Analysis of Volatiles from the New Zealand Scented Plant-Mairehau (*Leionema nudum*)

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ATTESTATION OF AUTHORSHIP

I hereby declare that this submission is my own work. Materials (including descriptions, conclusions, data, viewpoints or figures) from other people, publications and websites, appearing in this submission, were well cited, and their origins were listed in REFERENCE at the end of this submission.

Signature: 

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ABSTRACT

New Zealand native scented plants may possess potential industry value as perfume ingredients. To provide the overall impression of New Zealand scented plant resource and to guide the future researches in this area, this submission begins with a literature review on chemical and sensory studies of several commonly found New Zealand scented plants.

A New Zealand scented plant, mairehau (*Leionema nudum*) was chosen for compositional analysis of volatiles. First, during the 15 days natural dehydration after cutting from plants, mairehau twigs decreased in weight, but increased in the number of volatile compounds and their concentration. A negative linear regression with R^2=75.2% was observed between twigs weight and the number of volatile compounds. It was found that the metabolic change significantly altered the volatile characteristics from day 5 to day 9 during natural dehydration. The total 71 volatile compounds were grouped into 5 clusters in multivariate analysis according to their similarity in behaviour during 15 days of natural dehydration. For comparison between volatiles from different mairehau parts, a total of 33 (3 unidentified) and 37 (4 unidentified) compounds were found in samples of fresh mairehau twigs and mairehau flowers, respectively, and there were 15 compounds found in both. For the volatiles of homogenized mairehau samples, there were 99 and 92 compounds found in fresh samples and dry samples, with 73 and 55 compounds identified, respectively. Adding liquid Nitrogen during homogenization of fresh mairehau would give the volatiles a different dynamic status from those prepared under room condition; however, the number of volatile compounds did not significantly vary.

The volatiles were sampled by SPME. In this study, the 100 µm PDMS fibre showed the best reproducibility and was chosen for the experiment. The regression models of adsorption amount on fibre predicted by adsorption time (at 25°C and 35°C) agreed with the theoretic ones. In addition, increasing the adsorption temperature from 25°C to 35°C would promote the adsorption amount on the fibre.

GC-MS and NIST MS Database similarity searching were used for the qualitative and quantitative analysis of mairehau volatile compounds. To overcome the peak overlapping problem in GC-MS and improve the accuracy of the results, a chemometric algorithm, MCR-ALS coupled with EFA was applied. The algorithm showed a better efficiency in volatile samples with low complexity. The whole algorithm was successfully coded and ran in R software, suggesting this open-source software was capable for carrying out chemometric process, as well as MATLAB (a mathematic tool that most of publications used for carrying out chemometrics). R scripts were supplied in appendixes of this submission, providing a good reference to those who want to apply this technique with R software, in processing their GC-MS data.

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ABBREVIATION

GC-FID	*Gas Chromatography- Flame Ionization Detetor*
GC-MS	*Gas Chromatography–Mass Spectrometry*
SPME	*Solid Phase Micro Extraction*
TIC	*Total Ion Chromatogram*
EFA	*Evolving Factor Analysis*
MCR-ALS	*Multivariate Curve Resolution-Alternating Least Squares*
SVD	*Singular Value Decomposition*
OPA	*Orthogonal Projection Approach*
OVI	*Overall Volume Integration*
RI	*The Kovats Retention Index*
RT	*Retention Time*
UNOP	*the UNresolved Overlapped Peaks by chemometrics*
CV	*Coefficient of Variation*
NoC	*the Number of total detected volatile Components*
TPA	*the Total Peak Area*
PCA	*Principle Component Analysis*
PC	*Principle Component*
MTD1	*the Mairehau Twigs of Day 1*

Chapter 1 INTRODUCTION AND OBJECTIVES

1.1 Introduction

1.1.1 Background information about perfume

The word perfume is derived from the Latin phrase “per fume” meaning “through or by smoke”. The use of perfume has thousands years of history. The early use of perfume was in the form of burning aromatic herbs, which were usually incorporated with religion services (Bellis, n.d). Another method of using perfume is applying to the skin with aromatic gums, balms or ointments from trees. These can include products that are of mixed naturally sourced fragrances with bases such as honey and olive oil (Bellis, n.d; Keville & Green, 1995). They are found in the history of many cultures, including the ancient Maori society in New Zealand (Riley, 1994).

In the modern world, perfume is often thought of a fragrant liquid that humans put on skin or hair to give their body a pleasant odor. The modern perfume is associated with the advance in organic chemistry. It is normally composed of about 78% to 95% solvent, usually ethyl alcohol, and odorant oil or perfume ingredients, the amount of which varies, depending on the perfume types: 22% in Perfume Extract, 15% to 22% in Eau de Parfum (EDP), 8% to 15% in Eau de Toilette (EDT), about 4% in Eau de Cologne (EDC) and 1% to 4% in Eau Fraiche (EF) (P. Weston, n.d).

Materials of natural origin serve as the important inspiration for perfume ingredients in perfume industry. Perfume ingredients can be made directly from natural materials. These include a few from animal sources, such as the glandular secretion from male musk, and ambergris produced by sperm whales, however, most of the ingredients are sourced from botanical species (Sell, 2006).

There is always a search for new perfume ingredients which could be associated with novel odor. Perfume fragrances more or less model those from nature (Sell, 2006), as indicated by the major perfume fragrance types: citrus, floral, fruity, green, woody, oceanic, oriental and spicy. The impressions of the first five are suggested by their names deriving from botany. Oceanic uses synthetic ingredients to feature natural

scents like mountain air, ocean spray and clean linen. Oriental uses materials such as musk, ambergris and amber not from botanical materials. Spice includes some other pleasant scents (Helbig, n.d). When botanical materials are used for perfume manufacture, there is a strong commercial pressure to identify the key components that are present. Its volatile composition information is important to tell what the compounds are. This provides hints for artificial synthesis of these compounds to reconstitute that characteristic odor for low cost (Clery, 2006).

1.1.2 Scented plants from New Zealand

Because of geographical isolation, New Zealand possesses a large number of native vascular plants. Of these about 82% are endemic to the country (Dawson, 1988). New Zealand native resident Maori have accumulated thorough knowledge of local plants, especially those with nice scent. In ancient times, scented plants in New Zealand played important roles in Maori social customs, such as courtship, marriage and funeral. Maori also used their traditional ways to extract essential oils and gums for body perfumery purposes (Riley, 1994).

The value of these scented plants for perfume was earlier noticed by (Stanley G Brooker, Cambie, & Cooper, 1989). Also there has been interest in essential oils and extracts from selected New Zealand plants, including both native and introduced species (Perry, Douglas, & Porter, 1993). Since the scented plants traditionally used by Maori include species that are endemic to New Zealand, they may be the source of new odor that are specific to New Zealand, which will further facilitate the development of unique New Zealand products. So it is desirable to explore the volatile composition of these scented plants. In the present work, a New Zealand scented plant, mairehau (*Leionema nudum*), is chosen for the volatile compositional analysis.

1.1.3 Mairehau

Genus *Leionema* includes nearly 24 species, with 23 distributed in eastern Australia. The remained one species, mairehau, is only found in the North Island of New Zealand (Duretto, 2009; Wilson, 1998)(Figure 1-1,Figure 1-2).

Figure 1-1 The leaves of mairehau.Mairehau used in this study was purchased from ***Oratia Native Plant Nursery, NZ****. The website is www.oratianatives.co.nz*

Figure 1-2 The flowers of mairehau.

As described in (Riley, 1994), the whole mairehau plant is highly aromatic, with white flowers yielding a perfume, and glands on leaves and branchlets producing greenish-yellow oil. Mairehau extract was necessary ingredient of scented oils for Maori. Reports of mairehau essential oil include a publication dating back to 1928 from which the major compounds of the oil were understood as citronellal, citral, limonene and camphene (Ghishlberti, 1998), and a publication reporting the "sweet scent" of mairehau was attributed to terpinyl acetate, limonene, camphene, citronellal, citral, sesquiterpenes, and a sesquiterpene alcohol contained in its essential oil (Stanley G Brooker et al., 1989). It was reported that the furoquinoline alkaloid was extracted from mairehau (Bevalot, Armstrong, Gray, & Waterman, 1988). A systematic investigation on mairehau bark has isolated 21 compounds with 17 being identified (L. H. Briggs & Cambie, 1958).

Previous chemical studies on mairehau focused on the composition of mairehau essential oil and extract from mairehau bark. Limited by the technology of the time, the contained constituents may not have been thoroughly isolated and identified. In addition, there was little information about the volatile of the fresh mairehau. So it is timely to revisit the plant and investigate the compounds its volatile contains using new technology.

1.2 Objectives

1.2.1 Literature review of New Zealand scented plants

New Zealand scented plants used by Maori may have the commercial perfumery value for modern products. To give some idea of the range of scented plants available, Chapter 2 provides a literature review of common ones used by Maori. It also discusses some related plant species. These will guide the future study on other species of scented plants.

1.2.2 Analysis of mairehau volatile

In order to gain the broadest view of volatile compounds in mairehau scent, first, the effect of natural dehydration on mairehau volatiles was studied. This was followed by a comparison of volatiles in mairehau twigs and mairehau flowers. In addition, the

volatiles from homogenized mairehau prepared in three different ways (dry mairehau, fresh mairehau under room condition and fresh mairehau with liquid Nitrogen cooling) were studied.

1.2.3 Methodology

Headspace Solid Phase Micro Extraction (SPME) coupled with Gas Chromatography -Mass Spectrometry (GC-MS) was used for the volatiles sampling and identification. To improve the accuracy of the result, chemometric algorithm was developed and applied to the GC-MS data. This was the first time that chemometric methods was applied to studies on New Zealand scented plants. In addition, the chemometric method in this study was coded in R software, providing an alternative tool for chemometrics. This would encourage other researchers to use this method, since most of chemometric publications were based on MATLAB software which is no universally available.

Chapter 2 LITERATURE REVIEW AND BACKGROUND

This chapter contains a literature review on chemical and sensory studies of New Zealand scented plants from 12 genera. The emphasis is also on studies of closely-related species from the same genus.

2.1 A review on New Zealand scented plant

2.1.1 Genus Leionema

Genus *Leionema* was firstly suggested by (Wilson, 1998). Before that, all species under *Leionema* were classified in genus *Phebalium*. This may cause confusion in reading some old literature, for example, phytochemistry of some *Leionema* species can be indirectly obtained from a phytochemistry review on *Phebalium* species (Ghishlberti, 1998).

Similar as mairehau, other *Leionema* species from Australia also commonly have aromatic characteristics. *L scopulinum* was identified in 2003 from New South Wales and was described as having strong aroma (Horton, Crayn, Clarke, & Washington, 2004). However a chemical analysis on its scent could not be found in the literature. Another example is *L ambiens*, with typical aromatic foliage of *Leionema* species. By GC and GC-MS, 8 major compounds from its essential oil were identified as E)-β-ocimene, (Z)-β-ocimene, viridiflorene, bicyclogermacrene, globulol, viridiflorol, δ-cadinene and (E,E)-farnesol. The distinctive aroma of *L ambiens* was not due to these 8 compounds, but to a series of sequiterpenes and aliphatic esters presented in trace amounts. Hexanoic acid together with alcohols from C4-C6 were thought to be responsible for the "fruit salad" scent of *L ambiens* leaf oil. In addition, the oil did not contain α-pinene, which were usually found in *L elatius*, *L coxii* and *L dentatum* (Brophy & Golf, 2006; Ghishlberti, 1998).

2.1.2 Genus Pittosporum

Under genus *Pittosporum*, there are three species native to New Zealand valued as scented plants by Maori*: P eugenioides*, *P tenuifolium* and *P crassifolium*, as called by Maori, tarata, kohuhu and karo.

Tarata is also called lemon wood, because a fragrance of lemon is given off when crushing its yellow, sweet-scented flowers or leaves. In Maori traditional culture, the aromatic tarata was thought to be aphrodisiac, which can explain why tarata is included in many Maori perfume recipes (Riley, 1994).

Kohuhu is nowadays known as a common garden plant in New Zealand. It is an evergreen plant with attractive appearance of green, wave margined leaves and very black colored stem. The small and brownish-purple flower, although not showy, has a delicious honey scent (Miller, 2006). Maori in ancient time may have obtained the aromatic scent from kohuhu gum, leaves or greenish-yellow resin (Riley, 1994).

Karo is thought to be one of New Zealand's most aromatic plants with pleasantly pungent flowers which are good source of honey (Castro & Robertson, 1997; Riley, 1994).

Chemical studies on these three plants are rare. However, there are a number of chemical studies on other *Pittosporum* species. *P tobira* is a flowering plant native to Japan, and now grows throughout the world. A total of 49 compounds were identified in the oils that were distillated from leaves, flowers and fruit of *P tobira* growing in Lisbon, Portugal. In all three, n-nonane was the most abundant component, and myrcene was the second. There was no significant compositional difference in three oils (Rodrigues et al., 2007). The flower and fruit oils of *P tobira* growing in Iran were identified with 16 and 17 compounds, and α-pinene was the compound with the highest amount, and n-nonane was less than half amount of that. (Nickavar, Amin, & Yosefi, 2004). *P tobira* leaves oil from South Korea was confirmed with larvicidal effect against *Aedes aegypti*. The major constituents were undecane, 4-methyl-1,3-pentadiene, benzene and L-limenene (Chung, Seo, Kang,

Park, & Moon, 2010). Based on these, the constituents of *P tobira* oil may be affected by geographic location.

Extracts from the leaves of some *Pittosporum* species have been examined for their antimicrobial effects (Seo et al., 2002). *P undulatum* was traditionally used as a medicinal herb in Australia. Three triterpenoid spogenins were obtained from *P undulatum* leaves by enzymatic hydrolysis, and mono- and tri-terpenes were identified from *P undulatum* fruits (Higuchi, Komori, Kawasaki, & Lassak, 1983). The leaves oil of *P undulatum* in Brazil was rich in mono- and sesquiterpenes. The level of the main constituent, (+)-limonene, was affected by the collection time of the year (Lago, Favero, & Romoff, 2006). *P phillyraeoides* was reported with tri-terpenoid sapogenins in its leaves (Errington & Jefferies, 1988). *P viridiflorum* was reported with volatile mono- and sesquiterpenes responding for antimicrobial effects and a number of saponins, including a novel type with an unusual 2,3,4-trisubstituted glycosidic linkage, which showed weak cytotoxic activity on the A2780 human ovarian cancer cell line (Seo et al., 2002). *P napaulensis* is a rare medicinal tree endemic to Himalayas. Its bitter taste-pleasant aroma bark was used by the indigenous people for treating inflammation and rheumatic swellings. Research on miropropagation was carried out for its geographic limitation and rareness (Dhar, Upreti, & Bhatt, 2000). *P neelgherrense* from Western Ghats of India was used as herbs against snake bite and hepatic disorders by locals. A recent study examined the methanolic extracts from stem bark showing the hepatoprotective effects against liver damage (Shyamal et al., 2006).

2.1.3 Genus Aciphylla

There is a term called “The Grand Maori Perfume” which is used when Maori perfumes are discussed (Riley, 1994; Rob, 2007; Sly, n.d). “The Grand Maori Perfume” recipe consists of many New Zealand scented plants, and taramea (*Aciphylla squarrosa*) serves as the basis of that perfume (Riley, 1994). Although some authorities place taramea to *A colensoi* (Ellis, 1927), I will assume taramea is *A squarrosa*, as most authors have done.

Taramea grows in the North Island and the North tip of South Island of New Zealand from coast to 4,000ft altitude (Olive, 1956). The base of the spear-grass-like leaves were the materials for Maori making a semi transparent gum, the scent of which was described as “a mixture of honey and toffee” (Marvin, 2010; Riley, 1994). Maori leaders and young women wore the gum around their necks to get the pleasant odor, as a Maori song described (Ellis, 1927):

> "My little neck-satchel of sweet-scented moss.
>
> My little neck-satchel of fragrant fern.
>
> My little neck-satchel of odoriferous gum.
>
> My sweet-smelling neck-locket of sharp-pointed *taramea*."

Constituents of taramea essential oil from its seeds had been examined in a study that originally aimed to compare the composition of essential oils from 10 New Zealand plants (R. J. Weston, 2010). The most abundant constituent was dillapiole, a naturally occurred compound also found in many species from different families, such as *Apiaceae, Lauraceae, Lamiaceae* and *Piperaceae*. When used with certain insecticides, it can serve as a synergist to enhance the killing ability (Belzile et al., 2000). Myristicin was also present at a significant level. It was reported to have psychotropic effect and anti-cancer property (Alexander T Shulgin, 1966; Zheng, Kenney, & Lam, 1992). Scent evaluation has not yet been carried out on these two compounds. It should be pointed out that this essential oil of taramea was from the seeds, which may not totally reflect the constituents of taramea gum used by Maori, that is, there is uncertainty whether the scent of seed oil is identical to the gum from taramea leaves.

Commercial development on perfume based on taramea, although at its initial stage, has been reported in New Zealand (Marvin, 2010). Investigator has made effort to popularize the taramea cultivation as a farm crop for the cosmetic industry (Taramea Speargrass Research and Development group, L09/072/2010).

A total of 42 species had been confirmed under the genus *Aciphylla* up to 1986. These species are endemic to New Zealand except for two in Australia. (Mitchell,

Webb, & Wagstaff, 1998). Thus it would be interesting to investigate other *Aciphylla* species in New Zealand to answer whether there are another scented species like taramea.

2.1.4 Genus Hierochloe

Sweet grass refers to grass plants from the genus *Hierochloe*. Usually they have a very pleasant scent. In New Zealand, the one called karetu (*H redolens*) is known to be used in the past by Maori both for social customs and body cosmetics (Riley, 1994). Little research has been carried out on karetu, except a few on botanical viral studies (Davis & Guy, 2001; Delmiglio, Pearson, Lister, & Guy, 2010).

Other *Hierochloe* species in New Zealand include *H novae-zelandiae, H recurvata, H equiseta, H cuprea, H fusca, H brunonis* and *H arenaria* (Zotov, 1973). Their scent characteristics have not yet been described, neither have their chemical studies, which indicate that investigations on this area maybe fruitful.

Although *Hierochloe* species are well known for their sweet smell, actual chemical analysis and essential oils studies lag behind. *H odorata* is nearly the only one for which the scent, volatiles, medicinal and essential oil studies have been well carried out. The plant is distributed in North America ranging from Alaska to New Mexico (Winslow, 2000). It can also grow in northern Europe (Pukalskas et al., 2002). Warm temperature and rich, moist soil suits its growth (Winslow, 2000). American tribes used aromatic *H odorata* to purify air (mostly by burning), or as a perfume in special occasions (Winslow, 2000).

The volatile constituents of ethanol extracts of the root and the aerial parts of *H odorata* were examined with a total of 169 volatile compounds. The volatile oil was rich in coumarin, which accounted for 10.3 % of the root oil and 24.9% of the aerial oil. Quantitative analysis by HPLC showed that ethanol extract of the roots contained coumarin at 3.57% (m/m), and an extract for the aerial parts is 3.72% (m/m) (Ueyama, Arai, & Hashimoto, 1991). The sweet scent of *H odorata* was mainly from coumarin, which was similar to vanilla. Raining on or burning *H odorata* can enhance this sweet scent (Casey & Wynia, 2010).

H odorata was also known with herbal usage. It can be made into a tea or soup for colds, coughs, fevers or congested nasal passages (Casey & Wynia, 2010). The acetone extract of *H odorata* was shown with antioxidant activity (Bandoniene, Pukalskas, Venskutonis, & Gruzdiene, 2000). The extract can retard lipid oxidation, the compounds responsible for this were thought to be 5,8-dihydroxybenzopyranone, and 5-hydroxy-8-O-β-D-glucopyranosyl-benzopyranone (Pukalskas et al., 2002).

2.1.5 Genus Raukaua

Leaves of raukawa (*Raukaua edgerleyi*) possess an aromatic scent, and Maori used this material to produce scented oil. Such oil can be used in different ways: serving as an ingredient for "taramea scent", or being a preferred perfume on its own account. When mixed with other herbs, fat or gums, a "raukawa scent" was created (Riley, 1994). Raukawa is distributed in New Zealand with a wide range from Northern Island to the Stewart Island. Raukawa leaves can be aromatic when crushed (New Zealand Plant Conservation Network, 2011), it was described as "entire, glossy, distinctly veined, strongly aromatic adult leaves"(Heenan, 1998).

The genus *Raukaua* obtained its name from the species raukawa. It consists of three species, all endemic to New Zealand, *R anomalus, R edgerleyi* and *R simplex* (R. J. Weston, 2004b). Because hybridization occurred between these three species, some discussions exist in the taxonomy of the generated varieties (Heenan, 1998). Essential oils extracted by hydro distillation from these three species had been examined. The essential oils of raukawa and *R simplex* were abundant with monoterpenes, whereas sesquiterpenoids germacrene-B and γ -muurolene dominated the *R. Anomalus* oil. The high level of limonene resulted in the aroma of the raukawa oil. However, direct using of these oils in perfume industry were limited by their low yield (R. J. Weston, 2004b).

The family Araliaceae consists of 65 genera and six of them occurred in New Zealand: *Meryta, Stilbocarpa, Schefflera, Kirkophytum, Pseudopanax* and *Raukaua*. These six genera are all found with small number of species, mostly less than ten, in New Zealand. Oils of the three *P seudopanax* species, *P arboreus, P discolor* and *P lessonii* contained significant proportions of viridiflorol and a closely related

unidentified hydroazulene alcohol. In addition, the oil of *P arboreus* contained bicyclogermacrene, linalool and long chain hydrocarbons. *P discolor* oil contained nerolidol in abundance together with linalool and epi-α-muurolol. *P lessonii* oil contained a complex mixture of sesquiterpene alcohols including epi-α-muurolol and a mixture of long chain hydrocarbons. Nerolidol and linalool provided the *P discolor* oil with a pleasant floral aroma, but the yield of oil was very low (R. J. Weston, 2004a).

2.1.6 Genus Prumnopitys

Miro (*Prumnopitys ferruginea*) is an evergreen tree endemic to New Zealand. It can grow to 25m in height and 100cm in abh (diameter at breast height), with a round crown. Although its distribution is throughout the three main islands of New Zealand from lowland forests to 1000m altitude (Earle, 2012b), it is more frequently found in lowland forest (Lorimer, Mawson, Perry, & Weavers, 1995). The miro berry is about 12-15mm in diameter with a large seed surrounded by red color flesh outer layer (Clout & Tilley, 1992). The berry has a strong smell of turpentine (Taranaki Educational Resource: Research, n.d). New Zealand pigeon (*Hemiphaga novaeseelandiae*) likes to eat miro berry, however, they are unable to digest the seed and the defecated seed will germinate when environmental conditions are suitable, which may affect miro distribution (Clout & Tilley, 1992). Maori mainly used miro berry to snare bird and extracted scented oil (Riley, 1994).

From 25 specimens of the foliage oil of miro, there have been identified with ten sesquiterpene hydrocarbons (delta-elemene, alpha-cubebene, alpha-copaene, beta-cubebene, germacrene-D, beta-selinene, bicyclogermacrene, germacrene-A, delta-cadinene, gamma-cadinene), acorenone, seven diterpene hydrocarbons (rimuene, beyerene, pimaradiene, sandaracopimaradiene, phyllocladene, kaurene and abietatriene) and a diterpene alcohol (8 beta-hydroxyisopimarene). The terpene levels varied between specimens (Clarke, Perry, & Weavers, 1994).

In addition to the usage in perfume, miro supplied certain medicinal benefits. Foliage extracts can act against a wide range of parasites, such as fungus *Trichophyton mentagrophytes*, yeast *Candida albicans*, gram-positive bacterium *Bacillus subtilis*,

and gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. It showed cytotoxicity towards mammal cells, such as monkey kidney cells (Clarke et al., 1994; Lorimer et al., 1995). The antimicrobial active compound miroside (3-[(β-D-glucopyranosyloxy)-methyl]-2(5H)-furanone)), originally isolated from miro, has been artificially synthesized (Lorimer et al., 1995). A gin-like spirit flavored with miro fruit was reported in AUT university, and researchers there were in progress to develop a geographically-distinct alcoholic drink (Chaipongrattana, 2008).

Genus *Prumnopitys* is widely distributed around the pacific area including Australia, New Caledonia, New Zealand and South America, and it comprises nine species (Earle, 2012a). The only *Prumnopitys* species in Australia is *P ladei*. *P ladei* is a large tree up to 30m in height. It grows within a restricted area of northeastern Queensland. The fresh *P ladei* leaves oil yield was 0.07% w/w by hydro distillation. Mono-, di- and sesquiterpenes were presented at a significant level. The primary compounds were α-pinene, β-caryophyllene, rimuene and kaure (Brophy, Goldsack, & Forster, 2006).

2.1.7 Genus Macropiper

The Piperaceae family consists of nearly 1150 species widely distributed around the world. It is also known as the pepper family as most species are used in medicine, rituals and cooking (Rito, 2005). There are two genus including five species found in New Zealand: *Macropiper melchior, Macropiper hooglandii, Macropiper excelsum Peperomia tetraphylla* and *Peperomia urvilleana* (*Flowering seed plants: Off-shore Islands*, n.d; Gardner, 1997). We will discuss *M excelsum* further since it has been extensively used on many occasions by Maori.

There appears to be very little information on the use of *M excelsum* by other ethnic groups. Maori commonly called *M excelsum* kawakawa. There are three sub species: *M excelsum ssp. psittacorum* (distributed in Lord Howe Island, Norfolk Island and Kermadec Island); *M excelsum ssp. peltatum* (distributed in Islands offshore from the north coast of North Island); and *M excelsum ssp. excelsum* (distributed in North Island, South Island and Chatham Islands) (Bock, 2000). Kawakawa is actually

specific to *M excelsum ssp. excelsum* growing on the mainland of New Zealand where Maori settled.

Kawakawa sustains the herbivory of the larvae of a geometrid moth. The leaf damage by the moth feeding did not increase the chlorophyll content to compensate. This meant that kawakawa "has evolved to tolerate frequent and severe feeding damage on its leaves (S Hodge, Keesing, & Wratten, 2000)" without possessing an inducing system for chemical defense (Simon Hodge, Barron, & Wratten, 2000). Essential oil by steam distillation of kawakawa leaves and terminal branchlets contained up to 41.3% myristicin, 3.1% elemicin, and trace amounts of other compounds: α-pineme, β -phellandrene, aromadendrene, cadinenes, Camphene, Ketonic & non- ketonic oil, β-phellandrene, n-hexyl acetate, Oxygenated monoterpere, Azulenic oil, Ester, Palmitic acid and other unidentified compounds (L. A. Briggs, 1941; L. A. Briggs, Kingsford, Leonard, & White, 1975; Rito, 2005). The pleasant scented kawakawa oil was similar in composition to nutmeg oil, which was used as fragrant material. Nutmeg oil was thought to be psychoactive and hallucinogenic. The aromatic fraction in nutmeg oil contained a large amount of myristicin, elemicin and safrole plus trace amounts of methyleugenol, methylisoeugenol, eugenol, isoelemicin and isomyristicin. The myristicin and elemicin were responsible for the hallucinogenic effects (A. Shulgin & Shulgin, 1991; A T Shulgin, Sargent, & Naranjo, 1967).

Kawakawa was frequently used by Maori in many ways, both in the traditional and modern contexts. There was a tea drink made from kawakawa leaves (Riley, 1994; Rito, 2005). Medicinal usage of kawakawa was highly valued. It was almost one of the most used cures known to Maori for the healing property towards many ailments, as shown in Table 2-1. Kawakawa essential oil contained biological active compounds. The presence of myristicin gave kawakawa antiseptic properties and it was used as painkiller by Maori. Myristicin was chemically similar to eugenol from clove oil (S G Brooker & Cooper, 1959). Kawakawa products, such as essential oil, palm, kawakawa soap, or other form of kawakawa extract, can be found on market, with advertisements highlighting the healing and health benefits.

Parts	Function
Leaves & bark	Cuts, wounds, stomach pains, gonorrhea, steam baths
Leaves	Boils, toothache, stomach pains, skin disease, kidney trouble, eczema, V.D., bruises, rheumatism, wounds, worms, blood impurity, and bladder complaints.
Roots	Toothache, urinary complaints
Fruits and seeds	Excite the salivary glands, kidneys and bowels. Slightly diuretic and aphrodisiac.

Table 2-1 Healing effects from kawakawa (orally intake or chewing). (taken from(S G Brooker & Cooper, 1959; Rito, 2005))

2.1.8 Genus Leptospermum

There are about 28 species including trees and shrubs under genus *Leptospermum* from family Myrtaceae. Manuka (*L scoparium*) is the only *Leptospermum* species found in New Zealand, and it also grows in southeast Australia (Stephens, Molan, & Clarkson, 2005). A review on manuka in New Zealand was carried out in 2005 , and it contained botanical discussion of manuka, insect involvement in manuka growth, traditional and historic uses of manuka (Stephens et al., 2005). Here in this work the focus of manuka is on Maori traditional use, essential oil, manuka honey and a related species kanuka (*Kunzea ericoides*).

Manuka is among the most frequently used plants by Maori for food, medicine and timber. Its young branches occasionally produce a sugary gum that is safe enough to feed infants, or to relief cough (Crowe, 1981). Manuka wood used to be made into canoe deckings, canoe poles and fish traps, as well as weapons such as spears by

Maori (Riley, 1994). It was used as traditional medicine as sedative and astringent. Tannins and flavonoids were assumed to be partly associated with manuka medicinal properties. Eight methylated flavonoids were identified in dichloromethane extract of manuka (Haberlein & Tschiersch, 1994).

Manuka is also commonly valued as ornamental garden plant in New Zealand, for it can flower for almost nine months of the year. When cultivated in Europe, strategies such as hybridization were applied to improve flowers as described by (Stephens et al., 2005). The first description on manuka by European was from Captain Cook who brewed the dried manuka leaves into a tea. Thus manuka was also named as tea tree (Stanley G Brooker et al., 1989)

Essential oils from *Leptospermum* species involved with enormous amount of work. These work had been carried decades ago (Perry et al., 1993). Up to 1959, these species included *L scoparium*, *L flavescens var grandiflorum, L flavescens var microphyllum, L flavescens var leptophyllum, L odoratum, L lanigerum, L liversidgei, L citratum* and *L ericiodes* (R E Corbett & Gibson, 1959). Manuka oil constituents fell into four groups: sesquiterpene hydrocarbons, monoterpenes, oxygenated sesquiterpenes and triketones. The predominant section was sesquiterpene hydrocarbons accounting for more than 60% of the compounds. This section included cubebene, copaene, elemene, gurjunene, aromadendrene, farnesene, caryophyllene, selinene, calamenene and cadinene skeletons (Porter & Wilkins, 1998).

Due to their activity against Gram-positive bacteria including antibiotic-resistant strains (Douglas et al., 2004), the pleasant-scented manuka oil steam distillated from leaves is highly valued in New Zealand, and is attracting increasing international interest. Such activity was associated with six triketones as shown in Figure 2-1 (Haberlein & Tschiersch, 1994; Porter & Wilkins, 1998).

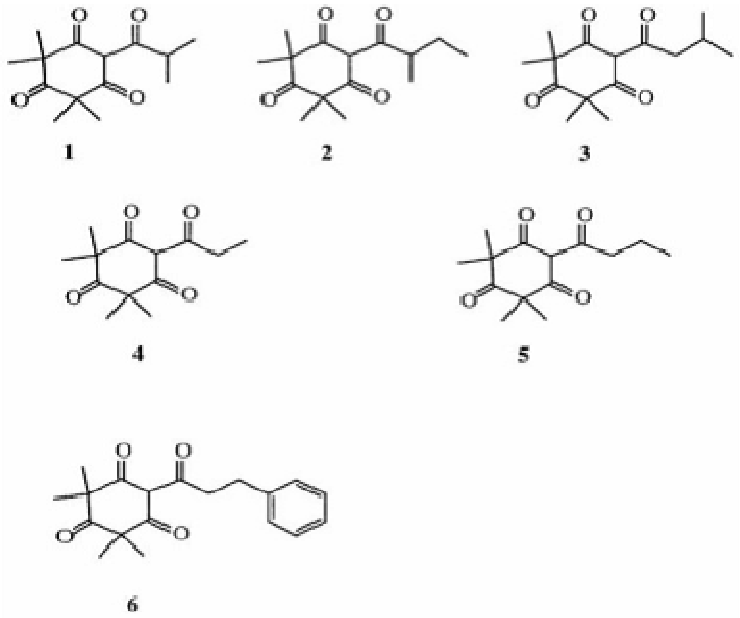


Figure 2-1 Triketones from manuka oil.(taken from (Porter & Wilkins, 1998))

Profiles of oil constituents may differ between manuka populations from different areas. Compared with New Zealand oil, manuka oil from Australia had a higher level of monoterpene, however the triketone content was lower or absent. Within New Zealand, the wild manuka population can be divided into five essential oil chemo-types: α-penene-rich oil from far North, triketones-rich oil from the East Cape, sesquiterpenes-rich oil from places other than North and South Islands, linalol/eudesmols-rich oil and myrcene/eudesmols-rich oil both from Nelson area of the South Island (Douglas et al., 2004). The further medicinal tests to determine the different performance of the chemo-types have not been carried out. In addition, there were no previous studies reported on the scent of manuka oil.

Before a taxonomic revision of genus *Leptospermum*, there were three species thought to be endemic to New Zealand: *L scoparium* (manuka), *L ericoides*, and *L sinclairii*. Now only manuka is left. The other two were re-classified as *Kunzea ericoides* (kanuka) and *K sinclairii* (kirk) (Bloor, 1992; Stephens et al., 2005). These three species have similar geographic distribution. Kanuka can also produce scented oil (Perry et al., 1993). At least eight compounds had been identified since 1959 (R E Corbett & Gibson, 1959): α-pinene, β-pinene, 1:8-cineole, dipentene, β-terpinene, linalool, α-terpineol and aromadendrene. Four acyl-phloroglucinol derivarives isolated from kanuka and kirk showed antiviral activity (Bloor, 1992). Compared with manuka oil, the kanuka oil featured lower level of oxygenated sesquiterpenes,

viridiflorol, viridiflorene and high level of α -pinene (Perry et al., 1993; Porter & Wilkins, 1998). Oils from these three were aromatic, and can be used in the formulations for soap (Stanley G Brooker et al., 1989).

In addition to essential oil, another important product is manuka honey. For a long time manuka honey was observed with antibacterial activity due to the contained peroxide, but the antibacterial activity of certain manuka honey cannot be attributed to peroxide (Adams, Boult, et al., 2008). Investigations had attempted to isolate such non-peroxide antibacterial compound, and confirmed the compounds for antibacterial property of manuka essential oil were not detected in manuka honey (Stephens et al., 2005). In 2008, methylglyoxal was identified as the dominant antibacterial compounds of manuka honey (Mavric, Wittmann, Barth, & Henle, 2008). Methylglyoxal was involved in the formation of advanced glycation endproducts (AGEs) (Adams, Boult, et al., 2008). It was a toxic electrophile towards bacteria by interacting with the nucleophilic centers of macromolecules such as DNA (Ferguson, Totemeyer, MacLean, & Booth, 1998). Methylglyoxal in manuka honey was related with manuka flowers; however, it did not come directly from the flowers. Manuka flower nectar was rich in dihydroxyacetone but contained little or no methylalyoxal. Dihydroxyacetone may react with amino acids, proteins, together with some other non-enzymatic conversions to form methylglyoxal. During storage of manuka honey, there is a decrease of dihydroxyacetone and increase of methylalyoxal. Adding dihydroxyacetone into other honey such as clover honey results in a similar methylalyoxal level to those of manuka honey (Adams, Manley-Harris, & Molan, 2008).

2.1.9 Genus Dacrydium

Rimu (*Dacrydium cupressinumm*) is endemic to New Zealand, mainly growing in lowland to mountain areas throughout North, South and Stewart islands. It is a conifer species, dioecious, evergreen and can be up to 35-60 meters tall (network, n.d). The rimu foliage oil abounds with diterpenes and sesquiterpenes.

The diterpene fraction mainly contained rimuene, phyllocladene, isophyllocladene and laurenene (R Edward Corbett, Lauren, & Weavers, 1979). Rimuene was first

isolated from rimu and that why it was so named. The foliage oil was the only source for a new diterpene laurenene (Perry et al., 1993). Laurenene was unusual due to the rosette-like structure with three five-membered rings, one seven-membered ring, one secondary and four tertiary methyl groups. Laurenene shared some common physical properties with rimuene, and this information was provided with more details in an introductory paper (R Edward Corbett et al., 1979). The diterpene fraction from *D intermedium* foliage contains rimuene, entrosadiene, ent-beyerene, phyllocladene, ent-kaurene, sclarene and ent-sclarene. Their identification, variation and biosynthesis were studied by (Perry & Weavers, 1985).

The sesquiterpene fraction mainly contained α-longipiene, longfolene, longibornyl, acetate, caryophyllene, caryophyllene oxide, humulene, α- and β-elemene, aromadendrene and a rare compound 9βH-caryophyllene. The biosynthetic discussion was presented in (Berry, Perry, & Weavers, 1985). A high level of 9βH-caryophyllene was found in some rimu specimens, and had attracted interest: its structure and stereochemistry were presented in (Hinkley, Perry, & Weavers, 1994).

Both diterpenes and sesquiterpenes were varied between trees, however, the variation was largely independent from environmental factors, and thus genetic control was proposed (Berry et al., 1985; Perry & Weavers, 1985).

Twenty native conifer species endemic to New Zealand have been studied in detail for their essential oils by the Universities of Auckland and Otago. Generally, the oils were rich with different types of terpenes (Perry et al., 1993). The essential oil of *D colensoi* gave at least 16 compounds by column separation, and 10 of them were confirmed as α-pinene, myrcene, limonene, terpinolene, longifolene, muurolene, cadinene, macrocarpol, phyllocladene and isophyllocladene (Briasco & Murray, 1952).

There was a lack of scent description and studies on rimu. Recent studies focused on rimu effect on ecology. The eruption of house mouse population was correlated with rimu seed fall in forest. Mice can open the rimu nut to eat the seeds leaving the husk. The nutritional content of rimu seeds were sufficient for healthy growth and

reproduction of the mouse (Harper, 2005; Ruscoe, Wilson, McElrea, McElrea, & Richardson, 2004).

2.1.10 Genus Agathis

Kauri (*Agathis australis*) is distributed in North Island of New Zealand. It is a giant tree with larger volume than other trees from New Zealand, although is not the tallest. It is an excellent source of timber, and the diameter of the bole is commonly 3 m, even up to 7 m. The tree can survive to great age, often hundreds of years. The relationship between diameter and age is weak, for a 10cm difference in diameter can mark a difference in age by 300 years (Ahmed & Ogden, 1987). Kauri is now defined as an endangered species. In addition to timber, another natural product is kauri gum. The gum is the subfossil resin from dead kauri wood buried underground for thousands of years. It contains terpenes, α-pinene, limonene, alcohols and diterpenoid acids. The special properties lead kauri gum to have a coating function and it was firstly used by varnish makers, and later used to make linoleum. Commercial utility of kauri gum dated back to 1830s, and kauri gum in Northland was described as "plentiful" until 1989 (Stanley G Brooker et al., 1989).

2.1.11 Genus Passiflora

Kohia (*Passiflora tetrandra*) is also known as New Zealand passion fruit. It is a vigorous climber, bearing dark green leaves, white aromatic flowers and orange-like fruit. The edible fruits are used for flavoring certain jellies made from seaweed. Both seeds and pulps of the fruit can produce the valuable cosmetic and medicinal oil (Riley, 1994). Oil can be extracted from seeds by cooking the fruits in a hāngi which is a traditional Maori method of cooking food using heated rocks buried in the ground in a pit oven. The oil is helpful in treating rheumatism, skin ailments, sore nipples and flatulence (Epuro Hands International Limited, 2005; *Maori uses: Medicinal plants, Climbers Kōhia, kaimanu, New Zealand passion flower*, n.d; Natural medicinal herbs, n.d). Further chemical studies specific to kohia has not been reported.

Genus *Passiflora* consists of more than 500 species distributed worldwide. There are only about 20 of them bear edible fruit, and further, only four are largely cultivated:

P edulis, P ligularis, P molissima and *P quadrangularis*. A hybrid strain by *P edulis* var *flavicarpa* and *P edulis* var *edulis* grown in Uganda had an oil yield between 18.5% and 28.3%. The fatty acid profile of that oil was confirmed with linoleic dominant by 67.8%-74.3%, the rests were oleic, palmitic, stearic and α-linolenic. The unsaturated fatty acid accounted for 85.4%-88.6% of total (Nyanzi, Carstensen, & Schwack, 2005). For *P caerulea* grown in Argentina, the oil yield is 29.9%, and the major oil fatty acids were 18:2 (63.1%), 18:1 (17.6%) and 16:0 (10.1%) (Quiroga, Bou, Vigo, & Nolaco, 2000). Thus, instead of been called as essential oil, the fruit oil of *Passiflora* was more properly seemed as diet oil. These fatty acids constituents may not be relevant to scent or volatile properties.

For more details about *Passiflora* species, one can refer the review by (Dhawan, Dhawan, & Sharma, 2004).

2.1.12 Genus Lophocolea

Kopuru (*Lophocolea semiteres*) is a delicate, translucent, pale green leafy liverwort, and the leaves are slightly aromatic. It is used by Maori to scent various oils. It is usually prepared by drying and powdering. This species has been found in New Zealand and Britain (Riley, 1994).

L brookwoodiana is a newly found species in Britain, described as "flavovirescentes, aromaticae" (Paton & Sheahan, 2006). Essential oil of *L bidentata* contained β-barbatene, α-selinene, diplophyllolide and (+)-(4S,4aS,5R,8aS)-trans-4,8a-dimethyl-4a,5-epoxy -decalin, which were the main highly fragrant compounds (Figure 2-2) (Rieck, Bolow, & Konig, 1995). Furano-eudesma- 4(15), 7, 11-trien-5α-ol identified in *L heterophylla* displayed a structural relationship with lindestrene, which was one of the fragrant constituents in myrrh oil and Lindera strychnifolia (Rieck & Konig, 1996) (Figure 2-3).

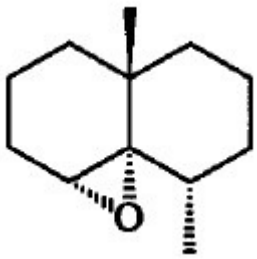


Figure 2-2 The highly fragrant compound from essential oil of L bidentata. (taken from (Rieck et al., 1995))

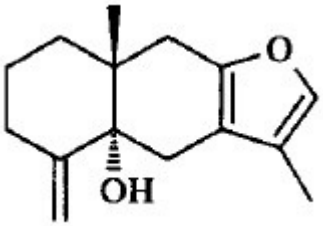


Figure 2-3 A fragrant compound from L heterophylla. (taken from (Rieck & Konig, 1996))

Liverworts synthesize a number of lipophilic terpenoids, aromatic compounds and acetogenins, thus most liverworts present with characteristic scent, pungency, bitterness, bioactivities and medicinal properties. The characteristic scents of liverworts are listed in Table 2-2. The corresponding volatile compounds from liverworts for a given scent were shown by Table 2-3. Especially, the bicyclohumulenone and tamariscol were commercially important, used as perfumes or as perfume components (Asakawa, 2007).

Species	Odor
Asterella species	Indole- or skatole-like, and higher plant *Houttuynia cordata*-like
Bazzania japonica	Sweet, balsamic, tree moss-like
B. pompeana	Oak moss-like
Cheilolejeunea imbricata	Strong milky smell
Chiloscyphus pallidus	Intense smell reminiscent of stink bug
Conocephalum conicum	Camphoraceous, strong mushroomy, and lactone-like
Conocephalum japonicum	Higher plant *Houttuynia cordata*-like
Frullania davulica	Mossy
F. tamarisci subsp. *tarmarisci*	Oak moss-like
Jungermannia obobata (*Solenostoma obovata*)	Carrot-like
Leptolejeunea elliptica	Intense and sweet phenol-like Mixed smell of naphthalene, and dried bonito
Lophocolea heterophylla	Strong and distinct mossy
L. bidentata	Strong and distinct mossy
L. minor	Strong moss-like
Lophozia bicrenata	Pleasant odor like cedar oil
Makinoa crispata	Rooty, earthy, woody, and amber-like
Mannia fragrans	Strong sweet-mossy
Odontoschisma denudatum	Civet, animal-like
Pellia endiviifolia	Dried seaweed-like
Plagiochila sciophila (*P. acanthophylla* subsp. *japonica*)	Sweet-mossy and woody
Porella gracillima	Woody-earthy
P. vernicosa	Malty, earthy
Radula perrottetii	Castor-like, animal-like
Takakia lepidozioides	Mixed smell of cinnamon and burnt wheat powder
Targionia hypophylla	Sweet turpentine
Trichocolea tomentella	Sulfur-like
Wiesnerella denudata	Strong sweet-mushroomy, green, and citrus

Table 2-2 Characteristic scent of liverworts. (taken from (Asakawa, 2007))

Species	Compounds	Scent
General	Volatile terpenoids, simple aromatic compounds	sweet-woody turpentine sweet-mossy fungal-like carrot-like mushroomy seaweed-like scents
Almost all liverworts	oct-1-en-3-ol and its acetate	mushroomy
Asterella species	skatole	unpleasant odor
Cheilolejeunea pallidus	(E)-dec-2-enal, (Z)-dec-2-enal (E)- and (Z)-pent-2-enals	stink bug smell
Leptolejeunea elliptica	*p*-ethylanisol *p*-ethylphenol *p*-ethyl phenyl acetate	fragrant
Cheilolejeunea imbricata	*(R)*-dodec-2-en-1,5-olide *(R)*-tetradec-2-en-1,5-olide	strong milk-like fragrance
Plagiochila sciophila	Bicyclohumulenone	patchouli vetiver cedar wood iris moss carnations ....
Frullania tamarisci subsp. *tamarisci* *F tamarisci* subsp. *obscura,* *F nepalensis* *F asagrayana*	Tamariscol	Woody and powdery green notes of mosses, hay, costus, violet leaf, and seaweeds...
Conocephalum conicum	(–)-sabinene or (+)-bornyl acetate or methyl cinnamate	characteristic odor
Jungermannia obobata	tris-normonoterpene ketone, 4-hydroxy-4-methylcyclohex-2-en-1-one	carrot-like odor
Lophocolea heterophylla *L bidentata*	(-)-2-methylisoborneol geosmin	strong and distinct mossy odor
Symphyogyna brongniartii	Geosmin	strong earthy-musty odor
Mannia fragrans	cuparene-type sesquiterpene ketone, grimaldone	strong sweet-mossy note
Targionia hypophylla	cis- and trans-pinocarveyl acetates	sweet turpentine-like odor

Table 2-3 Characteristic scents of liverworts species and the corresponding compound (Asakawa, 2007).

Chapter 3 MATERIALS AND METHOD

Section 3.1 gives the preparation of GC-FID and GC-MS. Section 3.2 discusses the optimisation of the Solid Phase Micro-extraction (SPME) variables. The preparation of different mairehau samples and methods for analysis their volatiles are given in section 3.3. Section 3.4 gives an overview of the software used for data handling.

3.1 GC-FID and GC-MS preparation

After SPME sampling, the SPME fibre carrying volatile components was injected into the injection port (in splitless mode) of the GC-FID or GC-MS for analysis. The injection port temperature was 200°C, and injection time was 2min.

GC-FID: A Shimadzu GC 2010 was equipped with a Flame Ionization Detector (FID) (Japan). The column was ZB-5 with length of 30.0m, film thickness of 0.25µm and inner diameter of 0.25mm. The temperature was 300°C for FID. The column initiated at 40°C for 3min, and increased at 5°C/min to 220°C holding for 2min. Nitrogen was the carrier gas. The total pressure was 74.3kPa and the total flow was 14 ml/min.

GC-MS: The Trace GC Ultra (Thermo Scientific, USA) was equipped with a Mass Spectrophotometer (Thermo Scientific, DSQ Series, USA).The DB-5 capillary column was installed. The setting of Trace GC Ultra was same as those of Shimadzu GC 2010 except Helium was the carrier gas (1.5ml/min). The mass spectrometer scanned masses from m/z 40 to m/z 650 at 2 scans per second. The mass spectrometer operated in the electron impact mode with the ion source temperature of 200 °C, an ionising voltage of 70eV, and the transfer line temperature of 250°C.

3.2 Effects of SPME variables on volatile extraction

3.2.1 Reproducibility of SPME fibre adsorption

Three types of SPME fibres were compared in reproducibility. They were 100 µm PDMS fibre, 60 µm PDMS/DVB fibre and 50/30 µm DVB/CAR/PDMS fibre

(Supelco Co., Bellefonte, USA). Fibres were pre-conditioned following the instructions given by the manufacturer, and then exposed for 30 min in the headspace of the 1130 ml conical flask containing 700ml condensed water (with strong aroma) from hydro distillation of mairehau (The headspace was pre-equilibrated for 1 h at room temperature ($20 \pm 2°C$)).The fibre was then directly inserted into injection port of GC-FID. Each type of fibre was repeated for 6 times.

3.2.2 Sample amount

To determine the optimum amount of sample in a 20 ml flat bottom headspace vial, 2.5g and 3.0g of homogenized dry mairehau were separately contained in two headspace vials (Chromatography Research Supplies, Inc, USA), and sealed (using PTFE/Silicone septum and crimp cap (Supleco, USA)). The headspaces were pre-equilibrated for 1 h at 25°C controlled by water bath. 100 µm PDMS fibre was used for headspace SPME. The fibre was then directly inserted into injection port of GC-FID. The process was repeated for 4 times for each vial.

3.2.3 Effects of adsorption time and temperature

To determine the optimum adsorption time and temperature, 3.0g homogenized fresh mairehau (the volume was about 10ml) was transferred into 20ml headspace vials and sealed, and pre-equilibrated for 1h. The 100 µm PDMS fibre was exposed in headspace for different times (5min, 10min, 15min, 20min, 30min, 40min, 50min, 60min and 90min). Adsorption temperatures were set as 25°C or 35°C. Then the fibre was injected into GC-FID injection port.

3.3 Mairehau sample preparation

3.3.1 Mairehau dehydration

In addition to the solvent free extraction, another advantage of SPME is that it can absorb the volatiles from samples like flowers in the field without destroying the sample. The field sampler carrying SPME fibres can be held for minutes near an object to absorb the released volatiles, and then the fibre can be taken into Laboratory for analysis. To minimize the effect of ambient air flow and to increase

the concentration of volatile, the object and SPME can be surrounded by a sealed container (Song, Xiao, Deng, Zhang, & Hu, 2006). Another option is to surround the field plants by a flask, and once the volatiles have reached equilibrium, the flask is removed and quickly sealed and then taken to the laboratory for SPME adsorption (An, Haig, & Hatfield, 2001).

To explore the potential change in mairehau volatile during natural dehydration after cutting the sample from plants, three similar weight mairehau twigs were cut from plants and placed in open tubes at room temperature (A-Figure 3-1).

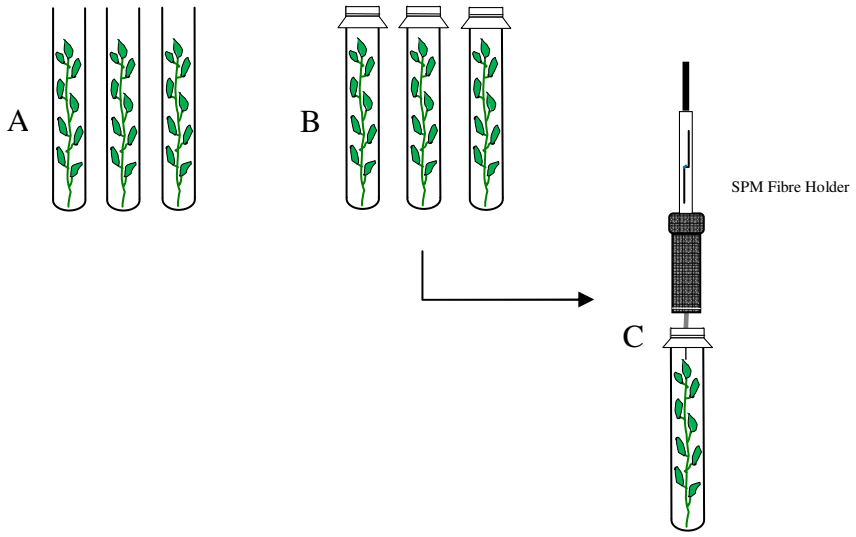


Figure 3-1 Volatile sampling on mairehau twigs. (may not equal to the real scale)

Then on days 1, 2, 5, 7, 9, 11, 13 and 15 after cutting from plants, the three tubes containing twigs were weighted. The tubes were sealed by foil for 1h before inserting the SPME fibre (B-Figure 3-1). Then the volatiles were sampled (C-Figure 3-1). Sampling time was 10 min. The SPME fibre was injected into GC-MS afterwards.

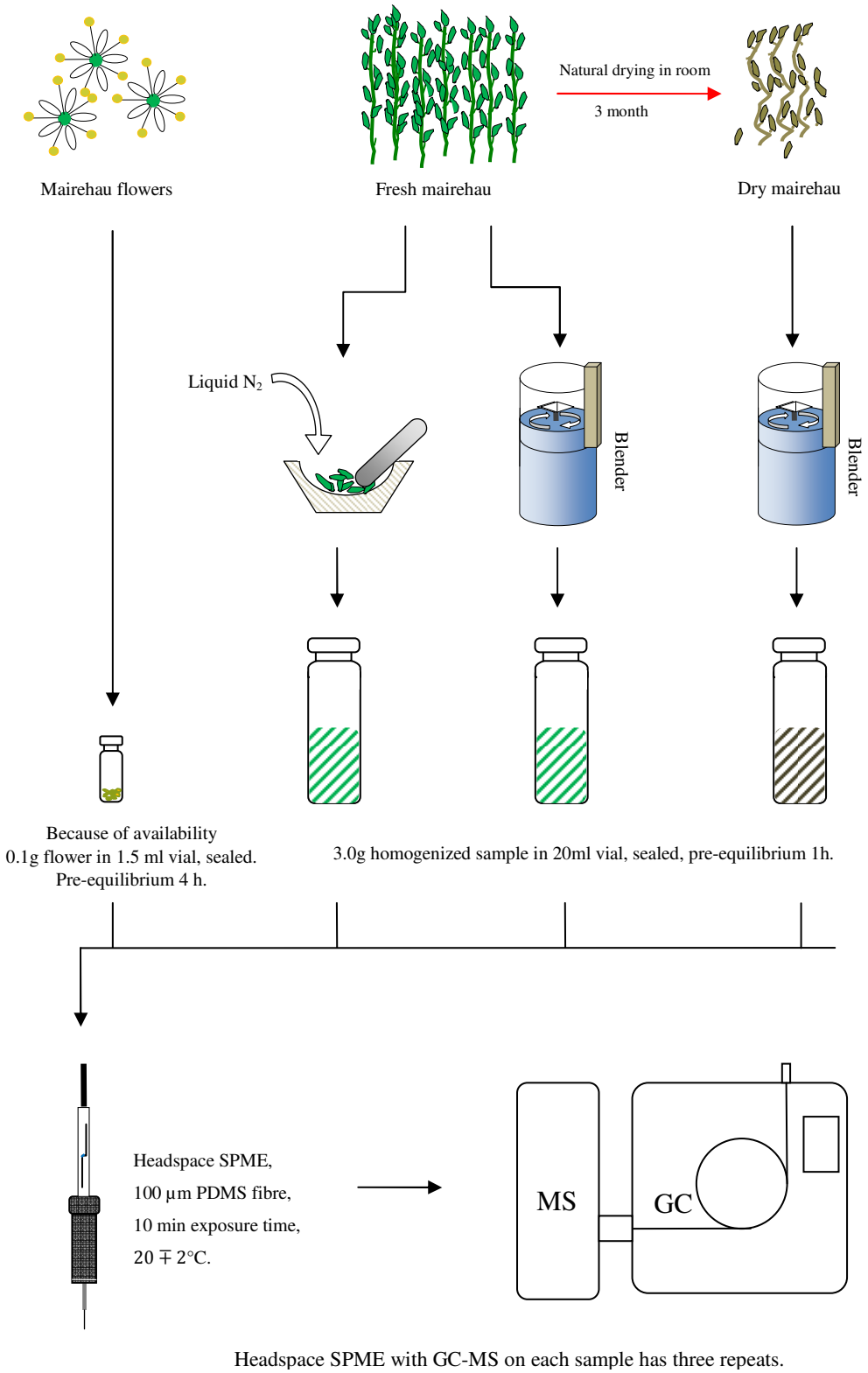


Figure 3-2 Sample preparation and analysis by headspace SPME with GC-MS.

3.3.2 Homogenized mairehau and mairehau flower

The homogenized mairehau samples were prepared in three ways (Figure 3-2):

1. Homogenized dry mairehau-fresh mairehau was naturally dried at room conditions for three months, and then homogenized using a coffee blender.
2. Homogenized fresh mairehau-fresh mairehau was homogenized directly using a coffee blender at 20 ± 2°C, and stored at 4°C.
3. Liquid Nitrogen cooling homogenized mairehau-fresh mairehau was manually ground with liquid Nitrogen cooling, and stored at -20°C.

Samples were placed in headspace vials and sealed. They were normalized and equilibrated at room condition for 1h before sampled by 100 µm PDMS fibre (The fibre exposure time was 10min).. The fibre was injected into GC-MS after sampling. The volatile of mairehau flowers was also analyzed (Figure 3-2).

3.4 Computer preparation

Chemometric algorithm (Details are shown in Chapter 4) of GC-MS data was carried out on R version 2.15.1(R Core Team, 2012) for Window 7. R scripts for the chemometric algorithm can be found in Appendixes of this submission. In addition to the default installed packages with R, Table 3-1 lists some other packages that were required for the algorithm (further packages based on which these packages relied are not listed here). Software ProteoWizard was used for converting the mass spectrum data format. The NIST MS Database (Version 2.0d, 2005) and the NIST Mass Spectral Search Program were used in peak identification in GC-MS. Other software for data sorting and analysis included Microsoft Office Excel 2007 and Minitab 16.

Package	Contributor
mzR	Bernd Fischer, Steffen Neuman, Laurent Gatto
msdata	Steffen Neumann
TargetSearch	Alvaro Cuadros-Inostroza, Jan Lisec, Henning Redestig, Matt Hannah
signal	Tom Short, Uwe Ligges, Sarah Schnackenberg, Hans Werner Borchers, Sebastian Krey, Olaf Mersmann
ChemometricsWithR	Ron Wehrens
ALS	Katharine M. Mullen
OrgMassSpecR	Nathan G Dodder, Katharine M Mullen

Table 3-1Additional R packages required for data analysis.These packages can be downloaded from http://cran.r-project.org/ or http://bioconductor.org/packages/. The packages are for R version 2.15.1.

Chapter 4 CHEMOMETRICS ON GC-MS DATA

4.1 Use of chemometrics to analyze GC-MS data from mairehau samples

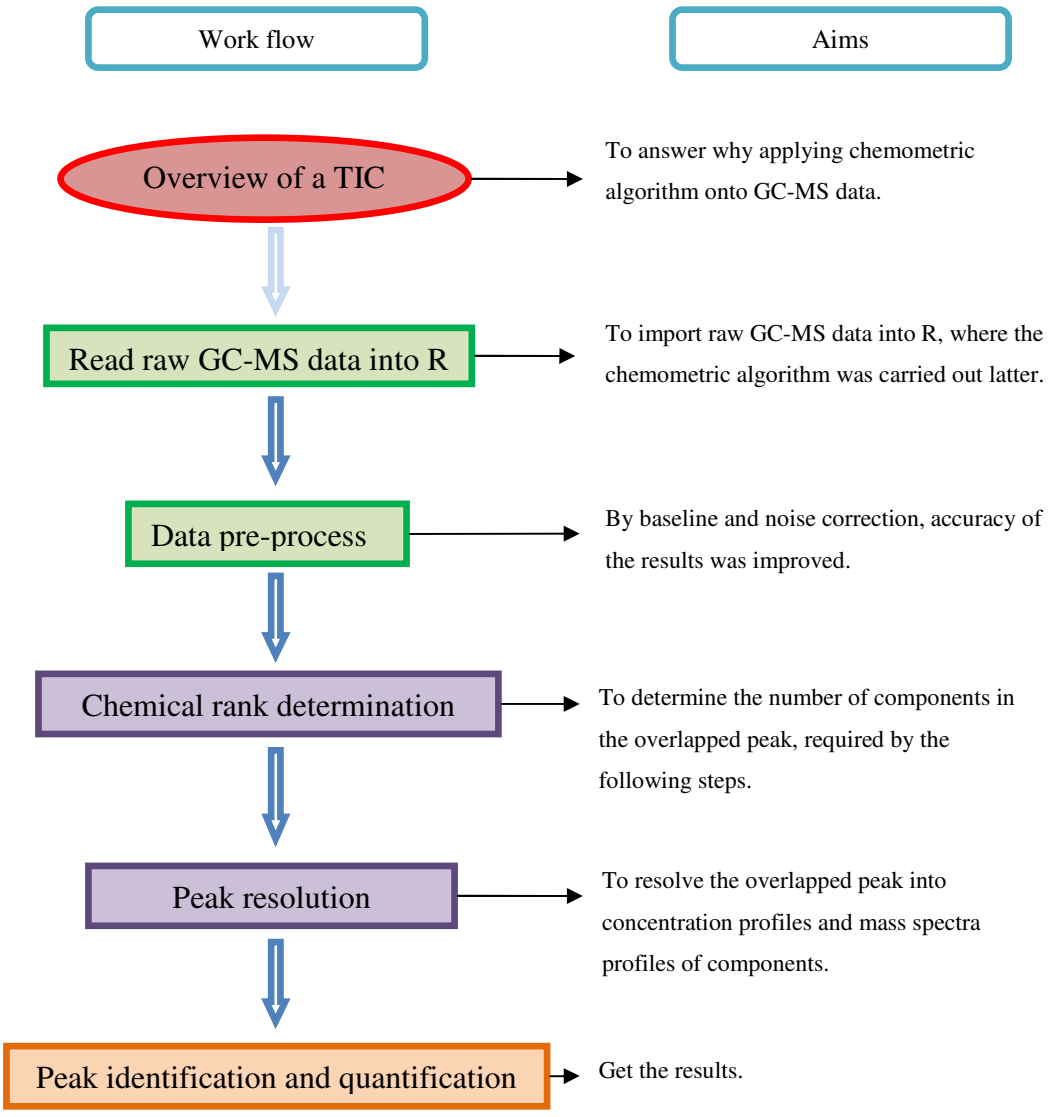


Figure 4-1 A work flow of chemometric method.

Chemometrics is a technique that can be used to extract as much information from chemical systems to better understand what their nature. In case of the system of GC-MS, the aim is to better identify the compounds in the sample by de-noising and

teasing out the data contained in overlapped peaks to identify the compounds they contained.

Figure 4-1 shows the overall work flow of applying chemometric algorithm on GC-MS data from mairehau volatile samples in this study. The aim of each step is also given. Each step is expanded with details throughout the following sections under this chapter.

4.1.1 Overview of a TIC

Figure 4-2 shows the Total Ion Chromatogram (TIC) from GC-MS of a dried mairehau volatile sample. It can be noticed that, a number of peaks are overlapping.

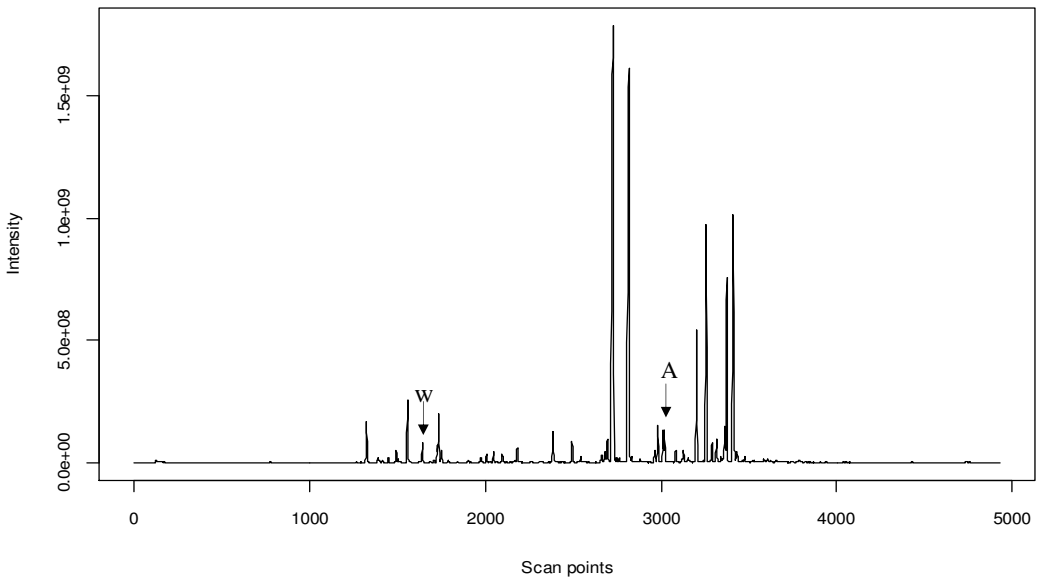


Figure 4-2 TIC of a dried mairehau volatile sample.

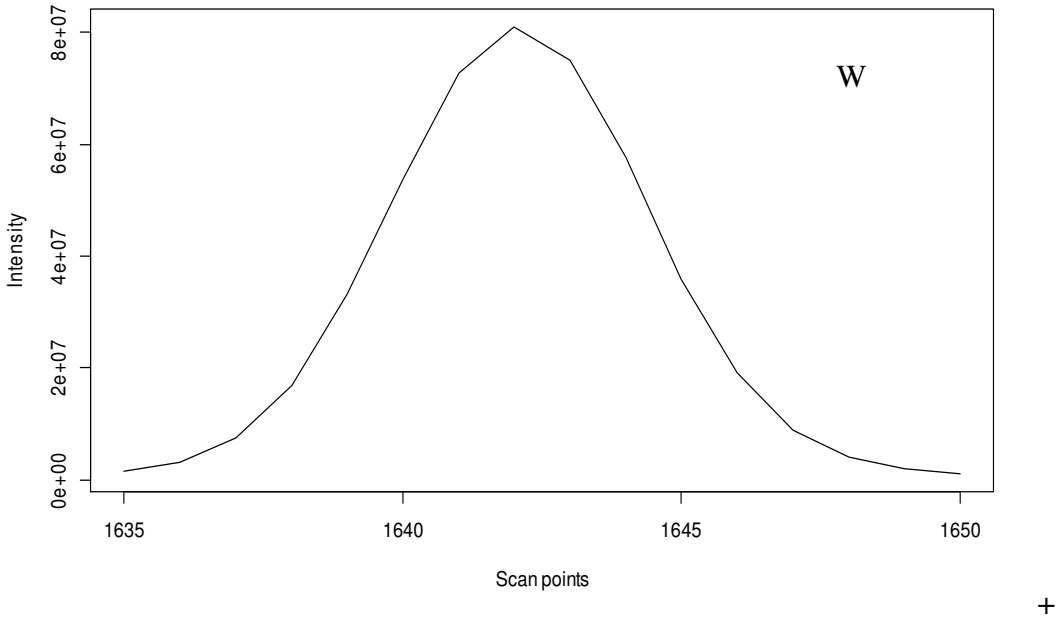


Figure 4-3 The zoomed in view of peak W as marked in TIC in Figure 4-2.

A well separated component in GC-MS would present its peak (ion chromatogram) with Gaussian curve, as shown in Figure 4-3. The mass-spectra at each scan point within the peak are conform to the mass-spectra of that component from MS library. The component can thus be identified by utilizing the mass-spectra (from any scan points within its peak) to search for the best match in MS library. Peak area (Curve integration) provides the quantitative information.

However, due to high sample complexity, and / or poor chromatographic separation, components may not be separated well, but co-eluted to each other, causing peak overlapping, as shown in Figure 4-4.

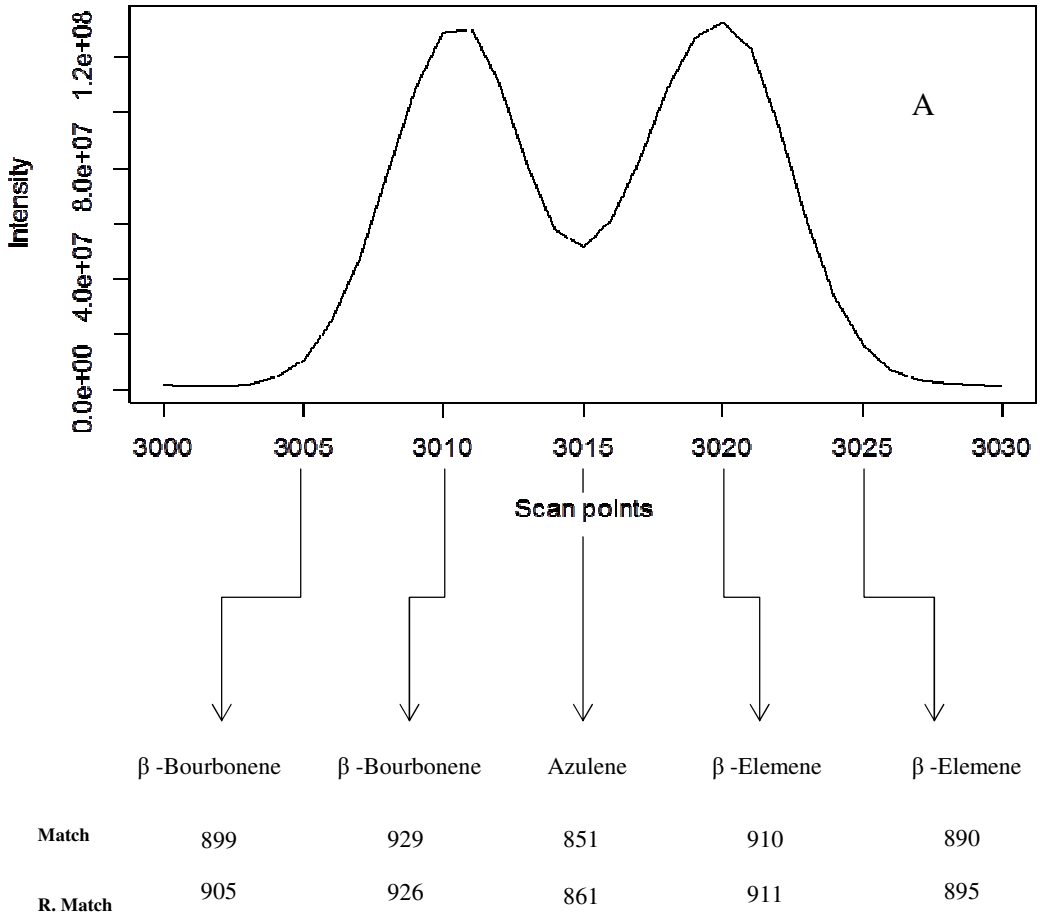


Figure 4-4 An overlapped peak of co-eluted compounds. This is the zoomed in view of the peak cluster A marked in TIC in Figure 4-2. The result of direct match searching in NIST MS library for mass spectra from five scan points is also shown.

In the case of co-elution system as shown in Figure 4-4, confusion from two aspects would occur, causing failure in components identification and inability to extract as much information as possible from the data.

First, from the two summits and high match and reverse match factors (out of 1000), we can roughly judge by similarity search in MS library that β-Bourbonene and β-Elemene were presented in peak cluster A. However one cannot be sure what happened around scan point 3015. It can be either merely overlapped area of two real peaks (in this case, mass spectra here was the combination of the two, and MS library

search gave a pseudo-component Azulene), or with a third component eluted between β-Bourbonene and β-Elemene. Match and reverse match factors of Azulene were not low enough to make a rejection.

In another case, when the retention time of two components were very close, the judge of component number based on the number of summits in overlapped peak would be inaccurate, as shown by the summit B in Figure 4-5.

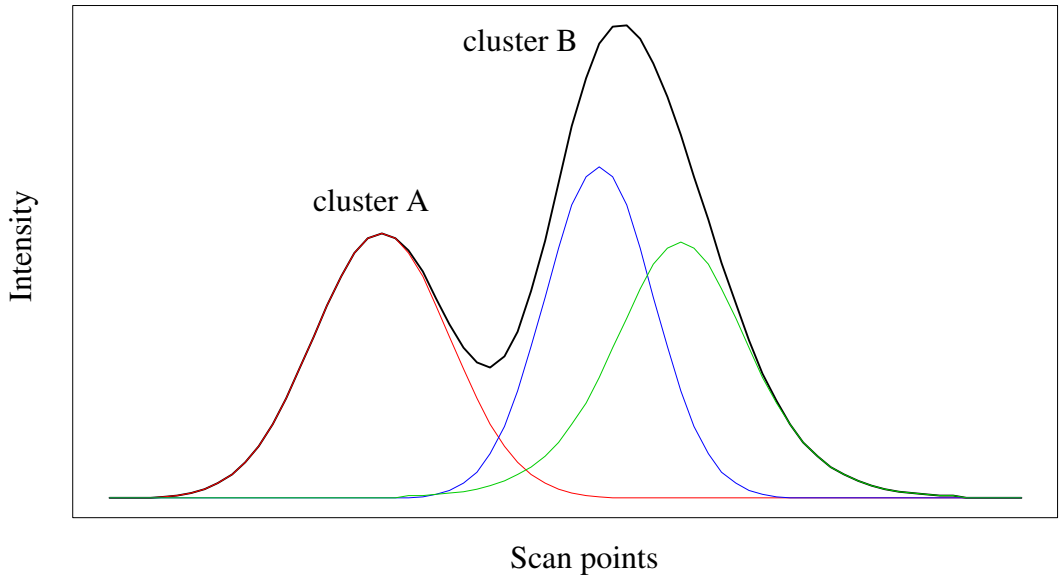


Figure 4-5 A system of three co-eluted components (GC-MS). The black curve is the ion chromotogram from a simulate TIC data, and it contains three components(red,blue and green curves). However, from the two summits of the ion chromotogram (black curve), we judge there are two components, which is incorrect.

The mass spectra at each scan point of cluster B in Figure 4-5 was from the combination of three components (blue, green, and part of the red curves). They varied in relative amount between different scan points of cluster B, and one can get different components at different scan point of cluster B using a traditionally MS library search, which were hardly identical to the true components plus with high match and reverse match factors. Thus, the component identification failed.

Second, In general, quantitative analysis was carried out with peak area. When components were co-eluted, the overlapped peak was directly split to obtain peak areas of the object components, as shown in Figure 4-6. This was done for simplicity in most of case (Gong, Liang, Cui, Chau, & Chan, 2001). In Figure 4-6, the two split peaks were not Gaussian curve shaped, thus the two areas were not the true peak areas of the components (β-Bourbonene and β-Elemene) in peak cluster A. Furthermore, if Azulene was really there, its peak area information was totally missed.

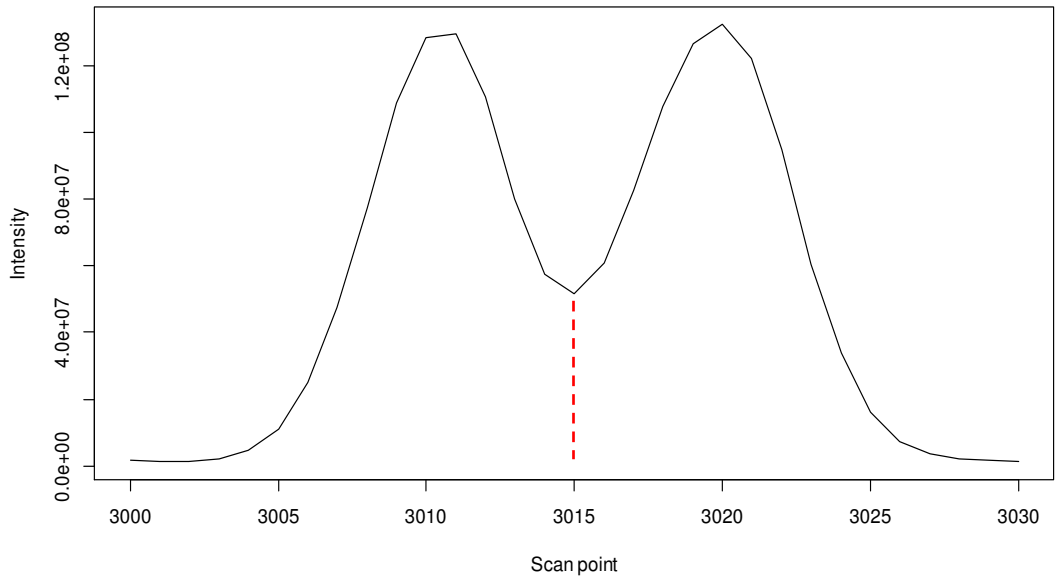


Figure 4-6 Peak areas determined by direct split (peak cluster A).

There are two solutions, *priori* and *posteriori*, for handling the problems resulted from overlapped peak in GC-MS data, as shown in Figure 4-7 (Amigo, Skov, & Bro, 2010)

***Priori* solution**: To separate the overlapped peak by resetting the classical chromatographic parameters, such as column type, flow speed and temperature program. The drawbacks are quite obvious for it is time consuming. In addition, it is common to find that overlapped peaks may occur at other retention time after reprogramming the method.

***Posteriori* solution**: Since the GC-MS data is three dimensional in terms of time (scan point), mass to charge ratio (m/z) and intensity, by chemometric algorithms, the original data can be resolved into contributions of independent chemical components, consequently, the single component signal becomes accessible for identification and quantitative analysis.

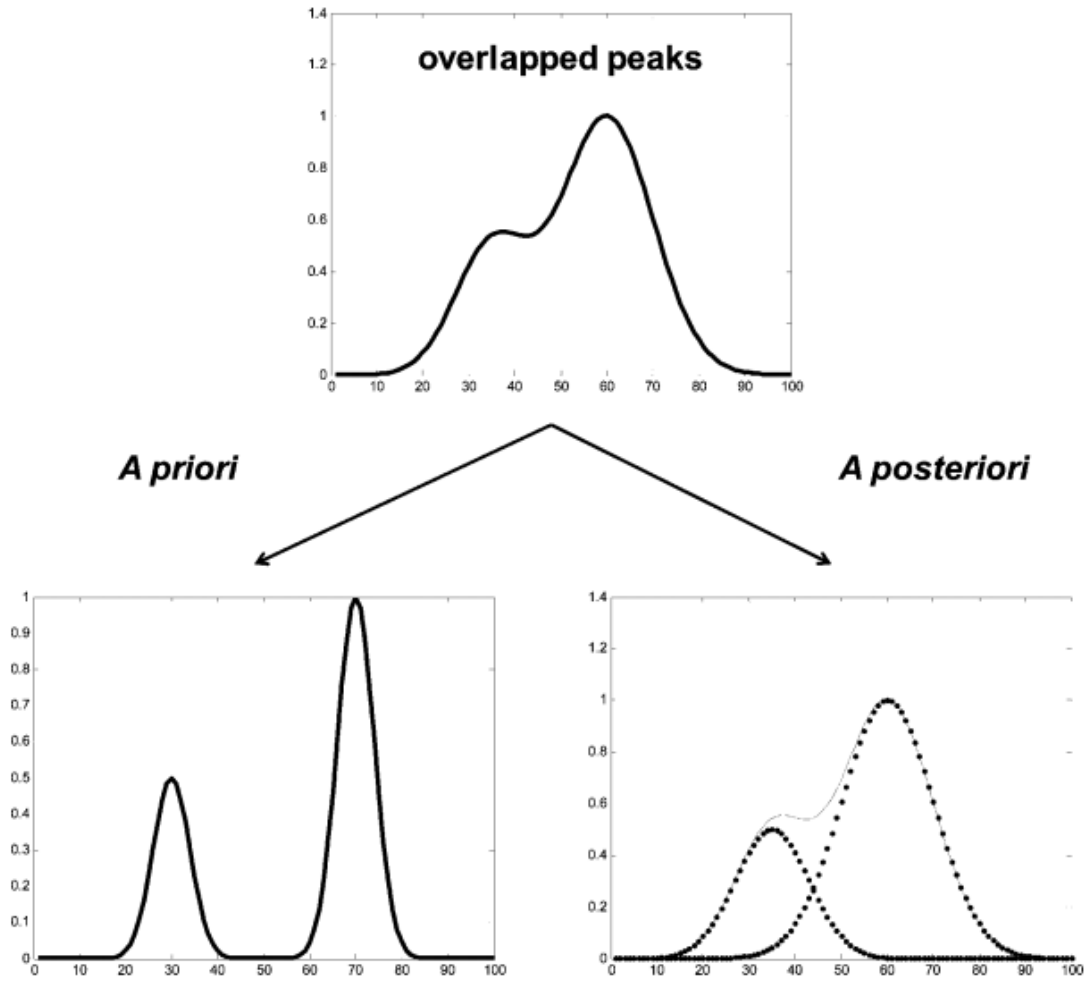


Figure 4-7 Two solutions for component co-elution problem.To the left, chromatographic setting is changed to improve the separation ability, to the right, chemometric algrithms are used to resolve the overlapped peaks. (taken from (Amigo et al., 2010))

In this study, *Posteriori* solution was chosen for handling the overlapped peaks. A chemometric algorithm, Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) coupled with Evolving Factor Analysis (EFA) was chosen.

4.1.2 Read the raw GC-MS data into R

The GC-MS device is normally connected to a computer where the data system software is installed to control and monitor the device and store the collected data into a file with a format that is developed by different manufacturers. These manufacturer-proprietary data formats ban the external manipulating of the GC-MS data. To address this limitation, before data analysis, our GC-MS data with Thermo Xcalibur file format .RAW was converted into an open, XML-based file format .mzxml (Lin, Zhu, Winter, Sasiowski, & Kibbe, 2005). The conversion process was completed by ProteoWizard Software in centroid mode (peak picking).

The TIC was divided into peak clusters by zero-component areas along the elution sequence (Jalali-Heravi, Moazeni, & Sereshti, 2011). The following chemometric process was carried out on each peak cluster. Peak cluster A shown in Figure 4-2 was selected to illustrate the chemometric process (Figure 4-4 shows the zoomed in view of cluster A).

The scan point range of peak cluster A read from the Xcalibur data system connected with the Thermo CG-MS device, was used for importing the cluster A into R. The peak cluster A was imported and stored in matrix X by the command:

```
X<-datamatrix(3000,3030, data="DMpdms2.mzxml")
```

The function datamatrix() is defined in Appendix A. Peak cluster A, from the .mzxml formatted GC-MS data file DMpdms2.mzxml (originally converted from the file DMpdms2.RAW by ProteoWizard Software), started at scan point 3000 and terminated at 3030. Since the NIST MS search program dealt with only integer m/z values when searching the NIST MS Database (The NIST Mass Spectrometry Data Center, 2008) for component identification, this importation process rounded the original exact m/z values into integers at the same time, and intensities of same integers were added up.

There were 610 rows and 31 columns of matrix X, representing the particle range (m/z: 40~649) and scan point range (3000~3030) of peak cluster A. Each element of

matrix X represented the intensity of an m/z at a scan point. 3D view of matrix X is shown in Figure 4-8, and the matplot is shown in Figure 4-9. Both illustrate the data structure.

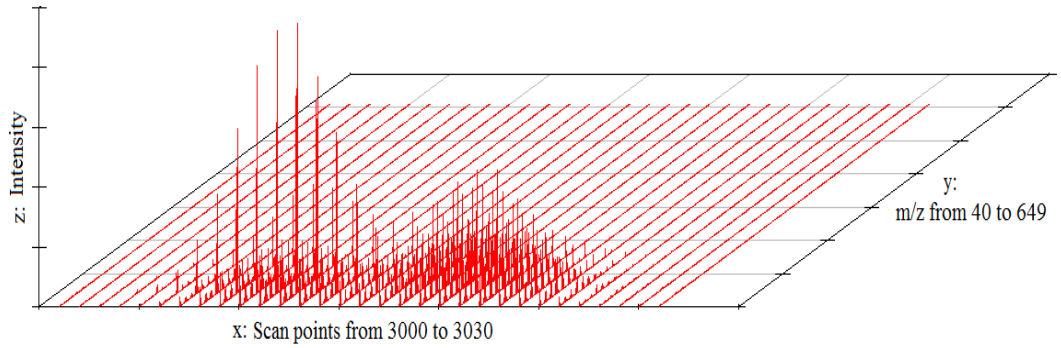


Figure 4-8 3D view of the data matrix X of peak cluster A. The zero component areas along elution sequence are about 3000~3004 and 3027~3030. The intensity signals are about gathered between m/z:40~250.The signals distributed along x axis in two neighbouring domains,although without a clear border.

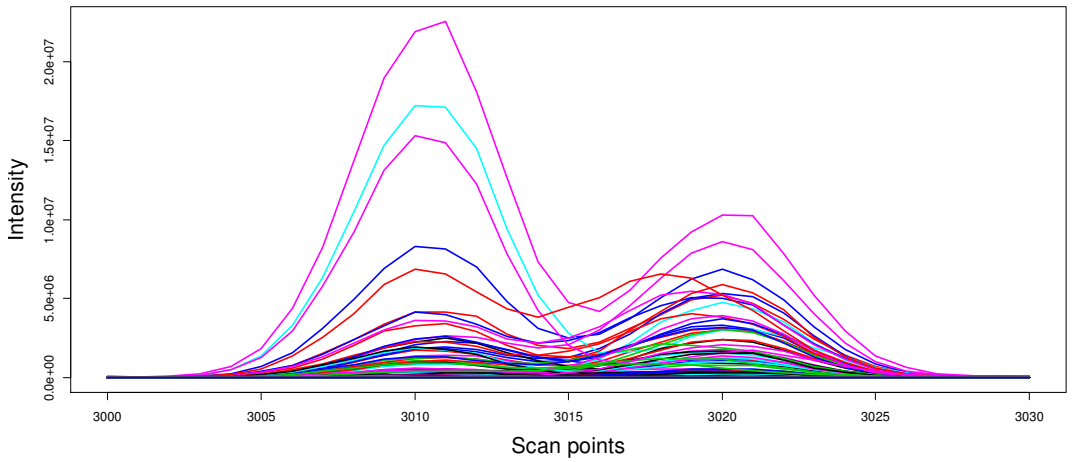


Figure 4-9 Matplot of the data matrix X of peak cluster A. Each curve represents the intensity changing by scan points for a m/z value from range 40~649.

4.1.3 Pre-processing of data

The matrix X can be modelled as:

$$X = \boldsymbol{CS^T} + \boldsymbol{D} + \boldsymbol{N} \qquad \textit{Equation 1}$$

In the case of A components in peak cluster A, *Equation 1* can be rewritten as:

$$X = \sum_{a=1}^{A} \boldsymbol{c_a s_a^T} + \boldsymbol{D} + \boldsymbol{N} \qquad \textit{Equation 2}$$

The bilinear matrix X can be represented by the product of concentration profiles **C**, spectrum profiles **S**, background **D** and measurement noise **N**. **T** denotes the matrix transpose. The chemometric resolution process aimed to recover the response profile **c** and **s** of each chemical component from the given X (Jalali-Heravi & Vosough, 2004). Then each chemical component was accessible for quantification and identification.

Before carrying out the resolution, it was necessary to minimize the effect of background **D** and measurement noise **N,** for improving the accuracy of final result.

The background **D** was also known as baseline. The baseline typically shifted up as GC column incrementally bleeding as temperature rising. During the short time span 24.965min~25.214min (corresponding to scan point range 3000~3030), the baseline shifting can be treated as a linear variable depending on time change. Zero component area from two sides of the peak cluster A would provide sufficient information to establish a linear regression of baseline versus time (or scan point), and the baseline was thus corrected from the matrix X (Jalali-Heravi et al., 2011; Jalali-Heravi & Vosough, 2004). However in this study, the baseline correction method was based on the work of (Chang, Banack, & Shah, 2007). The algorithm of this method was incorporated in the function baselineCorrection() from the R package "TargetSearch". Appendix B shows the application of baselineCorrection() on matrix X. The algorithm was applied on each row of matrix X, and the resulted baseline corrected matrix was denoted as matrix V.

The measurement noise **N** was corrected by applying the Savitzky–Golay filter (Jalali-Heravi et al., 2011; Schafer, 2011) on each row of matrix V. The function sgolayfilt() from the R package "signal" was required for this process. The noise correction procedure is shown in Appendix C, and the final baseline and measurement noise corrected matrix was denoted as matrix W. The result of baseline and measurement noise correction is exemplified in Figure 4-10.

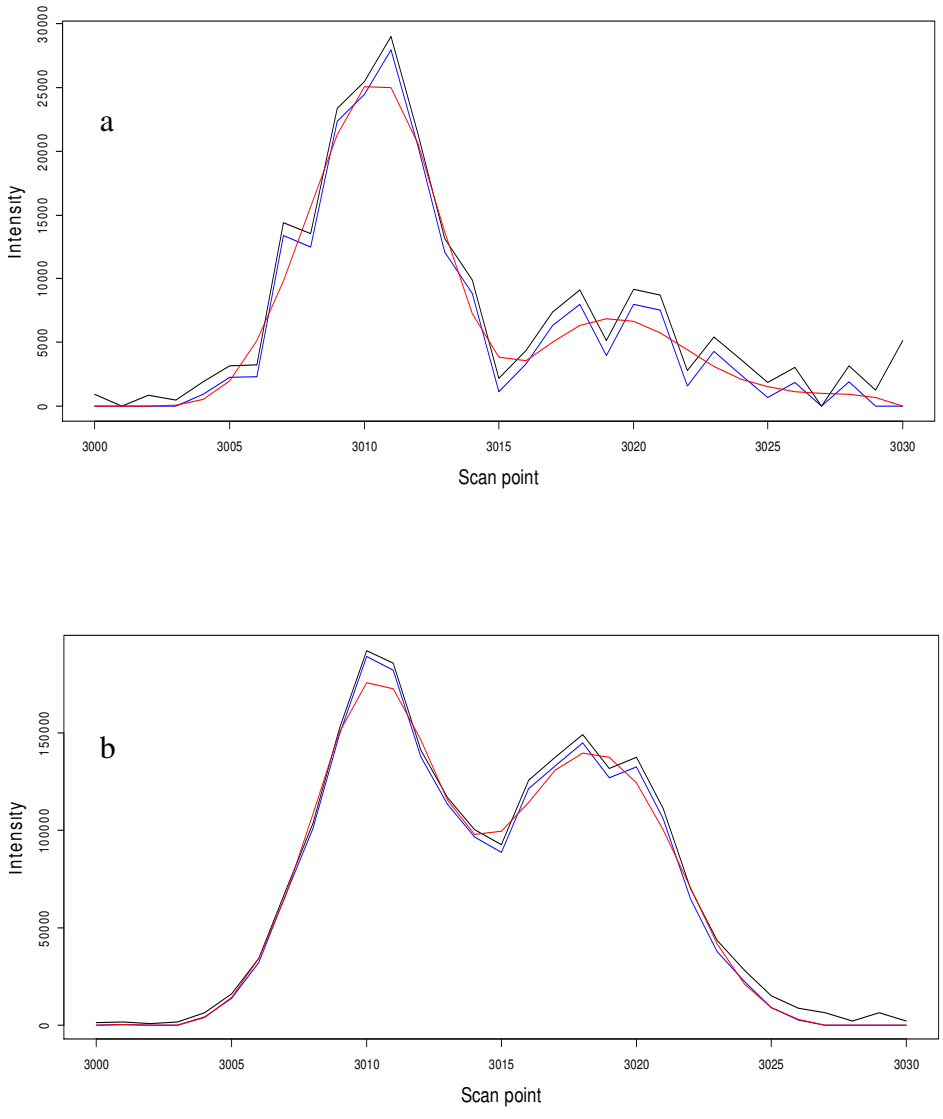


Figure 4-10 Baseline and noise correction. Figure a and b represent the intensities of two particles (m/z= 126 and m/z= 129 at row 87 and 90 of matrix X) changed along scan points. It can be noticed that original baseline-shifted and sharp curve was corrected into balaned and smooth curve. The pocedure was applied on all rows of matrix X.(black-original data from matrix X; blue-only baseline corrected data from matrix V; red-final baseline and noise corrected data from matrix W).

4.1.4 Determination of the chemical rank

Chemical rank is defined as the number of components in a co-eluted system (or a peak cluster of GC-MS data in this study). Theoretically, chemical rank equals to the rank of matrix from GC-MS data given absolutely zero error (effects of background and noise), which was unlikely possible for data collected in the laboratory.

Chemical rank determination is the first step in peak resolution. A simplest method is based on Singular Value Decomposition (SVD) of matrix. The procedure starts to give the objective matrix an assumption of the chemical rank. Usually the assumed chemical rank is larger than the reasonable value, in order not to lose useful information. For instance, 7 was an assumed chemical rank of matrix W (This assumption meant that peak cluster A contained 7 components, and the peak cluster A may not likely contain 7 components).

The top 7 largest eigenvalues from SVD of the first 3 rows of matrix W was obtained firstly, and then the second group of the 7 largest eigenvalues was obtained in the same way but from the first 4 rows of matrix W, and this procedure continued until all rows of matrix W were covered. In the end, these groups of 7 eigenvalues against scan points were line-plotted, as shown in Figure 4-11. Appendix D shows the scripts for doing these in R. The component emergence alone the eluting direction was read from the plot directly. The chemical rank of matrix W was 4 as indicated from the number of lines above zero level in Figure 4-11; however, final peak cluster A resolution based on 4 components lead similarity of mass spectra among 2 components, thus 3 was deemed as the optimized chemical rank.

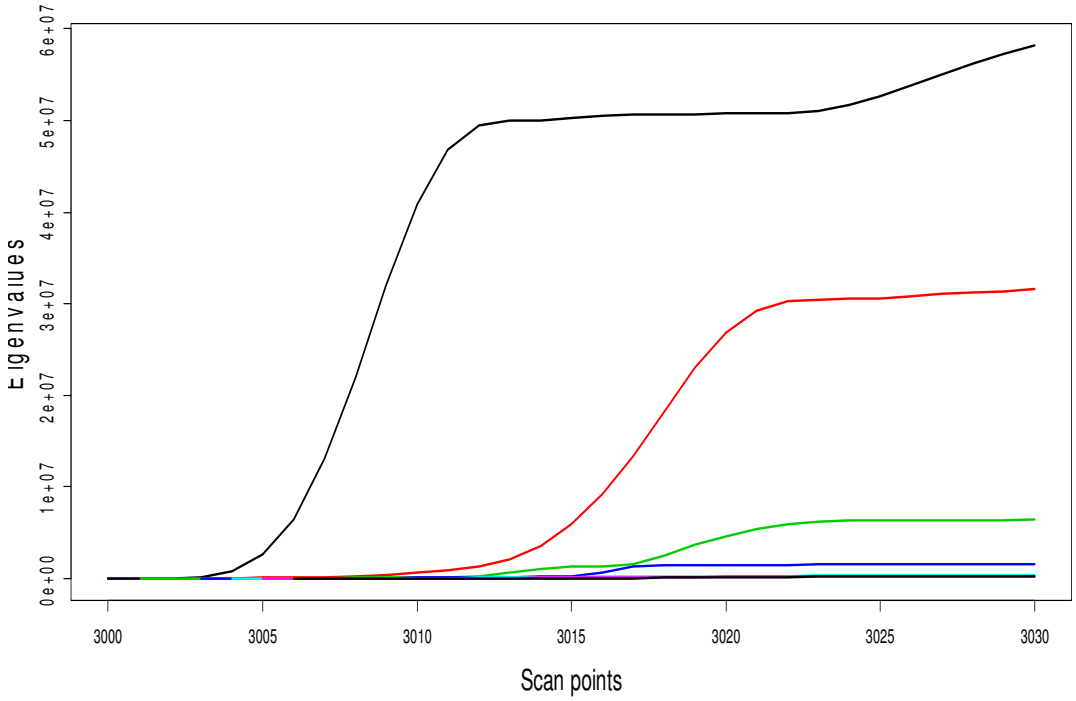


Figure 4-11 SVD eigenvalues plot for Chemical rank determination.

4.1.5 Peak resolution by MCR-ALS coupled with EFA

MCR-ALS: The theory of MCR-ALS and EFA are frequently documented in many chemometric publications, in order to ensure this submission logically fluent, they are briefly restated here.

Data pre-processing step can minimize, but not totally remove baseline and noise effects. The residual can be denoted as matrix **E** in Equation 3:

$$\mathbf{W} = \mathbf{C}\mathbf{S}^{\mathbf{T}} + \mathbf{E} \qquad \textit{Equation 3}$$

MCR-ALS takes **W** and an initial value (estimated **C** or **S**), for example an estimated **C** here in this study, and by repeated application of multiple least squares regression to produce an **S** that minimizing the residual **E.** In second round, the obtained **S** in turn serves as the initial value for MCR-ALS to produce a better estimated **C** which

will further serve as initial value for an even better estimated **S**. These steps alternate until no more improvement is made or desired number of iterations is reached (Wehrens, 2011).

However, there exist rotation matrixes **R** making the results ambiguous as *Equation 4* shows

$$\mathbf{W} = \mathbf{C}\mathbf{R}^{-1}\mathbf{R}\mathbf{S}^{\mathbf{T}} + \mathbf{E} \qquad \textit{Equation 4}$$

Adding constraints ensured a best suitable R that leads the concentration profile $\mathbf{C}\mathbf{R}^{-1}$ and spectra profile $\mathbf{R}\mathbf{S}^{\mathbf{T}}$ unique. In GC-MS data, constraints could be non-negativity of both concentration and spectra values, and unimodality of concentration profile (Jalali-Heravi et al., 2011; Wehrens, 2011).

The MCR-ALS (includes constraints adding) algorithm is incorporated in function als() from R package "ALS". The R script in Appendix E shows the processing of carrying out MCR-ALS on R.

Initial values for MCR-ALS: The initial value, either the estimated **C** or **S**, to start the MCR-ALS algorithm can be obtained by EFA (for the estimated **C**) or Orthogonal Projection Approach (OPA, for the estimated **S**). In this study, the estimated C was chosen as the initial value. The method of EFA is introduced in this section.

As shown in Figure 4-12, the EFA consisted of forward pass (A) and backward pass (B). Given the data matrix W and a relatively larger estimated number of components, for the forward pass, iteratively the singular values of sub matrices containing first two rows, three rows…and till the same rows of matrix W were calculated. The backward pass was similar but with the opposite iterative direction from the last two rows to the first row of matrix W. The estimated C was obtained by combining the singular values from both passes given an assumption that "the first compound to come up is also the first one to disappear"(Liang, 1996; Wehrens, 2011) . The coding for EFA in R is supplied in Appendix E.

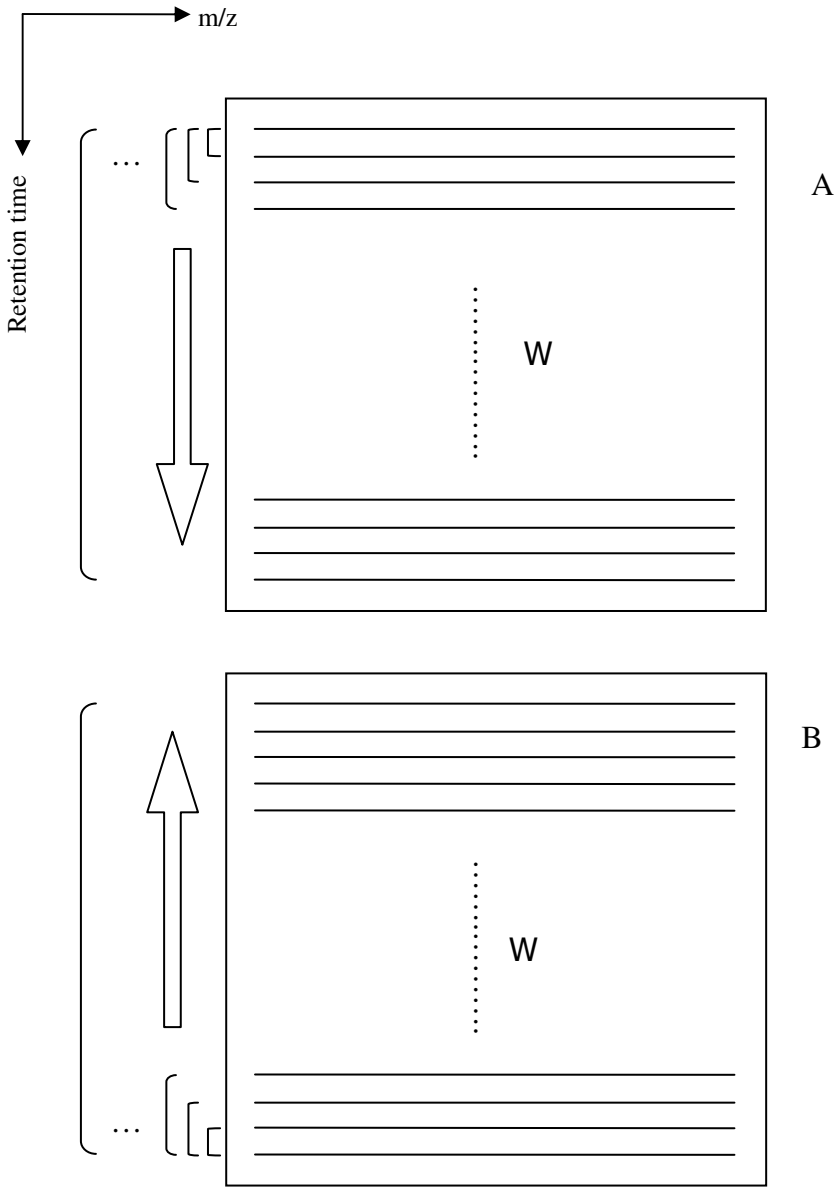
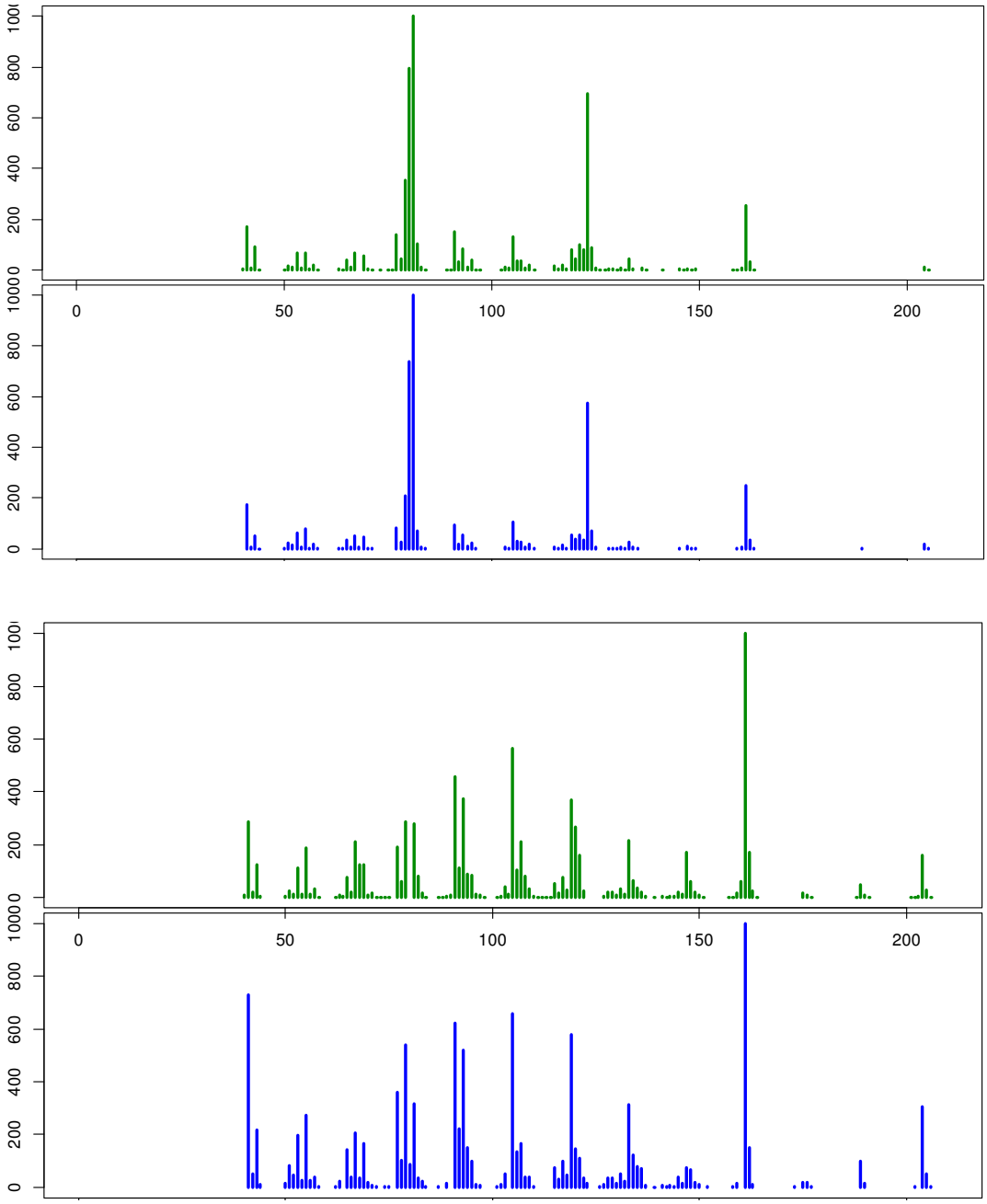


Figure 4-12 Illustration of EFA.

4.1.6 Identification of components

The three resolved mass spectra and their standard mass spectra from NIST Database are shown in Figure 4-13. The resolved mass spectra data was written into .MSP format files that can be recognized by NIST Database searching program (Appendix G). The resolved concentration profiles of peak cluster A is shown in Figure 4-14. (Appendix F).



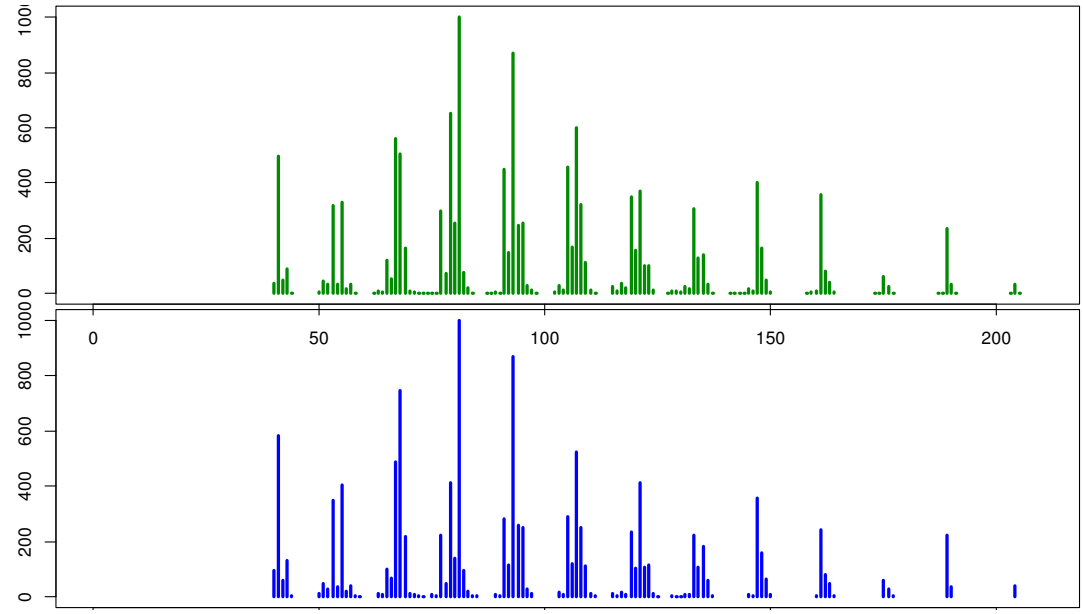


Figure 4-13 Three resolved mass spectra(green) and their standards from NIST Database(blue). m/z<40 of standards are omitted in plot, for GC-MS in selected ion monitoring (SIM) of m/z>=40. The high similarity between resolved mass spectra and standard mass spectra suggested the success of the resolution. Three compounds are β-Bourbonene(Mach 919; R.Match 922), γ -Muurolene(Match 886;R.Match 894) and β-Elemene (Match 920; R.Match 922). The second compound was not Azulene from Figure 4-4.

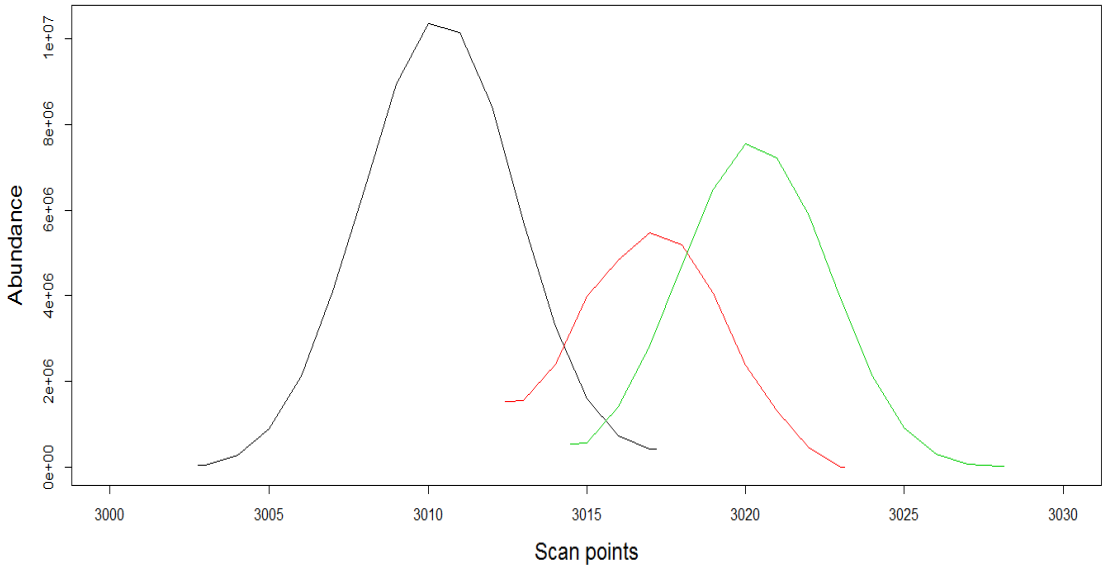


Figure 4-14 Resolved concentration profile of peak cluster A.

4.1.7 Quantitation of components

Quantitation analysis of each component was done by a technique called Overall Volume Integration (OVI) (Gong et al., 2001). After the peak cluster A being resolved into pure mass spectra and concentration profiles of three components, $\boldsymbol{c_a s_a^T}$ for ath ($1 \leq a \leq 3\ for\ peak\ cluster\ A$) component was known, and used as OVI value to get its ion chromatogram peak area (Appendix H) which was directly proportional to its mass.

Table 4-1 shows the relative mass of three components determined by methods of direct peak cluster split (Figure 4-6) and OVI.

Components	Peak cluster split	OVI
β-Bourbonene	1.00	0.94
γ-Muurolene	NA	0.32
β-Elemene	1.08	0.83

Table 4-1 Relative masses of determined components by different methods.Information of γ-Muurolene was not avaliable in direct peak cluster split.

4.2 A short summary

As shown in section 4.1, the method of MCR-ALS coupled with EFA were efficiently to resolve the overlapped GC-MS peak into profiles of each single component which was usually not accessible by traditional methods. It was also superior to *Priori* strategy (Figure 4-7) which was not always efficient and time consuming. For a faster peak resolving process, steps in section 4.1 were packed into functions determrank() and mresolution(), which can be found in Appendix I.

Chapter 5 VOLATILE COMPONENTS OF SAMPLES

Since the peak resolution method being chosen and coded in R, in this chapter, it was applied to GC-MS data of all samples to obtain the component profiles which are presented in tables under this chapter. The data analysis and discussion based on these are presented in next chapter.

Annotation:

a-Time unit is minute; **b**-The Kovats Retention Index (RI); **c**-Compounds identified by MS+RI or MS (match factor >800, while RI different from the NIST standard). For those unknowns that both MS and RI are not matching while their peaks in TIC are obvious and constantly appearing in three repeats. The compounds with long name (name 1~7) are placed below for aesthetic consideration. UNOP represents the unresolved overlapped peaks by chemometrics; **d**-Cas or NIST numbers of the identified compounds. The compounds present between two homogenized samples (Table 5-4, Table 5-5) are double underline marked; **e**-Mass proportion indicated by the ratio of integrated peak area to TIC total peak area. The values are means of three repeats. The top 10 values of each column are single underline marked; **f**-The days after twinges executed from plants. **g**- **C**oefficient of **V**ariation between three repeats, and they can be compared between components and samples for the unit effect are eliminated.

name 1:	1-Isopropyl-4-methylenebicyclo[3.1.0]hex-2-ene
name 2:	(3E,5E)-2,6-Dimethyl-1,3,5,7-octatetraene
name 3:	α-Methyl-α-[4-methyl-3-pentenyl]oxiranemethanol
name 4:	trans-5-Ethenyltetrahydro-α,α,5-trimethyl-2-furanmethanol
name 5:	5-Cyclopropylidene-1-pentanol
name 6:	1,1-Dimethyl-3-methylene-2-vinylcyclohexane
name 7:	(-)-trans-Pinocarvyl acetate
name 8:	1-Isopropyl-4,7-dimethyl-1,2,4a,5,6,8a-hexahydronaphthalene
name 9:	1-Isopropyl-4-methylenebicyclo[3.1.0]hex-2-ene
name 10:	trans-5-Ethenyltetrahydro-α,α,5-trimethyl-2-furanmethanol
name 11:	1,1-Dimethyl-3-methylene-2-vinylcyclohexane
name 12:	Bicyclo[3.1.1]hept-2-en-4-ol, 2,6,6-trimethyl-, acetate
name 13	4-Isopropyl-1,6-dimethyl-1,2,3,4,4a,7-hexahydronaphthalene
name 14:	1-Isopropyl-4,7-dimethyl-1,2,4a,5,6,8a-hexahydronaphthalene

Table 5-1 Volatile components of mairehau during dehydration.

No.	RT[a]	RI[b]	Compounds[c]	Cas No.[d]	Formula	%[e]							
						1[f]	2	5	7	9	11	13	15
1	3.36	654	1-Penten-3-ol	616-25-1	C5H10O	~	~	~	~	~	0.15	0.09	~
2	3.62	695	Unknown	~	~	~	~	~	~	~	0.07	0.07	~
3	3.92	710	Acetoin	513-86-0	C4H8O2	~	~	~	~	~	0.17	0.17	0.18
4	6.49	802	Hexanal	66-25-1	C6H12O	~	~	~	~	0.08	0.23	0.11	~
5	8.33	856	trans-2-Hexenal	6728-26-3	C6H10O	~	~	~	~	0.18	0.98	0.40	0.07
6	8.44	859	trans-3-Hexenol	928-97-2	C6H12O	~	~	~	~	0.03	~	~	~
7	8.80	870	trans-2-Hexenol	928-95-0	C6H12O	~	~	~	~	~	0.08	0.04	~
8	8.88	872	1-Hexanol	111-27-3	C6H14O	~	~	~	0.15	0.08	0.14	0.07	0.03
9	9.32	885	4-Penten-1-ol acetate	1576-85-8	C7H12O2	~	~	~	~	~	~	0.06	~
10	11.05	933	1R-α-Pinene	7785-70-8	C10H16	5.46	3.12	3.71	3.72	4.09	3.26	3.80	3.90
11	11.65	951	Camphene	79-92-5	C10H16	~	~	~	0.10	0.08	0.09	0.11	0.06
12	11.81	955	name 1	36262-09-6	C10H14	~	~	~	0.11	0.07	0.06	0.07	0.05
13	12.07	963	name 2	460-01-5	C10H14	0.52	~	0.69	0.85	0.84	0.80	0.94	0.64
14	12.45	973	Sabinene	3387-41-5	C10H16	0.77	~	0.54	0.60	0.72	0.67	0.83	0.68
15	12.64	979	(-)-β-Pinene	18172-67-3	C10H16	~	~	~	~	0.07	0.05	0.07	0.05

16	12.98	989	β-Pinene	127-91-3	C10H16	5.54	2.58	8.12	10.11	11.32	12.57	13.86	12.48
17	13.53	1004	Unknown	~	~	~	~	~	~	~	0.09	0.08	0.06
18	13.58	1006	cis-3-Hexenyl Acetate	3681-71-8	C8H14O2	~	~	~	0.52	~	~	~	~
19	13.60	1007	α-Phellandrene	99-83-2	C10H16	~	~	~	~	~	0.15	0.12	0.11
20	13.68	1009	3-Carene	13466-78-9	C10H16	5.15	1.06	~	~	~	0.30	0.42	0.27
21	14.23	1026	o,m or p-Cymene	NA	C10H14	~	~	~	~	0.04	0.05	0.07	0.06
22	14.38	1031	D-Limonene	5989-27-5	C10H16	0.82	0.75	1.47	1.75	1.84	1.96	2.32	2.26
23	14.45	1033	β-Phellandrene	555-10-2	C10H16	3.01	1.54	3.45	4.00	4.30	4.60	5.31	5.15
24	14.55	1036	Eucalyptol	470-82-6	C10H18O	0.58	~	~	0.14	0.10	0.12	0.16	0.07
25	14.90	1047	β-cis-Ocimene	3338-55-4	C10H16	0.39	0.45	1.33	1.79	1.60	1.95	2.20	2.08
26	15.34	1060	γ-Terpinene	99-85-4	C10H16	~	~	~	0.08	0.06	0.05	0.05	0.03
27	15.81	1074	name 3	132130 NIST	C10H18O2	1.58	1.25	3.21	3.69	3.00	2.63	2.20	1.57
28	15.84	1075	cis-Linalyl Oxide	5989-33-3	C10H18O2	~	~	~	1.08	0.93	~	~	~
29	16.24	1087	Terpinolene	586-62-9	C10H16	~	~	~	~	0.08	0.09	0.07	~
30	16.32	1090	name 4	34995-77-2	C10H18O2	0.45	~	1.05	1.44	1.34	0.89	0.70	0.47
31	16.41	1093	name 5	162377-97-1	C8H14O	0.32	~	0.86	1.63	1.20	0.87	1.12	0.78
32	16.70	1101	Linalool	78-70-6	C10H18O	2.13	1.25	13.88	25.96	16.51	9.38	5.92	3.38
33	16.84	1106	Unknown	~	~	~	1.26	0.66	0.16	0.12	0.17	0.09	0.08

34	17.05	1113	name 6	95452-08-7	C11H18	3.30	13.63	2.67	0.14	0.09	0.10	0.09	0.08
35	17.17	1117	Unknown	~	~	~	~	~	~	0.05	0.06	0.07	0.05
36	17.81	1138	cis-Limonene oxide	4680-24-4	C10H16O	~	~	~	~	0.04	~	0.05	0.03
37	17.93	1142	Limonene epoxide	1195-92-2	C10H16O	~	~	~	0.57	0.37	0.28	0.37	0.29
38	18.14	1149	L-pinocarveol	547-61-5	C10H16O	13.54	5.83	5.25	2.64	1.38	0.87	0.63	0.35
39	18.81	1170	Pinocarvone	30460-92-5	C10H14O	0.41	~	~	~	0.08	0.07	0.06	~
40	18.86	1172	Unknown	~	~	~	~	~	~	0.02	0.06	0.03	~
41	19.65	1198	Dodecane	112-40-3	C12H26	0.55	1.41	~	0.05	~	~	~	~
42	19.75	1201	α-Terpineol	10482-56-1	C10H18O	~	~	~	0.36	0.31	0.24	0.19	0.14
43	19.82	1203	Myrtenol	515-00-4	C10H16O	2.86	1.23	0.69	0.53	0.38	0.32	0.36	0.29
44	19.94	1208	Decanal	112-31-2	C10H20O	~	3.74	2.02	0.22	0.09	0.25	0.16	0.13
45	20.74	1236	Unknown	~	~	0.38	~	~	0.10	0.09	0.07	0.10	0.10
46	22.08	1283	Unknown	~	~	~	~	~	0.27	0.31	0.17	0.11	0.08
47	22.13	1285	Unknown	~	~	~	~	~	~	~	~	0.03	0.03
48	22.29	1291	Unknown	~	~	~	~	~	0.08	0.05	~	~	~
49	22.36	1293	Unknown	~	~	~	~	~	0.18	0.18	0.11	0.12	0.12
50	22.59	1301	name 7	149857 NIST	C12H18O2	5.87	2.99	11.75	19.41	17.74	13.46	13.56	15.42
51	22.70	1305	Unknown	~	~	1.60	~	~	~	~	~	~	~

52	22.84	1310	Unknown	~	~	~	~	~	~	0.08	0.09	0.09	0.11
53	23.35	1329	Myrtenyl acetate	1079-01-2	~	3.37	1.82	7.33	12.27	11.83	9.51	9.11	10.76
54	24.69	1378	Geraniol acetate	16409-44-2	C12H20O2	~	~	~	~	~	~	~	0.06
55	24.72	1379	Unknown	~	~	~	~	~	~	0.09	0.18	0.14	0.06
56	24.80	1382	Copaene	3856-25-5	C15H24	~	~	~	~	~	~	~	0.05
57	25.04	1391	β-Bourbonene	5208-59-3	C15H24	0.72	~	~	~	0.05	0.07	0.12	0.28
58	25.11	1393	β-Cubebene	13744-15-5	C15H24	0.58	~	~	~	0.03	0.05	0.06	0.10
59	25.21	1397	Tetradecane	629-59-4	C14H30	0.90	2.07	0.60	~	0.03	0.03	~	~
60	25.66	1415	α-Gurjunene	489-40-7	C15H24	1.12	~	~	~	~	~	~	~
61	26.01	1429	Caryophyllene	87-44-5	C15H24	1.03	~	~	~	0.05	0.07	0.10	0.17
62	26.63	1453	β-Farnesene	18794-84-8	C15H24	22.03	24.81	15.25	3.93	16.43	29.22	30.61	34.07
63	26.94	1465	Unknown	~	~	0.46	~	~	~	~	~	0.04	0.07
64	27.57	1490	Germacrene D	23986-74-5	C15H24	8.43	4.33	1.10	0.19	0.60	1.04	1.33	1.65
65	27.81	1499	Varidiflorene	21747-46-6	C15H24	0.70	~	~	~	~	0.04	0.04	~
66	27.95	1505	Elixene	3242-08-8	C15H24	1.81	0.99	~	~	0.10	0.14	0.17	0.18
67	28.35	1520	Unknown	~	~	~	1.09	~	~	0.08	0.11	0.13	0.15
68	28.42	1523	Unknown	~	~	~	0.91	~	~	~	0.05	0.05	0.05
69	31.35	1649	Unknown	~	~	~	~	~	~	~	~	0.03	0.03

70	36.51	1889	Unknown	~	~	~	~	~	~	~	~	0.06	0.05
71	39.40	2033	Manoyl oxide	596-84-9	C20H34O	3.61	21.90	14.39	1.18	0.76	0.71	0.47	0.55

Table 5-2 Volatile components of homogenized dry mairehau.

No.	RT	RI	Compounds	Cas No.	Formula	%	CV[g]
1	3.98	712	Acetoin	513-86-0	C4H8O2	0.003	0.06
2	5.37	762	Toluene	108-88-3	C7H8	0.002	0.05
3	6.49	802	Hexanal	66-25-1	C6H12O	0.010	0.11
4	6.77	810	Unknown	~	~	0.002	0.11
5	9.18	880	Unknown	~	~	0.003	0.08
6	9.22	881	1-(1-Methyl-2-cyclopenten-1-yl)ethanone	68752-16-9	C8H12O	0.003	0.03
7	9.77	897	Nonane	111-84-2	C9H20	0.002	0.07
8	9.97	902	Unknown	~	~	0.002	0.08
9	10.01	903	Unknown	~	~	0.002	0.04
10	10.57	919	Unknown	~	~	0.008	0.05
11	10.65	922	Unknown	~	~	0.002	0.18
12	10.76	925	α-Thujene	2867-05-2	C10H16	0.010	0.02
13	11.04	933	1R-α-Pinene	7785-70-8	C10H16	1.374	0.05
14	11.58	948	d-Camphene	5794-03-6	C10H16	0.172	0.06
15	11.64	950	Camphene	79-92-5	C10H16	0.068	0.07

16	11.79	954	1-Isopropyl-4-methylenebicyclo[3.1.0]hex-2-ene	36262-09-6	C10H14	0.057	0.04
17	12.07	962	(3E,5E)-2,6-Dimethyl-1,3,5,7-octatetraene	460-01-5	C10H14	0.157	0.03
18	12.44	973	Sabinene	3387-41-5	C10H16	0.382	0.06
19	12.63	979	(-)-β-Pinene	18172-67-3	C10H16	0.015	0.14
20	12.98	989	β-Pinene	127-91-3	C10H16	1.861	0.02
21	13.52	1004	psi.-Limonene	499-97-8	C10H16	0.009	0.19
22	13.56	1006	2,3-Epoxycarane, (E)-	20053-58-1	C10H16O	0.003	0.14
23	13.68	1009	3-Carene	13466-78-9	C10H16	0.608	0.07
24	13.95	1017	α-Terpinene	99-86-5	C10H16	0.004	0.05
25	14.06	1021	o-Cymene	527-84-4	C10H14	0.027	0.06
26	14.24	1026	m-Cymene	535-77-3	C10H14	0.072	0.06
27	14.39	1031	Limonene	138-86-3	C10H16	0.494	0.05
28	14.45	1033	β-Phellandrene	555-10-2	C10H16	1.497	0.06
29	14.58	1037	Eucalyptol	470-82-6	C10H18O	0.344	0.05
30	14.9	1046	β-cis-Ocimene	3338-55-4	C10H16	0.055	0.06
31	15.34	1060	γ-Terpinene	99-85-4	C10H16	0.012	0.05
32	15.86	1076	cis-β-Terpineol	7299-41-4	C10H18O	0.070	0.05
33	16.23	1087	Terpinolene	586-62-9	C10H16	0.004	0.40

34	16.4	1092	Unknown	~	~	0.023	0.15
35	16.45	1094	5-Cyclopropylidene-1-pentanol	162377-97-1	C8H14O	0.144	0.03
36	16.72	1102	Linalool	78-70-6	C10H18O	0.248	0.23
37	16.88	1107	Unknown	~	~	0.014	0.05
38	17.04	1113	1,1-Dimethyl-3-methylene-2-vinylcyclohexane	95452-08-7	C11H18	0.309	0.06
39	17.2	1118	Unknown	~	~	0.009	0.09
40	17.83	1138	Unknown	~	~	0.011	0.07
41	17.97	1143	trans-Limonene oxide	6909-30-4	C10H16O	0.038	0.09
42	18.06	1146	1-Methyl-5,6-divinyl-1-cyclohexene	61141-78-4	C11H16	0.079	0.14
43	18.17	1150	L-pinocarveol	547-61-5	C10H16O	0.474	0.07
44	18.31	1154	Unknown	~	~	0.008	0.07
45	18.84	1171	Pinocarvone	30460-92-5	C10H14O	0.030	0.06
46	19.15~19.44		UNOP	~	~	0.008	0.07
47	19.85	1205	Myrtenol	515-00-4	C10H16O	1.124	0.07
48	20.68	1234	Unknown	~	~	0.030	0.10
49	20.76	1237	Unknown	~	~	0.650	0.06
50	20.99~21.58		UNOP	~	~	0.231	0.07
51	22.18	1287	Unknown	~	~	0.406	0.06

52	22.32	1292	1,7,7-Trimethylbicyclo[2.2.1]hept-2-yl acetate	92618-89-8	C12H20O2	0.328	0.12
53	22.43	1296	Unknown	~	~	1.006	0.08
54	22.7	1305	(-)-trans-Pinocarvyl acetate	149857NIST	C12H18O2	28.225	0.06
55	22.87	1312	Unknown	~	~	0.150	0.31
56	23	1316	Unknown	~	~	0.128	0.08
57	23.44	1332	(-)-Myrtenyl acetate	149856NIST	C12H18O2	20.087	0.07
58	23.58	1338	cis-Carvyl Acetate	1205-42-1	C12H18O2	0.111	0.06
59	23.98	1352	α-Cubebene	17699-14-8	C15H24	0.083	0.04
60	24.67	1377	(+)-Cyclosativene	22469-52-9	C15H24	0.404	0.06
61	24.81	1382	Copaene	3856-25-5	C15H24	1.172	0.06
62	24.93	1387	Unknown	~	~	0.016	0.10
63	25.05	1391	β-Bourbonene	5208-59-3	C15H24	0.975	0.08
64	25.11	1393	β-Cubebene	13744-15-5	C15H24	0.333	0.04
65	25.14	1395	β-Elemene	515-13-9	C15H24	0.908	0.01
66	25.29	1400	Unknown	~	~	0.014	0.10
67	25.44	1406	(+)-Sativene	3650-28-0	C15H24	0.010	0.08
68	25.52	1409	(Z)-β-Farnesene	28973-97-9	C15H24	0.013	0.31
69	25.67	1415	α-Gurjunene	489-40-7	C15H24	0.617	0.74

70	25.95	1426	Ylangene	14912-44-8	C15H24	0.108	0.05
71	26.03	1429	Caryophyllene	87-44-5	C15H24	0.428	0.19
72	26.26	1438	Unknown	~	~	0.193	0.04
73	26.64	1453	β-Farnesene	18794-84-8	C15H24	5.404	0.63
74	26.96	1466	α-Caryophyllene	6753-98-6	C15H24	0.044	0.47
75	27.1	1471	(-)-Alloaromadendrene	25246-27-9	C15H24	8.413	0.08
76	27.37	1482	γ-Muurolene	30021-74-0	C15H24	0.709	0.00
77	27.59	1491	Germacrene D	23986-74-5	C15H24	1.211	0.74
78	27.8	1499	β-Chamigrene	18431-82-8	C15H24	0.074	0.08
79	27.97	1506	α-Muurolene	31983-22-9	C15H24	1.310	0.11
80	28.08	1510	γ-Cadinene	39029-41-9	C15H24	6.293	0.04
81	28.37	1523	(+)-Epi-bicyclosesquiphellandrene	54324-03-7	C15H24	8.441	0.10
82	28.44	1525	Unknown	~	~	0.188	1.10
83	28.55	1530	L-calamenene	483-77-2	C15H22	0.292	0.06
84	28.81	1541	Unknown	~	C15H24	0.061	0.06
85	28.91	1545	Name 8	24406-05-1	C15H24	0.186	0.33
86	29.29	1561	Unknown	~	~	0.043	0.03
87	29.38	1565	Isoaromadendrene epoxide	159366NIST	C15H24O	0.048	0.09

88	29.46	1568	Unknown	~	~	0.022	0.20
89	29.58	1573	α-Calacorene	293023NIST	C15H20	0.019	0.02
90	29.83	1583	Unknown	~	~	0.118	0.02
91	29.95	1588	Germacrene D-4-ol	72120-50-4	C15H26O	0.076	0.58
92	30.01	1591	(-)-Spathulenol	77171-55-2	C15H24O	0.106	0.06
93	30.09~31.68		UNOP	~	~	0.352	0.03
94	31.8	1669	α-Cadinol	481-34-5	C15H26O	0.032	0.03
95	33.54~33.94		UNOP	~	~	0.038	0.09
96	39.41	2033	Manoyl oxide	596-84-9	C20H34O	0.044	0.08

Table 5-3 Volatile components of mairehau flower.

No.	RT	RI	Compounds	Cas No.	Formula	%	CV
1	11.04	933	1R-α-Pinene	7785-70-8	C10H16	0.277	0.23
2	12.97	988	β-Pinene	127-91-3	C10H16	0.548	0.36
3	13.28	997	Decane	124-18-5	C10H22	0.046	0.31
4	13.68	1009	3-Carene	13466-78-9	C10H16	0.164	0.09
5	14.36	1030	(+)-Sylvestrene	1461-27-4	C10H16	0.110	0.20
6	14.44	1032	β-Phellandrene	555-10-2	C10H16	0.123	0.20
7	14.54	1035	β-trans-Ocimene	3779-61-1	C10H16	0.142	0.35
8	14.89	1046	β-cis-Ocimene	3338-55-4	C10H16	1.695	0.46
9	15.79	1074	cis-Linalyl Oxide	5989-33-3	C10H18O2	0.088	0.11
10	16.22	1087	Terpinolene	586-62-9	C10H16	0.591	0.08
11	16.57	1098	Undecane	1120-21-4	C11H24	0.304	0.43
12	16.66	1100	Linalool	78-70-6	C10H18O	0.502	0.21
13	17.03	1112	1,1-Dimethyl-3-methylene-2-vinylcyclohexane	95452-08-7	C11H18	2.543	0.07
14	18.12	1148	L-pinocarveol	547-61-5	C10H16O	0.106	0.17
15	19.63	1197	Dodecane	112-40-3	C12H26	0.710	0.29
16	22.5	1298	Tridecane	629-50-5	C13H28	0.874	0.14

17	22.55	1300	(-)-trans-Pinocarvyl acetate	149857NIST	C12H18O2	0.439	0.19
18	23.32	1328	(-)-Myrtenyl acetate	149856NIST	C12H18O2	0.175	0.23
19	23.56	1337	Unknown	~	~	0.066	0.04
20	23.94	1351	α-Cubebene	17699-14-8	C15H24	0.067	0.05
21	24.78	1381	Copaene	3856-25-5	C15H24	1.854	0.08
22	25.02	1390	β-Bourbonene	5208-59-3	C15H24	1.039	0.08
23	25.08	1392	β-Cubebene	13744-15-5	C15H24	0.918	0.10
24	25.4	1404	Eugenol methyl ether	93-15-2	C11H14O2	0.390	0.31
25	25.58	1411	Unknown	~	~	0.203	0.23
26	25.65	1414	α-Gurjunene	489-40-7	C15H24	0.441	0.08
27	25.94	1426	Ylangene	14912-44-8	C15H24	0.147	0.06
28	26	1428	Caryophyllene	87-44-5	C15H24	0.506	0.02
29	26.25	1438	Unknown			0.146	0.04
30	26.61	1452	(Z)-β-Farnesene	28973-97-9	C15H24	49.350	0.06
31	26.93	1465	α-Caryophyllene	6753-98-6	C15H24	0.285	0.15
32	27.04	1469	(-)-Alloaromadendrene	25246-27-9	C15H24	3.196	0.01
33	27.56	1489	Germacrene D	23986-74-5	C15H24	17.000	0.00
34	27.79	1498	Varidiflorene	21747-46-6	C15H24	0.902	0.09
35	27.93	1504	Germacrene B	15423-57-1	C15H24	12.926	0.03
36	28.32	1520	Unknown	~	~	0.284	0.01

37	28.41	1524		(-)-β-Cadinene	523-47-7	C15H24	0.844	0.11

Table 5-4 Volatile components of fresh homogenized mairehau under room temperature.

No.	RT	RI	Compounds	Cas No.	Formula	%	CV
1	1.6		Methyl acetate	79-20-9	C3H6O2	0.041	0.03
2	2.24		Ethyl Acetate	141-78-6	C4H8O2	0.157	0.08
3	3.41	663	Ethyl acetone	107-87-9	C5H10O	0.220	0.05
4	4.68	737	Isopentyl alcohol	123-51-3	C5H12O	0.016	0.06
5	5.36	762	Toluene	108-88-3	C7H8	0.005	0.07
6	8.3	854	3-Hexen-1-ol	544-12-7	C6H12O	0.013	0.06
7	8.45	859	cis-3-Hexenol	928-96-1	C6H12O	1.091	0.01
8	8.83	869	trans-2-Hexenol	928-95-0	C6H12O	0.021	0.06
9	8.94	873	1-Hexanol	111-27-3	C6H14O	0.263	0.03
10	9.12	878	Cyclofenchene	488-97-1	C10H16	0.004	0.04
11	9.57	891	Styrene	100-42-5	C8H8	0.046	0.08
12	9.76	896	Nonane	111-84-2	C9H20	0.004	0.01
13	9.97	902	Bornylene	464-17-5	C10H16	0.003	0.11
14	10.55	919	Unknown	~	~	0.066	0.02
15	10.75	925	α-Thujene	2867-05-2	C10H16	0.012	0.03
16	11.03	933	1R-α-Pinene	7785-70-8	C10H16	3.378	0.03

17	11.57	948	d-Camphene	5794-03-6	C10H16	0.730	0.03
18	11.63	950	Camphene	79-92-5	C10H16	0.126	0.05
19	11.78	954	name 9	36262-09-6	C10H14	0.105	0.03
20	12.06	962	(3E,5E)-2,6-Dimethyl-1,3,5,7-octatetraene	460-01-5	C10H14	0.104	0.01
21	12.43	973	Sabinene	3387-41-5	C10H16	0.247	0.04
22	12.62	978	(-)-β-Pinene	18172-67-3	C10H16	0.045	0.05
23	12.96	988	β-Pinene	127-91-3	C10H16	1.231	0.02
24	13.5	1004	(4Z or 4E)-4-Hexenyl acetate	42125-17-7 or 72237-36-6	C8H14O2	0.026	0.12
25	13.6	1007	cis-3-Hexenyl Acetate	3681-71-8	C8H14O2	1.060	0.03
26	13.68	1009	3-Carene	13466-78-9	C10H16	1.700	0.02
27	13.83	1014	Hexyl acetate	142-92-7	C8H16O2	1.142	0.01
28	13.92	1017	(2E)-2-Hexenyl acetate	10094-40-3	C8H14O2	0.273	0.03
29	14.05	1020	m-Cymene	535-77-3	C10H14	0.060	0.01
30	14.24	1026	o-Cymene	527-84-4	C10H14	0.095	0.02
31	14.38	1031	Limonene	138-86-3	C10H16	0.397	0.02
32	14.45	1033	β-Phellandrene	555-10-2	C10H16	0.857	0.02
33	14.6	1037	Eucalyptol	470-82-6	C10H18O	0.999	0.02
34	14.9	1046	β-cis-Ocimene	3338-55-4	C10H16	0.045	0.03
35	15.17	1055	α-Phellandrene	99-83-2	C10H16	0.009	0.02
36	15.34	1060	γ-Terpinene	99-85-4	C10H16	0.056	0.00

37	15.82	1075	Unknown	~	~	0.028	0.06
38	15.87	1076	cis-Linalyl Oxide	5989-33-3	C10H18O2	0.144	0.00
39	15.98	1080	trans-p-Mentha-2,8-dienol	139653NIST	C10H16O	0.016	0.02
40	16.24	1087	Terpinolene	586-62-9	C10H16	0.063	0.03
41	16.4	1092	name 10	34995-77-2	C10H18O2	0.052	0.02
42	16.42	1093	Unknown	~	~	0.030	0.02
43	16.75	1103	Linalool	78-70-6	C10H18O	3.576	0.02
44	16.9	1108	cis-β-Terpineol	7299-41-4	C10H18O	0.023	0.02
45	17.05	1113	name 11	95452-08-7	C11H18	0.071	0.01
46	17.22	1119	4-t-Pentylcyclohexene	51874-62-5	C11H20	0.038	0.00
47	17.32	1122	4,7,7-Trimethylbicyclo[4.1.0]heptan-2-ol	6909-22-4	C10H18O	0.014	0.04
48	18.01	1144	2,5-Dimethyl-2-vinyl-4-hexen-1-ol	50598-21-5	C10H18O	0.015	0.02
49	18.06	1146	3-Methyl-3,4-divinyl-1-cyclohexene	61141-77-3	C11H16	0.036	0.05
50	18.25	1152	L-pinocarveol	547-61-5	C10H16O	15.706	0.02
51	18.34	1155	cis-Verbenol	18881-04-4	C10H16O	0.251	0.03
52	18.6	1164	Pinocarveol	5947-36-4	C10H16O	0.027	0.02
53	19.03	1178	Unknown	~	~	0.076	0.03
54	19.16	1182	Unknown	~	~	0.042	0.02
55	19.22~19.76		UNOP	~	~	0.395	0.00
56	19.93	1207	Myrtenol	515-00-4	C10H16O	11.350	0.00

57	20.78	1237	name 12	195660NIST	C12H18O2	0.939	0.00
58	21.18	1252	Unknown	~	~	0.159	0.01
59	21.26	1254	Unknown	~	~	0.082	0.02
60	21.4	1259	Unknown	~	~	0.149	0.01
61	21.82	1274	3-Caren-10-al	151860NIST	C10H14O	0.084	0.03
62	21.87	1276	cis-Myrtanol	292845NIST	C10H18O	0.039	0.01
63	22.27	1290	Unknown	~	~	2.040	0.03
64	22.35	1293	Bornyl acetate	76-49-3	C12H20O2	0.389	0.02
65	22.69	1305	(-)-trans-Pinocarvyl acetate	149857NIST	C12H18O2	17.390	0.01
66	22.85	1311	Myrcenylacetat	149845NIST	C12H20O2	0.082	0.00
67	23.01	1317	Unknown	~	~	0.052	0.00
68	23.39	1331	(-)-Myrtenyl acetate	149856NIST	C12H18O2	1.302	0.03
69	23.98	1352	α-Cubebene	17699-14-8	C15H24	0.036	0.02
70	24.68	1378	Isoledene	156108NIST	C15H24	0.032	0.01
71	24.82	1383	Copaene	3856-25-5	C15H24	0.233	0.02
72	25.06	1392	β-Bourbonene	5208-59-3	C15H24	0.837	0.01
73	25.13	1394	β-Elemene	515-13-9	C15H24	0.180	0.02
74	25.41	1405	Eugenol methyl ether	93-15-2	C11H14O2	0.052	0.04
75	25.53	1409	β-Farnesene	18794-84-8	C15H24	0.097	0.01
76	25.64	1414	Unknown	~	~	0.093	0.02

77	25.67	1415	α-Gurjunene	489-40-7	C15H24	0.374	0.01
78	25.73	1417	Unknown	~	~	0.099	0.02
79	25.97	1427	Unknown	~	~	0.450	0.01
80	26.27	1439	β-Cubebene	13744-15-5	C15H24	0.084	0.01
81	26.53	1449	(+)-Aromadendrene	489-39-4	C15H24	0.027	0.08
82	26.69	1455	(Z)-β-Farnesene	28973-97-9	C15H24	18.555	0.01
83	27.08	1470	(-)-Alloaromadendrene	25246-27-9	C15H24	1.213	0.01
84	27.39	1483	Unknown	~	~	0.303	0.01
85	27.6	1491	Germacrene D	23986-74-5	C15H24	1.383	0.01
86	27.83	1500	Varidiflorene	21747-46-6	C15H24	0.427	0.07
87	27.97	1506	α-Muurolene	31983-22-9	C15H24	1.523	0.00
88	28.07	1510	γ-Cadinene	39029-41-9	C15H24	1.005	0.01
89	28.36	1522	(+)-Epi-bicyclosesquiphellandrene	54324-03-7	C15H24	1.480	0.03
90	28.45	1526	(-)-β-Cadinene	523-47-7	C15H24	1.285	0.01
91	28.56	1530	L-calamenene	483-77-2	C15H22	0.047	0.02
92	28.82	1541	name 13	16728-99-7	C15H24	0.024	0.01
93	28.92	1545	name 14	24406-05-1	C15H24	0.064	0.01
94	29.3	1561	Unknown	~	~	0.084	0.03
95	29.38	1565	ñ-trans-Nerolidol	40716-66-3	C15H26O	0.052	0.02
96	30.02	1591	(-)-Spathulenol	77171-55-2	C15H24O	0.061	0.05

97	30.10~31.40		UNOP	~	~	0.074	0.03
98	31.49	1655	tau.-Cadinol	5937-11-1	C15H26O	0.054	0.05
99	31.54	1657	Unknown	~	~	0.050	0.02
100	31.81	1669	α-Cadinol	481-34-5	C15H26O	0.057	0.02
101	39.43	2034	Manoyl oxide	596-84-9	C20H34O	0.354	0.05

Table 5-5 Volatile components of homogenized fresh mairehau cooled by liquid Nitrogen.

No.	RT	RI	Compounds	Cas No.	Formula	%	CV
1	3.56	686	2-Ethylpentane	589-34-4	C7H16	0.033	0.19
2	3.62	695	2-Ethylfuran	3208-16-0	C6H8O	0.009	0.47
3	5.39	763	Toluene	108-88-3	C7H8	0.007	0.03
4	6.43	800	cis-3-Hexenal	6789-80-6	C6H10O	0.015	1.09
5	6.5	802	Hexanal	66-25-1	C6H12O	0.024	0.97
6	8.11	849	Unknown	~	~	0.003	0.65
7	8.34	855	trans-2-Hexenal	6728-26-3	C6H10O	0.106	0.71
8	8.44	858	cis-3-Hexenol	928-96-1	C6H12O	0.120	0.11
9	8.82	869	Unknown	~	~	0.012	0.17
10	8.94	873	1-Hexanol	111-27-3	C6H14O	0.036	0.07
11	9.18	880	Unknown	~	~	0.022	0.19
12	9.61	892	Unknown	~	~	0.006	0.12
13	10	903	Bornylene	464-17-5	C10H16	0.012	0.03
14	10.4	915	Unknown	~	~	0.012	0.02
15	10.57	919	Unknown	~	~	0.212	0.11

16	10.77	925	α-Thujene	2867-05-2	C10H16	0.054	0.07
17	11.07	934	1R-α-Pinene	7785-70-8	C10H16	8.853	0.09
18	11.6	949	d-Camphene	5794-03-6	C10H16	1.807	0.10
19	11.66	951	Camphene	79-92-5	C10H16	0.391	0.10
20	11.8	955	1-Isopropyl-4-methylenebicyclo[3.1.0]hex-2-ene	36262-09-6	C10H14	0.139	0.01
21	12.08	963	(3E,5E)-2,6-Dimethyl-1,3,5,7-octatetraene	460-01-5	C10H14	1.714	0.09
22	12.46	974	Sabinene	3387-41-5	C10H16	2.086	0.09
23	12.64	979	(-)-β-Pinene	18172-67-3	C10H16	0.140	0.03
24	12.8	983	Unknown	~	~	0.015	0.13
25	13.03	990	β-Pinene	127-91-3	C10H16	11.747	0.16
26	13.54	1005	Unknown	~	~	0.018	0.13
27	13.6	1007	cis-3-Hexenyl Acetate	3681-71-8	C8H14O2	0.162	0.04
28	13.7	1010	3-Carene	13466-78-9	C10H16	4.418	0.07
29	13.81	1013	Hexyl acetate	142-92-7	C8H16O2	0.037	0.27
30	13.96	1018	α-Terpinene	99-86-5	C10H16	0.025	0.03
31	14.07	1021	m-Cymene	535-77-3	C10H14	0.318	0.06
32	14.24	1026	o-Cymene	527-84-4	C10H14	0.345	0.08
33	14.41	1031	D-Limonene	5989-27-5	C10H16	3.675	0.10

34	14.48	1034	β-Phellandrene	555-10-2	C10H16	5.040	0.06
35	14.59	1037	Eucalyptol	470-82-6	C10H18O	1.545	0.13
36	14.91	1047	β-cis-Ocimene	3338-55-4	C10H16	1.687	0.08
37	15.17	1055	α-Phellandrene	99-83-2	C10H16	0.106	0.05
38	15.34	1060	γ-Terpinene	99-85-4	C10H16	0.065	0.09
39	15.61	1068	Unknown	~	~	0.009	0.15
40	15.85	1076	5-Isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol	15537-55-0	C10H18O	0.150	0.14
41	15.94	1078	Unknown	~	~	0.024	0.16
42	16.11	1083	Unknown	~	~	0.039	0.05
43	16.24	1087	Terpinolene	586-62-9	C10H16	0.868	0.05
44	16.44	1094	5-Cyclopropylidene-1-pentanol	162377-97-1	C8H14O	0.439	0.18
45	16.74	1103	Linalool	78-70-6	C10H18O	3.031	0.10
46	16.88	1107	cis-β-Terpineol	7299-41-4	C10H18O	0.029	0.11
47	17.05	1113	1,1-Dimethyl-3-methylene-2-vinylcyclohexane	95452-08-7	C11H18	0.076	0.09
48	17.19	1118	4-t-Pentylcyclohexene	51874-62-5	C11H20	0.051	0.10
49	17.6	1131	Unknown	~	~	0.131	0.38
50	17.63	1132	Unknown	~	~	0.034	0.22
51	17.84	1139	cis-Limonene oxide	4680-24-4	C10H16O	0.014	0.28

52	17.94	1142	Unknown	~	~	0.070	0.21
53	17.97	1143	trans-Limonene oxide	6909-30-4	C10H16O	0.089	0.14
54	18.05	1146	1-Methyl-5,6-divinyl-1-cyclohexene	61141-78-4	C11H16	0.024	0.13
55	18.17	1150	L-pinocarveol	547-61-5	C10H16O	0.632	0.30
56	18.3	1154	cis-Verbenol	18881-04-4	C10H16O	0.024	0.46
57	18.83	1171	Pinocarvone	30460-92-5	C10H14O	0.020	0.15
58	19.03	1178	4-Terpineol	562-74-3	C10H18O	0.014	0.03
59	19.26	1185	Unknown	~	~	0.041	0.20
60	19.41	1190	Unknown	~	~	0.053	0.15
61	19.85	1205	Myrtenol	515-00-4	C10H16O	1.229	0.36
62	20.76	1237	name 15	195660NIST	C12H18O2	0.571	0.01
63	21.04	1247	Unknown	~	~	0.008	0.01
64	21.17	1251	Unknown	~	~	0.145	0.05
65	21.24	1254	Unknown	~	~	0.072	0.02
66	21.32~21.57		UNOP	~	~	0.090	0.06
67	21.79	1273	Unknown	~	~	0.017	0.35
68	21.84	1275	Solanone	54868-48-3	C13H22O	0.009	0.06
69	22.04~22.50		UNOP	~	~	1.547	0.04

70	22.7	1305	(-)-trans-Pinocarvyl acetate	149857NIST	C12H18O2	22.148	0.05
71	22.86	1311	Unknown	~	~	0.188	0.01
72	23	1316	Unknown	~	~	0.076	0.02
73	23.44	1332	(-)-Myrtenyl acetate	149856NIST	C12H18O2	13.763	0.07
74	23.57	1337	Unknown	~	~	0.046	0.05
75	24.19	1360	Nerol acetate	141-12-8	C12H20O2	0.041	0.01
76	24.74	1380	Unknown	~	~	0.027	0.00
77	24.82	1383	Unknown	~	~	0.183	0.03
78	25.05	1391	β-Bourbonene	5208-59-3	C15H24	0.136	0.24
79	25.22	1397	Cuminyl acetate	59230-57-8	C12H16O2	0.014	0.18
80	25.4	1404	Eugenol methyl ether	93-15-2	C11H14O2	0.021	0.09
81	25.52	1409	β-Farnesene	18794-84-8	C15H24	0.007	0.88
82	25.63	1413	Unknown	~	~	1.836	0.11
83	25.96	1426	Unknown	~	~	0.068	0.29
84	26.27	1439	β-Cubebene	13744-15-5	C15H24	0.016	0.22
85	26.65	1454	(Z)-β-Farnesene	28973-97-9	C15H24	5.870	0.27
86	27.07	1470	(-)-Alloaromadendrene	25246-27-9	C15H24	0.093	0.29
87	27.58	1490	Germacrene D	23986-74-5	C15H24	0.258	0.31

88	27.82	1500	Varidiflorene	21747-46-6	C15H24	0.073	0.39
89	27.96	1505	Unknown	~	~	0.225	0.28
90	28.06	1510	γ-Cadinene	39029-41-9	C15H24	0.074	0.30
91	28.34	1521	(+)-Epi-bicyclosesquiphellandrene	54324-03-7	C15H24	0.111	0.32
92	28.43	1525	δ-Cadinene	483-76-1	C15H24	0.069	0.29
93	29.28	1560	Unknown	~	~	0.011	0.18
94	29.37	1564	Nerolidol	7212-44-4	C15H26O	0.006	0.09
95	29.95	1588	Germacrene D-4-ol	72120-50-4	C15H26O	0.027	0.01
96	36.52	1889	Unknown	~	~	0.006	0.45
97	39.4	2033	Manoyl oxide	596-84-9	C20H34O	0.046	0.23

Chapter 6 DATA ANALYSIS AND DISCUSSION

6.1 Studies on SPME variables

6.1.1 Comparison between SPME fibres

Usually, fibre type selection is the first critical step in an experiment involving SPME. Criteria for the selection include the fibre's ability to absorb polar or non-polar compounds, reproducibility, sampling types (field or in Lab), cost et al. For simplification, in this study, the selection of fibre was based on its reproducibility.

The reproducibility of three types of SPME fibres was examined by comparing CV (Coefficient of Variance = (standard deviation/mean)*100%). The CVs of SPME fibres based on the total peak areas of six repeats (section 3.2.1) were 3.8% (PDMS fibre), 10.3% (PDMS/DVB fibre) and 14.5% (DVB/CAR/PDMS fibre). PDMS fibre associated with better reproducibility and was chosen for following experiment.

6.1.2 SPME variables: Sample amount

The optimized sample amount for putting into 20ml headspace vials to maximum SPME adsorption varied, because the samples were different. However, the resulting data from section 3.2.2 showed that with sample (homogenized dry mairehau) amounts of 2.5g and 3.0g, the total peak areas were significantly similar. Thus, the increase in sample amount from 2.5g to 3.0g would not bring much extra improvement in PDMS fibre adsorption. It was assumed that for all samples which were contained in 20ml headspace vial, 3.0g was a suitable sample amount.

6.1.3 SPME variables: Time and Temperature

The effects of SPME time and temperature were analysed by regression. In the regression model, both time and temperature were predictors, and the response was the total peak area which indicated the adsorption amount on fibre.

The collected data from section 3.2.3 was presented in Table 6-1.

Time (min)	Total peak area (from GC-FID)	
	25°C	**35°C**
5	23422368	29570105
10	34287806	46197522
15	43463565	56736822
20	50655185	65714150
30	60780237	72291982
40	67244945	81953058
50	72574315	89750309
60	78343315	102111351
90	80977930	104884217

Table 6-1 Total peak areas of adsorptions under different time and temperature.

By fitted line plotting the data in Table 6-1 in Minitab, Figure 6-1 and Figure 6-2 were obtained:

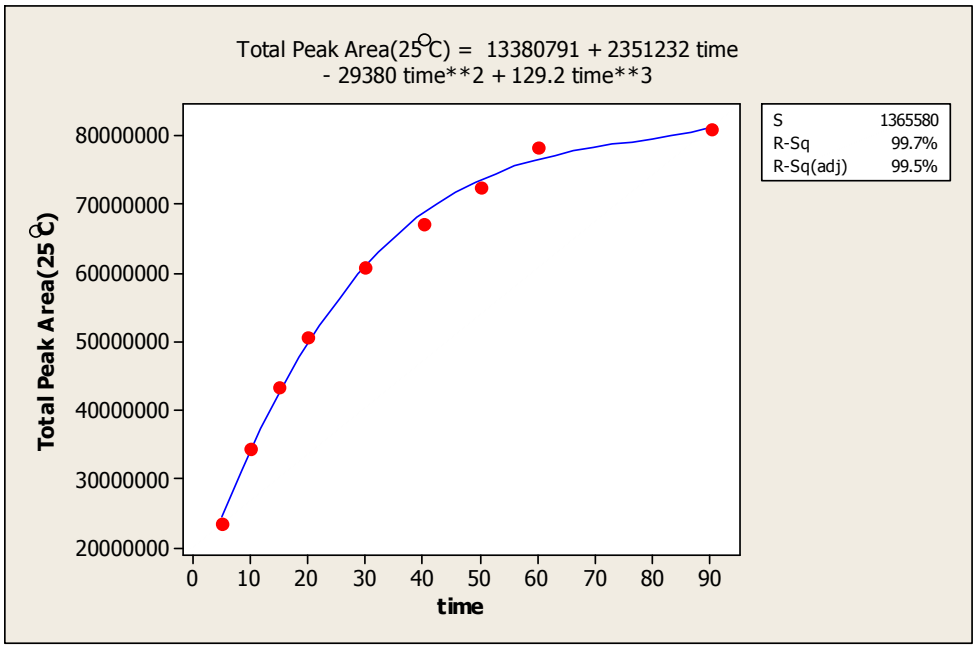


Figure 6-1 Fitted line plot at 25°C.

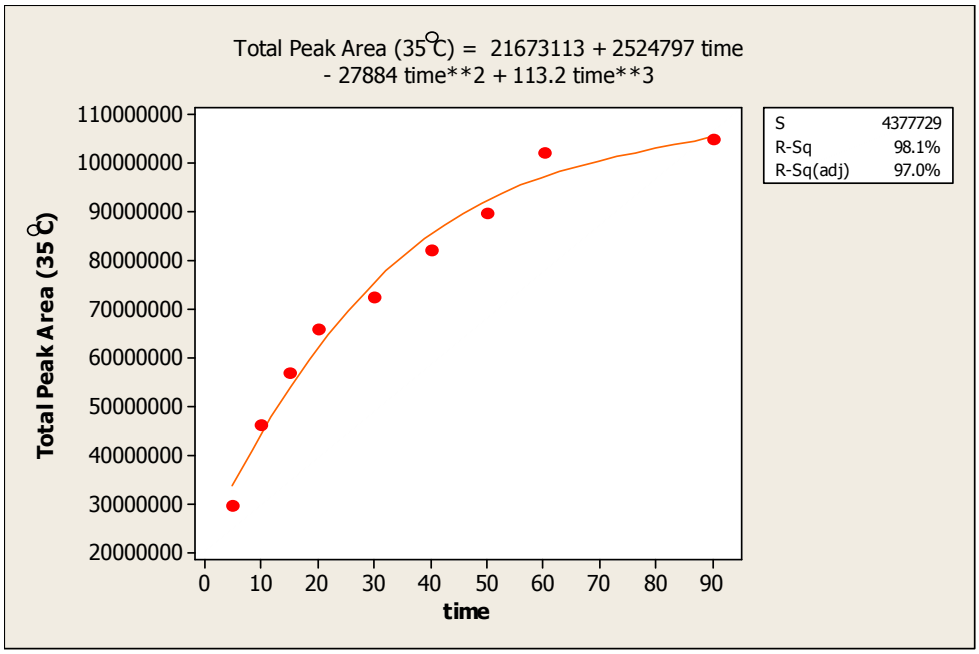


Figure 6-2 Fitted line plot at 35°C.

As shown in Figure 6-1and Figure 6-2, both at 25°C and 35°C, fibre adsorption amounts were cubic associated with time. The R^2 (coefficients of determination) were 99.5% and 97.0%, respectively. The appearances of the cubic regression lines agreed with the theoretical model of SPME adsorption (Pawliszyn, 1997).

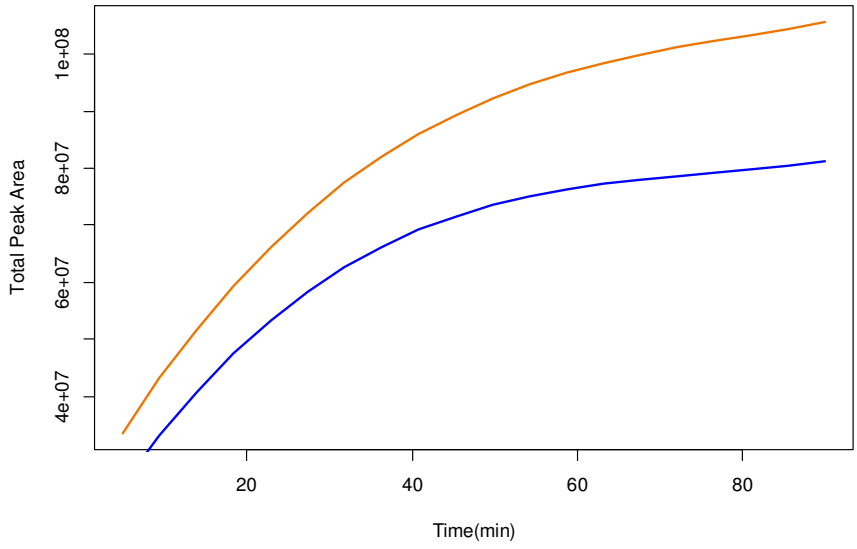


Figure 6-3 SPME fibre adsorption curves at 25°C(blue) and 35°C(Orange).

The adsorption models for 25°C and 35 °C were plotted together in Figure 6-3. It was interesting to notice that a higher temperature associated with a higher adsorption amount. However, this was true for only a relative low temperature, and when the temperature was high enough, the distribution constant between headspace and fibre decreased, causing a reducing in adsorption amount (Pawliszyn, 1997).

6.2 Volatiles of mairehau twigs during natural dehydration.

6.2.1 NoC and TPA

Dehydration has been reported to change the odor constitutes of plants, especially those edible plants such as fruit, vegetable and condiment. The plant odor constituents were from secondary metabolites (Sell, 2006). These volatile compounds could be changed both qualitatively and quantitatively by the changes in metabolic pathways and plant tissue structure, resulted from dehydration.

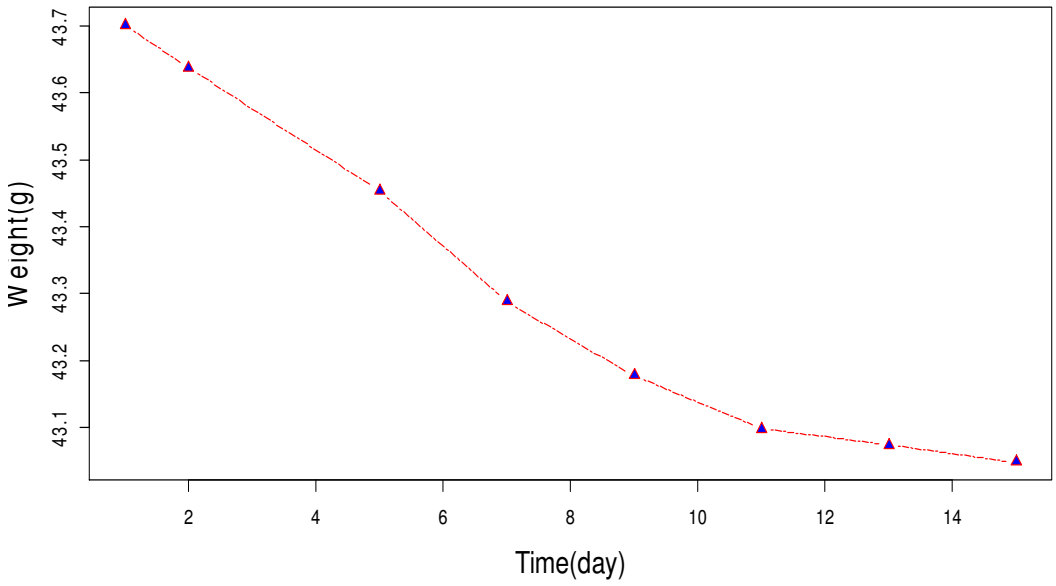


Figure 6-4 The weight of mairehau twigs (including the tubes) in a 15 days period after cutting from plant. Each value was the average of three twigs measured at the same day.

Based on the collected data of section 3.3.1, during the 15 days period after cutting from plants, the mairehau twigs showed a constant decrease in weight from day 1 to day 11 and a gentle dipping from day 11 to day 15(Figure 6-4). The weight lost can roughly be attributed to natural dehydration. From a simple sensory evaluation in this study (Using untrained humans to smell the sample), the odor of the mairehau twigs could be broadly described as "getting stronger".

To assess the change in volatile during the natural dehydration, Number of total detected volatile Components (NoC) and Total Peak Area (TPA) in Table 6-2 were plotted against the number of days (Figure 6-5).

Days	NoC	Scaled NoC	TPA	Scaled TPA
1	33	-0.57	468127803	-0.97
2	23	-1.22	108865367	-1.10
5	22	-1.29	184671096	-1.07
7	35	-0.44	1855995370	-0.47
9	51	0.59	5711207531	0.94
11	56	0.91	4661263263	0.56
13	61	1.24	5615376105	0.90
15	54	0.78	6469486043	1.21

Table 6-2 NoC and TPA of volatiles of mairehau twigs during 15 day period. Both NoCs and TPAs were z-score scaled to transform the means and variables to 0 and 1, while not changing the variability between observations. This was to facilitate the plot of two groups of data in one figure (Figure 6-5) for easily viewing and comparing their trends.

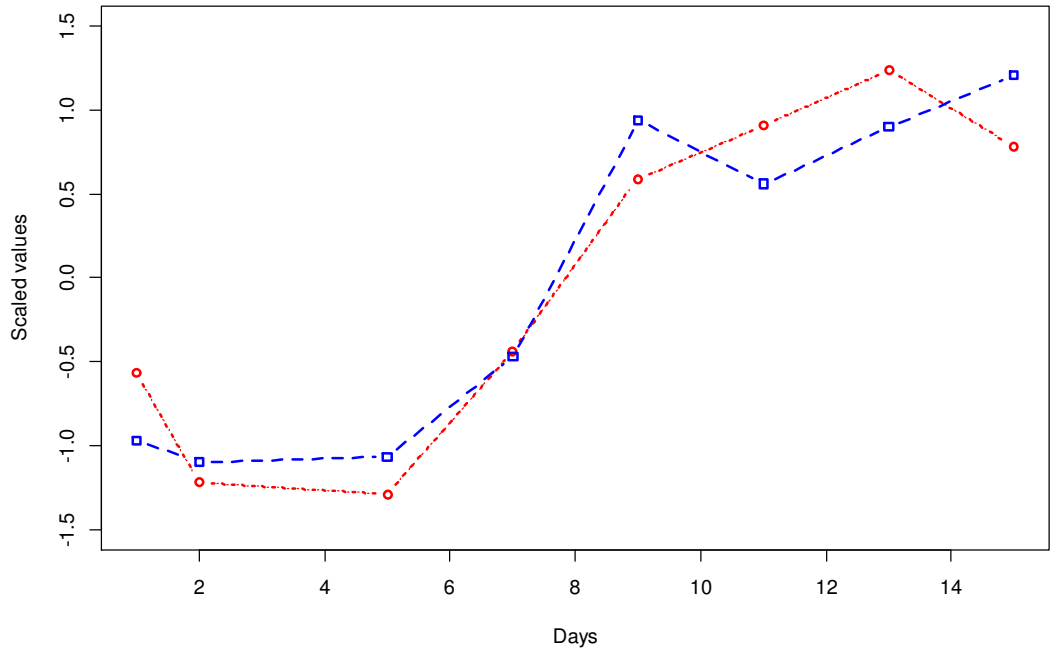


Figure 6-5 Plot of NoC and TPA against measuring days. Red- NoC; Blue- TPA.

As shown in Figure 6-5, NoC was decreased during the first five days followed by a steep increase to the maximum at day 13. Only 4 components, 1R-α-Pinene, β-Pinene, (-)-trans-Pinocarvyl acetate (name 7) and β-Farnesene were constantly among the top 10 values through the measuring days, in contrast, the rest of the top 10 values varied greatly (Table 5-1). Meanwhile, those gradually disappearing components during the first five days included 3-Carene, Eucalyptol, Pinocarvone, Dodecane, β-Bourbonene, β-Cubebene, α-Gurjunene, Caryophyllene, Varidiflorene, Elixene and two unknowns. The total mass percentage of these in day 1 was 13.85%, with only 3-Carene (5.51%) among the top 10 values. Most of the components that disappeared were those present with small amounts, partly causing a relative smaller variable in TPA during the same time span (some other components with increasing percentage may introduce counteract effect on TPA).

The TPA indicated the amount of injected components that were adsorbed on SPME fibre. This was positively related with the volatile concentration in headspace (Since 10 min of SPME fibre exposure time may not likely ensure the adsorption equilibrium). From Figure 6-5, it was concluded that the concentration of volatiles of mairehau twigs increased during the 15 days period, except a slightly decrease from day 1 to day 2. This would support the fact that the smell of mairehau generally became stronger because of dehydration going on during 15 days period. The abnormality of TPA at day 9 suggested a further measurement was necessary to distinguish whether it was from error or real concentration change.

6.2.2 Relationship between twigs weight and NoC (or TPA)

From Figure 6-4 and Figure 6-5, weight, NoC and TPA were almost linearly related with the number of days. A further plot of weight versus NoC is shown by Figure 6-6.

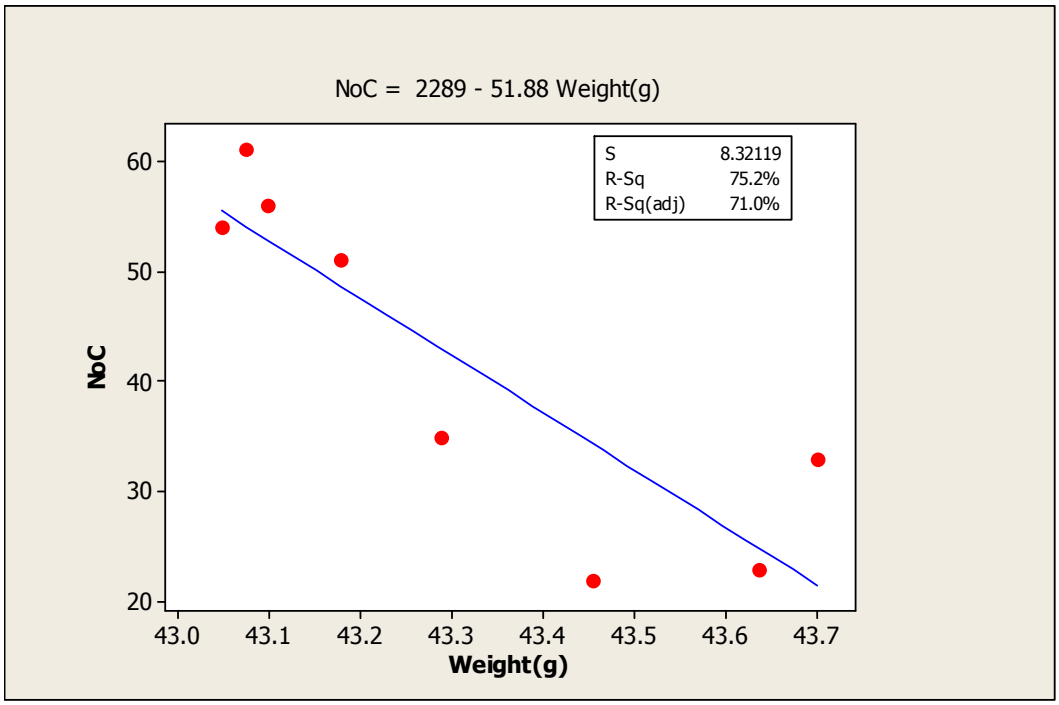


Figure 6-6 The regression plot of NoC versus mairehau twigs weight. A highly similar hehavior founding in NoC and TPA (Figure 6-5) suggested the similar information was carried by them, in addition, an uncertainity was shown at day 9 of TPA, thus it was wise to use only NoC not TPA to plot versus mairehau twigs weight.

As shown in Figure 6-6, generally as weight increased (meaning approaching to day 1), NoC decreased. A negative linear regression NoC=2289-51.88Weight (Shown by the blue line in Figure 6-6) can explain 75.2% of the relationship between the two.

6.2.3 Multivariate analysis of the mairehau twigs volatile

Principle Component Analysis (PCA) was carried out to assess the overall variability of all volatile components during the 15 day period. It should be pointed out that the value for PCA was the real component peak areas (z-score scaled) from GC-MS, rather than the percentage values from Table 5-1. This was valid because the same batch of mairehau twigs was used for all measuring days which enabled the inter-group comparison. In addition, during the 15 day period, the change in the percentage value of a component could be either resulted from its own behaviour, or the change in the other components. For carrying out PCA using Minitab software, the peak number (the first column of Table 5-1) was used as the component ID, for example point 62 was β-Farnesene which is in 62nd row. The bi-plot is shown in Figure 6-7.

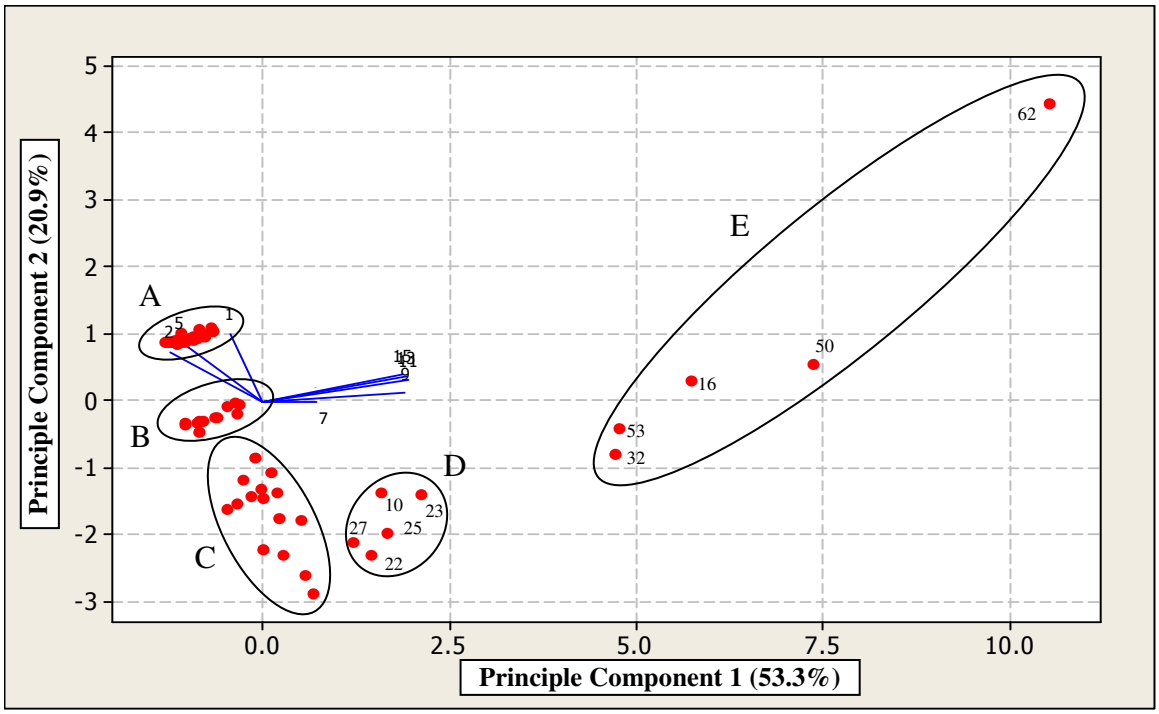


Figure 6-7 Bi-plot of PCA. cluster A,B,C were zoomed in by Figure 6-8.

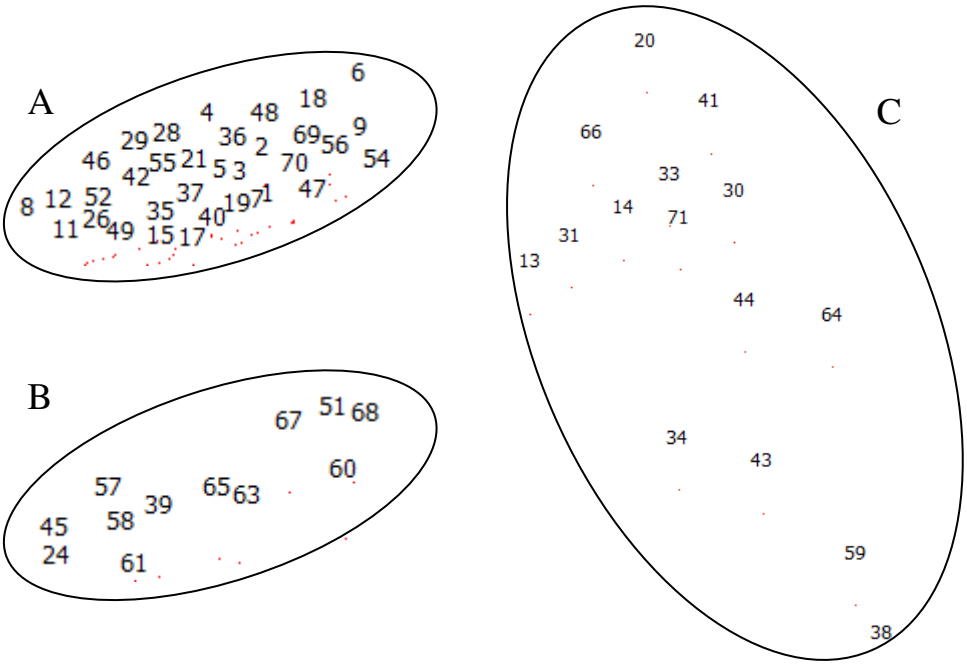


Figure 6-8 Zoomed in view of cluster A,B and C from Figure 6-7.

In Figure 6-7, the PC1 (principle component 1) and PC2 (principle component 2) explained 53.3% and 20.9% of total variation, respectively.

Loading: The blue lines, also called loadings, in Figure 6-7 were the projections of original variables (different measuring days) onto the new two dimensional coordinate system defined by PC1 and PC2. Since the loadings that were close to each other suggested their original variables essentially carried the similar information, the overall information was assigned into three directions determined by days 1, 2, 5 and days 9, 11, 13, 15 and day 7 (day 7 showed less significance to the PC1*PC2 system, because it was close to origin 0). It can be concluded that:

a) The volatile profiles were generally included two types. The first was shared among day 1, 2, 5 while the other was shared among day 9, 11, 13, 15;

b) The volatile profile of day 7 appeared to be an intermediate of those two types. It was reasonably to interpret that the change in metabolism during day 5 and day 9, caused the significant change of one volatile type into the other volatile type. Further sensory evaluation could be carried out.

Scores: The red points, also called scores, in Figure 6-7 were the projections of original peak areas of each compound onto the two dimensional coordinate system defined by PC1 and PC2. The original peak area of a compound was in an eight dimensional (corresponding to days 1, 2, 5, 7, 9, 11, 13 and 15) system which made spatial plotting impossible. By PCA, the system was reduced in dimension from eight to two to facilitate the plotting while retaining most of the information (PC1+PC2=74.2%), meanwhile, the pattern behind the original data was also easily observed. In Figure 6-7, the total volatile components were separated into five clusters. Within a cluster, the scores were close to each other, meaning the original observations were similar. Components from cluster A and B (total of 34 and 12 compounds respectively) were highly associated with days1, 2, 5, 7. Clusters D and E (total of 5 compounds for each) were associated with days 7, 9, 11, 13, 15. Other compounds from cluster C (total of 15 compounds) were associated with all measuring days, meaning they were the compounds that were less affected by dehydration from day1 to day 15.

6.3 Comparison in volatiles from different mairehau parts

Since the whole plant of mairehau being described as “highly aromatic” (Riley, 1994), in addition to the mairehau twigs, it was also essential to take the flower into observation (section 3.3.2). When combined with sensory evaluation in the future, the difference in volatile composition between mairehau twigs and flowers concluded from this study should help to identify the key components that are responsible for their characteristic fragrance notes. The mairehau twigs on day 1 (MTD1) were chosen as the counterpart of mairehau flowers in consideration of their equal freshness. The two volatile profiles reflected the original fragrant note before affected by dehydration or other processes.

6.3.1 Comparison in total number of compounds

A total of 33 and 37 compounds were found in volatiles of MTD1 and mairehau flowers, respectively. These two volatile types had 3 and 4 compounds unidentified, accounting for 2.44% and 0.7% of the mass. It thus can be concluded that, while containing more compounds, the volatiles of the mairehau flower was associated

with slightly higher certainty in mass, and low certainty in the number of compounds identified from this study.

6.3.2 Recurring compounds from two volatiles

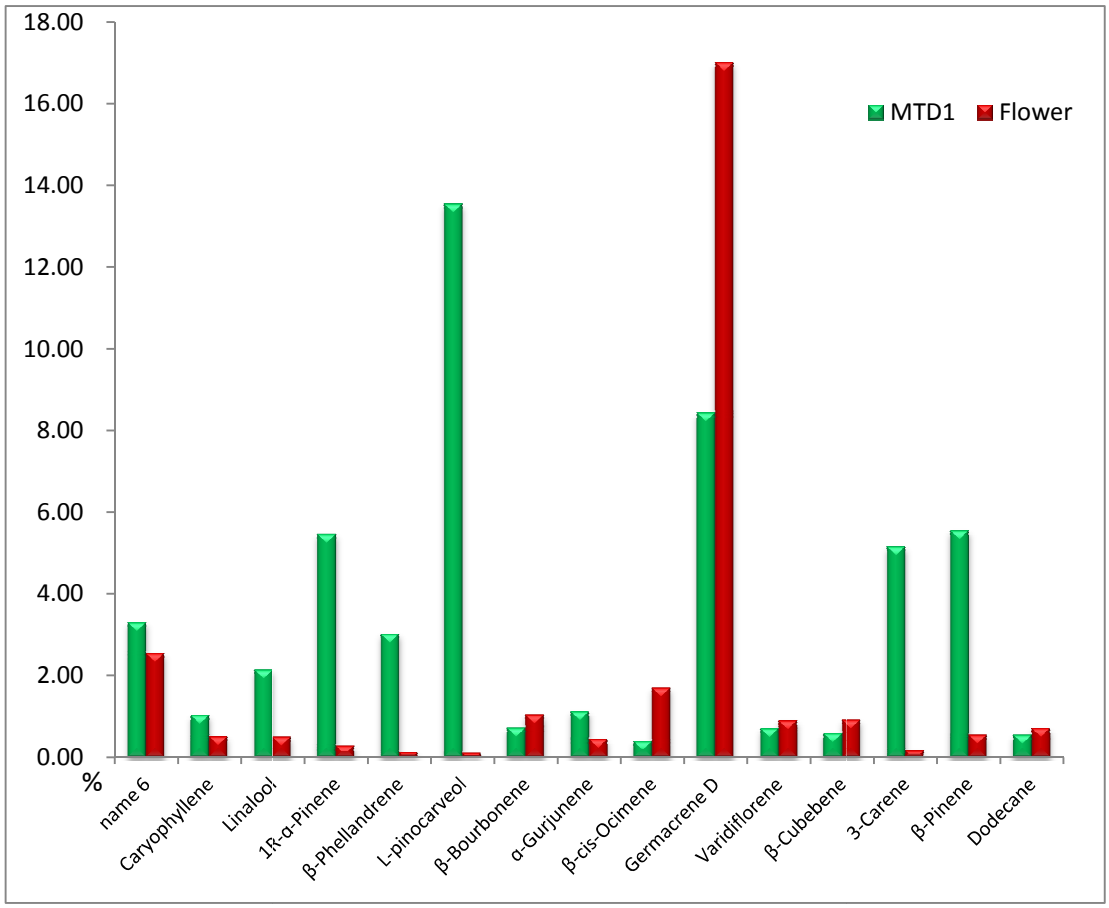


Figure 6-9 Compounds that were found both in MTD1 and mairehau flower. The height represented the mass percentage.

Figure 6-9 shows that a total of 15 compounds were found in both MTD1 and mairehau flower. These recurring compounds accounted for 51.66% and 27.47% of the volatile masses, thus the mass of origin-specific compounds were 49.34% and 72.53% in volatiles of MTD1 and mairehau flower, respectively.

Nine compounds (name 6, Caryophyllene, Linalool, 1R-α-Pinene, β-Phellandrene, L-pinocarveol, α-Gurjunene, 3-Carene, β-Pinene) were found with higher mass percentage in MTD1 than in mairehau flower. Among them, mass percentages of 1R-α-Pinene, β-Phellandrene, L-pinocarveol, 3-Carene and β-Pinene, were

dramatically higher. Among the remaining six compounds that were higher in mairehau flower, β -cis-Ocimene and Germacrene D were dramatically higher compared to those from MTD1.

6.3.3 Compounds with top 10 largest mass percentages

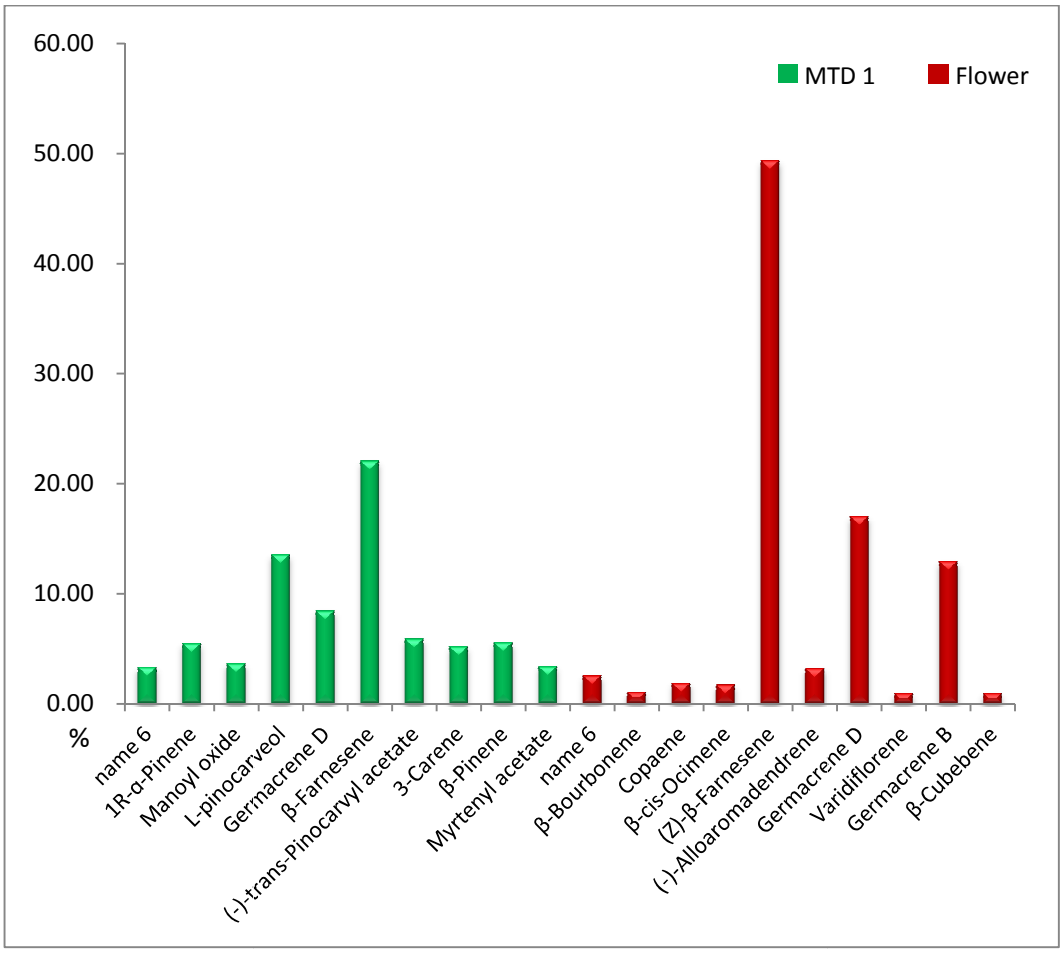


Figure 6-10 Top 10 compounds (mass percentage) in volatiles of MTD1 and mairehau flower.

Figure 6-10 shows the compounds with the top 10 largest mass percentages in volatiles of MTD1 and mairehau flower. Name 6 and Germacrene D were among them in both samples. The variable in percentage of the 10 from MTD1 (between 3.30% and 22.03%) was relatively smaller than those from mairehau flower (between 0.9% and 49.35%). These ten compounds accounted 76.3% and 91.42% in the total mass of the volatiles of MTD1 and mairehau flowers, respectively.

6.4 Volatiles of homogenized mairehau

The discussion in this section was based on data presented by Table 5-2, Table 5-4 and Table 5-5.

6.4.1 Efficiency of the selected chemometric method

The efficiency of MCR-ALS coupled with EFA peak resolution method can be assessed by the resolved concentration profiles. An example is shown in Figure 4-14 in which the resolved concentration profiles of components show nearly Gaussian curve, highly suggesting the real concentrations.

The method was effective in resolving overlapped peaks from relatively simple samples such as volatiles of mairehau twigs and flowers (Table 5-1, Table 5-3). Both the concentrations of the volatiles and number of compounds were relative low compared to those from homogenized mairehau.

For homogenized mairehau samples, MCR-ALS coupled with EFA peak resolution method was incapable for some elution periods (min): 19.15~19.44, 20.99~21.58, 30.09~31.68 and 33.54~33.94 from homogenized dried mairehau sample (Table 5-2), 19.22~19.76 and 30.10~31.40 from fresh homogenized mairehau sample (Table 5-4), 21.32~21.57 and 22.04~22.50 from fresh homogenized mairehau (Nitrogen cooled) sample (Table 5-5). The resolved concentration profiles of overlapped peaks during these elution periods did not give Gaussian curves, suggesting a failure in peak resolution.

The failure in peak resolution could result from both the sample and EFA. Firstly, the high complexity of the homogenized mairehau samples would cause severe overlap of several peaks (usually ≥5), and the zero component areas were not seen on the two sides of the peak cluster. However, during the chemometric algorithm, MCR-ALS coupled with EFA peak resolution method would still assume two zero component areas to correct the baseline. In addition, the coding from Appendix I was designed for resolving peak clusters containing maximum four components. Secondly, when applying EFA, it should be pre-assumed that, within a peak cluster, the component

that eluted first would disappear first (as shown in Figure 4-5). However, when components are embedded as shown in Figure 6-11, the EFA would not give the correct estimated initial values for MCR-ALS. In this study, this could cause the failure in peak resolution.

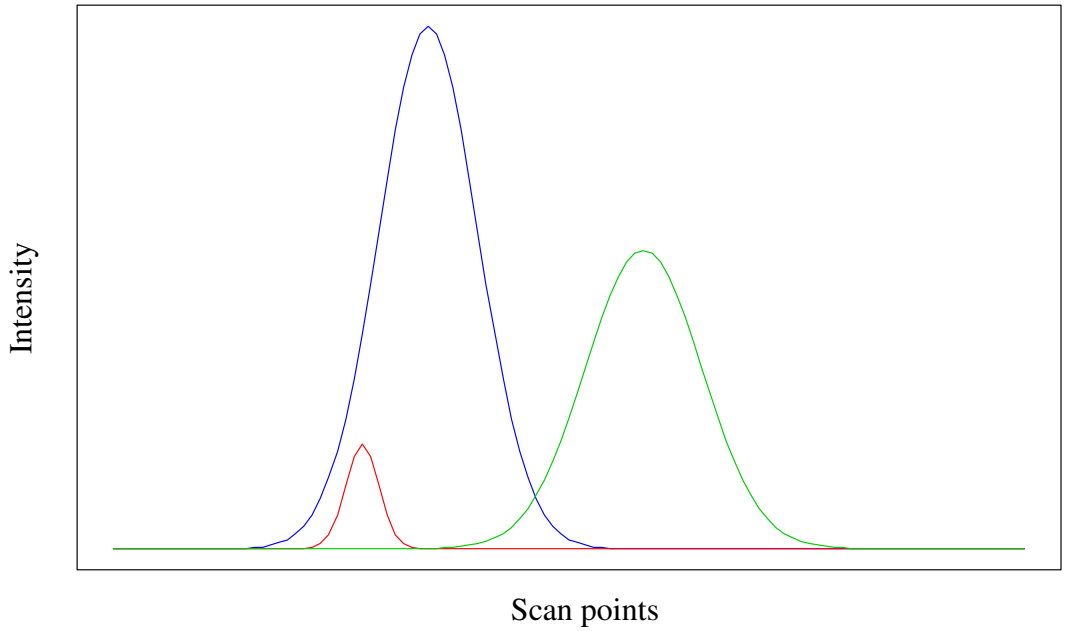


Figure 6-11 The embedded peaks. The red peak elutes later but disappear earlier than blue peak, and thus it was embedded.The EFA would not give a correct estimated concentration profile when this occurred.

The sample-caused reason can be eliminated by shortening the SPME adsorption time to reduce the adsorbed volatile amount on the fibre and optimizing the GC parameters setting for a better compound separation. When embedding was deemed to be occurring, OPA, instead of EFA can be chosen to provide the initial value for MCR-ALS.

6.4.2 Volatiles from dry and fresh mairehau

Homogenized mairehau samples prepared in three different ways (Figure 3-2) all gave a strong aroma. The difference in volatiles of homogenized fresh and dry mairehau samples that were prepared under room condition were discussed in this section based on data from Table 5-2 and Table 5-4.

Compounds	CAS No.	Fresh mairehau(%)	Dry mairehau (%)
(-)-β-Pinene	18172-67-3	0.045	0.015
(Z)-β-Farnesene	28973-97-9	18.555	0.013
name 9	36262-09-6	0.105	0.057
1R-α-Pinene	7785-70-8	3.378	1.374
3-Carene	13466-78-9	1.700	0.608
α-Cadinol	481-34-5	0.057	0.032
α-Muurolene	31983-22-9	1.523	1.310
α-Thujene	2867-05-2	0.012	0.010
Camphene	79-92-5	0.126	0.068
d-Camphene	5794-03-6	0.730	0.172
Eucalyptol	470-82-6	0.999	0.344
Germacrene D	23986-74-5	1.383	1.211
Linalool	78-70-6	3.576	0.248
L-pinocarveol	547-61-5	15.706	0.474
Manoyl oxide	596-84-9	0.354	0.044
Myrtenol	515-00-4	11.350	1.124
Nonane	111-84-2	0.004	0.002
o-Cymene	527-84-4	0.095	0.027
Terpinolene	586-62-9	0.063	0.004
Toluene	108-88-3	0.005	0.002
γ-Terpinene	99-85-4	0.056	0.012
(-)-Alloaromadendrene	25246-27-9	1.213	8.413
(-)-Myrtenyl acetate	149856NIST	1.302	20.087
(-)-Spathulenol	77171-55-2	0.061	0.106
(-)-trans-Pinocarvyl acetate	149857NIST	17.390	28.225
(+)-Epi-bicyclosesquiphellandrene	54324-03-7	1.480	8.441
name 2	460-01-5	0.104	0.157
name 6	95452-08-7	0.071	0.309
name 8	24406-05-1	0.064	0.186
α-Cubebene	17699-14-8	0.036	0.083
α-Gurjunene	489-40-7	0.374	0.617
cis-β-Terpineol	7299-41-4	0.023	0.070
Copaene	3856-25-5	0.233	1.172
L-calamenene	483-77-2	0.047	0.292
Limonene	138-86-3	0.397	0.494
m-Cymene	535-77-3	0.060	0.072
Sabinene	3387-41-5	0.247	0.382
β-Bourbonene	5208-59-3	0.837	0.975
β-cis-Ocimene	3338-55-4	0.045	0.055
β-Cubebene	13744-15-5	0.084	0.333
β-Elemene	515-13-9	0.180	0.908
β-Farnesene	18794-84-8	0.097	5.404
β-Phellandrene	555-10-2	0.857	1.497
β-Pinene	127-91-3	1.231	1.861
γ-Cadinene	39029-41-9	1.005	6.293

Table 6-3 Compounds that were found both in fresh mairehau sample and dry mairehau sample. The values show their mass percentages. The unidentified compounds and UNPAs are excluded from this table.

There are 99 and 92 compounds (excluding UNPA) found in volatiles of fresh mairehau samples and dry mairehau samples, with 73 and 55 compounds identified, respectively. Compounds that were found in both samples were listed in Table 6-3.

As shown in Table 6-3, a total of 45 compounds were repeatedly found in both fresh and dry mairehau samples, accounting for 87.26% and 93.58% (by weight) of the total volatile compounds of the two samples, respectively. As shown by the broken line centrally through Table 6-3, the level of 21 compounds (above the broken line) was decreased from fresh mairehau sample to dry mairehau sample, in contrast with increased level of the rest 24 compounds. Among the "level decrease" compounds, Myrtenol, L-pinocarveol and (Z)-β-Farnesene changed significantly from >10% to <1%. For those "level increase" compounds, the significant level change were observed in (-)-Myrtenyl acetate, β-Farnesene and (+)-Epi-bicyclosesquiphellandrene.

6.4.3 Effect of adding liquid Nitrogen on mairehau volatile

The volatile composition of homogenized fresh mairehau under room conditions may be the complicated results from oxidation, enzymatic activity since the broken plant tissue by homogenization facilitating the contact of enzymes and compounds, and molecular structure alternation by the heat that generated from high speed shearing force. To assess the effects of those factors, another homogenized fresh mairehau was prepared by low speed manual grounding. During the preparation, liquid nitrogen was added to maintain the low temperature, to inhibit potential chemical reactions (Figure 3-2). The discussion in this section was based on Table 5-4 and Table 5-5.

The compounds with top 10 levels and repeated compounds found in both samples were marked in Table 5-4 and Table 5-5. There was 95 compounds (excluding UNPA) found in volatile of homogenized mairehau sample prepared with adding liquid Nitrogen, with 55 identified.

The dynamic status of volatiles of fresh homogenized mairehau samples (with or without adding liquid Nitrogen) during nearly 4 hours (The time covering pre-

equilibrium, SPME adsorption and on waiting while GC-MS running) under room condition was compared. The two-sample t test (independent samples, unequal variances) was carried out on CVs of compounds from Table 5-4 and Table 5-5. Since the *p value* < α (0.05), the null hypothesis that the CVs from two samples were essentially the same was rejected. It thus be concluded that the dynamic status was significantly different during nearly 4 hours between the two volatiles, indicating that adding liquid Nitrogen during sample preparation had obvious effect on the volatile characteristics.

Chapter 7 CONCLUSION

SPME: 100 μm PDMS fibre for SPME showed the best reproducibility in this study. The established regression models for total amount adsorbed on the fibre (indicated by total peak area from GC-FID) versus fibre adsorption time (min) was *Total Peak Area=13380791+2351232*time-29380*time^2+129.2*time^3 (under 25 °C)*, and *Total Peak Area=21673113+2524797*time-27884*time^2+113.2*time^3 (under 35 °C).* Both agreed with the theoretic SPME fibre adsorption model. With the same fibre adsorption time, increasing temperature from 25 °C to 35 °C promoted the adsorption amount on the fibre.

CHEMOMETRICS: MCR-ALS coupled with EFA coded in R efficiently resolved the overlapped GC-MS peaks from mairehau samples with low complexity (including samples of mairehau flowers and mairehau twigs). However, the method was incapable for handling certain overlapped GC-MS peaks from samples with high complexity (including those homogenized mairehau samples). The problems could be solved by shortening the SPME fibre adsorption time to lower the sample complexity, or using OPA instead of EFA, because fewer pre requirements for the data is required.

NATURAL DEHYDRATION: The weights of mairehau twigs decreased during the 15 days natural dehydration after cutting from plants, while both the number of volatile compounds and volatile concentration increased with days adding. The negative linear relation between mairehau twigs weights and the number of volatile compounds had R^2= 75.2%.

By multivariate analysis, volatile profiles during the 15 days natural dehydration can be classified into three types: type 1 (covering days 1, 2, and 5), type 2 (covering day 7), and type 3 (covering 9, 11, 13 and 15). It can be interpreted that significant metabolic transformation affecting the volatile composition, occurred during day 6 to day 8. The total volatile compounds clustered into 5 groups (A, B, C, D and E) according to their similar behavior over the 15 days (Figure 6-7 and 6-8). Groups A and B (containing 34 and 12 compounds respectively) were highly associated with

type 1 and type 2. Groups D and E (containing 5 compounds for each) were associated type 2 and type 3. Group C (containing 15 compounds) were associated with all measuring days, meaning the compounds from cluster C was less affected by natural dehydration from day 1 to day 15.

COMPARISON BETWEEN DIFFERENT SAMPLES: Overall, the number of volatile compounds from mairehau twigs and flowers (33 and 37, respectively) was less than those from homogenized mairehau samples (99, 92 and 95 from homogenized dry mairehau, fresh mairehau and fresh mairehau adding liquid Nitrogen, respectively). 15 compounds were repeated, although with unequal levels, in volatiles of mairehau twigs and flowers, while for homogenized dry and fresh mairehau under room conditions, this was 45. Between the volatiles of the fresh mairehau homogenized with or without cooling by liquid Nitrogen, the number of repeated compounds was also 45. It was concluded from two sample t test on CVs of compounds that adding liquid Nitrogen during sample preparation had obvious effect onto volatile characteristics.

APPENDIX A

```
datamatrix<-function(m,n,data="my mzxml file") {
    library(mzR)
    library(msdata)
    q<-system.file(data, package="msdata")
    pdms<-openMSfile(q)
    s<-peaks(pdms, c(m:n))

#get the min m\z value
       e<-rep(0, times=length(m:n))
       i<-1; while(i<=length(m:n))
                {j<-apply(s[[i]], 2, min)
                 h<-j[1]
                 e[i]<-h
                 i<-i+1}
           from=min(round(e))

#get the max m\z value
       e<-rep(0, times=length(m:n))
       i<-1; while(i<=length(m:n))
                {j<-apply(s[[i]], 2, max)
                 h<-j[1]
                 e[i]<-h
                 i<-i+1}
           to=max(round(e))
           mass<-seq(from, to, by=1)

# round the list s
       i=1
       while(i<=length(m:n))
          { s[[i]]<-round(s[[i]])
            i<-i+1 }

#create an empty matrix telling the information of size
       mm<-matrix(rep(0, times=length(mass)*length(m:n)),
                ncol=length(m:n),nrow=length(mass))

#function to enlarge
     enlarge<-function(x){
                i=from
                last<-to
                y<-c(NULL,NULL)
                while(i<=last)
                  { n1<-x[x[,1]==i]
```

```
                  n2<-matrix(n1, ncol=2, byrow=FALSE)
                  t<-sum(n2[,2])
                  n3<-c(i,t)
                   y<-rbind(y,n3)
                    rownames(y)<-NULL
                    colnames(y)<-NULL
                    i<-i+1
                 }
                    return(y) }

#feed the mm
     i=1
     while(i<=length(m:n))
          {s[[i]]<-enlarge(s[[i]])
          mm[,i]<-s[[i]][,2]
          i<-i+1}

colnames(mm)<-c(m:n)
rownames(mm)<-c(from:to)
return(mm) }
```

APPENDIX B

```
library(TargetSearch)
v<-baselineCorrection(x, threshold = 0.5, alpha = 0.95, bfraction = 0.2,
    segments = 100, signalWindow = 17, method = "linear")
```

APPENDIX C

```
library(signal)
w<-t(v)
for(i in colnames(w))
     { a<-w[,i]
      sg<-sgolayfilt(a)
      for (c in 1:4)
      sg<-sgolayfilt(sg)
      w[,i]<-sg }
w[w<0]<-0
```

APPENDIX D

```
ma<-matrix(0,nrow(w),7)
for(i in 3:nrow(w))
    { svd<-svd(scale(w[1:i,], scale = FALSE)); ma[i,]<-svd$d[1:7] }

matplot(c(3000:3030),ma, xlab="Scan points",ylab="Eigenvalues",
            cex.lab=1.5, type="l",lty=1,lwd=2)
```

APPENDIX E

These three R scripts for illustrating the algorithm procedure of MCR-ALS, EFA and OPA, are originally taken from *SECTION 11.5- Multivariate Curve Resolution* of the reference (Wehrens, 2011) without any adaptation. The R package "ChemometricsWithR" developed by the same author Ron Wehrens contains functions mcr(),efa() and opa() doing the same algorithm also.

```
mcr <- function(x, init, what = c("row", "col"),
convergence = 1e-8, maxit = 50)
{
what <- match.arg(what)
if (what == "col") {
CX <- init
SX <- ginv(CX) %*% x
} else {
SX <- init
CX <- x %*% ginv(SX)
}
rms <- rep(NA, maxit + 1)
rms[1] <- sqrt(mean((x - CX %*% SX)^2))
for (i in 1:maxit) {
CX <- x %*% ginv(SX)
SX <- ginv(CX) %*% x
resids <- x - CX %*% SX
rms[i+1] <- sqrt(mean(resids^2))
if ((rms[i] - rms[i+1]) < convergence) break;
}
list(C = CX, S = SX, resids = resids, rms = rms[!is.na(rms)])
}
```

```
#### #### #### #### #### #### ####
```

```
efa <- function(x, ncomp)
{
nx <- nrow(x)
Tos <- Fros <- matrix(0, nx, ncomp)
for (i in 3:nx)
Tos[i,] <- svd(scale(x[1:i,], scale = FALSE))$d[1:ncomp]
```

```
for (i in (nx-2):1)
Fros[i,] <- svd(scale(x[i:nx,], scale = FALSE))$d[1:ncomp]
Combos <- array(c(Tos, Fros[,ncomp:1]), c(nx, ncomp, 2))
list(forward = Tos, backward = Fros,
pure.comp = apply(Combos, c(1,2), min))
}
```

####

```
opa <- function(x, ncomp)
{
Xref <- colMeans(x)
Xref <- Xref / sqrt(sum(crossprod(Xref))) # scaling
selected <- rep(0, ncomp)
for (i in 1:ncomp) {
Xs <- lapply(1:nrow(x),
function(ii, xx, xref) rbind(xref, xx[ii,]),
x, Xref)
dissims <- sapply(Xs, function(xx) det(tcrossprod(xx)))
selected[i] <- which.max(dissims)
newX <- x[selected[i],]
if (i == 1) {
Xref <- newX / sqrt(crossprod(newX))
} else {
Xref <- rbind(Xref, newX / sqrt(sum(crossprod(newX))))
}
}
dimnames(Xref) <- NULL
list(pure.comp = t(Xref), selected = selected)
}
```

APPENDIX F

The resolved mass spectra are stored in t$S, and the resolved concentration profile are stored in t$C[[1]].

```
z<-efa(w,3)

t <- als(CList = list(z$pure.comp),
 PsiList = list(w), S = matrix(0, 610, 3),
 nonnegS = TRUE, nonnegC = TRUE,
 optS1st = TRUE, uniC = TRUE)
```

APPENDIX G

```
filename<-c("for test")
sample<-c("sample 1")
instrument<-c("thermo")
x<-min(as.numeric(rownames(w)))
y<-max(as.numeric(rownames(w)))
for(i in 1:ncol(t$S))
{
   a<-paste(x,"to",y,"peak",i,".msp")
   compound<-c(a)
   meta<-data.frame(compound,filename,sample,instrument)
   mz<-c(40:649); intensity<-t$S[,i]
   spectra<-data.frame(filename, mz, intensity)
   WriteMspFile(spectra, meta, filename = a, comment = "")
}
```

APPENDIX H

```
#compound1
c1<-t$C[[1]][,1]%*%t(t$S[,1])
tic1<-apply(c1, 1, sum)
b<-as.matrix(tic1)
q1<-0
for(i in 1:(nrow(b)-1))
{
h<-(b[i,]+b[(i+1),])/2
q1<-q1+h
}

#compound2
c2<-t$C[[1]][,2]%*%t(t$S[,2])
tic2<-apply(c2, 1, sum)
bb<-as.matrix(tic2)
q2<-0
for(i in 1:(nrow(bb)-1))
{
h<-(bb[i,]+bb[(i+1),])/2
q2<-q2+h
}

#compound3
c3<-t$C[[1]][,3]%*%t(t$S[,3])
tic3<-apply(c3, 1, sum)
bbb<-as.matrix(tic3)
q3<-0
for(i in 1:(nrow(bbb)-1))
{
h<-(bbb[i,]+bbb[(i+1),])/2
q3<-q3+h
}
```

APPENDIX I

For a quick peak resolve process without trivial resetting parameters, the operation can follow those:

1). Read the selected peak cluster (scan points ranging m~n) into a matrix X in R software, by command X<-datamatrix(m,n,data="my mzxml file").(the definition of datamatrix() is in Appendix A)

2). Determine the chemical rank of the peak cluster by command determrank(X, p), which produces a SVD eigenvalues plot similar as Figure 5-11, from which one can read the peak cluster's chemical rank, denoted as r. p is a pre-assuming chemical rank value.

3). Given r, the command mresolution(X,r,"names") will pre-process X first, and resolve the peak cluster by MCR-ALS method initialed from EFA. The outputs contain mass spectra plots, resolved concentration profile plot, resolved TIC plot, .msp formated mass spectra files for NIST searching and peak areas of each single components. The resolved concentration profile plot can be used to assess the resolving quality. In addition, mresolution() is designed for hanling r<5, which suit most of cases in this study.

determrank() and mresolution() are defined by following scripts:

```
library(TargetSearch)
library(signal)
library(ChemometricsWithR)
library(ALS)
library(MASS)
library(OrgMassSpecR)

                        #To determine the chemical rank
determrank<-function(x, p)
{
      #baseline correction
      v<-baselineCorrection(x, threshold = 0.5, alpha = 0.95, bfraction = 0.2,
                        segments = 100, signalWindow = 17, method = "linear")

      #smooth
      w<-t(v)
      for(i in colnames(w))
        {     a<-w[,i]
              sg<-sgolayfilt(a)
              for (c in 1:4)
              sg<-sgolayfilt(sg)
              w[,i]<-sg
         }
      w[w<0]<-0

      #chemical rank plot
      z<-efa(w,p)
      matplot(z$forward,type="l",lty=1)
}


              #### #### #### #### #### #### ####

               #To resolve the peak given chemical rank setted

mresolution<-function(x, r, names)
{

#baseline correction
v<-baselineCorrection(x, threshold = 0.5, alpha = 0.95, bfraction = 0.2,
    segments = 100, signalWindow = 17, method = "linear")
```

```
#smooth
w<-t(v)
for(i in colnames(w))
  {
  a<-w[,i]
  sg<-sgolayfilt(a)
  for (c in 1:4)
  sg<-sgolayfilt(sg)
  w[,i]<-sg
  }
w[w<0]<-0

z<-efa(w,r)
z$pure.comp[is.na(z$pure.comp)]<-0
t <- als(CList = list(z$pure.comp),
           PsiList = list(w), S = matrix(0, ncol(w), r),
           nonnegS = TRUE, nonnegC = TRUE,
           optS1st = TRUE, uniC = TRUE)

# using loop to generate .msp file with batch
filename<-c("for test")
sample<-c("sample 1")
instrument<-c("thermo")
x<-min(as.numeric(rownames(w)))
y<-max(as.numeric(rownames(w)))
xx<-min(as.numeric(colnames(w)))
yy<-max(as.numeric(colnames(w)))

for(i in 1:ncol(t$S))
   {
   a<-paste(x,"to",y,names,"peak",i,".msp")
   compound<-c(a)
   meta<-data.frame(compound,filename,sample,instrument)
   mz<-c(xx:yy); intensity<-t$S[,i]
   spectra<-data.frame(filename, mz, intensity)
   WriteMspFile(spectra, meta, filename = a, comment = "")
   }


if(r==1)
{
#compound1
c1<-t$C[[1]][,1]%*%t(t$S[,1])
tic1<-apply(c1, 1, sum)
b<-as.matrix(tic1)
q1<-0
for(i in 1:(nrow(b)-1))
```

```
        {
           h<-(b[i,]+b[(i+1),])/2
           q1<-q1+h
        }

dev.new()
plot(c(xx:yy),t$S[,1]/max(t$S[,1])*100,type="h",col="blue",
     ylab="Abundance",xlab="m/z",main="MS of component 1") #ms plot

dev.new()
matplot(c(x:y),t$C[[1]],type="l",lty=1,ylab="Intensity",xlab="Scan points",
          main="Resolved concentration profile")  #concentration plot

dev.new()
plot(c(x:y),tic1,type="l",ylab="Intensity",xlab="Scan points", main="The
     resolved peak cluster")                          #resolved TIC

area<-matrix(q1,1,1,dimnames=list(c("Peak area"),c("component1")))
return(area)

}


if (r==2)
{
#compound1
c1<-t$C[[1]][,1]%*%t(t$S[,1])
tic1<-apply(c1, 1, sum)
b<-as.matrix(tic1)
q1<-0
for(i in 1:(nrow(b)-1))
       {
       h<-(b[i,]+b[(i+1),])/2
       q1<-q1+h
       }

#compound2
c2<-t$C[[1]][,2]%*%t(t$S[,2])
tic2<-apply(c2, 1, sum)
bb<-as.matrix(tic2)
q2<-0
for(i in 1:(nrow(bb)-1))
       {
       h<-(bb[i,]+bb[(i+1),])/2
       q2<-q2+h
       }
```

```
dev.new()
plot(c(xx:yy),t$S[,1]/max(t$S[,1])*100,type="h",col="blue",
     ylab="Abundance",xlab="m/z",main="MS of component 1") #COMP1

dev.new()
plot(c(xx:yy),t$S[,2]/max(t$S[,2])*100,type="h",col="darkorchid2",
     ylab="Abundance",xlab="m/z",main="MS of component 2")

dev.new()
matplot(c(x:y),t$C[[1]],type="l",lty=1,ylab="Intensity",xlab="Scan points",
          main="Resolved concentration profile")

dev.new()
plot  (c(x:y),tic1,type="l",ylab="Intensity",xlab="Scan points",
       main="The resolved peak cluster")
points(c(x:y),tic2,type="l",col="darkorchid2")

area<-matrix(c(q1,q2),1,2,
               dimnames=list(c("Peak area"),c("component1","component2")))
return(area)

}


if (r==3)
{
#compound1
c1<-t$C[[1]][,1]%*%t(t$S[,1])
tic1<-apply(c1, 1, sum)
b<-as.matrix(tic1)
q1<-0
for(i in 1:(nrow(b)-1))
{
h<-(b[i,]+b[(i+1),])/2
q1<-q1+h
}
#compound2
c2<-t$C[[1]][,2]%*%t(t$S[,2])
tic2<-apply(c2, 1, sum)
bb<-as.matrix(tic2)
q2<-0
for(i in 1:(nrow(bb)-1))
{
h<-(bb[i,]+bb[(i+1),])/2
q2<-q2+h
}
#compound3
```

```
c3<-t$C[[1]][,3]%*%t(t$S[,3])
tic3<-apply(c3, 1, sum)
bbb<-as.matrix(tic3)
q3<-0
for(i in 1:(nrow(bbb)-1))
{
h<-(bbb[i,]+bbb[(i+1),])/2
q3<-q3+h
}

dev.new()
plot(c(xx:yy),t$S[,1]/max(t$S[,1])*100,type="h",col="blue",
     ylab="Abundance",xlab="m/z",main="MS of component 1") #COMP1

dev.new()
plot(c(xx:yy),t$S[,2]/max(t$S[,2])*100,type="h",col="darkorchid2",
     ylab="Abundance",xlab="m/z",main="MS of component 2")

dev.new()
plot(c(xx:yy),t$S[,3]/max(t$S[,3])*100,type="h",col="springgreen4",
     ylab="Abundance",xlab="m/z",main="MS of component 3")

dev.new()
matplot(c(x:y),t$C[[1]],type="l",lty=1,ylab="Intensity",xlab="Scan points",
          main="Resolved concentration profile")

dev.new()
plot(c(x:y),tic1,type="l",ylab="Intensity",xlab="Scan points",
     main="The resolved peak cluster")
points(c(x:y),tic2,type="l",col="darkorchid2")
points(c(x:y),tic3,type="l",col="springgreen4")

area<-matrix(c(q1,q2,q3),1,3,
                dimnames=list(c("Peak area"),
                c("component1","component2","component3")))
return(area)

}


if (r==4)
{
#compound1
c1<-t$C[[1]][,1]%*%t(t$S[,1])
tic1<-apply(c1, 1, sum)
b<-as.matrix(tic1)
q1<-0
```

```
for(i in 1:(nrow(b)-1))
     {
     h<-(b[i,]+b[(i+1),])/2
     q1<-q1+h
     }
#compound2
c2<-t$C[[1]][,2]%*%t(t$S[,2])
tic2<-apply(c2, 1, sum)
bb<-as.matrix(tic2)
q2<-0
for(i in 1:(nrow(bb)-1))
     {
     h<-(bb[i,]+bb[(i+1),])/2
     q2<-q2+h
     }
#compound3
c3<-t$C[[1]][,3]%*%t(t$S[,3])
tic3<-apply(c3, 1, sum)
bbb<-as.matrix(tic3)
q3<-0
for(i in 1:(nrow(bbb)-1))
     {
     h<-(bbb[i,]+bbb[(i+1),])/2
     q3<-q3+h
     }
#compound4
c4<-t$C[[1]][,4]%*%t(t$S[,4])
tic4<-apply(c4, 1, sum)
bbbb<-as.matrix(tic4)
q4<-0
for(i in 1:(nrow(bbbb)-1))
     {
     h<-(bbbb[i,]+bbbb[(i+1),])/2
     q4<-q4+h
     }

dev.new()
plot(c(xx:yy),t$S[,1]/max(t$S[,1])*100,type="h",col="blue",
     ylab="Abundance",xlab="m/z",main="MS of component 1")

dev.new()
plot(c(xx:yy),t$S[,2]/max(t$S[,2])*100,type="h",col="darkorchid2",
     ylab="Abundance",xlab="m/z",main="MS of component 2")

dev.new()
plot(c(xx:yy),t$S[,3]/max(t$S[,3])*100,type="h",col="springgreen4",
     ylab="Abundance",xlab="m/z",main="MS of component 3")
```

```
dev.new()
plot(c(xx:yy),t$S[,4]/max(t$S[,4])*100,type="h",col="red",
     ylab="Abundance",xlab="m/z",main="MS of component 4")

dev.new()
matplot(c(x:y),t$C[[1]],type="l",lty=1,ylab="Intensity",
          xlab="Scan points", main="Resolved concentration profile")

dev.new()
plot(c(x:y),tic1,type="l",ylab="Intensity",xlab="Scan points",
     main="The resolved peak cluster")
points(c(x:y),tic2,type="l",col="darkorchid2")
points(c(x:y),tic3,type="l",col="springgreen4")
points(c(x:y),tic4,type="l",col="blue")

area<-matrix(c(q1,q2,q3,q4),1,4,
            dimnames=list(c("Peak area"),
            c("component1","component2","component3","component4")))

return(area)

}

}
```

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