80 cm long glass condenser tube. Glass marbles were placed inside the tube in order to increase the surface that the vapour needed to pass through. The purpose of the increased surface was to improve separation of volatile flavour components within the vapour. A cooling system



**Figure 2.** Distillation apparatus

of water was used to condense the vapour. Fractions were collected in 50 and 100 ml round bottle flasks which were enclosed by glass caps and further cooled in an ice-bath. Temperature of evaporates was recorded as fractions were collected. New fractions were aimed to be collected for every 1°C increase to ensure separation and isolation of volatile flavour compounds; it is known that volatile compounds evaporate at different temperatures and this is dependent on its molecular weight. Distillation proceeded until at least one fraction had been collected at a temperature of 100°C; the boiling point of water. Fractions were collected for approximately 3-5 hours per fruit extract. Collected fractions were stored sealed in a domestic fridge (4°C) until further analysis could be performed.

### **2.1.3 Organoleptic evaluation**

An organoleptic evaluation was performed of seed, flesh and skin fractions by making 1% dilutions with water of each fraction. Fractions were sniffed and tasted to identify differences of flavour within each fraction of seed, flesh and peel separately. Fractions of similar flavour features were grouped and analysis was carried out before gas chromatography and gas chromatography – mass spectrometry was commenced.

## ***2.2 Identification of flavour components***

### **2.2.1 Gas Chromatography (GC)**

Gas chromatography (GC) was used to separate and identify volatile components of feijoa fruit distillates of seed, flesh and skin. Samples representing all fractions of distilled fruit flesh, peel and seed extracts were analysed separately to obtain an overall view of their flavour profile.

Further GC was used to determine if the distillation technique was effective by analysing whether the flavour compounds had been successfully separated. Results of the organoleptic evaluation were used; grouped fractions of fruit flesh, seeds and skin distillates were analysed separately. Gas chromatography analysis was performed using a Hewlett Packard model 5890 capillary column gas chromatograph equipped with a flame ionization detector (FID). Separation was carried out using a 30 m x 0.25 mm DB wax column. A 5 µl sample at room temperature (25°C) was injected for each run. The syringe was cleaned with ethanol in between injections followed by a flush through with sample before a new injection. The column temperature program for analysis was set to an initial temperature of 50°C which was held for 10 minutes. The temperature was then increased 5°C/minute up to a temperature of 220°C which was held for 20 minutes.

To be able to identify peaks of the obtained GC – chromatograms and to get an approximate quantification reference compounds were analysed. Reference compounds were grouped into four samples using Kovats retention indices (Kovats, 1958) to obtain separated peaks and thereby simplify analysis of the chromatograms. Chosen reference compounds (see Appendix 1) were added in the ratio of 1:1 in each sample. A similar GC set up to the previously mentioned analysis was used, the only difference being a reduction of the amount of sample being injected to the column which was decreased to 3 µl. The purpose of decreasing the amount of sample for injection was to adjust the height of peaks in the resulting chromatograms. Identification of flavour compounds was obtained by comparing peaks of the analysed fruit distillates with peaks of analysed reference compounds.

### **2.2.2 Gas Chromatography-Mass Spectrometry (GC-MS)**

To determine the total contents of flavour compounds in feijoa fruit distillates GC-MS analysis was performed. GC-chromatograms of single fractions of flesh, skin and seeds were analysed separately and those of different profiles from a flavour point of view were chosen for further analysis using the GC-MS technique.

A SPME (solid-phase micro extraction) column was used to prepare the sample for injection into the GC-MS. Ethanol extract (20  $\mu$ l) was pipetted into a 20 mL SPME vial containing 8.98 mL of deionised water. Further 3 g of sodium chloride was added and the vial was capped. The volatiles were extracted from the headspace of the SPME vial at a temperature of 50°C for 40 minutes. Desorption time was 5 minutes at 50°C. The SPME fibre used was a DVB/CAR/PDMS 2 cm version Supelco (Sigma-Aldrich Pty. Ltd., Sydney, Australia).

The injection port was set to 250°C and the oven was initially set at 30°C and held for 5 minutes. The temperature was then increased 5°C/ 5 minutes up to a temperature of 240°C.

The flow rate used was 1 mL/min linear velocity (29.3 cm/s) with a splitless initial injection of 3 minutes and then a 20:1 split. The mass spectra interface was set to 250°C with the ion source set to 200°C. The scan range was 33 m/z – 450 m/z. The NIST standard reference database 05 (National Institute of Standards and Technology, Gaithersburg, MD, USA) was used to identify the output peaks.

## **3. Results and discussion**

### ***3.1 Extraction method of flavour components***

#### **3.1.1 Sample preparation and distillation**

To extract volatile flavour components of fresh feijoa fruit two trials were performed. The first trial failed due to an excess of fruit material in the sample which got caramelized and burnt during heating. The distillation could not be carried out. Even if the sample had not been burnt the caramelization would still have had a negative influence on the results due to a development of new flavour compounds from the caramelization process. The second trial was successful and the fruit material was removed by filtration; heating could be performed without the extracts becoming burnt. As a result fractions could be collected at different temperatures. Data from the collection of fractions can be found in Table I-III in Appendix 2. Analysis of chromatograms representing the extracts of flesh, seeds and skin (See Appendix 3, Fig. I-III) indicates a successful distillation; volatile flavour components of the feijoa fruit extracts have been extracted and peaks of the chromatograms have been successfully separated. Data analysis of chromatograms representing the last distilled fraction of each fruit extract of seeds, flesh and skin (see Appendix 4a-c) indicated a successful extraction of volatile flavour components; only a few peaks were recorded in these chromatograms and they consisted of a high proportion of solvents.

#### **3.1.2 Organoleptic evaluation**

The aim of the organoleptic evaluation was to distinguish flavour differences between fractions of distilled fruit extracts of skin, seeds and fruit peel. Fractions of similar flavour features were grouped to reduce the number of samples for further evaluation using gas chromatography. The organoleptic evaluation resulted in six new fractions per fruit extract being prepared (see Table 1). The last fraction of each extract had an odour characterized as a still-odour; a smell that can be compared to gunpowder. This is a common phenomenon using the distillation technique and is well known in the analysis of essential oils (Arctander, 1960). The problem could have been avoided by leaving the samples to age in a temperature of 12-

15°C for one to several months or by aerating the samples in open containers (Arctander, 1960). Due to time constraints and the risk of losing volatile flavour compounds of interest this was not carried out.

**Table 1.** Results of the organoleptic evaluation presenting grouped fractions and sample numbers

Fruit extract	Fraction no.	Sample no.
<b>Skin</b>	1-5	S1
	6-7	S2
	8	S3
	9-13	S4
	14-16	S5
	17-18	S6
<b>Seeds</b>	1-4	F1
	5-9	F2
	10-12	F3
	13-15	F4
	16	F5
	17-18	F6
<b>Flesh</b>	1-5	K1
	6-12	K2
	13	K3
	14-16	K4
	17-18	K5
	19	K6

## ***3.2 Identification of flavour components***

### **3.2.1 Gas Chromatography (GC)**

Gas chromatography (GC) was performed to determine whether the separation of volatile flavour compounds using the distillation technique had been successful and to detect volatile flavour compounds that contribute to the flavour of fresh feijoa fruit. GC-analysis was used to investigate whether flavour compounds of feijoa fruit extracts had been successfully separated and to detect which flavour compounds there were within the different extracts of skin, flesh and seeds.

Samples representing all fractions of distillates were analysed to obtain an overall view of the flavour profile for fruit flesh, peel and seeds separately resulting in three GC-chromatograms (see Appendix 3, Fig. I-III) which suggested a large number of flavour compounds in each extract. The retention times of the peaks in these chromatograms were compared with retention times of reference compounds (see Appendix 1) to identify flavour compounds. Experimental data and identified flavour compounds for fruit flesh, seeds and peel respectively are presented in Table 2-4.

**Table 2.** Data from GC- analysis of combined seed fractions and identified flavour compounds

Peak (no.)	Retention time (min)	Area (%)	Compound
1	2.797	0.00042	<i>Unidentified</i>
2	4.065	0.00054	<i>Unidentified</i>
3	4.171	0.00096	<i>Unidentified</i>
4	5.462	99.9726	Ethanol
5	22.940	0.00269	2-nonanone
6	30.191	0.01778	Methyl benzoate
7	31.345	0.00425	Ethyl benzoate
8	35.579	0.00083	Geraniol

**Table 3.** Data from GC- analysis of combined flesh fractions and identified flavour compounds

Peak (no.)	Retention time (min)	Area (%)	Compound
1	4.148	0.00112	<i>Unidentified</i>
2	5.507	99.97722	Ethanol
3	20.318	0.00088	Cis-3-hexenyl acetate
4	22.905	0.00024	2-nonanone
5	25.242	0.00174	<i>Unidentified</i>
6	25.681	0.00101	<i>Unidentified</i>
7	30.151	0.01340	Methyl benzoate
8	31.305	0.00197	Ethyl benzoate
9	39.918	0.00070	<i>Unidentified</i>
10	41.950	0.00182	<i>Unidentified</i>

**Table 4.** Data from GC- analysis of combined skin fractions and identified flavour compounds

Peak (no.)	Retention time (min)	Area (%)	Compound
1	3.240	0.0002	<i>Unidentified</i>
2	4.142	0.00088	<i>Unidentified</i>
3	5.293	99.83523	Ethanol
4	17.615	0.00157	<i>Unidentified</i>
5	22.894	0.00498	Cis-3-hexanol
6	25.245	0.00100	<i>Unidentified</i>
7	25.669	0.00130	<i>Unidentified</i>
8	27.819	0.00468	Linalool
9	30.147	0.01496	Methyl benzoate
10	31.300	0.00444	Ethyl benzoate
11	31.952	0.00393	<i>Unidentified</i>
12	35.537	0.00279	Geraniol
13	39.476	0.00125	<i>Unidentified</i>
14	39.826	0.00170	<i>Unidentified</i>
15	39.927	0.00876	<i>Unidentified</i>
16	40.387	0.00614	<i>Unidentified</i>
17	40.513	0.00153	<i>Unidentified</i>
18	40.642	0.00455	<i>Unidentified</i>
19	40.852	0.00755	<i>Unidentified</i>
20	41.373	0.00248	<i>Unidentified</i>
21	41.495	0.00316	<i>Unidentified</i>
22	41.679	0.00215	<i>Unidentified</i>
23	41.839	0.00300	<i>Unidentified</i>
24	41.960	0.02478	<i>Unidentified</i>
25	42.582	0.00667	<i>Unidentified</i>
26	42.777	0.00621	<i>Unidentified</i>
27	43.103	0.00770	<i>Unidentified</i>
28	43.328	0.00531	<i>Unidentified</i>
29	43.895	0.00541	<i>Unidentified</i>
30	44.026	0.01981	<i>Unidentified</i>
31	44.193	0.00238	<i>Unidentified</i>
32	44.520	0.00149	<i>Unidentified</i>
33	55.491	0.00227	<i>Unidentified</i>

To detect flavour compounds samples of fruit flesh, seeds and skin (see Appendix 1, Table 1) were analysed separately resulting in 18 GC-chromatograms; six chromatograms per fruit fraction (see Appendix 4a-c). Using the GC-technique 13 of 23 reference compounds were identified within the single fractions of flesh, skin and seed extracts (see Appendix 4a-c). There were several peaks of which there were no matches to the analysed reference

compounds (see Appendix 1 for analysed reference compounds). Further there were peaks within the chromatograms of the various samples that were not detected by the GC-equipment; this was probably due to very low concentrations of volatile flavour compounds within these samples that could not be identified using this method.

Among the samples of gathered fractions feijoa peel resulted in the highest number of detected compounds (33 peaks) followed by flesh (15 peaks) and seeds (10 peaks), see Appendix 4a-c. These results were expected since similar results have been presented by Shaw *et al.* (1989) who reported that the skin oil fraction of feijoa fruit was more complex than the flesh fraction.

#### **3.2.1.1 Seeds**

Analysis of the GC - chromatogram presenting the overall content of flavour compounds within the seed extract (see Table 2) resulted in five identified flavour compounds. Meanwhile analysis of chromatograms presenting the single fractions resulted in seven detected compounds in total (see Appendix 4c). This indicates that it is necessary to analyse samples of higher concentrations to assure detection and identification of as many flavour compounds as possible. Ethyl acetate was a unique finding in this extract (see Table I, Appendix 4c).

#### **3.2.1.2 Fruit Flesh**

Analysis of the GC – chromatogram presenting the overall content of flavour compounds within the fruit flesh extract (see Table 3) resulted in four identified flavour compounds whilst analysis of chromatograms of single fractions resulted in nine detected compounds (see Appendix 4a). Among the detected compounds were *cis*-3-hexenyl butyrate (see Table I-V, Appendix 4a) and 2-nonanone (see Table II, Appendix 4a) unique findings to the flesh extract.

#### **3.2.1.3 Skin**

Analysis of the GC – chromatogram presenting the overall content of flavour compounds within the skin extract (see Table 4) resulted in five detected flavour compounds in total whilst analysis of chromatograms presenting the single fractions (see Appendix 4b) resulted in

seven detected compounds. Linalool (see Table I-III, Appendix 4b) and ethyl propionate (see Table VI, Appendix 4b) were unique findings to this extract. The detected compounds within this extract (see Appendix 4b) have also been identified in a previous study performed by Shaw *et al.* (1989) in their analysis of the skin oil of feijoa fruit where they identified 36 flavour compounds using the GC and GC-MS technique.

The low frequency of detected compounds was not expected; results presenting the overall content of flavour compounds within the skin fractions (see Table 4) indicated a higher frequency of flavour components in comparison to the extracts of flesh (15 identified compounds) and seeds (10 identified compounds). This indicates a limitation using reference compounds and GC as a single method to identify every peak within the chromatograms. This method is very time consuming.

### **3.2.2 Gas Chromatography-Mass Spectrometry (GC-MS)**

GC-MS analysis was performed to identify flavour compounds of the feijoa fruit extracts of seeds, skin and flesh. Due to time constraints a limited number of samples were analysed using the GC-MS technique. Which fractions of the seed, flesh and skin distillates were to be subjected to further investigation was determined by analysis of GC – results (see Appendix 4a-c). Chosen fractions presented different flavour profiles by showing peaks at different retention times which is very interesting from a flavour point of view. The criteria was fulfilled by two fractions of the flesh distillates; K1 and K4 (see Appendix 4a, Table I & IV), one fraction of the skin distillate; S2 (see Appendix 4b, Table II) and one fraction of the seed distillates; F2 (see Appendix 4c, Table II). Results of the GC-MS analysis were analysed using Kovats Indices (Kovats, 1958). The identity of compounds that had a similarity match less than 90% was treated with caution. Data of unidentified compounds were excluded from the results.

#### **3.2.2.1 Seeds**

In sample F2 there were 76 compounds identified. The flavour compound dominant in this fraction was ethyl benzoate which had a peak area of 11.64%. Gas chromatography suggested that ethyl acetate was present in the seed extract. However, this could not be confirmed as

GC-MS analysis showed that ethyl acetate was not present in the sample. What seemed to be ethyl acetate in the GC analysis was probably mistaken for isoamyl alcohol as it has a similar molar mass which is approximately 88 g/mol. A previous study of the flavour profile of fruit flesh (including seeds) supports this theory since ethyl acetate was not a reported finding (Shaw, Allen, Yates & Franich, 1990).

Citronellyl acetate was detected within the seed extract of feijoa fruit, a unique finding since it was not detected in the extracts of flesh and skin and it has not been previously reported as a flavour constituent of feijoa fruit (Hardy & Michael, 1970; Shaw *et al.*, 1983; Shaw *et al.*, 1989; Shaw *et al.*, 1990). Its odour is described as floral, green, rose, fruity, citrus, woody and tropical fruit (The good scents company, a).

Cis-rose oxide and trans-rose oxide were detected within the seed fraction. It is only the cis-isomer that contributes to the odour (Alters *et al.*, 2010) which is described as red rose, green, floral and slightly spicy (The good scents company, b). Cis-rose oxide was only found within the seed fraction and this is the first time that this flavour compound has been detected in feijoa fruit.

Geraniol acetate and isoamyl acetate are other compounds that are unique findings to the seed extract of this study and are further reported for the first time as flavour compounds of feijoa fruit (Hardy & Michael, 1970; Shaw *et al.*, 1983; Shaw *et al.*, 1989; Shaw *et al.*, 1990).

The great amount of new findings of flavour compounds in feijoa is because it is the first time extraction and GC-MS analysis have been performed on feijoa seeds separately; in previous work extracts have been a mix of fruit flesh and seeds (Hardy & Michael, 1970; Shaw *et al.*, 1983; Shaw *et al.*, 1990). Further the improved concentration of volatile flavour compounds has enabled detection of flavour compounds which are present in very low concentrations.

### 3.2.2.2 Fruit flesh

In fraction K1 73 compounds were identified and in fraction K4 80 compounds were identified. The flavour compound dominant in fraction K1 was ethyl butanoate with a peak area of 18.04% followed by cis-3-hexenyl acetate (9.23%). In fraction K4 was methyl benzoate dominant with a peak area of 8.42% followed by 3-hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate (5.13%). Ethyl butanoate, ethyl benzoate and methyl benzoate are known to contribute to a green or fruity odour (Shaw *et al.*, 1990) and were all found within the fruit flesh extract. Methyl benzoate is also associated with the characteristic sweet note of fresh feijoa fruit (Shaw *et al.*, 1989) and has been found in previous work presented by Hardy & Michael (1970), Shaw *et al.* (1989) and Shaw *et al.* (1990). Ethyl octanoate was detected in one of the fractions of the flesh (see Appendix 4a, Table I) an unique finding in this study and this is reported for the first time as a flavour compound of feijoa fruit. Its odour is described as waxy, sweet, musty, pineapple and fruity with a creamy, dairy nuance and it has similar taste properties (The good scents company, c).

### 3.2.2.3 Skin

In the analysed fraction S2, 88 compounds were identified. Acetaldehyde was not found in this study in contrast to work presented by Shaw *et al.* (1989) who reported findings within the skin oil of Feijoa *Sellowiana*. Shaw *et al.* (1989) and Shiota, Minami & Tsuneya (1980) reported finding of octan-3-one which was not found within the peel extract of this study whilst 1-octen-3 one was detected. This could be explained by an oxidative reaction taking place during extraction or storage of the extract in this study resulting in a transformation from octan-3-one to 1-octen-3 one, thus creating an artefact. Differences within the extraction method used in this study and the study performed by Shaw *et al.* (1989) and Shiota *et al.* (1980) could be another reason to the different results.

The flavour compounds with quantitative dominance within the peel extract were cis-3-hexenyl benzoate (13.95%) and humulane-1.6-dien-3-ol (11.34%). Cis-3-hexenyl benzoate is also known to contribute a sweet, floral and spicy flavour (The good scents company, d). It has

been previously reported within the skin oil of feijoa fruit (Shaw *et al.*, 1989) and within its fruit flesh (Shaw *et al.*, 1990). Humulane-1,6-dien-3-ol is described to contribute a spicy flavour (El-Sayed, 2012) and is reported for the first time as a volatile flavour compound of feijoa fruit. C-5 – C-8 alcohols are known to contribute with a ‘greenish’ odour which was distinct in the peel extract of this study (Shaw *et al.*, 1989). In sample S2 was five C-5 – C-8 alcohols found: 2-heptanol, 1-hexanol, (Z)-hex-3-en-1-ol, n-octan-3-ol and 2-ethyl-1-hexanol.

### **3.2.3 GC versus GC – MS**

Results of the gas chromatography should only be used to get an indication of which flavour compounds that can be found within the fruit extracts of this study. This conclusion is based on the results where the fruit flesh extracts resulted in a higher frequency of detected compounds in comparison to the seed and peel extract which was found to be incorrect since results of the GC-MS analysis showed the peel extract to contain the highest amount of detected flavour compounds. Further D-limonene was detected within the feijoa extracts performing GC-MS (see Appendix 4a-c) but not by performing GC as a single method. D-limonene is reported for the first time as a flavour compound of feijoa fruit (Hardy & Michael, 1970; Shaw *et al.*, 1983; Shaw *et al.*, 1989; Shaw *et al.*, 1990).

## **4. Conclusions**

Analysis of results performing gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) indicates that the extraction method used has been successful; chromatograms obtained contained a great number of peaks which were shown to be interesting in a flavour point of view. Using GC-analysis 13 of 23 reference compounds was identified. Results from the GC-MS were shown to be most successful and this technique presented a great amount of data of which seven flavour compounds are reported for the first time.

Due to the magnitude of detected compounds using the GC-MS technique analysis of these results could not be completed since there were time constraints and limitations on the size of this project. There is a need for further analysis to complete the analysis of results to determine the extent of compounds that can be classified as flavour compounds and further determine the extent of unique findings within these results. Further investigation could also be performed to examine the flavour properties of these compounds and in which extent they contribute to the flavour of fresh feijoa fruit; more information is needed to produce a nature identical flavour mix for the food industry.

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(2012-03-10)

## Appendix

### *Appendix 1 - Identification of flavour compounds*

Reference compounds were analysed to be able to detect flavour compounds of the distilled fruit extracts. The table is presenting results of GC – analysis of chosen reference compounds with experimental data of retention times.

Standard (no.)	Retention time (min)	Compound
1	3.42	Methyl acetate
	7.6	Ethyl butyrate
	19.22	2-octanone
	25.356	Cis-3-hexenyl butyrate
	30.186	Methyl benzoate
	31.36	Ethyl benzoate
2	4.007	Ethyl acetate
	5.26	Ethanol
	9.447	Hexanal
	15.1	Limonene*
	17.063	Ocimene
	17.00	Ethyl hexanoate
	22.8	Cis-3-hexanol
	27.824	Linalool
	35.5	Geraniol
3	5.37	Ethyl propionate
	13.66	Myrcene
	20.44	Cis-3-hexenyl acetate
	22.95	2-nonanone
4	14.629	2-heptanone
	15.15	Limonene
	17.07	Ocimene
	21.7	Hexanol

\*Impurity of ocimene

## ***Appendix 2 – GC extraction***

**Table I.** Data from distillation of seed extract

Fraction (no.)	Time (min)	Temperature (°C)
1	11.33	78
2	11.39	79
3	12.40	80
4	13.28	80
5	14.21	81
6	14.28	82-83
7	14.33	84-85
8	14.36	86-87
9	14.43	88
10	14.48	89-91
11	14.54	92
12	15.01	93-94
13	15.04	96
14	15.10	96-98
15	15.15	99
16	15.22	100
17	16.45	100
18	17.15	*

\*The remaining extract

**Table II.** Data from distillation of flesh extract

Extract (no.)	Time (min)	Temperature (°C)
1	14.58	78
2	15.01	79
3	15.04	80
4	15.24	80
5	15.34	81
6	15.39	82
7	15.48	83.5
8	15.55	84
9	16.00	86
10	16.05	87
11	16.08	89-90
12	16.16	92-96
13	13.26	78
14	13.28	79-80
15	13.29	82-94
16	13.31	96
17	13.36	98
18	13.41	99
19	13.45-14.20	100
20	15.00	*
21	15.00	*

\*The remaining extract

**Table III.** Data from distillation of skin extract

Extract (no.)	Time (min)	Temperature (°C)
1	14.22	78
2	15.49	79-80
3	16.00	80
4	16.21	81
5	16.34	82
6	16.34	83
7	16.36	84
8	16.40	85-86
9	16.44	88
10	16.47	90
11	16.50	91-92
12	16.54	93-94
13	16.59	95-96
14	17.01	97
15	17.04	98
16	17.10	99-100
17	17.50	101
18	18.25-19.00	101
19	>19.00	*

\*The remaining extract

### Appendix 3 – GC-analysis results of combined fractions

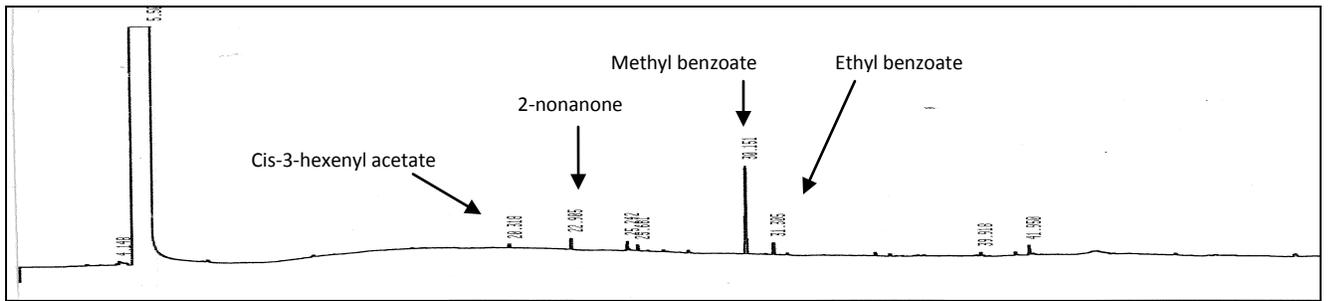


Fig. I. Chromatogram presenting results of GC-analysis of combined flesh distillates

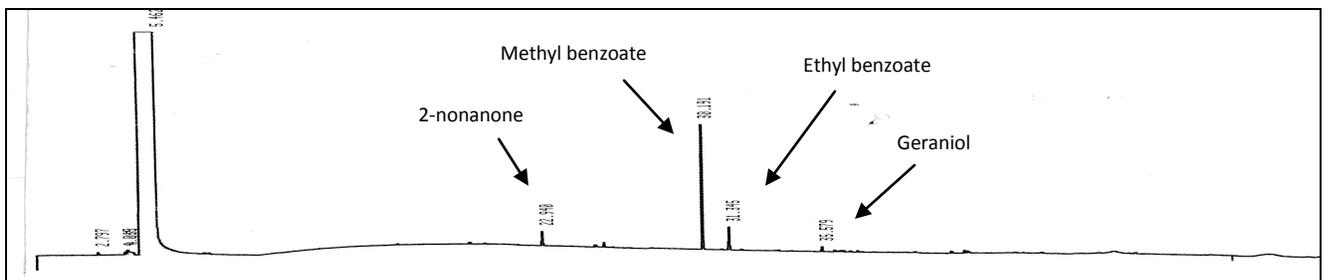


Fig. II. Chromatogram presenting results of GC-analysis of combined seed distillates

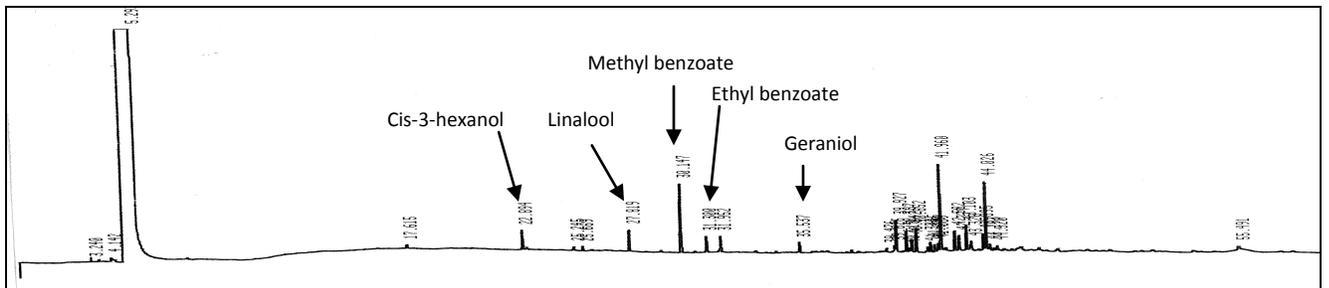


Fig. III. Chromatogram presenting results of GC-analysis of combined skin distillates

## Appendix 4

### Appendix 4a - Experimental data and identified compounds for fractions of the flesh extract

**Table I.** Results of GC- analysis for fraction K1 of the flesh extract.

Peak (no.)	Retention time (min)	Area (%)	Constituent
1	2.550	0.00677	<i>Unidentified</i>
2	2.745	0.00337	<i>Unidentified</i>
3	2.920	0.13147	<i>Unidentified</i>
4	3.313	0.00040	<i>Unidentified</i>
5	3.380	0.00110	Methyl acetate
6	3.615	0.00043	Methyl acetate
7	3.982	0.00499	<i>Unidentified</i>
8	5.585	88.67008	Ethanol
9	5.660	11.17728	Ethanol
10	7.612	0.00210	Ethyl butyrate
11	20.385	0.00053	Cis-3-Hexenyl acetate
12	24.444	0.00071	<i>Unidentified</i>
13	30.113	0.00085	Methyl benzoate

**Table II.** Results of GC- analysis for fraction K2 of the flesh extract.

Peak (no.)	Retention time (min)	Area (%)	Constituent
1	4.175	0.00041	<i>Unidentified</i>
2	5.595	93.97350	Ethanol
3	5.629	6.00508	Ethanol
4	20.489	0.00072	Cis-3-hexenyl acetate
5	22.937	0.00151	2-nonanone
6	30.230	0.01474	Methyl benzoate
7	31.385	0.00231	Ethyl benzoate

**Table III.** Results of GC- analysis for fraction K3 of the flesh extract.

Peak (no.)	Retention time (min)	Area (%)	Constituent
1	2.754	0.01938	<i>Unidentified</i>
2	3.328	0.00187	<i>Unidentified</i>
3	3.385	0.00175	Methyl acetate
4	3.634	0.00051	<i>Unidentified</i>
5	4.123	0.00188	<i>Unidentified</i>
6	5.233	39.62101	Ethanol
7	5.339	12.40088	Ethanol
8	5.500	21.94171	Ethanol
9	5.660	26.00954	Ethanol
10	12.027	0.00069	<i>Unidentified</i>
11	25.146	0.00081	Cis-3-hexenylbutyrate

**Table IV.** Results of GC- analysis for fraction K4 of the flesh extract.

Peak (no.)	Retention time (min)	Area (%)	Constituent
1	2.745	0.00340	<i>Unidentified</i>
2	4.095	0.00096	<i>Unidentified</i>
3	5.429	99.98262	Ethanol
4	12.057	0.00173	<i>Unidentified</i>
5	22.786	0.00120	Cis-3-hexenol
6	25.129	0.00312	Cis-3-hexenylbutyrate
7	25.592	0.00177	Cis-3-hexenylbutyrate
8	30.091	0.00334	Methyl benzoate
9	57.760	0.00192	<i>Unidentified</i>

**Table V.** Results of GC- analysis for fraction K5 of the flesh extract.

Peak (no.)	Retention time (min)	Area (%)	Constituent
1	2.754	0.00209	<i>Unidentified</i>
2	4.113	0.00481	<i>Unidentified</i>
3	4.911	99.98349	Ethanol
4	25.601	0.00394	Cis-3-hexenylbutyrate
5	35.478	0.00570	Geraniol

**Table VI.** Results of GC- analysis for fraction K6 of the flesh extract.

Peak (no.)	Retention time (min)	Area (%)	Constituent
1	4.739	100.00	Ethanol

## Appendix 4 b - Experimental data and identified compounds for fractions of the skin extract

**Table I.** Results of GC- analysis for fraction S1 of the skin extract.

Peak (no.)	Retention time (min)	Area (%)	Constituent
1	4.684	1.47951	Ethanol
2	5.012	98.48022	Ethanol
3	17.958	0.00707	<i>Unidentified</i>
4	27.915	0.00548	Linalool
5	30.265	0.01460	Methyl benzoate
6	30.992	0.00744	<i>Unidentified</i>
7	42.035	0.00571	<i>Unidentified</i>

**Table II.** Results of GC- analysis for fraction S2 of the skin extract.

Peak (no.)	Retention time (min)	Area (%)	Constituent
1	3.035	0.00068	<i>Unidentified</i>
2	3.411	0.00045	<i>Unidentified</i>
3	3.4162	0.00078	<i>Unidentified</i>
4	5.406	55.51758	Ethanol
5	5.509	14.06192	Ethanol
6	5.645	19.54613	Ethanol
7	5.715	10.70852	Ethanol
8	22.851	0.00537	Cis-3-hexanol
9	26.090	0.00048	<i>Unidentified</i>
10	27.832	0.00578	Linalool
11	30.166	0.02151	Methyl benzoate
12	31.325	0.00446	Ethyl benzoate
13	31.430	0.00097	<i>Unidentified</i>
14	31.957	0.00543	<i>Unidentified</i>
15	34.463	0.00077	<i>Unidentified</i>
16	35.540	0.00215	<i>Unidentified</i>
17	36.277	0.00061	<i>Unidentified</i>
18	36.787	0.00079	<i>Unidentified</i>
19	37.667	0.00083	<i>Unidentified</i>
20	37.895	0.00090	<i>Unidentified</i>
21	38.205	0.00080	<i>Unidentified</i>
22	39.220	0.00047	<i>Unidentified</i>
23	39.473	0.00171	<i>Unidentified</i>
24	39.831	0.00237	<i>Unidentified</i>
25	39.925	0.01230	<i>Unidentified</i>
26	40.268	0.00047	<i>Unidentified</i>
27	40.391	0.00772	<i>Unidentified</i>
28	40.515	0.00177	<i>Unidentified</i>
29	40.638	0.00591	<i>Unidentified</i>

30	40.855	0.00744	<i>Unidentified</i>
31	41.290	0.00115	<i>Unidentified</i>
32	41.497	0.00304	<i>Unidentified</i>
33	41.680	0.00220	<i>Unidentified</i>
34	41.845	0.00185	<i>Unidentified</i>
35	41.963	0.03592	<i>Unidentified</i>
36	42.166	0.00133	<i>Unidentified</i>
37	42.587	0.00167	<i>Unidentified</i>
38	42.781	0.00375	<i>Unidentified</i>
39	43.115	0.00612	<i>Unidentified</i>
40	43.326	0.00192	<i>Unidentified</i>
41	43.892	0.00212	<i>Unidentified</i>
42	44.027	0.00778	<i>Unidentified</i>
43	44.190	0.00207	<i>Unidentified</i>
44	44.368	0.00038	<i>Unidentified</i>
45	44.518	0.00102	<i>Unidentified</i>
46	44.828	0.0093	<i>Unidentified</i>

**Table III.** Results of GC- analysis for fraction S3 of the skin extract.

Peak (no.)	Retention time (min)	Area (%)	Constituent
1	3.388	0.00059	<i>Unidentified</i>
2	3.561	0.00040	<i>Unidentified</i>
3	4.736	4.79989	Ethanol
4	5.552	88.21379	Ethanol
5	5.587	6.87370	Ethanol
6	22.823	0.00359	Cis-3-hexanol
7	25.635	0.00049	<i>Unidentified</i>
8	27.810	0.00168	Linalool
9	30.141	0.01095	Methyl benzoate
10	31.935	0.00405	Ethyl benzoate
11	34.448	0.00054	<i>Unidentified</i>
12	39.456	0.00084	<i>Unidentified</i>
13	29.814	0.00186	<i>Unidentified</i>
14	39.906	0.00265	<i>Unidentified</i>
15	40.366	0.00545	<i>Unidentified</i>
16	40.494	0.00072	<i>Unidentified</i>
17	40.625	0.00320	<i>Unidentified</i>
18	40.831	0.00708	<i>Unidentified</i>
19	41.476	0.00259	<i>Unidentified</i>
20	41.660	0.00196	<i>Unidentified</i>
21	41.825	0.00207	<i>Unidentified</i>
22	41.944	0.01524	<i>Unidentified</i>
23	42.562	0.00361	<i>Unidentified</i>
24	42.760	0.00450	<i>Unidentified</i>
25	43.090	0.00710	<i>Unidentified</i>

26	43.310	0.00323	<i>Unidentified</i>
27	43.876	0.00418	<i>Unidentified</i>
28	44.006	0.01543	<i>Unidentified</i>
29	44.185	0.00215	<i>Unidentified</i>
30	44.361	0.00134	<i>Unidentified</i>
31	44.504	0.00188	<i>Unidentified</i>
32	44.795	0.00145	<i>Unidentified</i>
33	45.551	0.00076	<i>Unidentified</i>
34	45.615	0.00133	<i>Unidentified</i>

**Table IV.** Results of GC- analysis for fraction S4 of the skin extract.

Peak (no.)	Retention time (min)	Area (%)	Constituent
1	4.589	0.86120	Ethanol
2	5.242	58.26046	Ethanol
3	5.370	21.23956	Ethanol
4	5.471	19.56802	Ethanol
5	22.830	0.00284	Cis-3-hexanol
6	30.116	0.00498	Methyl benzoate
7	31.276	0.00030	Ethyl benzoate
8	31.920	0.00223	<i>Unidentified</i>
9	35.505	0.00132	Geraniol
10	40.361	0.00124	<i>Unidentified</i>
11	40.606	0.00120	<i>Unidentified</i>
12	40.826	0.00315	<i>Unidentified</i>
13	41.335	0.00155	<i>Unidentified</i>
14	41.465	0.00145	<i>Unidentified</i>
15	41.650	0.00098	<i>Unidentified</i>
16	41.810	0.00196	<i>Unidentified</i>
17	41.928	0.00328	<i>Unidentified</i>
18	42.554	0.00513	<i>Unidentified</i>
19	42.730	0.00354	<i>Unidentified</i>
20	43.077	0.00350	<i>Unidentified</i>
21	43.240	0.00139	<i>Unidentified</i>
22	43.295	0.00215	<i>Unidentified</i>
23	43.863	0.00404	<i>Unidentified</i>
24	43.997	0.01463	<i>Unidentified</i>
25	44.173	0.00151	<i>Unidentified</i>
26	44.369	0.00120	<i>Unidentified</i>
27	44.485	0.00149	<i>Unidentified</i>
28	46.381	0.00097	<i>Unidentified</i>
29	47.244	0.00194	<i>Unidentified</i>
30	53.365	0.00101	<i>Unidentified</i>
31	55.416	0.00206	<i>Unidentified</i>

**Table V.** Results of GC- analysis for fraction S5 of the skin extract.

Peak (no.)	Retention time (min)	Area (%)	Constituent
1	3.515	0.00105	Methyl acetate
2	4.115	0.00554	Ethanol
3	5.065	99.97402	Ethanol
4	25.620	0.00368	<i>Unidentified</i>
5	35.490	0.00210	Geraniol
6	41.325	0.00436	<i>Unidentified</i>
7	41.799	0.00286	<i>Unidentified</i>
8	42.538	0.00436	<i>Unidentified</i>
9	42.705	0.00207	<i>Unidentified</i>

**Table VI.** Results of GC- analysis for fraction S6 of the skin extract.

Peak (no.)	Retention time (min)	Area (%)	Constituent
1	4.771	98.06656	Ethanol
2	5.352	0.06322	Ethyl propionate
3	25.346	0.69802	<i>Unidentified</i>
4	25.753	0.53996	<i>Unidentified</i>
5	48.662	0.63223	<i>Unidentified</i>

## Appendix 4 c - Experimental data and identified compounds for fractions of the seeds extract

**Table I.** Results of GC- analysis for fraction F1 of the seeds extract.

Peak (no.)	Retention time (min)	Area (%)	Constituent
1	2.568	0.00971	Unidentified
2	2.768	0.00703	<i>Unidentified</i>
3	3.404	0.00070	Methyl acetate
4	4.013	0.00908	Ethyl acetate
5	5.430	65.60173	Ethanol
6	5.538	14.85498	Ethanol
7	6.661	19.51156	Ethanol
8	7.644	0.00179	Ethyl butyrate
9	20.437	0.00073	Cis-3-hexenyl acetate
10	30.162	0.00230	Methyl benzoate
11	31.325	0.00045	Ethyl benzoate

**Table II.** Results of GC- analysis for fraction F2 of the seeds extract.

Peak (no.)	Retention time (min)	Area (%)	Constituents
1	4.123	0.00061	<i>Unidentified</i>
2	5.452	74.87382	Ethanol
3	5.532	12.80242	Ethanol
4	5.603	12.26990	Ethanol
5	22.809	0.00284	Cis-3-hexanol
6	26.052	0.00056	<i>Unidentified</i>
7	30.127	0.03742	Methyl benzoate
8	31.285	0.00730	Ethyl benzoate
9	31.878	0.00088	<i>Unidentified</i>
10	33.368	0.00035	<i>Unidentified</i>
11	33.528	0.00067	<i>Unidentified</i>
12	36.225	0.00067	<i>Unidentified</i>
13	36.365	0.00061	<i>Unidentified</i>
14	37.112	0.00065	<i>Unidentified</i>
15	41.925	0.00083	<i>Unidentified</i>
16	42.069	0.00060	<i>Unidentified</i>

**Table III.** Results of GC- analysis for fraction F3 of the seeds extract.

Peak (no.)	Retention time (min)	Area (%)	Constituents
1	4.120	0.00224	<i>Unidentified</i>
2	5.159	69.32291	Ethanol
3	5.240	16.99464	Ethanol
4	5.295	13.67020	Ethanol
5	25.610	0.00121	<i>Unidentified</i>
6	30.102	0.00763	Methyl benzoate
7	35.486	0.00119	Geraniol

**Table VI.** Results of GC- analysis for fraction F4 of the seeds extract.

Peak (no.)	Retention time (min)	Area (%)	Constituents
1	3.512	0.00094	Methyl acetate
2	4.102	0.00246	<i>Unidentified</i>
3	5.143	99.99110	Ethanol
4	30.075	0.00253	Methyl benzoate
5	35.475	0.00298	Geraniol

**Table V.** Results of GC- analysis for fraction F5 of the seeds extract.

Peak (no.)	Retention time (min)	Area (%)	Constituents
1	1.121	0.00803	<i>Unidentified</i>
2	4.119	0.02108	<i>Unidentified</i>
3	4.783	99.86477	Ethanol
4	25.288	0.04687	<i>Unidentified</i>
5	25.679	0.02425	<i>Unidentified</i>
6	35.548	0.03498	Geraniol

**Table VI.** Results of GC- analysis for fraction F6 of the seeds extract.

Peak (no.)	Retention time (min)	Area (%)	Constituents
1	4.658	98.17619	Ethanol
2	25.190	1.82383	<i>Unidentified</i>