University of Reading

Culinary herbs: determining the basis of variation in herb flavour

PhD in Food and Nutritional Sciences

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Ana Cristina Contente

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Abstract

Culinary herbs such as basil (*Ocimum basilicum*), coriander (*Coriandrum sativum*) and rosemary (*Rosmarinus officinallis*) are crops grown across the world for their healthy characteristics and distinct flavours. They can be consumed fresh or dried, in salads or as garnish, in soups, sauces or curries, forming essential ingredients in many cuisines. Research investigating the aroma profile of these herbs often excludes information about the variety, production type and other growing conditions resulting in inaccurate data conclusions. These variables have been described in published literature to have an impact on the flavour profile of other crops such as celery and lettuce.

Basil, coriander and rosemary were grown using different production methods across several years (2018, 2019, 2020, 2021) and at multiple sites within the UK. The influence of factors including production methods, geographical location, production season and year on the aroma composition of these herbs was investigated. The aroma profile of the three herbs was determined using solid-phase microextraction gas chromatography-mass spectrometry. Differences in volatile composition and influence on sensory perception were analysed using sensory profiling with a trained panel (n = 11). Finally, basil and coriander samples were presented to a consumer panel (n = 117 and n = 106, respectively) to identify consumer acceptance and attribute preference.

Significant differences in the volatile composition were influenced by production method, plant maturity and environmental factors, leading to significant differences in the sensory profile. Temperatures between 10-20 ˚C resulted in higher proportions of monoterpenes and phenylpropanoids for rosemary and basil, and aldehydes for coriander, whilst the influence of soil, water source and lighting was herb specific. Consumer grouping identified two groups

exhibiting differences in hedonic response to basil and coriander samples. Aroma and flavour intensity were identified as drivers of liking. Further studying the relationship between production variables and flavour of herbs will increase information to guide growers on how to produce a more consistent product that meets consumers expectations.

Declaration of original authorship

Declaration: I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

Signed: Ana Cristina Contente

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List of abbreviations

A – Alcohol

ADH – Alcohol dehydrogenase

AHC – Agglomerative hierarchical cluster

AL – Aldehyde

AK – Alkane

AKE – Alkene

B – Basil

C – Coriander

DMAPP – Dimethylallyl diphosphate

E – Ester

FPP – Farnesyl pyrophosphate

GPP – Geranyl pyrophosphate

GC-MS – Gas chromstography mass spectrometry

HPL – Hydroperoxide lyase

HPS – High pressure sodium

HSP – Heat shock protein

Ir – Irrigation

IPP – Isopentenyl diphosphate

IS – Internal standard

JAR – Just-About-Right

LED – Light-emitting diode

LOX – Lipoxygenase

LRI – Linear retention index

M – Monoterpene

MEP – Non-mevalonate pathway

MFA – Multiple factor analyis

MIAPAE – Minimum Information about a Plant Aroma Experiment

MVA – Mevalonic acid pathway

nd – Not detected

ns – Not significant

O – other

P - Phenylpropanoid

PCA – Principal component analysis

R – Rosemary

Rf – Rainfall

ROS – Reactive oxygen species

S – Sesquiterpene

Sl – Sunlight

SPME – Solid phase microextraction

var. – Variety

U – Unknown

UV - Ultraviolet

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Project introduction and aims

Culinary herbs have been used for a long time and been used in many cuisines around the world. They are used for their many properties including essential oils, preservatives and aromatic contribution to food, and are used by pharmaceutical, cosmetics and culinary industries (Bower et al 2016, Tapsell et al 2006). Herbs can be consumed in a variety of formats such as dried or fresh, leaves or seeds, cut or pots, infused oil or in pre-made meals. Some of the most consumed culinary herbs are basil, coriander and rosemary. The aroma characteristics of basil are attributed to the presence of monoterpenes and phenylpropanoids, coriander aroma is due to the presence of aldehydes, and rosemary aroma is dominated mainly by monoterpenes. Basil gives a clove, spicy and herbal aroma, which is attributed to the presence of eugenol, estragole and linalool as the main compounds (Díaz-Maroto et al 2004, Padalia and Verma 2011). Coriander aroma is due to the presence of compounds such as (*E*)-2-decenal, *(E)*-2-dodecenal, decanal, dodecanal, *(E)*-2-tridecenal and tetradecenal, and these have been associated with aroma descriptors of green and soapy notes (El-Zaeddi et al 2016, Neffati and Marzouk 2008). However, soapy perception of coriander has been associated with human genetics and the propensity of receptors that perceive these compounds as soapy, pungent or dirt-like (Eriksson et al 2012). Rosemary aroma is constituted by monoterpenes including camphor, eucalyptol (1,8-cineole), borneol and alpha-pinene, which give a herbal, fresh, eucalyptus aroma (Pintore et al 2002, Socaci et al 2008).

Several studies have analysed culinary herbs and their volatile composition. Some compounds are commonly identified as key odorants compounds, however different relative abundances are reported including basil, coriander and rosemary (Anjum et al 2011, Calín-Sánchez et al 2012, El-Zaeddi et al 2016, Lee et al 2005, Salido et al 2003, Tamura et al 2013).

Few studies have considered how the variety of the analysed crop may impact the production of volatiles, or the conditions under which the samples were produced, factors that Turner et al (2021) suggested were essential in providing Minimum Information for a Plant Aroma Experiment (MIAPAE). Furthermore, the majority of these studies have analysed the essential oil of the herbs, after the plants were subject to an extraction procedure or after a drying process. Szumny et al. (2010) identified 34 compounds in rosemary using steam hydrolysation and drying of the samples (Szumny et al 2010). Conversely, Salido et al. (2003) detected 53 compounds using GC-MS after steam distillation of rosemary twigs (Salido et al 2003). A study analysing basil aroma identified 28 compounds after the steam distillation of the herb (Díaz-Maroto et al 2004), whereas a study using thermal desorption identified 22 aroma compounds (Chang et al 2007). Coriander essential oil was analysed by gas chromatography and reported to have 30 aroma compounds (Ravi et al 2007) whereas coriander leaves analysed using gas-chromatography after hydro-distillation revealed 28 volatile compounds (Shahwar et al 2012). Combining all of these data, it is obvious that the chemical composition of a herb will be dependent on the tissue studied, the variety grown, culture conditions, extraction methods and the sensitivity of the analysis methods.

The factors discussed in the paragraph above play an important role in the growth of herbs, so it is important to state this information otherwise data are incomplete and challenging to replicate. Few studies that have been previously completed include detail of any of the variables mentioned. Additionally, no study has been conducted looking at the aroma composition of the same herb in a multi-year and multi-site experiment, where the influence of environmental factors (temperature, light, water and soil) and internal factors (variety) are analysed. For these reasons, this project aims to analyse the influence of these factors on basil, coriander and rosemary in a multi-year (2018-2021) and multi-site (growers across UK) suite

of experiments. Culinary herbs are now cultivated using a variety of production methods which give rise to different environments in which the crop develops. These include pot herbs, which are characterised by high density planting under protected environment in a glasshouse, protected field grown in soil – which will be of different composition depending on the region of the UK growing the herbs – and herbs grown in open fields, which are also subject to soil variability and greater extremes of heat, light intensity and other abiotic and biotic stress stimuli. The aroma compositions of herbs in the experiments in this thesis were identified using solid phase microextraction gas chromatography-mass spectrometry and, by combining these data with sensory profiling using a trained panel, we were able to investigate the differences in the aroma profile and the perceived flavour and aroma.

Culinary herbs have been commonly used for centuries due to their characteristic properties, however the preference of the flavour is a topic that has not been much explored, and limited research has been done looking at consumer preference of basil, coriander and rosemary or the drivers of preference (Caracciolo et al 2020). Understanding the answer to these questions will help elucidate the herbs' desirable consumer qualities and by educating growers how production factors affect the flavour and quality of the crops. The project aims that were addressed in this thesis are listed below:

- To determine and identify aroma compounds of basil, coriander and rosemary
- To investigate the impact of different growing seasons on aroma profile over three years
- To examine the impact of production methods on aroma profile
- To investigate the effect of plant and leaf maturity on the flavour profile of rosemary

- To correlate the volatile profiles with sensory profiling data in order to associate flavour analysis with human sensory perception
- To identify consumer preference and drivers of liking of basil and coriander

The thesis is divided into seven chapters; the first chapter investigates the basil, coriander and rosemary aroma literature that has been completed and identified information that is missing. Following this, the second chapter contains results identifying the aroma compounds present in the profile of four basil (*var*. Sweet Genovese), five coriander (*var*. Cruiser) and six rosemary (*var.* unknown) varieties, produced in pots, open field, field under protected conditions or hydroponic system (only for basil samples). Chapter 3 elucidates how differences in the aroma composition were perceived by a trained sensory panel. Chapter 4 focuses on different environmental factors and their influence on the aroma composition using the same basil, coriander and rosemary samples throughout. In Chapter 5, rosemary plant maturity is investigated and its impact on the aroma composition and sensory perception. Chapter 6 uses basil and coriander samples that had been analysed throughout the study, and which were presented to a consumer panel to investigate preference and to identify what attributes consumers find desirable in basil and coriander. To conclude, the final chapter comprises an overall discussion, conclusions and potential future work.

The herbs material used in chapters 2,3,4,5 and 6 was in the form of fresh leaves for each of the herbs. Preliminary analysis was completed where fresh leaves were analysed and compared, where addition of saturated calcium chloride solution was used as a preservation method. The addition of this solution was observed to preserve the volatile profile for the complete time of analysis. Significant losses in the aroma profile have been reported in literature, when fresh leaves of culinary herbs were submitted to drying methods including oven

dried, freeze-dried and by microwave. Rosemary, when microwave dried, resulted in a significant loss of the volatile components, however colour retention was reported (Rao et al 1998). The effect of different drying methods on basil leaves was analysed, and significant losses on the aroma profile were reported with consequently differences in the sensory profile, however drying at room temperature for a longer period of time resulted in the least losses (Díaz-Maroto et al 2004). Additionally, few studies identified the temperature ranges that would cause the least losses in the aroma profile of thyme and coriander leaves, more substantial losses were detected when herbs were dried at higher temperatures (50 – 70 ˚C) and lower losses at lower (< 50 ˚C) temperatures, however significant losses were still reported (Łyczko et al 2021, Sárosi et al 2013). We therefore avoided drying methods in the present study and as such this thesis provides the most comprehensive analysis of fresh herb flavour and aroma chemistry to date. Limited prior research has been done using fresh herb material, so the use of fresh leaves fills a gap in our research understanding of flavour chemistry in addition to exploring the environmental factors driving flavour that were stated above.

Chapter 1: Literature review

1.1 Introduction

Culinary herbs have a long history and are used in many cuisines around the world providing flavour to dishes. Different parts of these plants can be used for their aromatic properties, including seeds, roots, leaves and stems, and can be used fresh or dried (Tapsell et al 2006). Culinary herbs have also been used for their essential oils in the pharmaceutical and cosmetics industry as well as for their antioxidant properties as preservatives (Bouzouita et al 2003, Hossain et al 2010, Singletary 2016). Encouragement to replace salt as a means of adding flavour to food by increasing the use of culinary herbs has caused an increase in the frequency of consumption of herbs (Bower et al 2016). Herbs are marketed in a variety of formats for consumption: the whole plant in a small pot (to be consumed as fresh as possible), prepacked herbs (freshly cut herbs, including leaves and stem) and dried herbs; these can also be found added to premade meals. Fresh herbs and prepacked herbs can usually be found in the vegetable section of the supermarket, at ambient temperature, however dried herbs are presented in the spices and food cupboard section.

This thesis focuses on three of the most commonly consumed fresh culinary herbs in the United Kingdom (UK): basil, coriander and rosemary (Anon 2016). Basil and coriander are grown as annual crops, often with multiple growing cycles per year, whereas rosemary is a woody perennial shrub that is often planted in permanent field sites that remain in place for several years. In 2016 the retail market for herbs was estimated to be £70-100 million (Anon 2016). Additionally, statistical data estimates that salt and spices market in the UK generates a revenue of £550 million, with an observed rise of £50 million (2018-2021), with a production volume (2021) of 48.9 million kilograms (Anon 2022).

Plants and algae use photosynthesis as a process to synthesize organic molecules using water, sunlight and carbon dioxide, additionally, plants will synthesise a range of other compounds classified as secondary metabolites (Sato 2014). These metabolites are necessary for plant survival in its ecosystem due to their interactions with the environment (Verpoorte 2000). Some secondary metabolites have been described to be involved in plant growth and development, as sources of energy, precursors of organic compounds and sources of nitrogen, furthermore, these compounds can offer protection to the plant (allomones), be involved with plant pollination (synomones) and to interact with organisms to locate the plant (kairomones) (Seigler 1998). Additionally, these chemical compounds have been reported to reduce feeding intensity of herbivores resulting in plant survival (Dziba and Provenza 2008).

Basil (*Ocimum basilicum)* is a member of the Lamiaceae (mint) family, and it is highly cultivated in Mediterranean areas. Basil can be consumed as fresh leaves, dried leaves and essential oil for flavouring other food products (Putievsky and Galambosi 1999). Chemical composition of the essential oil of basil is variable, composed of phenylpropanoids (~ 67 %) and monoterpenes (~ 25 %), with main compounds being linalool and estragole, accounting for around 66 % and 24 % (respectively) of the volatile composition (Padalia and Verma 2011). Basil has been described as having two types of glandular trichomes, peltate and capitate, on the surface of its leaves, responsible for the synthesis and storage of essential oils (Gang et al 2001). Drying basil will affect its appearance as well as aroma, since the process leads to changes in the volatile profile. Diaz-Maroto et al. reported a decrease in volatile content of 28.6 % and 27.4 % when using oven drying (at 45 ˚C) and freeze drying, respectively, and smaller losses (13 %) when air dried at ambient temperature, additionally an increase in some sesquiterpenes and decrease in linalool was reported (Díaz-Maroto et al 2004).

Coriander (*Coriandrum sativum*) is a plant from the Umbelliferae/Apiaceae family, highly produced in Mediterranean areas and in the Middle East. Coriander can be consumed as fresh or dried leaves and as dried seeds, as both parts of the plant store flavour compounds. Coriander aroma is mainly provided by aliphatic fatty acid derivatives including (*Z*)-2-decenal and dodecanal, suggesting that unlike most herbs, coriander aroma is attributed to primary compounds, carboxylic acids, sugars and amino acids which are involved in respiration and protein synthesis, suggesting that coriander's aroma will be less affected by environmental factors (Chadwick 2018, Geissman and Crout 1969, Neffati and Marzouk 2008). Both parts of the herbs are used for their oil content, however they have been described as having different composition, with leaves containing mainly aldehyde compounds including (*E*)-2-decenal, dodecanal and (*E*)-2-tridecenal, whereas seeds are mainly composed of monoterpenes including alpha-pinene and camphor with major compound being linalool (Neffati and Marzouk 2008, Shahwar et al 2012). Linalool can be found in both leaves and seeds of coriander, however this is present in different quantities, accounting for over 50 % of seed essential oil, whereas less than 15 % can be found in leaves essential oil (Shahwar et al 2012). Coriander essential oil with strong sweet floral odours has been attributed to the presence of geranyl acetate in higher amounts (Ravi et al 2007). Compounds like *(E)*-2-decenal, *(E)*-2-dodecenal, decanal, dodecanal, *(E)*-2-tridecenal and tetradecenal are responsible for most of the coriander oil composition, providing a characteristic green and soapy aroma, attributed to this herb (El-Zaeddi et al 2016).

Rosemary (*Rosmarinus officinallis*) is a woody aromatic herb from the family Lamiaceae (the same as basil); it is used fresh, dried and for its essential oil. It is produced worldwide, however the main area of production is the Mediterranean countries. The oil of rosemary is constituted mainly by monoterpenes and it can be described as primarily borneol and 1,8-

cineole, followed by camphor and limonene, depending on the variety of the herb (Pintore et al 2002).

Most research looking at culinary herbs flavour utilises their essential oils, and little research can be found in the literature that reports on studies using fresh culinary herbs.

1.2 Volatile compounds contributing to herbs aroma

Plants, through the process of photosynthesis, produce organic compounds called primary metabolites (Cruickshank 2012). The function of the primary metabolites is associated with the structure and physiology of the plant, and consist of carbohydrates, proteins, nucleic acids and lipids, and these metabolites are universal to the plants (Rosenthal and Berenbaum 2012). Secondary metabolites are complex compounds with high variation that result from further metabolization of the primary compounds (Cruickshank 2012). They are responsible for signalling mechanisms and plant defence, interacting with the environment around the plant and external organisms (Rosenthal and Berenbaum 2012). Some secondary metabolites are volatile compounds, which get dispersed through the air, allowing the plant to communicate with the environment and other living organisms around the plant, this includes attracting pollinators, inhibiting the activity of herbivores and microbes to aid plant survival (Dudareva et al 2004). There are two types of defence mechanisms, direct and indirect. Direct defence, can be physical or chemical, the first being thicker cuticles and thorns whereas the second involves the production of chemical compounds that will work as toxins and repellents in order to defend from pests (Dicke and Van Poecke 2002, Schwab et al 2008). Indirect defence, starts after a plant is damaged by, for instance an herbivore, which results in the synthesis and release of volatile compounds, these will attract other species that prey upon and parasitize what is threatening the plant or they will attract more herbivores to herbivore-infested plants,

additionally it has been hypothesised that that the release of volatiles will communicate to neighbouring plants and induce their defence mechanisms (Dicke and van Loon 2000, Dudareva et al 2004).

Volatiles are complex structures that consist of a hydrocarbon structure with oxygen, nitrogen and sulphur. The many different structures that these compounds can have makes them specific for their function. Within the secondary metabolites of relevance to herbs it is possible to divide them into terpenes, alkanes, phenolics and aldehydes (Rohloff 2006).

1.2.1 Terpenes

Terpenes are hydrocarbon compounds and components of essential oils. Isoprene is a hydrocarbon that can be found in every plant, and helps to maintain photosynthesis when the plant is exposed to high temperatures (31-46 ˚C) by dissolving into thylakoid membranes and interact with membrane fatty acids and stabilize the membranes by preventing the formation of water channels that occur when plant is exposed to temperatures of 31-45 ˚C (Sharkey et al 2001). Isoprene was also found to eliminate reactive oxygen species by reacting with these and lowering their concentrations on leaves (Loreto and Velikova 2001). Isoprene is made of five carbons (C_5) and is the base block for terpene synthesis of other compounds (Figure 1.1): monoterpenes, consist of two isoprene units (C_{10}), sesquiterpenes, three isoprene units (C_{15}) and diterpenes, four isoprene units (C_{20}) (Dudareva et al 2004, Parker 2015, Schwab et al 2008).

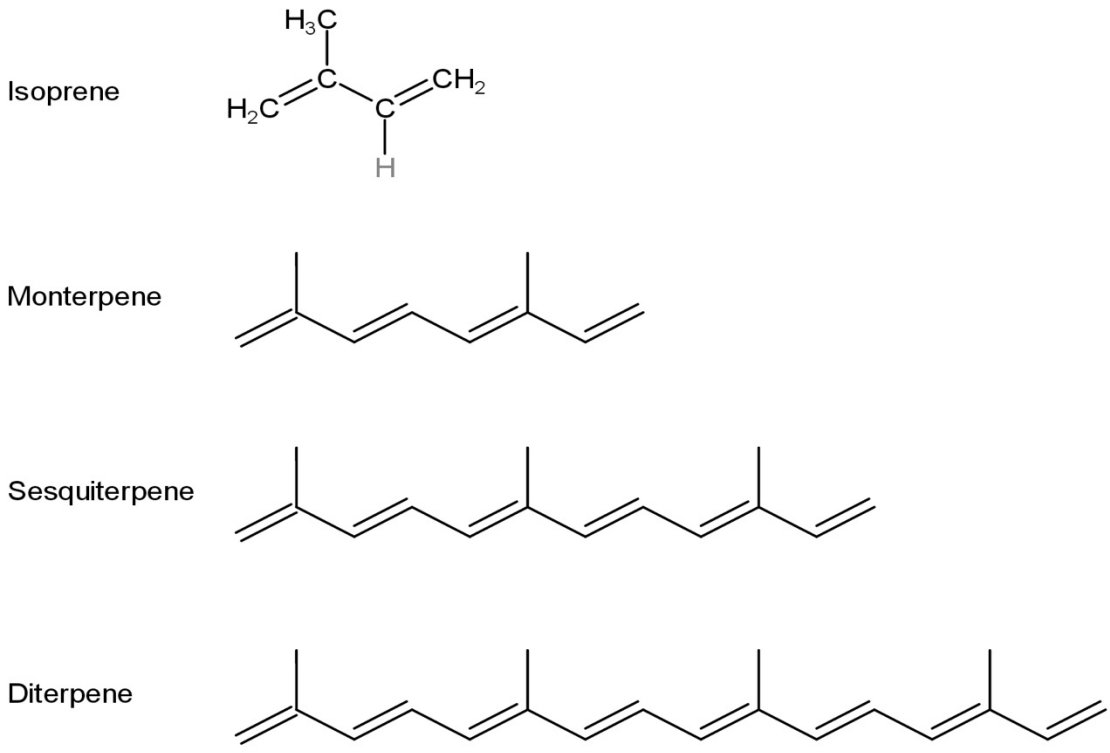


Figure 1.1: Isoprene and other terpene compounds with corresponding structure.

Volatiles such as isoprenoids are the result of an enzymatic process, contrary to compounds such as aldehydes and alcohols that can be formed through oxidation process, for instance to achieve geraniol, where the activity of geranyl diphosphate synthetase is required in the synthesis pathway (Valcourt 2014). The basic pathway for the synthesis of terpenes is formation of basic isoprene units, condensation of basic units and conversion of the units to the final compounds (Dudareva et al 2004). Terpenes can be formed from two pathways, mevalonic (MVA) pathway (Figure 1.2) or the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway (Figure 1.3), with starting compounds acetyl-CoA and pyruvate (respectively), resulting in isopentenyl pyrophosphate (IPP) and isomerised to dimethylallylpyrophosphate (DMAPP) (Dudareva et al 2004, Parker 2015, Schwab et al 2008). MVA pathway will produce precursors for sesquiterpenes and the MEP pathway will produce precursors for monoterpenes and diterpenes (Parker 2015).

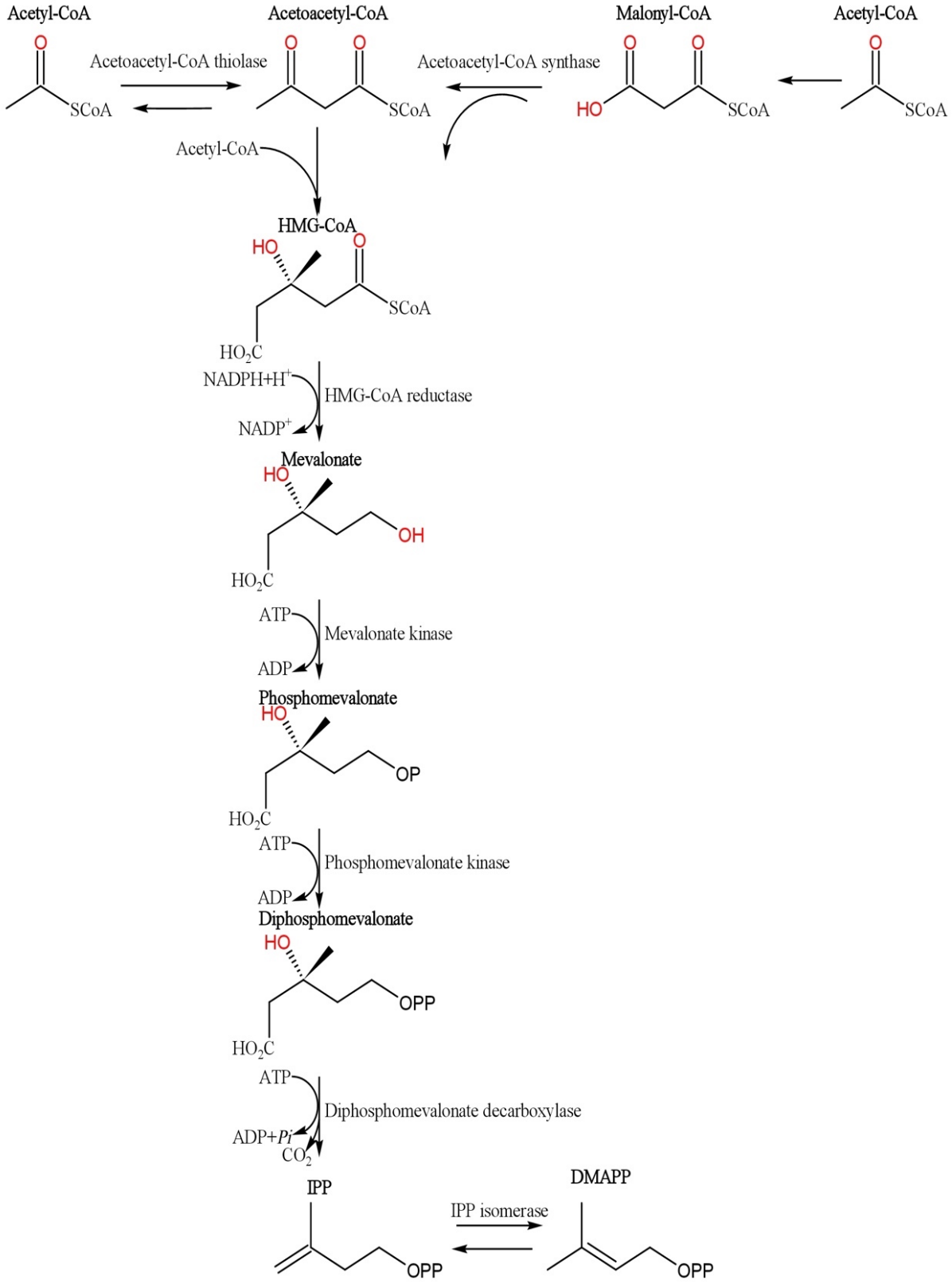


Figure 1.2: Schematic of mevalonate pathway for synthesis of isopentenyl pyrophosphate (IPP) and dimethylallylpyrophosphate (DMAPP).

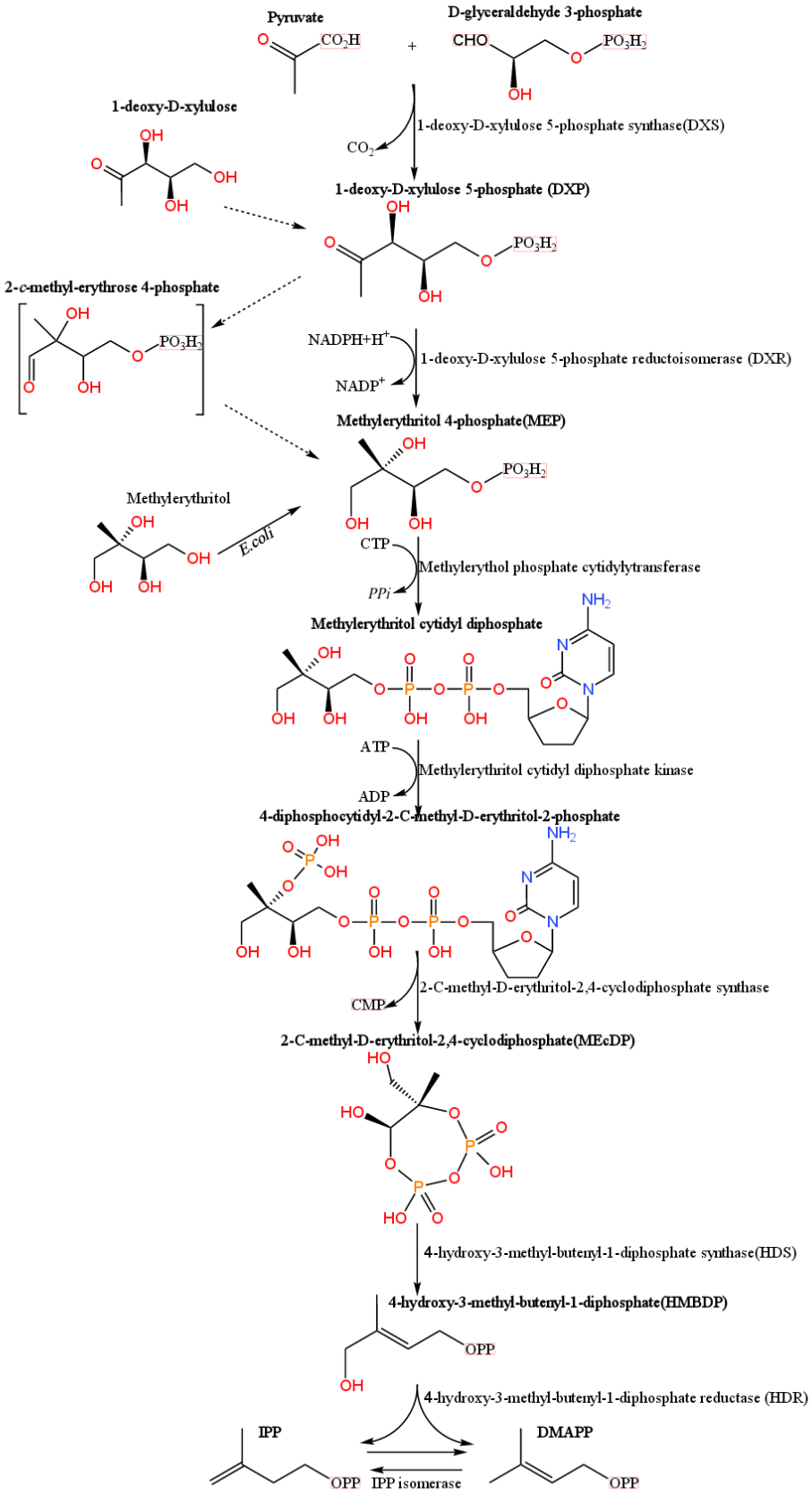


Figure 1.3: Schematic MEP (2-C-methyl-D-erythritol-4-phosphate) pathway for synthesis of isopentenyl pyrophosphate (IPP) and dimethylallylpyrophosphate (DMAPP).

Both pathways will form isopentenyl pyrophosphate (IPP) and dimethylallylpyrophosphate (DMAPP), following this (Figure 1.4), both compounds will be combined to form geranyl diphosphate (GPP) for monoterpene synthesis and farnesyl diphosphate (FPP) for sesquiterpenes synthesis. A sequence of reactions then begins including hydrolysis, ionisations, oxidoreductions or shifts that will result in compounds from the terpene family (Parker 2015).

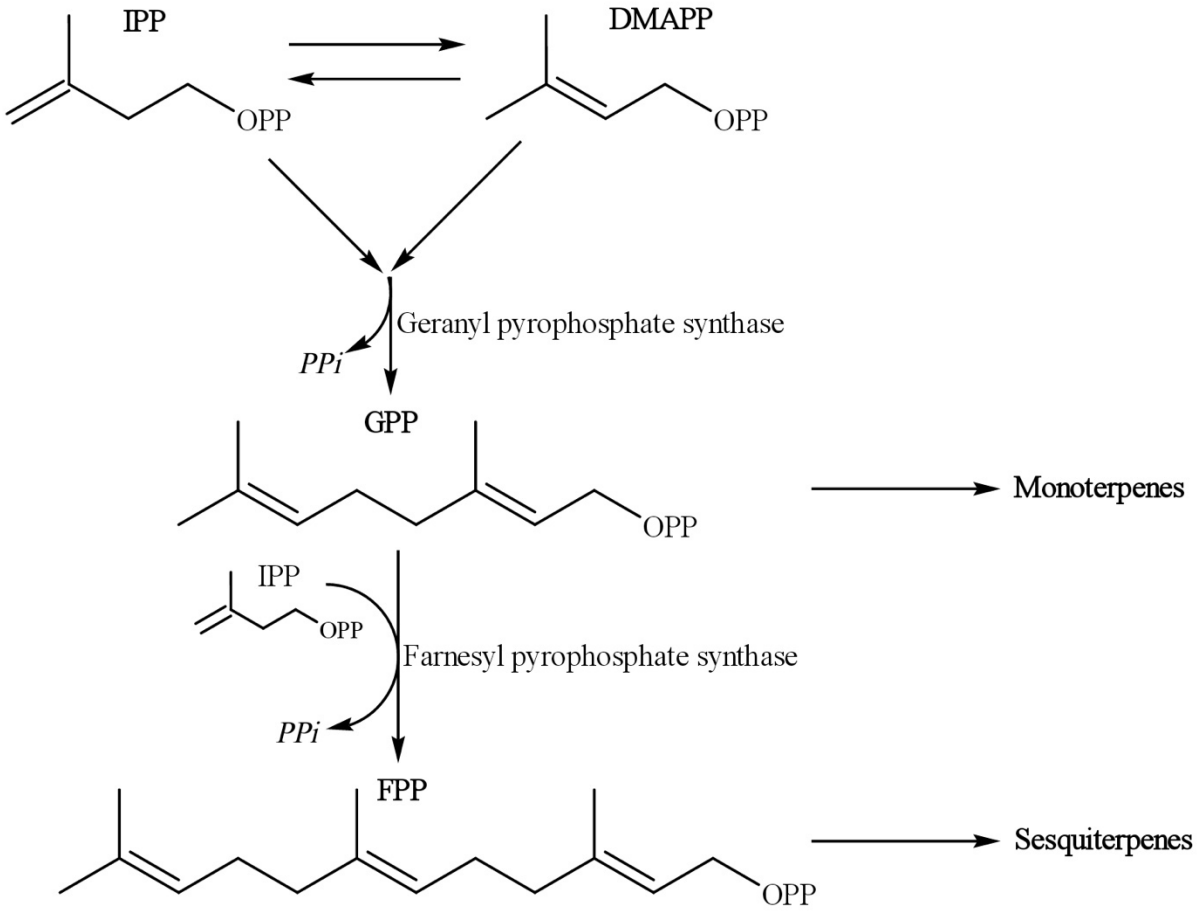


Figure 1.4: Schematic of pathway for monoterpenes and sesquiterpenes synthesis.

Terpenes have been found to reduce feeding intensity of herbivores (helping the plant survive) (Dziba and Provenza 2008), help during seed germination by maintaining forest fires due to high inflammability (Ormeño et al 2009) and acts as membrane stabilizers (Chang et al

2007). Terpenes are also involved in interactions between plants, pollinators and also in stress responses (Dicke and Van Poecke 2002, Schwab et al 2008).

Plants like herbs, which produce essential oils in their trichomes or glandular cells, have their essential oils primarily composed of terpenes (Schwab et al 2008). These are produced and stored in specific structures of leaves, flowers and seeds and only in certain types of plant families (Valcourt 2014). Terpenes have been recognised as the second largest group of compounds present in the essential oil of basil with 1,8-cineole and linalool identified (Figure 1.5) as some of the main contributors to the basil aroma (Chang et al 2007, Miele et al 2001). The majority of rosemary essential oil is constituted of terpenes, with the major compound being camphor followed by alpha-pinene and 1,8-cineole (Salido et al 2003).

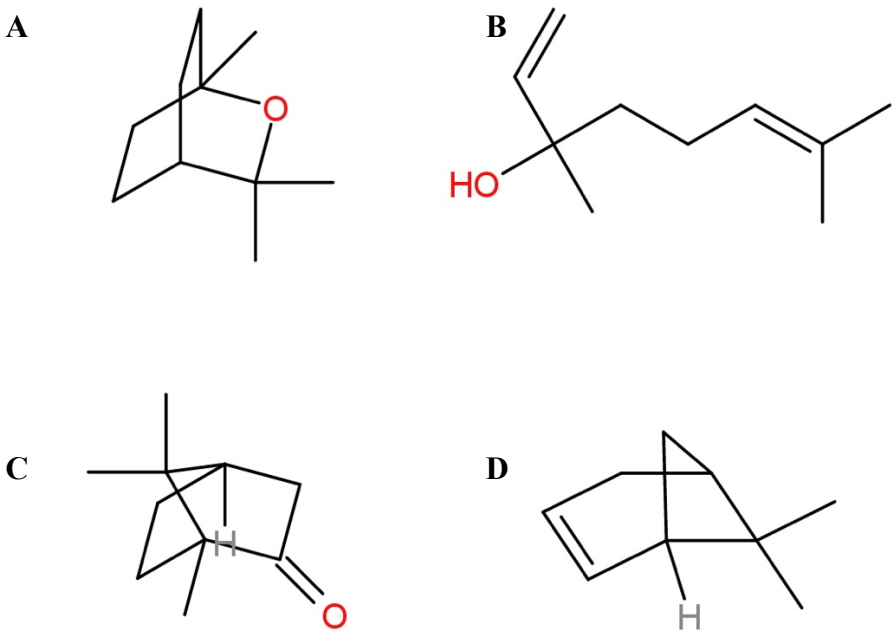


Figure 1.5: Terpenes that occur and contribute to the typical aroma of basil and rosemary; (A) 1,8-cineole, (B) linalool, (C) camphor, (D) alpha-pinene.

1.2.2 Alcohols and aldehydes

Volatile compounds like aldehydes and alcohols are derived from fatty acids, these compounds are commonly found in plants and are responsible for the green leaf aroma which attracts some parasitoid species (Dicke and Van Poecke 2002, Schwab et al 2008). Aldehydes, like other volatiles, are also produced as part of plants defence mechanism against predators like herbivores (Dicke and Van Poecke 2002). The production of these volatiles is induced when the plant is wounded or attacked by insects (Chehab et al 2008, Meerburg et al 2008). Aldehydes and alcohols originate from saturated and unsaturated fatty acids, as these undergo three different processes including alpha-oxidation, beta-oxidation and lipoxygenase pathway (Schwab et al 2008). Short chain (C6 and C9) aldehydes and alcohols are produced through lipoxygenase pathway (Figure 1.6) using enzymatic reactions, involving lipoxygenase (LOX), hydroperoxide lyase (HPL) and alcohol dehydrogenase (ADH) (Turner et al 2021).

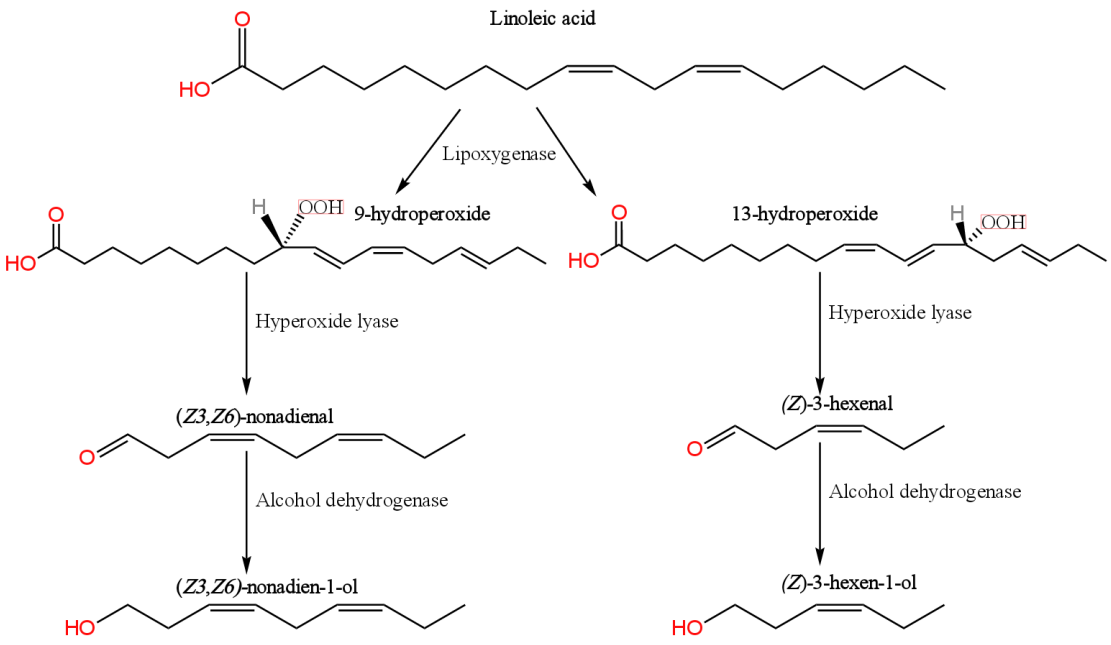


Figure 1.6: Schematic of lipoxygenase pathway for aldehydes and alcohol synthesis.

Besides being a defence mechanism, aldehydes also contribute to the aroma of the plant (Meerburg et al 2008), depending on the carbon chain length the compounds will contribute with different aromas, C3-C5 aldehyde impart a chemical aroma, C6 aldehydes provide green notes like fresh cut grass and higher chain the compounds transmit a fruity, floral and fatty aroma (Parker 2015). Alcohol compounds are formed after corresponding aldehydes are metabolized by alcohol dehydrogenase (Schwab et al 2008).

Shahwar et al (2012) identified aldehydes as some of the major compounds in the essential oil of coriander leaves, compounds like (*E*)-2-decenal, (*E*)-2-undecenal and (*E*)-2-dodecenal were amongst the main compounds identified (Shahwar et al 2012), and structures of these can be seen in Figure 1.7. Aldehyde compounds like (*E*)-2-hexenal (Figure 1.7), can be found in most plants including culinary herbs and are responsible for the green/grassy aroma (Parker 2015).

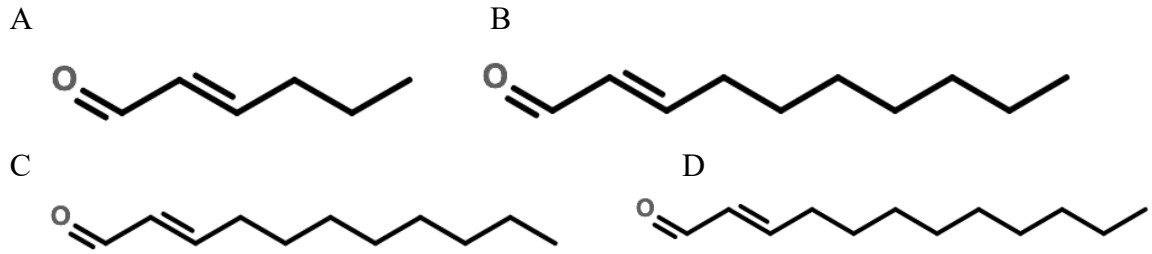


Figure 1.7: Aldehydes that occur and contribute to the typical aroma of basil, rosemary and coriander; (A) (*E*)-2-hexenal, (B) (*E*)-2-decenal, (C) (*E*)-2-undecenal, (D) (*E*)-2-dodecenal.

1.2.3 Phenylpropanoids

Phenylpropanoids are another secondary metabolite that can be found in plants. They are essential for plant survival as they are building units of lignin which is a major component of plant's secondary wall structure and protect against UV-B radiation as they remove reactive oxygen species (ROS) which are produced with UV exposure and will damage plant growth

(Deng and Lu 2017). These compounds usually consist of a six-carbon aromatic phenyl groups and a three carbon side chain (Deng and Lu 2017). The phenylpropanoid pathway is involved in plant growth, structure and response to environmental factors (Biała and Jasiński 2018). The synthesis of these compounds starts with aromatic amino acid phenylalanine, which with the use of three enzymes (Figure 1.8): phenylalanine ammonia-lyase, cinnamic acid 4-hydroxylase and 4-coumarate-CoA ligase, results in p-coumaryl CoA (Biała and Jasiński 2018, Deng and Lu 2017). This will then serve as a precursor for different phenylpropanoids like flavonoids, monolignols and phenolic acids (Deng and Lu 2017).

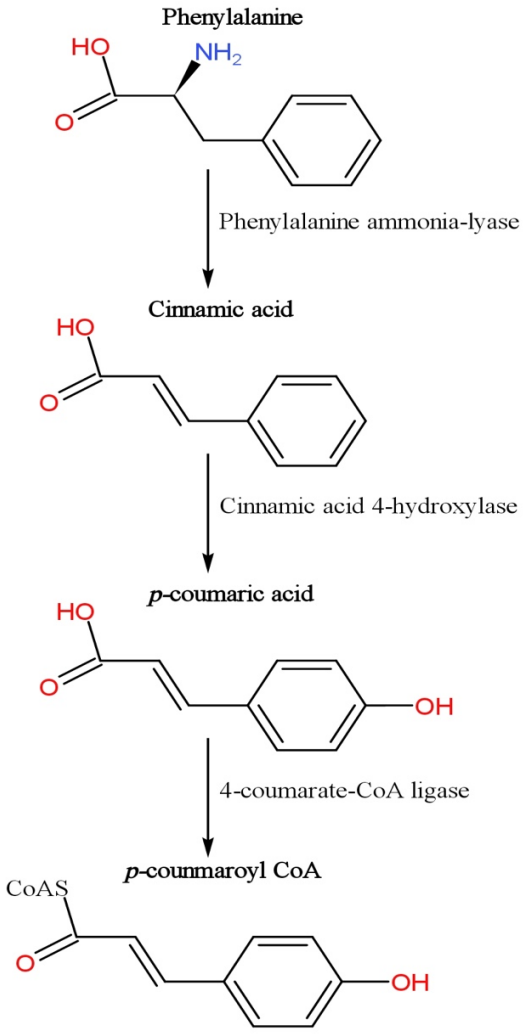


Figure 1.8: Schematic of phenylpropanoid pathway.

Phenylpropanoids are the main contributors to the aroma of some spices and herbs; compounds like eugenol, estragole and thymol, can be found in basil, thyme and cinnamon, among others (Umar Lule and Xia 2005). Phenylpropanoids like eugenol and estragole are the main contributors for the typical basil aroma (Lee et al 2005, Vallverdú-Queralt et al 2014), and the structures of these compounds are presented in Figure 1.9.

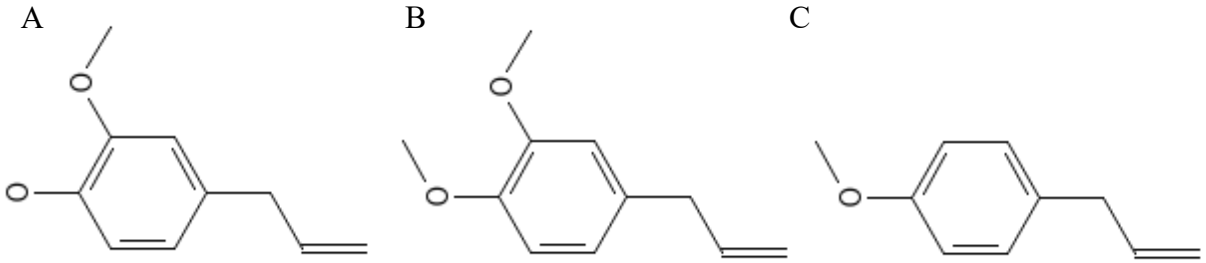


Figure 1.9: Phenylpropanoids that occur and contribute to the typical aroma of basil; (A) eugenol, (B) methyl eugenol, (C) estragole.

1.3 Environmental influences on the aroma of fresh herbs

Environmental factors have been described as having an influence on the flavour profile of the herbs and as a consequence on the production of the volatile compounds. An example of this are aldehydes and alcohols production which is induced after the plant is wounded by insect and herbivores (Scala et al 2013). Culinary herbs can be produced by different cultivation systems: open field, protected field (under polytunnel), pots and hydroponics in glasshouse. In open field production plants are exposed to various environmental conditions including direct sunlight, variable photoperiods, uncontrolled amount of rainfall, uncontrolled growth temperatures with differences between day and night, type of soil and pH, mineral and nutrient availability and pathogens exposure, however plants have more space to grow due to area of field. Crops grown under polytunnel will be protected against uncontrolled amount of rainfall, diffuse sunlight and provides more space for plants to grow, however plants will still be subject

to uncontrolled growth temperatures and subject to increases due to protected setting, type of soil and pH, nutrients availability and increased humidity due to the use of polytunnels. Producing herbs in pots under glasshouse allows for a controlled environment, which would allow to set growth temperature to desired range, controlled amounts of water and use supplemented light to increase crop yield, because plants are in a greenhouse they are protected from winds and less prone to predators, however greenhouse glass filters out UV radiation reducing a stressor that affects secondary metabolism, additionally plants grow under pressure due to the ratio of seeds per pot. Hydroponic production has similar conditions to pots due to glasshouse setting, however due to the absence of soil it allows for better control on amount of water and nutrients that are available to the plant, avoiding deficits of these factors, additionally usually used LED light system which can be manipulated (intensity, spectral wavelength and photoperiod) in order to benefit plants production. Various growing factors affect plants produced in open field and field under protected conditions which are dependent on geographical location of the production site. A variety of abiotic stresses including high and low temperature, drought, pH stress, UV stress and pathogen infection, can create stress in the plant thus triggering secondary metabolites synthesis for plant protection and consequently contribute to characteristic odours, flavours and colours (Akula and Ravishankar 2011). Therefore, differences in growing conditions can lead to abiotic stresses, influencing synthesis of aromatic compounds and consequently crops post-harvest characteristics.

1.3.1 Temperature

Temperature is an important factor when it comes to the production of herb crops, as it influences their yield and growth rate, but it also plays an important role in the production of the secondary metabolites like volatiles. The effect of low (5-10 ˚C) and high (26-35 ˚C) temperature stresses on plants have been studied, with these factor described as the bigger

influencer to plants, suggesting that optimal growth temperature ranges as the best option for plant production (Akula and Ravishankar 2011). However, limited information is available in the literature about the influence of growth temperature on the flavour of fresh herbs.

Crops grown at very high temperatures (above 30 ˚C) will lead to an increase of aroma compounds in comparison to crops grown in moderate temperatures (around 20 ˚C daily), which produce a less intense aroma and flavour (Jasper et al 2020). Jasper et al (2020) assessed the effects of growth temperature in rocket plants, where plants grown at 40 ˚C daytime (30 ˚C night-time) experienced slow germination and retarded plant growth and no plants were ready to be harvested within the same period as plants grown at lower temperatures (20 ˚C and 30 ˚C). Severe heat stress has been described to cause cell damage and death, this happens when plants are not able to produce a heat shock response, which is a momentary reprogramming of cellular activity leading to the synthesis of heat shock proteins (HSP) that provides thermotolerance to the plant, this inability is due to the lack of sensitivity of their proteins and enzymes to heat inactivation and denaturation (Schöffl et al 1998). Several studies analysed the effect of temperature during production on the crop oil yield, and concluded that aromatic crops such as dill, oregano and Spanish thyme, produce between three to five times higher essential oil yield when grown at temperatures between 20 ˚C and 29 ˚C compared to when grown at lower temperatures, between 10-17 ˚C, confirming the influence of temperature on the secondary metabolism and consequently the flavour profile (Hälvä et al 1993, Khalid and El-Gohary 2014, Kosakowska et al 2019).

Differences were seen between basil samples produced at different temperatures, where higher temperature (25 ˚C) led to higher aroma volatile content (70 % higher than samples produced at 15 ˚C) with a notable increase in the eugenol amount (Chang et al 2007). Another

study showed that the optimal growth temperature for basil was 25 ˚C, where it reached 49 cm of plant height, eight leaf-pairs on main stem and specific leaf area of 206 cm^2 g^{-1} compared to 38 cm of height, seven leaf-pairs and leaf area of 170 cm^2 g^{-1} observed in basil grown at temperatures of 15 ˚C, additionally higher temperature (25 ˚C) resulted in three times more essential oil content than lower temperature (15 ˚C) (Chang et al 2005). Growth temperatures of 25 ˚C also affected the aroma volatile composition of the of the oil, where eugenol and cis-ocimene increased however no effect was observed in eucalyptol and linalool (Chang et al 2005). An increase in the number of the peltate trichomes occurred when basil was grown at higher temperatures (25 ˚C), which have been described as the main producers and storage units of the plants essential oils, which potentially caused an increase of the oil content of the herb, however no analysis were made on this (Cruickshank 2012).

Coriander (*Coriandrum sativum*) grown in Turkey was studied in order to determine how growing conditions would affect the crop yield, essential oil yield and composition. The samples were harvested under two different seasons (spring vs summer), and therefore exposed to different average temperatures. Plants harvested during the spring experienced temperatures between 3 ˚C and 16 ˚C (grown from November to June), whilst coriander harvested during the summer season (grown from May to July) experienced temperatures between 17 ˚C and 23 ˚C. As a result, fresh herbage yield in the spring harvest was 24.8 t ha^{-1} whilst summer harvest herbage yield was 7.2 t ha^{-1}, this is attributed to longer (8 months compared to 3 months) to vegetation period experienced by the spring harvest. However, herbs harvested in the summer season produced 0.55 mL 100 g^{-1} of essential oil compared to 0.45 mL 100 g^{-1} (spring harvest), whilst relative abundance of *(E)*-2-decenal for summer harvest was 29 % compared to 25 % for spring harvest and linalool 0.67 % and 1.74 % (respectively), suggesting that average growth

temperature between 17-23 ˚C would be beneficial for the essential oil production (Telci and Hisil 2008).

A study conducted in Spain, looking at the seasonal variation of rosemary oil, concluded that herbs produced during summer season resulted in higher (1.8 %) oil yields compared to the ones produced during the winter (1.0 %) season (Salido et al 2003). Furthermore, some of the compounds responsible for the rosemary aroma, like camphor, beta-pinene and myrcene, were at higher levels in summer samples, the opposite was seen for alpha-pinene with highest levels for winter production (Salido et al 2003). Another study performed in the Balkan Peninsula, concluded that some of the major aroma components of the rosemary essential oil were affected by the growing temperatures, apart from borneol which was not significantly affected by the temperature. Compounds like 1,8-cineole and camphor were affected by the temperature but with opposite behaviour, with the first one observed to decrease from ~38 % to ~15 % in concentration when the temperature varied from 7 ˚C to 10 ˚C, whereas for the second compound the concentration increased from ~10 % to ~29 % with the increase of 7 ˚C to 10 ˚C, considering that these compounds have different production pathways, this suggests that 1,8-cineole pathway is favoured at lower temperatures whereas camphor if favoured at higher temperatures (Lakušić et al 2012).

Therefore, there is a gap in knowledge to explain the effect of temperature during growth on the subsequent flavour and aroma composition of herbs. Most of the published studies are focused on the impact of temperature on oil yield and total volatile content of herbs but little is known about the effect that temperature has during growth on the aroma composition of fresh culinary herbs.

1.3.2 Water

Irrigation practices can be used in order to manage the growth and development of a crop. In plants, when exposed to drought stress, stomata undergo closure, which reduces water losses and limits CO_2 uptake and by consequence reduces photosynthesis, affecting plant growth (Osakabe et al 2014). However, plants have developed mechanisms in order to adapt to environmental adversities like water stress, by changing metabolic and structural capacities, helping the plant to survive and adapt to new environmental conditions (Chaves et al 2002). Exposing plants to some water deficit (up to 70 % field capacity) has been reported to increase essential oil content (Ekren et al 2012, Khalid 2006, Omidbaigi et al 2003) and increase the oil content at 125 % field capacity constituents, however these increases were not significant and further investigation needs to be carried to understand if water deficit can significantly increase essential oil in the plant (Ekren et al 2012, Khalid 2006). Drought stress is another significant abiotic stress to the plant, however the extent varies with plant species, this will lead to oxidative stress, increasing the amounts of secondary metabolites such as phenolics and flavonoids (Akula and Ravishankar 2011). Water stress has also been described to affect the height of the plant, fresh and dry weight, causing a decrease of these attributes (Baher et al 2002). These findings suggest that water stress may protect the plant against other abiotic stresses and consequently change the post-harvest characteristics of the crop, however this is species specific and the extent of the stress needs to be determined for each crop.

Different levels of water stress can affect fresh and dry weight of the plants as well as essential oils constituents. In a study conducted by Khalid (2006), different water stress levels were tested across two seasons using two different varieties of *Ocimum*, and the effects on the plant and plant volatile composition were evaluated. The study showed that the greatest fresh and dry weight was obtained at 75 % field water capacity (water content held by the soil) and

the total essential oil content (g plant^{-1}) was also greater at this stress level. However, *O. basilicum* had its highest percentage of oil content at 50 % field water capacity (0.37 %) compared to 100 % (0.27 %) and 125 % (0.34 %) field water capacity. The volatiles linalool and 1,8-cineole were found to be at their highest percentage of essential oil (35.6 % and 9.9 %, respectively) under the 50 % field water capacity compared to 100 % (32.5 % and 8.7 %, respectively) and 125 % (33.4 % and 9,4 %, respectively) field water capacity, conversely estragole, which has been described as a key compound in some basil aroma, was greater at 125% field water capacity (35.0 %) compared to 100 % (34.0 %) and 50 % (34.5 %) field water capacity (Khalid 2006). Water stress (50 % field water capacity) can increase the percentage of volatiles in the oil , but it depends on the species, as it can also affect the volatile components, however different volatiles respond in different ways, without always showing any relation between water stress and type of compounds produced (Khalid 2006). A similar study evaluated purple basil (*Ocimum basilicum*) and the effect of different water treatments, where 125 % field water capacity led to taller plants (3.1 cm taller than 100 % field capacity) and greater fresh mass (double amount compared to 100 % field capacity), but with free draining field conditions (Ekren et al 2012). Ekren et al. (2012) concluded that essential oil percentage was at its greatest at 50% field water capacity (1.10 %) compared to 100 % (0.91 %) and 125 % (0.99 %) field water capacity, similar to what was reported by Khalid (2006). A mixed response to water deficit stress of reported for volatiles compounds, where the percentage of linalool was 64.3, 62.3, 62.3 and 63.5 % at 125, 100, 75 and 50 % field water capacity, respectively, whereas estragole percentage was 1.45, 6.03, 5.65 and 5.22 % at 125, 100, 75 and 50 % field water capacity, respectively, and eugenol percentage 7.39, 7.26, 7.04 and 6.50 % for 125, 100, 75 and 50 % field water capacity (Ekren et al 2012).

A study looking at the effect of water deficit stress on different varieties of coriander from Iran, concluded that exposing coriander plants to no irrigation after the start of flowering or after the start of flowering led to lower essential oil yield and seed yield. However, a significant increase in linalool and 2-pentadecanone when the plants irrigation was terminated at blooming or flowering stage (Nadjafi et al 2009). These findings led the authors to conclude that water stress had a negative effect of the plant yield but it led to a more aromatic profile (Nadjafi et al 2009). In Tunisia, a study looked at the effect of salinity and its effect on the essential oil yield and composition. It was concluded that under very high salt concentrations (75 mM NaCl) there was a reduction of 38 % in essential oil yield, however mild and moderate stress (25 and 50 mM NaCl), resulted in an increase of 18 % and 43 % (respectively) in the essential oil yield of coriander and a significant increase in (*E*)-2-decenal, (*E*)-2-dodecenal and dodecanal. Most of the essential oil compounds in coriander increased in abundance with mild to moderate salt stress, and significantly decreased at high levels of salt. However, increase in salt content resulted in the reduction of fatty acids which were hypothesised to affect the formation of plant membranes (Neffati and Marzouk 2008). Additionally, the application of NaCl also showed an impact in essential oil yield and composition, an increase of 0.05 % in essential oil yield was detected at 50 mM NaCl, with an increase of (*E*)-2-decenal, (*E*)-2-dodecenal and dodecanal content of 44, 29 and 41 %, respectively, compared to an increase of 30, 23 and 29 %, respectively, at 0 mM NaCl (Neffati and Marzouk 2008).

Rosemary, similarly, to basil, is affected by water supply, and water deficit stress results in a significant increase of essential oil, however crop yield is reduced. Water deficit stress also affected the relative abundance of individual compounds, causing an increase in alpha-pinene, 1,8-cineole and borneol but a decrease in linalool and camphor (Gharib et al 2016). A study looking at the effect of the concentration of soil solutions in rosemary, using a 5 L plastic pot

containing desert dune sand, applied different treatments over a period of four weeks after three weeks of basal solution (1.8 mM N, 0.35 mM P, 0.64 mM K, 1.0 mM Ca, 0.35 mM Mg, 0.35 mM S, 0.03 mM Fe, 0.4 µM Zn, 5 µM Mn, 0.1 µM Cu and 23 µM B), reported that plant growth was not affected by the combination of basal solution with 100 mM NaCl, however rosemary irrigated with combination of basal solution with 100 mM NaCl and 5-10 mM KCl or with the combination of basal solution, 100 mM NaCl and 4-8 mM $CaCl_2$, resulted in a significant ($p<$ 0.05) increase in the relative abundance of 1,8-cineole and camphor (Tounekti et al 2011).

These findings make clear that water plays an important role in the production of culinary herbs, affecting both yield and essential oil of the crop, furthermore, finding the appropriate level of water deficit stress could benefit crop yield, essential oil yield and volatile composition of the plant, allowing culinary herbs growers to produce more aromatic crops. However, this effect varies depending on the species of the crop and no association can be made with the volatile groups. Therefore, it makes it difficult for growers to adopt a system in order to improve both yield of the crop and flavour.

1.3.3 Light

Light is an important environmental variable due to its essential role in photosynthesis and also its influence on plant morphology, cellular functions and chemical composition (Cruickshank 2012). Light can be considered in the context of quantity (intensity), quality (wavelength composition) and photoperiod (period of light/dark over a 24 h cycle), both important factors that can impact on the essential oil content of an aromatic plant. Light intensity was reported to have a positive effect on secondary compounds production by stimulating their synthesis and accumulation of photosynthetic pigments (chlorophylls and

carotenoids) after daily doses of UV-radiation, suggesting this can be used to manipulate crops characteristics (Akula and Ravishankar 2011).

Rosemary (*Rosmarinus officinallis*) grown under pot conditions, when exposed to natural light (9 h) only (control) and supplemented with 3 h (at end of day) of red (660 nm) or far-red (730 nm) light, suffered detrimental effects on the essential oil content and its composition. Far-red light increased oil production (0.2 mL 100 g^{-1} dry weight), while the opposite was seen for red light (0.03 mL 100 g^{-1} dry weight), however both wavelengths stimulated plant growth (far-red: 9.11 cm; red: 7.23 cm; control: 5.15 cm) (Mulas et al 2006). Far-red light led to an increase of alpha-pinene (34.1 %), camphene (4.9 %) and p-cymene (1.5 %) compared to red light(16.2 %, 2.3 % and 0.0%, respectively) and control (29.5 %, 3.9 % and 1.3 %, respectively), conversely red light increased limonene (8.7 %), bornyl acetate (4.6 %), alpha-cedrene (5.0 %) and neryl acetate (6.9 %) compared to control (2.8 %, 3.0 %, 0.0 % and 0.9 %, respectively) and far-red (2.8 %, 6.8 %, 4.2 % and 1.9 %, respectively) lighting (Mulas et al 2006). The effect of light intensity on rosemary was analysed, where three light treatments (100 %, 50 % and 25 % sunlight) were applied by shading plants using black plastic nets with suitable cut-off, reduced light intensity (50 % and 25 %) resulted in higher leaf area (0.59 cm^2 and 0.67 cm^2, respectively) compared to 100 % sunlight (0.46 cm^2), whereas 50 % sunlight resulted in the highest essential oil yield per fresh biomass (0.40 % in comparison to 0.30 % in 100 % sunlight), additionally an increase of relative amounts of compounds including alpha-pinene (37.8 %), camphene (7.9 %) and eucalyptol (13.8 %) in comparison to at 100 % light intensity (30.5 %, 6.9 %, 13.5 %, respectively) and a decrease of camphor (5.6 %) and borneol (4.6 %), in comparison to at 100 % light intensity (7.4 % and 8.8 %, respectively) (Raffo et al 2020).

A similar study, examining basil (*Ocimum basilicum),* using white light on reflective wavelengths on mulches, concluded that blue (~ 400 nm) and red (~ 700 nm) wavelengths decreased the total volatile content by a quarter and half (respectively) compared to control conditions, which used black polyethylene that reflected 6 % of light. Leaf area was higher at red reflective mulch (785 cm^2) compared to other colour (black: 608 cm^2; blue: 591 cm^2; white: 687 cm^2), but their dry weight was smaller (red: 3.03 g) when compared to the control (white: 5.41 g) (Loughrin and Kasperbauer 2001, 2003). Litvin et al (2020) supplemented 16 h of light-emitting diodes (LED), low blue:red ratio of 7:93 and high blue:red ratio of 30:70 (blue-450 nm peak and red-670 nm peak), or high pressure sodium (HPS) (400 W) with controlled temperatures of 24 ˚C and 20 ˚C for day and night respectively, reported shorter basil plants (2.5 cm shorter) grown under LED compared to HPS lighting. However, increased photosynthesis (20 %) in LED than HPS was observed. Conversely, a change in the volatile content was described with myrcene increased by 130 % under LED conditions as well as higher concentrations of myricetin and orientin (Litvin et al 2020). Similarly, Carvalho et al (2016) described shorter (50 % shorter) plants under LED conditions than plant grown in natural light condition in greenhouse (over a photoperiod of 12 h light/12 h dark) apart from a combination of blue (450 nm), red (600 nm) and yellow (600 nm) (1:1:1) LED lighting. Further to this, blue and red LED lighting produced similar volatile content to greenhouse plants, however when a third wavelength (green-520 nm or yellow-600 nm) was added changes were registered, as adding green resulted in higher content of sesquiterpenoids (double amount than greenhouse), monoterpenoids (double amount than greenhouse), eugenol (nine times higher amount than greenhouse) and estragole (double amount than greenhouse), whereas when using yellow as the third wavelength resulted in an increase in sesquiterpenoids (double amount than greenhouse), monoterpenoids (double amount than greenhouse) and estragole (triple amount than greenhouse (Carvalho et al 2016). The effect of light intensity on clove basil (*Ocimum*

gratissimum) was studied, where increased light intensity resulted in higher essential oil yield however this was due to increase in leaf biomass, conversely no effect on the glandular trichome density and essential oil content was observed (Fernandes et al 2013).

Coriander (*Coriandrum sativum*) grown under greenhouse conditions with red LED lighting supplementation resulted in higher biomass (double the biomass) and taller plants (~ 17 cm) with higher content (~ 5 % higher) of decanal, dodecanal and (*E*)-2-tridecenal. Conversely, red and blue lighting produced shorter plants (~ 7 cm) and higher content (~ 7 % higher) of (*Z*)-3-hexenal, imparting a green, fatty and grassy aroma notes (McAusland et al 2020).

These findings show that light can be used to control crop yield and morphology as well as aroma composition, and in choosing the combination of light supplementation different results can be achieved. Further investigation on the manipulation of light quantity and quality needs to be investigated in order to further understand how this can be achieved.

1.3.4 Nutrition

Nutrients availability determine plant growth and development, and are involved in several metabolic pathways (Kumar et al 2021). In order to produce enough food to feed the rapidly growing human population, fertilisers have been used in crop production to improve yield and biomass, however this can lead nutrition stress which will affect photosynthesis and the production of secondary metabolites (Akula and Ravishankar 2011, Kumar et al 2021).

Nitrogen is one of the nutrients essential for vegetative growth of the crop since is required for the synthesis of starch in leaf, amino acid production for protein synthesis, crop

yield and synthesis of secondary metabolites (Kumar et al 2021). A study reported an decrease in the production of secondary metabolites of leaf mustard with increasing amounts of nitrogen applied (Juan Li et al 2008). The use of nitrogen for the production of basil helps produce a higher crop yield and essential oil yield. A study by Nurzynska-Wierdak et al (2013) analysed the effects of nitrogen and potassium on the essential oil yield and composition of basil, and the results showed that an increase in nitrogen in the soil resulted in an increase of the essential oil ($p < 0.05$) content as well as increase of 10 % in linalool abundance. A similar effect was seen with potassium with a significant ($p < 0.05$) increase of essential oil production and not significant increase of 1,8-cineole by 1.5 % were observed (Nurzynska-Wierdak et al 2013). This study concluded that the use of nitrogen not only promoted crop development but also increased the content of monoterpenoids, such as 1,8-cineole and linalool, as well as sesquiterpenes such as germacrene D and epi-alpha-cadinol (Nurzynska-Wierdak et al 2013). Another study, also in Italy, investigated the effect of nitrogen on basil crop yield and essential oil production. Similar conclusions were drawn, the application of nitrogen increased crop yield and produce a positive effect on the essential oil yield (Sifola and Barbieri 2006). No analysis on the effect of essential oil compounds was made. Coriander seed yield, essential oil and oil content was analysed after the use of increasing amounts of nitrogen, where seed yield was highest at 60 kg ha^{-1} and essential oil higher at 90 kg ha^{-1} (Akbarinia et al 2007). Additionally, an increase of 2.7 times of coriander's seed essential oil was reported with an application of 80 kg ha^{-1} of nitrogen (Moosavi et al 2015). This leads to the conclusion that the manipulation of nitrogen could have an impact on the culinary herbs under study, as the use of nitrogen resulted in different outcomes on the essential oil production for the three herb species investigated.

Iron is an important micronutrient involved in photosynthesis and secondary metabolites production, which can be found available in the majority of soils, however, due the neutral to

basic pH of the soil, iron intake by plants can be reduced leading to deficiency (Wenfeng Li and Lan 2017). Consequently, producers resort to the use of iron sprays to supplement plant growth. A study in Italy tested the effects of iron on rosemary essential oil yield and composition, after differences were seen with the water intake of the plant (Mulè et al 1996). Iron sprays were applied to the crops and no effect on oil yield was seen, however it caused an increase in the production of verbenone, suggesting an increase of the metabolic process which converts alpha-pinene to verbenone, would benefit market value of rosemary oil for perfume industry as it imparts a camphor and spicy aroma (Moretti et al 1998). The application of sprays of zinc and iron was reported to influence the volatile oil and volatile composition, applying 200 ppm of zinc and 200 ppm of iron significantly increased essential oil content and an increase of main compounds decanal, (*E*)-2-decenal and (*E*)-2-undecenal of 3 % in relative percentage (Said-Al Ahl and Omer 2009).

This suggests that the use of nutrients for crop production can be manipulated for yield but also flavour characteristics of the plant. More work is required to examine the impact of a range of environmental conditions on the profile of volatile and non-volatile compounds linked to flavour, and to move beyond previous experiments which have primarily focused on yield parameters.

1.4 Conclusion

The information gathered, suggests that the flavour profiles of different herb species are complex and consist of range of compounds that can be detected in different proportions. Types of compounds that determine the flavour of each herb have been consistent with basil aroma attributed to the presence of monoterpenes and phenylpropanoids, coriander to aldehydes and

alkanes, and rosemary to the presence of monoterpenes. However, profiles are less consistent and differences can be attributed to differences in variety or growing environment.

Several studies on herbs volatile composition have been conducted, however few studies have stated the variety of the herb produced, type of production or environmental conditions and if the study was completed in multiple time points or locations. Consequently, few studies are able to attribute flavour variance to environmental factors or the genetic influence of the variety. The few published studies that specify the variety used, demonstrated that environmental conditions impact the aroma of the herb since differences in the aroma profile composition were detected. Stating information about the production conditions will provide a better understanding on how the aroma and flavour of the crops are affected.

Consumer preference of the flavour of culinary herbs is lacking in the literature and this needs to be investigated to help improve herb quality in terms of consumer preference. Additionally, it is necessary to correlate flavour chemistry with sensory profiling and consumer liking, as this will inform growers how herbs are perceived and how sensory attributes such as flavour and aroma drive consumer preference.

Chapter 2: Aroma profiles of three culinary herbs: basil (*Ocimum basilicum* var. Sweet Genovese), coriander (*Coriandrum sativum* var. Cruiser) and rosemary (*Rosmarinus officinallis*)

2.1 Introduction

Culinary herbs have a long history and are used in many cuisines around the world, providing flavour to dishes. Different parts of these plants can be used for their aromatic properties, from the seeds to the berries, and can be used fresh or dried (Tapsell et al 2006). Replacing salt by using culinary herbs to add flavour to dishes in a more healthy way has led to an increase in the frequency of consumption of herbs (Bower et al 2016, Opara and Chohan 2014). Culinary herbs can be consumed in their fresh state or dried, either mixed with other ingredients in a cooking sauce or consumed uncooked. The form in which the herbs are consumed will affect their volatile compounds composition, in consequence determining their aroma profile (Chohan et al 2008, Díaz-Maroto et al 2004). In order to understand the aroma characteristics imparted by three of the most commonly consumed fresh herbs, the relative concentration of volatiles compounds was determined.

Basil (*Ocimum basilicum)* is a member of the Lamiaceae (mint) family, and it is highly cultivated in Mediterranean areas. Chemical composition of the essential oil of basil depends on the variety, with the main constituents being linalool, estragole, eugenol and 1,8-cineole (Miele et al 2001). A study looking at extracts of basil leaves additionally identified methyl cinnamate as one of the major aroma constituents (Lee et al 2005) and, unlike previous studies, Klimánková et al (2008) also identified bergamotene as a major compound. Linalool and estragole were described as being responsible for the typical basil aroma and for representing 60-70 % of the oil composition (Yousif et al 1999). Conversely, another study described

eugenol and beta-caryophyllene as the major compounds of basil aroma, responsible for ~ 60 % of the constituents (Sulochanamma et al 2009).

Coriander (*Coriandrum sativum*) is a plant from the Umbelliferae/Apiaceae family. Both seed and leaves of coriander can be used for their oil content, however they have been described as having different composition (Neffati and Marzouk 2008). Coriander with strong sweet, floral odour has been attributed to the presence of geranyl acetate in higher amounts (Ravi et al 2007). Additionally, El-Zaeddi et al (2016) identified compounds like (*E*)-2-decenal, (*E*)-2-dodecenal, decanal, dodecanal, (*E*)-2-tridecenal and 2-tetradecenal as being responsible for most of the coriander oil composition, providing a characteristic green and soapy aroma, attributed to this herb. Furthermore, (*E*)-2-decenal was identified as the most abundant volatile compound, followed by linalool, (*E*)-2-dodecenal, (*E*)-2-tetradecenal, 2-decen-1-ol, (*E)*-2-undecenal, dodecanal, (*E*)-2-tridecenal, (*E*)-2-hexadecenal, 2-pentadecenal, and α-pinene (Anjum et al 2011). Conversely, another study looking at coriander cultivar Jantar identified (*E*)-2-dodecenal, decanal, and (*E*)-2-decen-1-ol as the main compounds contributing to the aroma, followed by dodecanal, 1-decanol, phytol, undecanal, tetradecanal, (*E*)-2-undecenal, oleic acid, (*E*)-2-tridecen-1-ol, cubenol, and nonane (Nurzyńska-Wierdak 2013).

Rosemary (*Rosmarinus officinallis*) is a woody aromatic herb from the family Lamiaceae. It is produced worldwide, however the main area of production is the Mediterranean countries. The oil of rosemary is constituted mainly by monoterpenes and it can be described as primarily borneol and 1,8-cineole, followed by camphor and limonene, depending on the variety of the herb (Pintore et al 2002). Conversely, another study analysed the rosemary oil composition from plants grown in Spain identified 53 compounds, with the major constituents being camphor, alpha-pinene and 1,8-cineole, followed by camphene, borneol, beta-pinene, beta-

caryophyllene and limonene (Salido et al 2003). Furthermore, rosemary from the Balkan Peninsula was analysed and two types of essential oil were identified, 1,8-cineole and camphor types, with alpha-pinene and borneol as part of the main contributors for the aroma (Lakušić et al 2012).

In the present study, the aroma profiles of the culinary herbs rosemary, coriander, and basil, were analysed at a commercial harvest maturity using a number of different production methods. The same variety was grown at all sampling sites in the case of basil and coriander samples, however due to production practices, rosemary sample varieties were unknown. The main purpose was to assess and compare the aroma profile of each of the herbs using solid-phase microextraction (SPME), which is a solvent free sample preparation technique, isolation and concentration of volatiles compounds. For this study, the herbs were analysed fresh, to understand better what their aroma profile is when consumed in their native state.

2.2 Materials and methods

2.2.1 Plant material

Fresh leaf material was sourced and delivered by different growers across the United Kingdom (UK). This material was equivalent to fresh leaf products delivered to commercial chains and consumers. Samples of Rosemary (*Rosmarinus officinallis*), Coriander (*Coriandrum sativum* var. Cruiser) and Basil (*Ocimum basilicum* var. Sweet Genovese*),* were provided that were representative of UK's fresh culinary herb production sector.

Samples from different types of agronomic practice were provided (Table 2.1) herbs grown in pots under protected conditions (Pot), produced in soil protected under glass (Soil),

grown in open field subject to weather conditions (Field) and using a hydroponics system (Hydroponics). Growing conditions supplied by growers when records were available, with some information not shared due to commercial confidentiality. This chapter defines the key characteristics of the aroma profile of each herb for a single UK growing season; subsequent chapters will examine the contribution of seasonal variation on herb profile. For each herb sample supplied by the growers three subsamples were selected randomly, several sprigs of leaves from the sample were selected and equal proportions of young and older leaves (top and bottom respectively) were selected to create each replicate (n = 3) for the analysis.

Table 2.1: Location, type of production and environmental factors for each sample for the three herbs.

Herb	**Sample**	**Location**	**GPS**	**Production type**	**Av Temp[A]** (˚C)	**Soil type[B]**	**Water supply[C]** (mm day^{-1})	**Light source[D]** (h day^{-1})
Basil	B1	West Sussex	50.4848˚N, 0.4413˚W	Pot	20-25	Peat	Ir	15 h-Sl
	B2	Lincolnshire	52.7442˚N, 0.3779˚W	Pot	20-25	Mixture	Ir	16 h-Sl
	B3	West Sussex	50.4914˚N, 0.4445˚W	Hydroponics	20-25		Ir	12 h-LED
	B4	Worcester	52.0736˚N, 2.0345˚W	Soil covered	16-20	Loamy	Ir	14 h-Sl
Coriander	C1	West Sussex	50.4848˚N, 0.4413˚W	Pot	20-25	Peat	Ir	16 h-Sl
	C2	Lincolnshire	52.7442˚N, 0.3779˚W	Pot	20-25	Mixture	Ir	16 h-Sl
	C3	York	54.1345˚N, 1.2430˚W	Open field	16-20	Loamy Clay	2.0mm-Rf	16 h-Sl

							0.7mm-Ir	
	C4	West Sussex	50.8198˚N, 0.7807˚W	Open field	16-20	Sandy	2.5mm-Rf Ir	16 h-Sl
	C5	Worcester	52.0736˚N, 2.0345˚W	Open field	20-25	Loamy	2.3mm-Rf Ir	16 h-Sl
Rosemary	R1	West Sussex	50.4848˚N, 0.4413˚W	Pot	20-25	Peat	Ir	16h-Sl
	R2	York	54.1345˚N, 1.2430˚W	Soil covered	11-15	Loamy Clay	1.7mm-Ir	15h-Sl
	R3	York	54.1345˚N, 1.2430˚W	Open field	11-15	Loamy Clay	1.8mm-Rf 0.4mm-Ir	12h-Sl
	R4	Reading	51.3697˚N, 0.9556˚W	Open field	16-20	Loamy Clay	5.1mm-Ir	12h-Sl
	R5	Worcester	52.0736˚N, 2.0345˚W	Open field	16-20	Loamy	1.9mm-Rf	12h-Sl
	R6	Norwich	52.3927˚N, 1.2648˚E	Open field	16-20	Loamy	1.2mm-Rf 1.8mm-Ir	12h-Sl

[A] Average temperature over 24 h; [B] Type of soil used: mixture- composed of 90 % peat substrate and 10 % perlite [C] Average water amount and water source used: I- irrigation and Rf- rainfall; [D] Average photoperiod and light source used: Sl- sunlight, HPS-high pressure sodium and LED- light emitting diode (Philips Toplights-DRW/LB)

All the samples were harvested at commercial maturity (15-26 cm in height) and sent by a courier in boxes with cooling packs. Herbs were stored at 5 ºC (cut samples) or at room temperature (pot samples), and analysis was carried out within four days of receipt.

2.2.2 Preparation of samples

Fresh leaves were hand cut out from samples received from growers, including equal weights of young and old leaves (from top and bottom of herb sprig). Portions of 2 g of fresh herb material of each independent replicate were ground with 2.8 mL of saturated calcium chloride solution, using a pestle and mortar. Both ground leaves and solution were transferred to a 20 mL SPME vial fitted with a screw cap making a weight of 5 g and to this 50 µL of propyl propanoate (internal standard) at 100 ppm was added. Vials were stored at 4 ˚C until extraction. From each sample three biological replicates were prepared, extracted, and analysed once in the equipment (n = 3).

2.2.3 Chemical reagents

For fresh sample preparation, saturated calcium chloride solution was prepared using calcium chloride salt purchased from Sigma Aldrich (Gillingham, United Kingdom). An internal standard (IS), used for the volatile composition of each sample, was prepared using a neat propyl propanoate and diluted in pure methanol, both solutions were obtained from Sigma Aldrich. The alkane standards C_6-C_{25} (100 µg mL^{-1}) in diethyl ether were obtained from Merck (Poole, UK).

2.2.4 Solid Phase Micro-Extraction (SPME) followed by Gas Chromatography-Mass Spectrometry (GC-MS)

The volatile compounds of analysis was carried out by automated headspace SPME using an Agilent 110 PAL injection system and Agilent 7890 gas chromatograph with 5975C mass spectrometer (Agilent, Santa Clara, CA, USA). The SPME fibre stationary phase was composed of 75 µm divinylbenzene/Carboxen™ on polydimethylsiloxane, Supelco (Bellefonte, PA, USA). Samples were incubated at 35 ˚C with an agitation of 500 rpm for 10 min followed by 30 min fibre exposure to the headspace. After extraction, the fibre was inserted into the GC-MS injection port and desorbed for 5 min. An Agilent capillary column HP-5MS (30 m x 250 µm x 0.25 µm thickness) (Agilent, Santa Clara, CA, USA) was used chromatographic separation. The run started using the temperature programme: 5 min at 40 ˚C isothermal, an increase of 4 ˚C min^{-1} and 5 min at 260 ˚C isothermal, and injection mode was splitless. The data were recorded using HP G1034C Chemstation system.

The relative concentration for each volatile compound was analysed and relative concentration was calculated in relation to the IS (propyl propanoate). The volatile compounds were identified by comparing each mass spectra with the mass spectra of compounds analysed in our laboratory (The Flavour Centre, University of Reading) and from spectral databases (ADAMS, NIST and INRAMASS). Confirmation of the identification of the compounds was done using linear retention indices (LRIs) that were calculated using the retention times of known alkanes (C_6-C_{25}) and comparing with the LRI of compounds analysed in similar conditions.

2.2.5 Statistical analysis

The quantitative data of the volatiles compounds obtained from the GC-MS analysis, were analysed by one-way analysis of variance (ANOVA) and principal component analysis (PCA) using XLSTAT version 2020.5.1 (Addinsoft, Paris, France). Tukey's post hoc test was applied in order to detect which samples were significantly different ($p < 0.05$).

2.3 Results and Discussion

2.3.1 Basil

In total, 88 compounds were detected in the headspace of the three samples of basil (Table 2.2). The compounds detected included 28 monoterpenes, 18 sesquiterpenes, six other compounds, three phenylpropanoids, three esters, two aldehydes, two alcohols, one alkane and 25 unidentified compounds. Quantitative differences in the aroma profiles were observed between production type of the four basil samples of this study, confirmed by one-way ANOVA. Hydroponically produced basil (B3) produced the highest abundance of volatile compounds, however composition was observed with monoterpenes accounting for 44-56 % of composition and phenylpropanoids 14-24 % of composition. Basil samples produced in West Sussex (B1 and B3) showed similar total relative amounts of monoterpenes and sesquiterpenes, with lower and higher relative amounts (respectively) than the other basil samples (B2 and B4). Seven compounds showed no significant differences in relative amount between type of production and the majority were minor compounds, apart from methyl eugenol, indicating that the methods of production studied had limited influence on these.

Table 2.2: Relative abundance of aroma compounds identified in the headspace of fresh basil samples.

Code	Compound	LRI[a]	ID[b]	Relative abundance [c]				*p*-value
				B1 Pot	B2 Pot	B3 Hydroponics	B4 Soil	
	Alcohol							
A1	(*Z*)-3-Hexen-1-ol	856	A	3.5 ^{b}	5.3 ^{b}	10 ^{ab}	17 ^{a}	**
A2	1-Octanol	1072	A	nd	7.0	nd	16	ns
	Total (%)			0.1	0.4	0.2	1.0	
	Aldehyde							
AL1	(*E*)-2-Hexenal	853	A	6.3 ^{bc}	9.8 ^{ab}	11 ^{a}	4.1 ^{c}	**
AL2	Octanal	1002	A	26 ^{a}	6.8 ^{b}	2.3 ^{b}	10 ^{b}	**
	Total (%)			0.7	0.5	0.3	0.5	
	Alkane							
AK1	Decane	999	A	nd	0.9	nd	0.9	ns
	Total (%)			0.0	0.03	0.0	0.03	
	Ester							
E1	Hexyl hexanoate	1386	A	6.6 ^{a}	2.1 ^{b}	2.3 ^{b}	1.3 ^{b}	**
E2	Geranyl butyrate	1560	A	6.3 ^{bc}	2.8 ^{c}	19 ^{a}	10 ^{b}	***
E3	Cinnamyl butyrate	1643	A	5.3 ^{b}	3.5 ^{b}	18 ^{a}	7.0 ^{b}	***
	Total (%)			0.4	0.3	0.7	0.6	
	Monoterpene							
M1	alpha-Thujene	931	B[1]	14 ^{a}	9.5 ^{b}	12 ^{ab}	11 ^{ab}	*
M2	alpha-Pinene	940	A	34 ^{a}	18 ^{b}	19 ^{b}	16 ^{b}	**
M3	Camphene	956	A	9.2 ^{a}	5.1 ^{b}	5.5 ^{b}	3.9 ^{b}	**
M4	Sabinene	979	A	69 ^{a}	50 ^{b}	45 ^{bc}	35 ^{c}	***
M5	beta-Pinene	984	A	82 ^{a}	48 ^{b}	53 ^{b}	43 ^{b}	**
M6	beta-Myrcene	992	A	82 ^{ab}	61 ^{b}	97 ^{a}	87 ^{ab}	*
M7	alpha-Phellandrene	1010	A	7.8 ^{a}	4.6 ^{b}	7.4 ^{a}	6.0 ^{ab}	*
M8	delta-3-Carene	1017	A	11 ^{a}	3.6 ^{b}	nd	2.0 ^{b}	***
M9	alpha-Terpinene	1022	A	8.1 ^{bc}	5.3 ^{c}	12 ^{a}	9.5 ^{ab}	**
M10	Eucalyptol (1,8-cineole)	1040	A	578 ^{a}	455 ^{b}	540 ^{ab}	424 ^{b}	*
M11	Ocimene quintoxide	1051	A	204 ^{a}	139^{bc}	181 ^{ab}	125 ^{c}	**
M12	gamma-Terpinene	1064	B[2]	13 ^{a}	7.0 ^{a}	13 ^{a}	11 ^{a}	ns
M13	Terpinolene	1095	A	74 ^{a}	36 ^{b}	42 ^{b}	37 ^{b}	**
M14	Linalool	1105	A	672 ^{b}	562 ^{b}	1047 ^{a}	697^{ab}	*
M15	allo-Ocimene	1132	B[3]	1.5 ^{b}	1.2 ^{b}	2.4 ^{a}	1.8 ^{ab}	**
M16	Camphor	1160	A	169 ^{a}	101^{ab}	31 ^{b}	37 ^{b}	***
M17	(*Z*)-beta-Terpineol	1177	A	20 ^{a}	12 ^{b}	nd	9.2 ^{b}	***
M18	Isoborneol	1179	A	14 ^{b}	21 ^{b}	73 ^{a}	16 ^{b}	***
M19	Terpinen-4-ol	1188	B[4]	15 ^{a}	1.2 ^{a}	2.1 ^{a}	1.4 ^{a}	ns
M20	alpha-Terpineol	1200	B[5]	93 ^{a}	58 ^{b}	70 ^{ab}	45 ^{b}	*
M21	Nerol	1232	A	1.8 ^{c}	1.3 ^{c}	4.2 ^{a}	3.1 ^{b}	***
M22	Geraniol	1256	A	6.5 ^{b}	3.8 ^{b}	14 ^{a}	7.2 ^{b}	**
M23	Bornyl acetate	1297	A	54 ^{c}	44 ^{c}	78 ^{b}	116 ^{a}	***
M24	cis-Pinocarvyl acetate	1308	B[6]	nd	nd	nd	1.1	
M25	alpha-Terpinyl acetate	1355	A	10 ^{a}	4.4 ^{b}	nd	2.0 ^{bc}	***

M26	Dehydrocineole	995	B[7]	2.3 [b]	2.7 [ab]	3.4 [ab]	4.2 [a]	*
M27	p-Cymene	1030	A	nd	nd	nd	1.6	
M28	(*Z*)-Linalool oxide	1074	A	42 [a]	26 [a]	27 [a]	nd	**
	Total (%)		47	55	44	56		
	Other							
O1	Ethylbenzene	864	A	nd	nd	nd	1.1	
O2	Styrene	893	A	nd	nd	nd	7.5	
O3	Limona ketone	1139	B[8]	nd	nd	nd	1.1	
O4	Octyl acetate	1208	A	8.2 [a]	5.5 [a]	2.0 [a]	9.8 [a]	ns
O5	2-Hydroxycineol	1225	B[9]	nd	nd	3.3	nd	
O6	*(Z)*-Isoeugenol	1421	B[10]	1.9 [b]	1.4 [b]	2.8 [a]	nd	***
	Total (%)			0.2	0.2	0.2	0.6	
	Phenylpropanoid							
P1	Estragole	1204	A	4.4	3.5	2.1	1.0	ns
P2	Eugenol	1371	B[11]	704 [a]	459[ab]	735 [a]	296 [b]	**
P3	Methyl eugenol	1409	A	469	208	367	142	ns
	Total (%)			24	22	20	14	
	Sesquiterpene							
S1	alpha-Ylangene	1391	B[12]	nd	nd	3.6	2.4	
S2	alpha-Copaene	1396	B[13]	19 [bc]	8.9 [c]	36 [a]	24 [ab]	***
S3	beta-Elemene	1401	B[14]	8.6 [ab]	4.4 [b]	12 [a]	5.8 b	**
S4	trans-Bergamotene	1431	B[15]	9.3 [a]	4.6 [ab]	6.1 [a]	nd	**
S5	beta-Caryophyllene	1441	A	nd	nd	8.1 [a]	4.9 [b]	***
S6	alpha-Caryophyllene	1446	B[16]	16 b	5.2 [c]	28 [a]	11 [bc]	***
S7	Bergamotene	1452	B[17]	368 [a]	239 [b]	297 [ab]	22 [c]	***
S8	Aromadendrene	1457	A	nd	nd	73 [a]	50 [b]	***
S9	alpha-Farnesene	1463	B[18]	272 [a]	125 [a]	225 [a]	119 [a]	*
S10	alpha-Guaiene	1465	B[19]	nd	nd	42 [a]	26 [b]	***
S11	alpha-Humulene	1477	A	5.3 [a]	2.4 [b]	nd	nd	***
S12	alpha-Muurolene	1481	B[20]	77 [b]	26 [b]	130 [a]	49 [b]	**
S13	trans-Muurola-4(14)5-diene	1488	B[21]	20 [b]	8.9 [b]	36 [a]	18 [b]	***
S14	Germacrene D	1496	B[22]	3.3 [c]	nd	56 [a]	32 [b]	***
S15	Bicyclogermacrene	1514	B[23]	11 [c]	5.2 [c]	45 [a]	25 [b]	***
S16	trans-Calamenene	1547	B[24]	5.8 [bc]	2.8 [c]	12 [a]	8.1 [b]	***
S17	alpha-Calacorene	1567	B[25]	nd	nd	1.9[a]	1.3 [b]	***
S18	(*Z*)-Nerolidol	1570	A	1.9 [b]	nd	4.3[a]	1.6 [bc]	***
	Total (%)			17	14	19	13	
	Unknowns							
U1	unknown	1113		2.1	nd	nd	nd	
U2	unknown	1136		3.4	2.4	3.2	2.8	ns
U3	unknown	1146		4.9 [a]	3.0 [b]	5.3 [a]	4.3 [ab]	*
U4	unknown	1170		nd	nd	nd	1.7	
U5	unknown	1248		nd	nd	nd	1.1	
U6	unknown	1340		nd	nd	4.7 [a]	2.8 [b]	***
U7	unknown	1351		nd	nd	23 [a]	11 [b]	***
U8	unknown	1373		nd	nd	nd	1.9	***

U9	unknown	1415	29	2.0	8.0	4.2	ns
U10	unknown	1436	nd	nd	2.5	nd	
U11	unknown	1444	5.2 [a]	2.3 [b]	nd	nd	***
U12	unknown	1468	10 [bc]	4.9 [c]	29 [a]	14 [b]	***
U13	unknown	1472	17 [a]	7.4 [b]	6.2 [b]	3.1 [b]	**
U14	unknown	1483	nd	nd	nd	10	
U15	unknown	1499	18 [a]	8.1 [b]	nd	8.3 [b]	***
U16	unknown	1504	50 [a]	25 [b]	34 [ab]	nd	***
U17	unknown	1507	106 [ab]	39 [c]	131 [a]	61 [bc]	**
U18	unknown	1523	75 [b]	31 [c]	149 [a]	82 [b]	***
U19	unknown	1529	37 [c]	18 [c]	114 [a]	78 [b]	***
U20	unknown	1538	98 [b]	48 [b]	188 [a]	97 [b]	***
U21	unknown	1543	25 [c]	11 [c]	85 [a]	49 [b]	***
U22	unknown	1555	4.0[bc]	1.9 [c]	10 [a]	6.0 [b]	***
U23	unknown	1587	nd	nd	1.6	nd	
U24	unknown	1666	21[b]	13[b]	82[a]	27[b]	***
U25	unknown	1681	nd	nd	4.0[a]	1.7[b]	***
	Total (%)		10	7.1	16	15	

[a] Linear retention index (LRI) on a DB-5 column. [b] A, mass spectrum and LRI agree with those of authentic compounds; B, mass spectrum (spectral quality value >80 was used) and LRI agrees with reference spectrum in the NIST/EPA/NIH mass spectra database and LRI agree with those in the literature cited [1] (Adams et al 2006); [2] (Adams et al 2006); [3] (Sabulal et al 2007); [4] (Adams et al 2006); [5] (Baranauskiene et al 2003); [6] (Marongiu et al 2006); [7] (Baccouri et al 2007); [8] (Miyazaki et al 2011); [9] (Hamm et al 2004); [10] (Varlet et al 2007); [11] (Varlet et al 2007); [12] (Damon 2002); [13] (Le Quere and Latrasse 1990); [14] (Everaerts et al 1993); [15] (Alberdan S Santos et al 1998); [16] (Buchin et al 2002); [17] (Limberger et al 2003); [18] (G Flamini et al 2002); [19] (Marongiu et al 2005); [20] (Everaerts et al 1993); [21] (Adams and Nguyen 2005); [22] (Baranauskiene et al 2003); [23] (Silva et al 2012); [24] (Loayza et al 1995); [25] (Lazari et al 2000). [c] Estimated abundance collected in the headspace of basil samples containing 2.8 mL of saturated calcium chloride, calculated by comparison with of 100 ppm propyl propanoate used as internal standard; internal standard was used to normalise chromatograms; means of three replicate samples are shown; means not labelled with the same letters are significantly different (p<0.05) and Tukey's HSD multiple pairwise comparison; nd - not detected; ns - not significant probability obtained by ANOVA, * significant at the 5 % level; ** significant at the 1 % level; *** significant at 0.1 % level.

Many authors have reported eugenol, linalool and 1,8-cineole as the major compounds responsible for basil aroma and responsible for more than half of the composition of basil oil (Klimánková et al 2008, Lee et al 2005, Miele et al 2001, Yousif et al 1999). Aroma composition of samples produced in a pot (B1 and B2) cultivation system have 47-55 % monoterpenes, 22-24 % phenylpropanoid, 14-17 % sesquiterpenes and 7-10 % unidentified compounds. Conversely, hydroponically produced basil (B3) was comprised of 44 % monoterpenes, 20 % phenylpropanoids, 19 % sesquiterpenes and 16 % unidentified compounds. Basil grown under protected conditions (B4), developed 56 % monoterpenes, 14% phenylpropanoids, 13 % sesquiterpenes and 15 % unidentified compounds. Pot produced

samples and hydroponics were grown under similar temperature ranges (Table 2.1), both pot produced were grown under sunlight and similar photoperiod whereas hydroponics under LED lights, which has been reported to affect basil's height (shorter plants) and significantly ($p < 0.05$) increase the production of volatiles compounds, this would confirm why sample B3 expressed the highest abundance of volatiles compounds (Carvalho et al 2016, Litvin et al 2020). Basil grown on field under protected conditions was produced under lower temperature range (16-20 ˚C) and shorter photoperiod (14 h), this temperature has been reported to lead to shorter basil plants and lower volatiles abundances (Chang et al 2005, 2007), however results from the present study showed highest proportion of monoterpenes in this sample which are one of the main type of compounds for the basil aroma (Miele et al 2001).

Principal component analysis was used to visualise the chemical differences observed across the different basil productions (Figure 2.1), with volatile compounds identified that expressed significant differences between samples (Table 2.2). Principal component analysis dimension one (F1) and two (F2) explained 88.50 % of the total variation within the data. The first axis separated the samples by type of pot production (B1 and B2) from hydroponics and soil under polytunnel (B3 and B4), whilst the second axis separated the samples by location, West Sussex (B1and B3) from Lincolnshire and Worcester (B2 and B4). West Sussex produced basil was associated with majority of compounds detected (Figure 2.1), additionally compounds described as relevant to the basil aroma were highly associated with hydroponic production (B3), this could be due to this sample being produced under optimal temperature range (20-25 ˚C), the use of LED lighting which has been associated with higher volatile content and the hydroponic system allows better control of plant nutrition and hydration (Chang et al 2007, Ciriello et al 2021, Litvin et al 2020). Conversely, eugenol displayed low correlation with samples from West Sussex and a negative correlation with samples from Lincolnshire or

Worcester. Soil-grown plants under protected conditions (B4) were highly correlated with minor monoterpenes and some unknown compounds. Whereas pot produced samples from Lincolnshire were associated with alkane and aldehyde compounds, which are not associated with the typical basil aroma, contrary to other pot sample (B1) which was produced using a mixture substrate, which is composed of 90 % peat and 10 % perlite coarse (Table 2.1), however differences in aroma composition could be due to irrigation amounts or different plant density in the pot, since plants were grown under similar photoperiods, further investigation needs to be carried out where isolated variables are changed in order to analyse its effect on the volatile composition of the herbs. Most compounds were positively correlated with first (F1) and second (F2) dimension, and camphor (M16), ocimene quintoxide (M11) and terpinolene (M13) were negatively correlated with F1 but positively correlated with F2. These results suggest that differences in the overall abundance of volatile compounds were determined by temperature during growth and type of production, whereby hydroponics expressed higher volatile content.

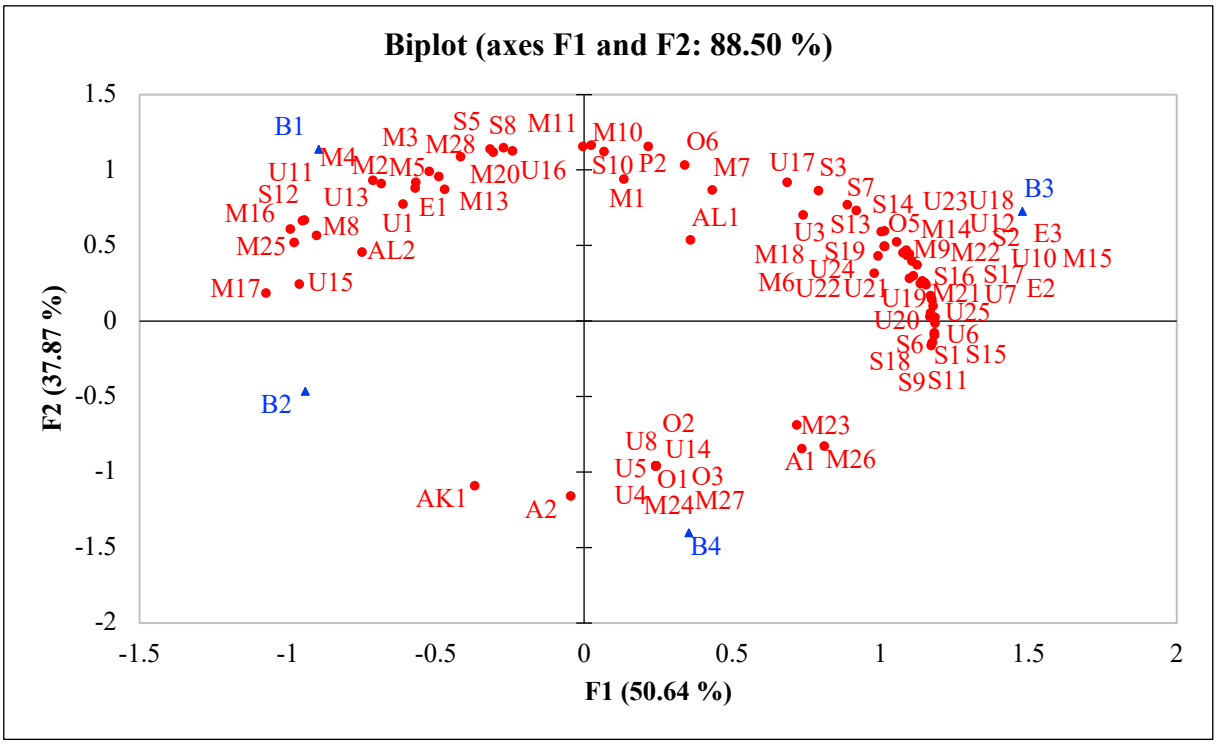


Figure 2.1:Principal component analysis of four basil samples showing correlations with volatile compounds (red circle) and samples (blue triangle): B1- pots; B2- pots; B3- hydroponics; B4- protected soil.

2.3.2 Coriander

In total, 76 compounds were detected in the headspace of five different coriander samples (Table 2.3). The compounds detected included 15 aldehydes, 11 monoterpenes, four alkanes, four alcohol, three alkene, two esters, one sesquiterpene, one other compound and 35 unidentified compounds. Quantitative differences in the aroma profiles were observed between coriander samples of this study, confirmed by one-way ANOVA. Open field produced samples of coriander (C1, C2 and C3) expressed the highest amounts of volatile compounds, furthermore the sample produced in York (C3) displayed the highest proportion of aldehydes, the sample produced in West Sussex (C4) the highest contents of unidentified compounds and the field sample from Worcester (C5) the highest proportion of monoterpenes. No significant differences between types of production were found in relative amount of three compounds, including (*Z*)-3-Hexen-1-ol, undecane and (*Z*)-3-Hexenyl acetate.

Table 2.3: Relative abundance of aroma compounds identified in the headspace of fresh coriander samples.

Code	Compound name	LRI[a]	ID[b]	Relative abundance [c]					*p*-value
				C1 Pots	C2 Pots	C3 Field	C4 Field	C5 Field	
	Alcohol								
A1	(*Z*)-3-Hexen-1-ol	857	A	12	45	27	27	29	ns
A2	(*E*)-2-Hexen-1-ol	866	A	1.6[b]	12 [a]	15 [a]	19 [a]	15 [a]	**
A3	2-Nonanol	1095	A	0.7[b]	nd	7.4 [b]	24 [ab]	65 [a]	**
A4	1-Nonanol	1171	A	nd	nd	13	13	13	ns
	Total (%)			4.4	12	1.1	2.0	2.2	
	Aldehyde								
AL1	Hexanal	800	A	2.9	2.7	nd	nd	nd	ns
AL2	(*E*)-2-Hexenal	854	A	13[a]	5.4 [a]	2.4 [a]	2.7 [a]	1.8 [a]	*
AL3	Octanal	1003	A	nd	nd	14 [a]	7.4 [b]	4.4 [b]	***
AL4	Nonanal	1104	A	nd	1.3 [b]	78 [a]	55 [a]	52 [a]	***
AL5	(*E*)-2-Nonenal	1161	A	nd	nd	39 [a]	4.4 [b]	2.3 [b]	***
AL6	Decanal	1208	A	9.6[c]	47 [c]	831 [b]	735 [b]	1249 [a]	***
AL7	(*E*)-2-Decenal	1268	A	nd	2.4 [b]	1515 [a]	1241 [a]	1122 [a]	***
AL8	Undecanal	1309	A	5.3[c]	14 [c]	217 [a]	92 [b]	117 [b]	***
AL9	(*E,E*)-2,4-Decadienal	1321	B[1]	nd	nd	6.6	6.6	5.2	ns

AL10	*(E)*-2-Undecenal	1369	A	1.7[c]	8.7 [c]	568 [a]	367 [b]	340 [b]	***
AL11	Dodecanal	1411	A	30 [c]	30 [c]	182 [a]	61 [c]	127 [b]	***
AL12	(*E*)-2-Dodecenal	1473	B[2]	52 [c]	97 [c]	782 [a]	548 [b]	772 [a]	***
AL13	Tridecanal	1512	B[3]	1.1[c]	2.1 [c]	13 [a]	6.2 [b]	6.8 [b]	***
AL14	(*E*)-2-Tridecen-1-al	1573	B[4]	6.4[b]	13 [b]	96 [a]	75 [a]	79 [a]	***
AL15	Tetradecanal	1614	B[5]	2.8[c]	3.8 [bc]	11 [a]	7.1 [ab]	10 [a]	***
	Total (%)			38	47	80	76	70	
	Alkane								
AK1	Nonane	900	A	82 [a]	69 [ab]	43 [bc]	14 [c]	29 [c]	***
AK2	Decane	1000	A	5.4[b]	4.7 [b]	13 [a]	3.7 [b]	3.9 [b]	***
AK3	Undecane	1100	A	7.7	7.7	16	9.3	8.0	ns
AK4	3-Methylnonane	972	B[6]	0.9	nd	nd	nd	nd	
	Total (%)			29	17	1.3	0.6	0.7	
	Alkene								
AKE1	(*E)*-2-Nonene	906	B[7]	0.7	nd	nd	nd	nd	
AKE2	1-Dodecene	1194	A	nd	1.3 [b]	26 [a]	29 [a]	nd	***
AKE3	1-Tridecene	1292	B[8]	nd	nd	nd	2.7	nd	
	Total (%)			0.2	0.3	0.5	0.7	0.0	
	Ester								
E1	(*Z*)-3-Hexenyl acetate	1005	A	nd	0.7	2.7	2.5	3.1	ns
E2	(*Z*)-3-Hexenyl hexanoate	1391	A	nd	0.8	nd	2.0	nd	ns
	Total (%)			0.0	0.3	0.1	0.1	0.1	
	Monoterpene								
M1	alpha-Pinene	940	A	1.0[b]	1.1 [b]	14 [b]	nd	70 [a]	**
M2	Camphene	956	A	nd	nd	5.3 [b]	nd	33 [a]	**
M3	beta-Pinene	984	A	nd	nd	6.2 [b]	nd	30 [a]	**
M4	beta-Myrcene	992	A	0.9[b]	1.2 [b]	28 [a]	5.2 [b]	27 [a]	***
M5	alpha-Phellandrene	1010	A	nd	nd	nd	1.9 [b]	47 [a]	**
M6	*p*-Cymene	1030	A	0.9[b]	nd	6.8 [b]	28 [b]	122 [a]	***
M7	Limonene	1035	A	0.9[b]	1.2 [b]	27 [b]	48 [ab]	107 [a]	**
M8	Eucalyptol (1,8-cineole)	1038	A	1.1[b]	nd	12[b]	9.8[b]	117[a]	***
M9	gamma-Terpinene	1064	A	2.2[b]	1.7 [b]	6.1 [b]	2.6 [b]	59 [a]	**
M10	1,3,8-para-Menthatriene	1119	B[9]	nd	nd	nd	20	38	
M11	L-Carvone	1255	A	nd	nd	nd	30	nd	
	Total (%)			2.2	1.1	2.0	3.5	12	
	Other								
O1	Styrene	893	A	nd	nd	4.3	2.4	4.3	ns
	Total (%)			0.0	0.0	0.1	0.1	0.1	
	Sesquiterpene								
S1	Caryophyllene	1446	B[10]	nd	nd	4.3	nd	3.6	ns
	Total (%)			0.0	0.0	0.1	0.0	0.1	
	Unknowns								
U1	unknown	838		0.8	nd	nd	nd	nd	
U2	unknown	932		nd	nd	12	nd	6.5	ns

U3	unknown	1022	nd	nd	nd	nd	11	
U4	unknown	1091	nd	nd	nd	1.8	nd	
U5	unknown	1147	1.1[c]	1.5 [c]	3.4 [bc]	7.3 [ab]	12 [a]	***
U6	unknown	1166	nd	nd	18 [ab]	30 [a]	26 [ab]	*
U7	unknown	1199	nd	nd	69 [a]	62 [a]	67 [a]	**
U8	unknown	1216	nd	nd	nd	2.5	2.8	ns
U9	unknown	1246	nd	nd	nd	nd	2.0	
U10	unknown	1251	nd	nd	117	110	90	
U11	unknown	1281	0.7[d]	1.2 [d]	5.9 [b]	3.5 [c]	8.1 [a]	***
U12	unknown	1299	nd	nd	33 [a]	19 [b]	19 [b]	***
U13	unknown	1337	nd	nd	2.4 [a]	1.2 [b]	2.6 [a]	***
U14	unknown	1347	nd	0.7 [b]	2.3 [ab]	1.9 [ab]	8.0 [a]	*
U15	unknown	1352	nd	1.3 [c]	32 [ab]	35 [a]	16 [bc]	***
U16	unknown	1359	nd	nd	5.5 [a]	nd	3.1 [ab]	**
U17	unknown	1375	nd	nd	nd	2.9	3.4	
U18	unknown	1400	2.2[c]	4.1 [c]	44 [a]	33 [b]	51 [a]	***
U19	unknown	1433	nd	nd	2.0	nd	2.2	ns
U20	unknown	1450	1.3[c]	2.1 [c]	9.0 [a]	5.6 [ab]	4.6 [bc]	***
U21	unknown	1455	1.6[c]	3.1 [c]	41 [a]	31 [b]	50 [a]	***
U22	unknown	1483	0.6	nd	nd	nd	nd	
U23	unknown	1501	nd	1.3 [c]	11 [a]	4.5 [b]	4.5 [b]	***
U24	unknown	1529	nd	nd	2.7 [a]	1.9 [b]	2.6 [a]	***
U25	unknown	1544	1.6[c]	1.9 [c]	5.5 [ab]	3.7 [bc]	6.1 [a]	***
U26	unknown	1552	nd	nd	4.2	2.9	3.5	ns
U27	unknown	1557	nd	nd	2.4 [a]	1.6 [b]	2.0 [ab]	***
U28	unknown	1602	3.1[c]	4.0 [c]	12 [b]	15 [ab]	17 [a]	***
U29	unknown	1659	1.6[c]	2.1 [c]	8.2 [b]	10 [a]	12 [a]	***
U30	unknown	1677	60 [b]	74 [b]	287 [a]	284 [a]	315 [a]	***
U31	unknown	1706	nd	nd	2.2	2.4	2.2	ns
U32	unknown	1755	nd	nd	nd	nd	3.9	
U33	unknown	1779	7.1[b]	14 [b]	44 [a]	49 [a]	41 [a]	***
U34	unknown	1840	0.7[d]	1.4 [cd]	2.7 [bc]	5.2 [a]	3.3 [b]	***
U35	unknow	1882	1.1[b]	1.7 [b]	6.8 [a]	8.0 [a]	6.9 [a]	***
	Total (%)		26	23	15	17	15	

[a] Linear retention index (LRI) on a DB-5 column. [b] A, mass spectrum and LRI agree with those of authentic compounds; B, mass spectrum (spectral quality value >80 was used) and LRI agrees with reference spectrum in the NIST/EPA/NIH mass spectra database and LRI agree with those in the literature cited [1] (Adams et al 2005); [2] (Morteza-Semnani et al 2007); [3] (Ramarathnam et al 1993a); [4] (Judžentienė and Būdienė 2008); [5] (Ramarathnam et al 1993a); [6] (Zhendi Wang et al 1994); [7] (Zaikin and Borisov 2002); [8] (Song et al 2003); [9] (Lucero et al 2006); [10] (Buchin et al 2002). [c] Estimated abundance collected in the headspace of basil samples containing 2.8 mL of saturated calcium chloride, calculated by comparison with of 100 ppm propyl propanoate used as internal standard; internal standard was used to normalise chromatograms; means of three replicate samples are shown; means not labelled with the same letters are significantly different ($p<0.05$) and Tukey's HSD multiple pairwise comparison; nd – not detected; ns – not significant probability obtained by ANOVA, * significant at the 5 % level; ** significant at the 1 % level; *** significant at 0.1 % level.

Many authors have reported aldehydes as the main compounds responsible for the coriander aroma, with (*E*)-2-dodecenal, dodecanal, (*E*)-2-decenal, (*E*)-2-undecenal and decanal identified as main contributors (Anjum et al 2011, El-Zaeddi et al 2016, Nurzyńska-Wierdak 2013). Aldehydes comprised 70-80 % of the composition of coriander produced in open field (C3, C4 and C5) followed by 15-17 % unidentified compounds and 0.6-1.3 % of alkane compounds. Conversely, pot produced coriander (C1 and C2) was comprised of 38-47 % aldehydes, 17-29 % alkanes and 23-26 % of unidentified compounds. Compounds such as decanal, (*E*)-2-undecenal, (*E*)-2-dodecenal and (*E*)-2-decenal displayed significantly ($p < 0.001$) higher abundances (Table 2.3) in open field coriander (C3, C4 and C5) compared to pot produced plants (C1 and C2), conversely (*E*)-2-hexenal and nonane showed significantly ($p < 0.05$) higher abundances in pot samples (C1 and C2). Pot samples were produced under a higher temperature range (20-25 ˚C) which has been reported as leading to higher volatile abundance (Hcini et al 2013), however other environmental factors such as plant density or soil type might have prevented an increase in volatiles due to limitation in uptake soil, inorganic elements and water micronutrients, which are necessary for plant growth and are involved pathways including the synthesis of secondary metabolites (Kader 2008). The compound (*E*)-2-decenal has been identified as an aroma impact compound, and was detected in highest amounts (~28%) in coriander produced in open field (C3 and C4) and under temperatures of 16-20 ˚C but different soils (loamy/clay and sandy, respectively). Coriander from Worcester produced in open field condition (C5) at higher temperatures (20-25 ˚C) in a loamy soil, led to a higher proportion of monoterpenes (12 %) than coriander samples C1, C2, C3 and C4 (~2 %), these compounds have not been described as relevant for the aroma of coriander (Anjum et al 2011, El-Zaeddi et al 2016).

Principal component analysis was used to visualise the chemical differences observed across the different coriander productions (Figure 2.2), with volatile compounds identified that expressed significant differences between samples (Table 2.3). Principal component analysis dimension one (F1) and two (F2) explained 82.41 % of the total variation within data. The first axis separated the samples by type of production pots (C1 and C2) from open field produced coriander (C3, C4 and C5), whilst the second axis separated the samples by growth temperature range: C1, C2 and C5 (20-25 ˚C) from C3 and C4 (16-20 ˚C). York (C3) and West Sussex (C4) produced coriander were associated with majority of compounds detected (Figure 2.2), additionally compounds described as relevant to the coriander aroma were highly correlated with these samples (C3 and C4), this could be due to similar growth temperatures (16-20 ˚C), similar photoperiod (16 h) and similar water amount (2.5-2.7 mm) (Table 2.1). Conversely, most monoterpene compounds such as p-cymene (M6), limonene (M7) and eucalyptol (M8), displayed association with coriander sample from Worcester (C5) which was grown at 20-25 ˚C in a loamy field. Temperatures between 15-22 ˚C were reported to increase coriander contents of (*E*)-2-decenal and linalool this would explain why this sample (C5) was associated with most monoterpenes (Telci and Hisil 2008). Whereas, pot produced coriander (C1 and C2) was produced at similar temperatures (20-25 ˚C) to sample C5, however different soil substrates (peat vs mixture vs loamy) which are nutrient rich soils, were highly associated with some minor aldehydes and alkane compounds such as (*E*)-2-hexenal (AL2), hexanal (AL1) and nonane (AK1). Additionally, samples C3 and C4, were associated with majority of unidentified compounds. This could be due to soil type, however these samples used different types (loamy/clay and sandy, respectively), or due to irrigation volumes and photoperiod (Table 2.1), which were similar between samples. These production factors have been reported to influence the aroma composition of coriander (McAusland et al 2020, Neffati and Marzouk 2008). Most compounds were positively correlated with first (F1) and second (F2) dimension, and (*E*)-2-

hexenal (AL2), hexanal (AL1) and nonane (AK1) were negatively correlated with F1 and with F2. These results suggest that differences in the main compounds were determined by temperature of growth, however method of production also played a determining factor on overall abundance of compounds, whereby open field produced at 16-20 ˚C expressed higher volatile content and proportion of aldehyde compounds. Ultimately, further investigation needs to be carried out in order to understand how individual variables will affect the production of volatile compounds.

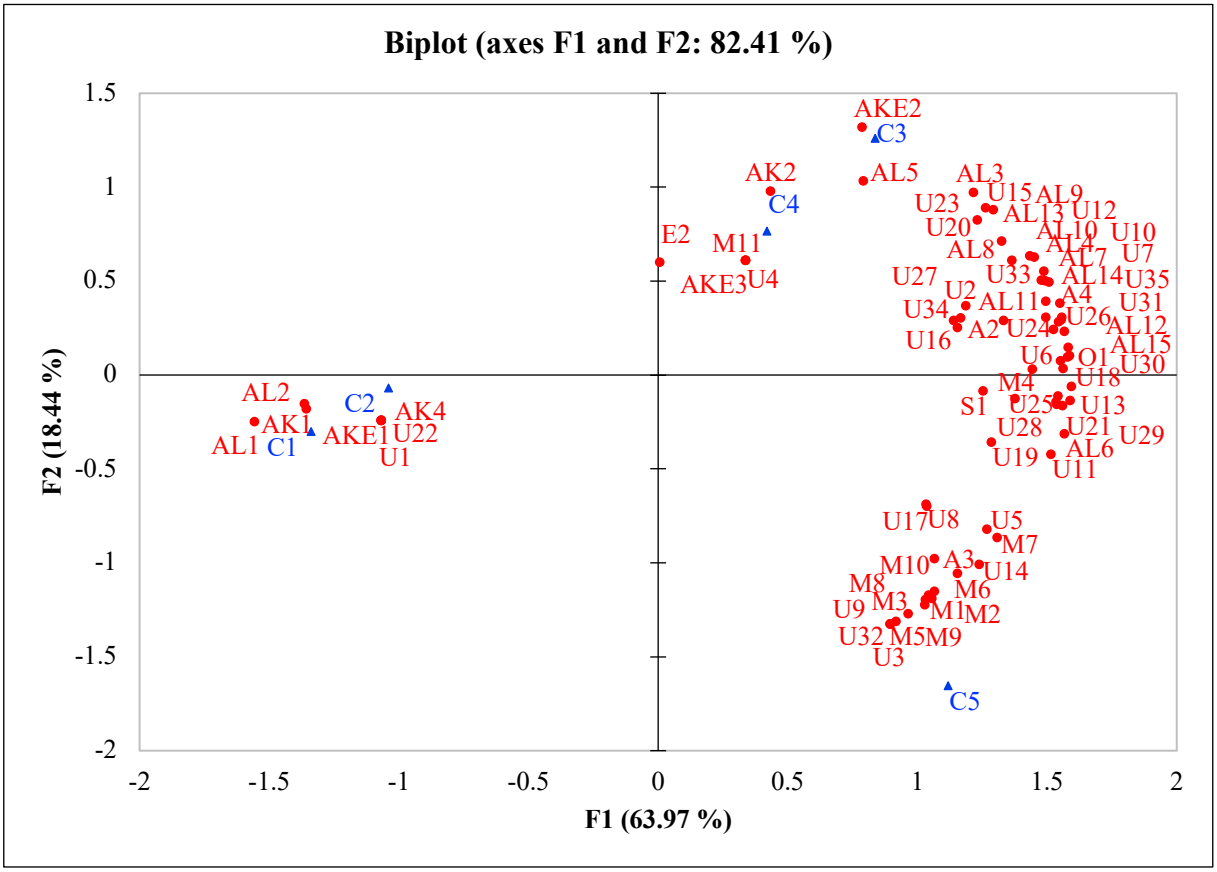


Figure 2.2:Principal component analysis of five coriander samples showing correlations with volatile compounds (red circle) and samples (blue triangle): C1- pots; C2- pots; C3- open field; C4- open field; C5- open field.

2.3.3 Rosemary

In total, 100 compounds were detected in the headspace of six rosemary samples (Table 2.4). Detected compounds included 38 monoterpenes, 11 sesquiterpenes, three alcohol, three aldehyde, three esters, three phenylpropanoid, three other compounds and 36 unidentified

compounds. Quantitative differences in the aroma profiles were observed for all the detected compounds between the six rosemary samples, confirmed by one-way ANOVA. York rosemary samples (R2 and R3) expressed the highest amounts of volatile compounds, furthermore similar composition was observed between the two samples produced in this location. Conversely, the highest contents of monoterpenes were detected in open field samples from Worcester (R5) and Norwich (R6), which have been identified as main aroma contributors for rosemary (Pintore et al 2002). Due to commercial practices, the variety of the crops is unknown by growers, and differences in genotype could be causing differences between samples.

Table 2.4: Relative abundance of aroma compounds identified in the headspace of fresh rosemary samples.

Code	Compound name	LRI[a]	ID[b]	Relative abundance [c]						*p*-value
				R1	R2	R3	R4	R5	R6	
				Pot	Soil protected	Open field	Open field	Open field	Open field	
	Alcohol									
A1	(*Z*)-3-Hexen-1-ol	856	A	4.7 [b]	nd	65	47	62	64	ns
A2	1-Hexanol	868	A	nd	nd	nd	nd	7.9	nd	
A3	3-Octanol	998	A	8.3 [c]	46 [b]	96 [a]	91 [a]	8.9 [c]	14 [c]	***
	Total (%)			0.3	0.2	0.6	0.5	0.5	0.4	
	Aldehyde									
AL1	(*E*)-2-Hexenal	854	A	31[b]	82[ab]	112[ab]	145[a]	94[ab]	145[a]	*
AL2	Neral	1247	A	nd	48 [a]	56 [a]	8.7 [b]	6.2 [b]	12 [b]	***
AL3	Geranial	1276	A	9.9 [b]	68 [a]	76 [a]	18 [b]	13 [b]	24 [b]	***
	Total (%)			0.9	0.8	0.8	0.7	0.8	0.9	
	Ester									
E1	(*Z*)-3-Hexenyl acetate	1007	A	nd	nd	nd	nd	12	nd	
E2	Citronellyl acetate	1353	A	nd	nd	nd	16	nd	nd	
E3	trans-Verbenyl acetate	1295	B[1]	51	nd	nd	nd	nd	nd	
	Total (%)			1.1	0.0	0.0	0.1	0.1	0.0	
	Monoterpene									
M1	Tricyclene	929	B[2]	5.5[c]	87 [ab]	103 [a]	73 [ab]	37 [bc]	56 [abc]	**
M2	alpha-Thujene	932	B[3]	8.1[d]	570[b]	931 [a]	208 [c]	112 [cd]	161 [cd]	***
M3	alpha-Pinene	942	A	257[c]	2563[a]	2943[a]	1411[b]	1160[b c]	1554[b]	***
M4	Camphene	958	A	131[c]	1713[a]	1897[a]	1450[a b]	852[bc]	1163[a b]	***
M5	Sabinene	980	A	31[c]	75[b]	126[a]	67 [b]	45[bc]	56[bc]	***
M6	beta-Pinene	986	A	72[c]	1560[ab]	1715[a]	1745[a]	699[bc]	980[ab]	***

M7	alpha-Phellandrene	1012	A	69[b]	126[b]	122[b]	783[a]	792[a]	948[a]	***
M8	beta-Phellandrene	1015	B[4]	111[a]	22[bc]	36[b]	16[bc]	7.5[c]	19[bc]	***
M9	delta-3-Carene	1018	A	nd	nd	nd	15	16	20	
M10	alpha-Terpinene	1024	A	43[c]	502[a]	379[ab]	279[b]	247[b]	303[b]	***
M11	m-Cymene	1028	A	2.3	nd	nd	nd	nd	nd	
M12	p-Cymene	1031	A	60	nd	nd	nd	nd	nd	
M13	Limonene	1040	A	296[b]	3713[a]	4620[a]	4240[a]	2672[a]	3582[a]	***
M14	Eucalyptol (1,8-cineole)	1044	A	317	343	nd	nd	nd	nd	
M15	gamma-Terpinene	1066	A	68 [c]	858 [a]	648 [ab]	684 [ab]	447 [b]	623 [ab]	***
M16	Terpinolene	1096	A	202[d]	898[a]	730[ab]	481[bc]	390[cd]	454 [cd]	***
M17	Linalool	1104	A	315[b]	541[b]	612[b]	1074[a]	284[b]	287 [b]	***
M18	cis-Sabinene hydrate	1107	B[5]	nd	94 [b]	182 [a]	nd	60 [b]	78 [b]	***
M19	Filifolone	1112	B[6]	7.3[b]	11 [b]	33 [a]	nd	nd	nd	***
M20	beta-Thujone	1126	A	4.7[b]	12 [a]	11 [a]	nd	nd	nd	***
M21	*cis-p*-Mentha-2,8-dien-1-ol	1132	A	nd	9.9 [bc]	15 [ab]	24 [a]	nd	nd	***
M22	Camphor	1162	A	509[c]	2466[ab]	2824[ab]	2993[a]	1805[b]	2337[a b]	***
M23	α-Phellandren-8-ol	1172	B[7]	6.3	nd	nd	nd	nd	nd	
M24	Pinocarvone	1177	B[8]	25[bc]	nd	nd	201[a]	41[bc]	64[b]	***
M25	Borneol	1182	A	395[c]	1056[ab]	1603[a]	1574[a]	816[bc]	1071[a b]	***
M26	1-Terpinen-4-ol	1190	A	192[b]	570 [a]	666 [a]	341 [b]	280 [b]	306 [b]	***
M27	alpha-Terpineol	1203	A	82 [c]	773 [ab]	998 [a]	802 [ab]	553 [b]	646 [b]	***
M28	gamma-Terpineol	1210	A	19 [b]	31 [ab]	49 [a]	36 [ab]	22 [b]	34 [ab]	**
M29	Verbenone	1226	B[9]	400[b]	939[a]	1068[a]	411[b]	468[b]	710[ab]	***
M30	Geraniol	1258	A	14 [b]	26 [ab]	28 [ab]	15 [b]	37 [a]	37 [a]	**
M31	Piperitone	1267	A	11	13	16	13	9.5	10	ns
M32	Bornyl acetate	1300	A	324[c]	1844[ab]	1917[ab]	2577[a]	1224[b c]	1429[b]	***
M33	Myrtenyl acetate	1337	B[10]	5.2	nd	nd	12[a]	nd	nd	
M34	Geranyl acetone	1458	B[11]	4.9	nd	nd	nd	nd	nd	
M35	Dehydrosabinene	951	B[12]	nd	nd	9.5	nd	nd	nd	
M36	Thuja-2,4(10)-diene	962	B[13]	63[ab]	34[c]	44[bc]	79[a]	29[c]	42[bc]	***
M37	Ocimene quintoxide	1051	A	10[c]	22[abc]	17[bc]	40[a]	36[ab]	13 [c]	**
M38	(*Z*)-Sabinene hydrate	1075	A	9.4[c]	182[b]	412[a]	214[b]	182[b]	263[b]	***
	Total (%)			84	85	85	86	90	90	
	Other									
O1	Styrene	895	A	nd	25	nd	nd	nd	nd	
O2	1-Octen-3-one	978	A	13	nd	nd	nd	nd	nd	

O3	Methyl jasmonate	1668	A	2.5^{c}	8.0^{c}	7.9^{c}	22^{a}	9.4^{bc}	16^{ab}	***
	Toatl (%)			0.3	0.1	0.03	0.1	0.1	0.1	
	Phenylpropanoid									
P1	Estragole	1207	A	nd	14 ^{b}	28 ^{a}	nd	9.2 ^{b}	11 ^{b}	***
P2	Eugenol	1368	A	5.2^{c}	15^{b}	12^{bc}	7.9 ^{c}	9.7^{bc}	30 ^{a}	***
P3	Methyleugenol	1408	A	20^{b}	29^{ab}	34^{a}	nd	31^{ab}	42 ^{a}	***
	Total (%)			0.5	0.2	0.3	0.03	0.3	0.4	
	Sesquiterpene									
S1	alpha-Copaene	1390	A	41^{ab}	61^{a}	84 ^{a}	7.7 ^{b}	nd	nd	***
S2	alpha-Ylangene	1397	B[14]	5.1	nd	nd	nd	nd	nd	
S3	beta-Caryophyllene	1447	A	75^{b}	521^{a}	417 ^{ab}	486^{a}	415 ^{ab}	589^{a}	*
S4	alpha-Humulene	1481	A	13 ^{b}	113 ^{b}	73 ^{b}	926^{a}	68^{b}	94^{b}	***
S5	Germacrene D	1498	A	3.3^{b}	9.6 ^{b}	36 ^{a}	7.5 ^{b}	nd	nd	***
S6	alpha-Amorphene	1502	A	nd	35	nd	nd	nd	nd	
S7	beta-Bisabolene	1522	B[15]	nd	17	19	9.1	nd	6.3	ns
S8	delta-Amorphene	1529	B[16]	nd	51	43	nd	nd	nd	ns
S9	(*Z*)-Nerolidol	1539	A	2.6^{b}	8.7 ^{a}	nd	nd	nd	nd	***
S10	trans-Calamenene	1547	B[17]	nd	9.9	nd	nd	nd	nd	
S11	Caryophyllene oxide	1617	B[18]	4.2^{c}	19^{bc}	26^{bc}	64^{a}	29^{bc}	48^{ab}	**
	Total (%)			3.0	3.3	2.4	5.9	3.5	3.8	
	Unknowns									
U1	unknown	925		nd	nd	11	nd	nd	nd	
U2	unknown	989		nd	631^{ab}	859^{a}	590^{b}	nd	nd	***
U3	unknown	994		125^{c}	1579^{a}	1656^{a}	666^{b}	474^{bc}	592^{b}	***
U4	unknown	1062		2.2	nd	nd	nd	nd	nd	
U5	unknown	1117		4.0^{ab}	nd	9.8	nd	nd	nd	
U6	unknown	1135		29^{b}	72^{b}	179^{a}	46 ^{b}	52 ^{b}	87 ^{b}	***
U7	unknown	1140		24	nd	nd	nd	nd	nd	
U8	unknown	1149		4.2^{b}	12 ^{a}	nd	12 ^{a}	nd	nd	***
U9	unknown	1151		nd	23 ^{b}	51 ^{a}	nd	nd	nd	***
U10	unknown	1154		28^{b}	20^{bc}	63^{a}	nd	8.4^{cd}	10^{cd}	***
U11	unknown	1175		27	nd	nd	nd	nd	nd	
U12	unknown	1184		nd	25	nd	nd	nd	nd	
U13	unknown	1194		7.2^{b}	24^{a}	30^{a}	23^{a}	nd	nd	***
U14	unknown	1213		50^{a}	13^{b}	19^{b}	17^{b}	11^{b}	13^{b}	***
U15	unknown	1229		nd	nd	nd	58	nd	nd	
U16	unknown	1234		11^{c}	126^{a}	68^{b}	70^{b}	45^{bc}	52^{bc}	***
U17	unknown	1240		nd	15^{a}	10^{ab}	7.3^{b}	5.3^{bc}	nd	***
U18	unknown	1252		33^{a}	nd	nd	14^{b}	nd	nd	***
U19	unknown	1260		71	nd	nd	nd	nd	nd	
U20	unknown	1285		6.4^{b}	12^{b}	31^{a}	7.6 ^{b}	7.6^{b}	14^{b}	**
U21	unknown	1311		nd	nd	nd	24	nd	nd	

U22	unknown	1321	3.5^{cd}	15^{a}	8.2^{b}	6.2^{bc}	nd	6.4^{bc}	***
U23	unknown	1343	13	nd	nd	nd	nd	nd	
U24	unknown	1375	17	nd	nd	nd	nd	nd	
U25	unknown	1401	15^{b}	18^{b}	63^{a}	13^{b}	13^{b}	29^{ab}	**
U26	unknown	1429	4.5^{bc}	nd	21^{a}	nd	nd	9.7^{b}	***
U27	unknown	1464	nd	29	36	nd	nd	nd	ns
U28	unknown	1474	nd	nd	nd	11	nd	nd	
U29	unknown	1516	nd	23	23	nd	nd	nd	ns
U30	unknown	1543	5.4	9.3	9.7	nd	nd	nd	ns
U31	unknown	1568	nd	11	12	nd	nd	nd	ns
U32	unknown	1644	nd	nd	nd	39	nd	nd	
U33	unknown	1665	nd	nd	nd	18	nd	nd	
U34	unknown	1686	3.5^{c}	15^{bc}	14^{bc}	41^{a}	17^{bc}	26^{b}	***
U35	unknown	1694	nd	nd	nd	14	nd	nd	
U36	unknown	1699	nd	nd	nd	32	nd	nd	
	Total (%)		10	10	11	6.7	4.3	4.4	

[a] Linear retention index (LRI) on a DB-5 column. [b] A, mass spectrum and LRI agree with those of authentic compounds; B, mass spectrum (spectral quality value >80 was used) and LRI agrees with reference spectrum in the NIST/EPA/NIH mass spectra database and LRI agree with those in the literature cited [1] (Lucero et al 2006); [2] (Adams et al 2006); [3] (Zoghbi et al 1998); [4] (El-Ghorab et al 2002); [5] (Marongiu et al 2006); [6] (Avato et al 2004); [7] (Morteza-Semnani et al 2005); [8] (Adams et al 2005); [9] (Gohari et al 2006); [10] (Hamm et al 2004); [11] (V A Isidorov et al 1998); [12] (Mosayebi et al 2008); [13] (Miyazaki et al 2011); [14] (Adams et al 2005); [15] (Baranauskiene et al 2003); [16] (Özel et al 2006); [17] (Loayza et al 1995); [18] (Chyau et al 2007). [c] Estimated abundance collected in the headspace of basil samples containing 2.8 mL of saturated calcium chloride, calculated by comparison with of 100 ppm propyl propanoate used as internal standard; internal standard was used to normalise chromatograms; means of three replicate samples are shown; means not labelled with the same letters are significantly different (p<0.05) and Tukey's HSD multiple pairwise comparison; nd – not detected; ns – not significant probability obtained by ANOVA, * significant at the 5 % level; ** significant at the 1 % level; *** significant at 0.1 % level.

Many authors have reported camphor, alpha-pinene, borneol, limonene and 1,8-cineole as the major compounds responsible for rosemary aroma and responsible for the majority of the composition of rosemary oil (Lakušić et al 2012, Pintore et al 2002, Salido et al 2003). Aroma composition of sample produced in a pot (R1) cultivation system displayed 82 % monoterpenes, 10 % unidentified compounds and 3 % sesquiterpenes. Conversely, open field produced rosemary (R3, R4, R5 and R6) were comprised of 83-89 % monoterpenes, 4-11 % unidentified compounds and 2-6 % sesquiterpenes. However, rosemary sample varieties are unknown, this means samples might be from varieties which predetermines the aroma profile of the crop, making it difficult to dissect the influence of growing factors such as type of production, growth temperature, soil type and water supply (Lucy Turner, Lignou, et al 2021b). Similar contents

of main compounds (Table 2.4) for rosemary aroma such as camphor, borneol, limonene and alpha-pinene, were detected between open field rosemary samples (R4, R5 and R6), which were produced at similar temperature range (16-20 ˚C), photoperiod (12 h) and soil type (loamy soil) (Table 2.1), and were found at higher temperatures than other samples from York and West Sussex which were produced at lower and higher temperatures ranges respectively. A study by Lakušić et al (2012) reported the influence of growth temperature on compounds such as camphor and eucalyptol, where the camphor expressed increase in abundance when produced at lower temperatures, this was observed in the present study with open field samples, which were produced between 16-20 ˚C, expressing significantly ($p < 0.001$) higher relative abundance compared to pot production. Conversely, eucalyptol expressed an increase in abundance at higher temperatures, this was confirmed in the present study with eucalyptol only detected ($p < 0.001$) in rosemary (R1) produced at 20-25 ˚C, however other factors might have been influenced by the method of production (pot) and other growing factors such as longer photoperiod (16 h) source and soil type (Lakušić et al 2012).

Principal component analysis was used to visualise the chemical differences observed across the different rosemary productions (Figure 2.3), with volatile compounds identified that expressed significant differences between samples (Table 2.4). Principal component analysis dimension one (F1) and two (F2) explained 69.97 % of the total variation within data. The first axis separated the samples by type of soil, loamy/clay soil (R2, R3 and R4) from loamy soil and peat substrate (R1, R5 and R6), whilst the second axis separated the samples by temperature during growth, samples R4, R5 and R6 (16-20 ˚C) from samples R1 (20-25 ˚C) and R2 and R3 (11-15 ˚C). Rosemary produced at medium temperature range (16-20 ˚C) was associated with half of compounds detected (Figure 2.3). Additionally compounds described as relevant to the rosemary aroma such as camphor (M22), borneol (M25), limonene (M13), camphene (M4) and

bornyl acetate (M32) were highly associated with open field production (R4). This could be due to this sample (R4) being produced under medium range temperature (16-20 ˚C), using a loamy/clay soil, using higher water amounts (5.1 mm) and shorter photoperiod (12 h). A study reported higher essential oil yield in samples produced in summer and higher abundance of alpha-pinene in winter produced rosemary, conversely results from the present study showed higher volatiles abundance in samples produced in York (R2 and R3) at 11-15 ˚C and similarly a high association (Figure 2.3) of these samples wit alpha-pinene (M3) (Salido et al 2003). Furthermore, producing rosemary at higher temperatures was reported to lead to higher abundance of eucalyptol (M14). Rosemary sample R1 (pots from West Sussex) was produced at the higher temperature range (20-25 ˚C) and displayed a high association with this compound (M14)(Figure 2.3). However, other conditions could be influencing this aroma composition such the variety of the plant, the type of production or the plant density experienced by plants in pots (Lakušić et al 2012, Pintore et al 2002). Rosemary results from the present study (Table 2.4, Figure 2.3) displayed differences between samples which might be influenced by the growing conditions of the crops, however the variety of these samples is unknown which might be the variable causing the differences between samples. Most compounds were positively correlated with first (F1) dimension, and eucalyptol (M14) was negatively correlated with F1 and with F2. These results suggest that differences in the main compounds were influenced by growth temperature, however observed differences may be due to variation in genotypes as each production site is unaware of the crop's variety being used.

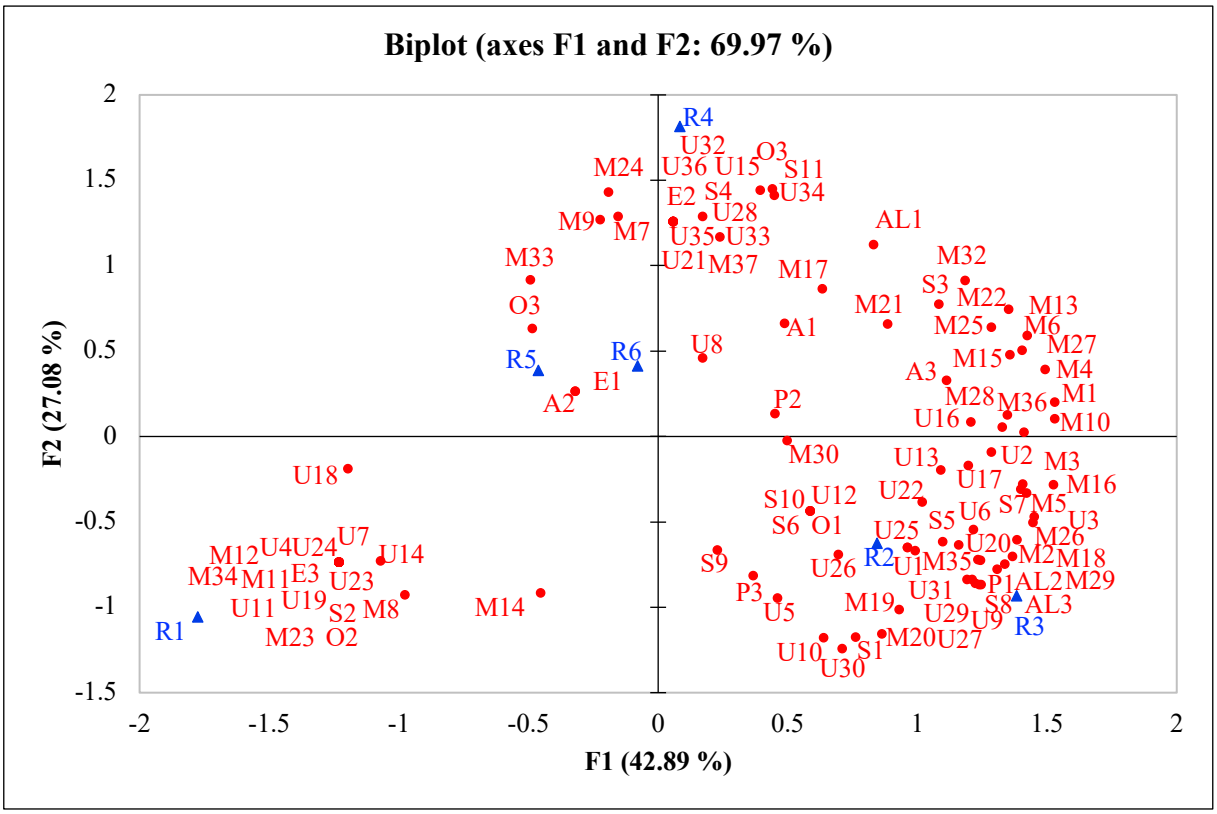


Figure 2.3: Principal component analysis of six rosemary samples showing correlations with volatile compounds (red circle) and samples (blue triangle): R1- pots; R2- protected field; R3- open field; R4- open field; R5- open field; R6- open field.

2.4 Conclusions

Type of production displayed a strong influence on the aroma composition of basil and coriander. Pot produced samples resulted in the lowest abundances of volatile compounds including compounds described in literature as relevant to the aroma of these herbs and higher proportions of minor compounds. Conversely, samples produced in open field (coriander) and hydroponics (basil) resulted in higher abundance of volatile compounds and higher proportions of main compounds and unidentified compounds. No influence of type of production was detected in rosemary composition, however due to unknown varieties of the samples this factor (type of production) might not be significant.

The three herbs were produced across several locations in the UK, which results in differences in environmental conditions and growing conditions, leading to significant differences expressed in several compounds for the three herbs. Temperature range during growth expressed the biggest influence on the aroma profile of the three herbs, where higher temperatures (20 -25 ˚C) range resulted in lower abundances of volatiles including most relevant compounds for the aroma of coriander and rosemary, conversely basil produced in this range resulted in higher volatile abundances including main compounds. Additionally, the type of soil in which plants were produced had an influence on the aroma profiles loamy type soils were associated with higher relative abundance of volatiles, which might due to higher availability of macronutrients and micronutrients. Similarly, hydroponic produced basil also resulted in higher relative abundance of volatile compounds. However, due to differences in more than one environmental factor is not possible to associate one of these to the differences observed between samples.

With apparent differences in the aroma, identifying the influence of growing conditions on the flavour and how these might be perceived when consumed is impossible without carrying out sensory profiling studies using a trained panel and consumer preference trials. Additionally, growing herbs in controlled environment and controlled crop variety would provide a deeper understanding how each environmental factor affects the aroma composition of each herb. The findings from this study will provide fresh herb producers with a deeper understanding how differences in environmental conditions will affect the aroma of the crop and how this might be perceived when consumed, allowing growers to guide their herbs production with aroma profiles in mind.

Chapter 3: An investigation of the relationship of volatile composition and sensory profile of three culinary herbs: basil (*Ocimum basilicum* var. Sweet Genovese), coriander (*Coriandrum sativum* var. Cruiser) and rosemary (*Rosmarinus officinallis*)

3.1 Introduction

Herbs are used as seasoning in food and are used in many cuisines around the world, providing flavour to dishes, therefore it is important to understand their flavour and organoleptic characteristics. Basil (*Ocimum basilicum*) is an aromatic herb belonging to the Lamiaceae family, which is widely used for culinary purposes due to its unique flavour. The characteristic aroma of basil can be attributed to specific aroma volatile compounds belonging to the chemical class of terpenes and phenylpropanoids (Bernhardt et al 2015). Studies that have analysed basil's essential oils and dried leaf material, have identified eucalyptol (1,8-cineole), linalool, estragole (methyl chavicol) and eugenol as the main contributors to the aroma of the herb (Díaz-Maroto et al 2004, Klimánková et al 2008, Lee et al 2005, Patel et al 2021). These have been described as responsible for the clove, floral, sweet, anise and eucalyptus aroma (Patel et al 2021).

Coriander (*Coriandrum sativum*) is an aromatic crop from the Apiaceae family, that can be used for its leaves or seeds. Essential oil from coriander leaves has been analysed using distillation and gas chromatography, and the main flavour components identified belong to the chemical class of aldehydes and alcohols (Singletary 2016). Compounds like (*E*)-2-decenal, (*E*)-2-dodecenal, decanol, dodecanol and decanal, have been identified as the main aroma compounds for coriander leaves (Anjum et al 2011, Łyczko et al 2021, Neffati and Marzouk 2008, Nurzyńska-Wierdak 2013). Coriander leaves (cilantro) have been described as imparting

a bitter, citrus, fatty, green and soapy flavour (Lawless et al 2012). Additionally, a study analysing character-impact compounds, identified a lower abundant compound, (*Z*)-3-hexenal as having a great impact on the aroma, responsible for the green aroma described in coriander leaves (Cadwallader et al 1999).

The culinary herb rosemary (*Rosmarinus officinallis*) belongs to the family of Lamiaceae (mint family), and its leaves are widely used as fresh or dried for their flavour in different foods and also perfumery. The essential oil of this herb is mainly composed of monoterpenes, with main characteristic compounds identified as camphor, eucalyptol (1,8-cineole), borneol, verbenone, alpha-pinene and camphene (Díaz-Maroto et al 2007, Hcini et al 2013, Lakušić et al 2012, Salido et al 2003). Fresh rosemary has been described as having a fresh, pine, herbaceous and woody aroma, provided by the main compounds described above (Szumny et al 2010).

Limited research has been conducted looking at establishing correlations between the volatile chemical composition and the sensory profile of fresh herbs, with most studies analysing foods enhanced with culinary herbs (Amoroso et al 2017, Gurkan and Hayaloglu 2017, Heck et al 2019, Marangoni and Moura 2011). Fresh thyme was analysed for its volatile composition and sensory evaluation was carried out, differences in sensory perception were detected between samples and these reflected differences in the essential oil content and composition (Kosakowska et al 2019). Similarly, a study evaluated differences in volatile content of essential oil of thyme and sensory profile after different drying techniques, where sensory data showed corresponding differences to essential oil content (Sárosi et al 2013). Additionally, essential oil of dried leaves of basil was analysed by GC-MS to determine the composition and sensory profile was determined by trained panel, where results from both

analysis showed high correlations with sensory attributes describing characteristics of compounds detected in the essential oil (Bernhardt et al 2015, Costa et al 2014). Further research needs to be carried out in order to understand if this relation is detected using fresh culinary herbs.

The aim of this study was to investigate the relationship between the volatile chemical composition and the sensory characteristics of fresh samples namely basil, coriander and rosemary grown in different locations in the UK. Sensory evaluation using a trained panel was used to understand how their chemical volatile composition was organoleptically perceived. Eventually, this information could help herb growers understand what affects the flavour and how these differences are perceived when consumed and use aroma profile targets to guide herb production.

3.2 Materials and methods

3.2.1 Plant material

Fresh herbs were sourced as described in Chapter 2 (subsection 2.2.1) with similar criteria. Samples of Rosemary (*Rosmarinus officinallis*), Coriander (*Coriandrum sativum* var. Cruiser) and Basil (*Ocimum basilicum* var. Sweet Genovese*),* were provided from growers during the summer season of 2019.

Growing conditions for the samples were provided (Table 3.1) by growers when records were available, with some information not shared due to commercial confidentiality. This paper defines the key characteristics of the aroma profile of each herb for a single UK growing season; subsequent publications will examine the contribution of seasonal variation on herb profile.

Table 3.1: Location, type of production and environmental factors for each sample for the three herbs.

Herb	Sample	Location	GPS	Production type	Av Temp[A] (˚C)	Soil type[B]	Water supply[C] (mm day^{-1})	Light source[D] (h day^{-1})
Basil	B1	West Sussex	50.4848˚N, 0.4413˚W	Pot	20-25	Peat	Ir	16h-Sl 0h-HPS
	B2	Lincolnshire	52.7442˚N, 0.3779˚W	Pot	16-20	Mixture	Ir	16h-HPS
	B3	West Sussex	50.4914˚N, 0.4445˚W	Hydroponics	20-25		Ir	12h-LED
Coriander	C1	West Sussex	50.4848˚N, 0.4413˚W	Pot	16-20	Peat	Ir	16h-Sl HPS
	C2	Lincolnshire	52.7442˚N, 0.3779˚W	Pot	16-20	Mixture	4.5mm-Rf Ir	HPS
	C3	York	54.1345˚N, 1.2430˚W	Open field	11-15	Loamy Clay	1.3mm-Rf 0.7mm-Ir	15h-Sl
	C4	West Sussex	50.8198˚N, 0.7807˚W	Open field	11-15	Sandy Loamy	1.4mm-Rf Ir	14h-Sl
Rosemary	R1	West Sussex	50.4848˚N, 0.4413˚W	Pot	20-25	Peat	Ir	15h-Sl HPS
	R2	York	54.1345˚N, 1.2430˚W	Soil covered	20-25	Loamy Clay	2.8mm-Ir	14h-Sl
	R3	York	54.1345˚N, 1.2430˚W	Open field	16-20	Loamy Clay	2.3mm-Rf	12h-Sl

	0.2mm-
	Ir

[A] Average temperature over 24 h; [B] Type of soil used:mixture- composed of 90 % peat substrate and 10 % perlite [C] Average water amount and water source used: I- irrigation and Rf- rainfall; [D] Average photoperiod and light source used: Sl- sunlight, HPS-high pressure sodium and LED- light emitting diode (Philips Toplights-DRW/LB)

Samples were stored as described in Chapter 2, and replication was also carried out as in study from previous chapter (Chapter 2:. Preparation of samples followed the method described in in Chapter 2: in subsection 2.2.2.

3.2.2 Chemical reagents

Chemical reagents used for preparation and analysis of samples of present study are described in Chapter 2: in subsection 2.2.3 (page 57).

3.2.3 Solid Phase Microextraction (SPME) followed by GC-MS

Herb samples were prepare as described in Chapter 2:,subsection 2.2.4 (page 58), and analysis was carried out as described in Chapter 2.

3.2.4 Sensory evaluation of fresh herbs samples

Quantitative descriptive analysis (QDA) was carried out in order to determine sensory characteristics of fresh samples of basil, coriander and rosemary and estimated quantitatively. A trained sensory panel at the Sensory Science Centre (University of Reading, n = 11, 10 female and 1 male), was used to develop a consensus vocabulary describing each of the three herbs. During the vocabulary development, panellists were asked to describe the appearance, aroma, taste, flavour, mouthfeel and aftereffects of the samples and produce the necessary descriptive terms. The terms were discussed as a group and a panel leader, which led to a consensus of 27,

31 and 31 attributes for basil, coriander and rosemary samples, respectively. Samples were assessed in a temperature-controlled room (22 ˚C) under artificial daylight and in isolated booths and with iPads. Leaves were washed and a sprig of leaves from each herb was served at room temperatures in similar quantities. The panellists scored each sample in duplicate, in separate sessions, and the data was collected using Compusense Cloud Software. Samples were presented using a random three-digit number, which were provided in a monadic balanced order, with samples sets allocated randomly to panellists. The panellists were asked to assess appearance first, break the leaves to assess the aroma, and to eat some leaf material to assess the flavour and mouthfeel; this was followed by a 30s delay to assess the aftereffects. The intensity of each attribute was scored on a 100 point unstructured line scale. Between each sample panellists were asked to cleanse their palate using water and plain yogurt.

3.2.5 Statistical analysis

Quantitative data obtained from the GC-MS analysis, were analysed by one-way analysis of variance (ANOVA) and multiple factor analysis (MFA) using XLSTAT version 2020.5.1 (Addinsoft, Paris, France). Tukey's post hoc test was applied in order to assess which samples were significantly different ($p< 0.05$). Only compounds with significant differences were included in the multiple factor analysis.

SENPAQ version 6.3 (Qi Statistics, Kent, UK) was used to analyse the data from the sensory panel, and ANOVA was used to check significant differences for each attribute. The means taken from the assessors were then correlated with the volatiles composition means using MFA.

3.3 Results and Discussion

3.3.1 Basil

3.3.1.1 Volatile composition

In total, 44 compounds were detected in the headspace of the three samples of basil (Table 3.2). The compounds detected included 16 monoterpenes, eight sesquiterpenes, two phenylpropanoids, two aldehydes and 13 unidentified compounds. Significant quantitative differences in the aroma profiles were observed between production type for the three basil samples confirmed by one-way ANOVA. Hydroponically produced basil (B3) contained the highest amounts of volatile compounds, particularly monoterpenes, phenylpropanoids and sesquiterpenes compounds. No significant differences were observed for 18 compounds, including methyl eugenol, suggesting that method of production has no influence on these.

Table 3.2: Relative abundance of aroma compounds identified in the headspace of fresh basil samples.

Code	Compound name	LRI [a]	ID [b]	Relative abundance [c]			*p*-value
				B1	B2	B3	
	Alcohol						
A1	1-Octanol	1077	A	12	8.7	14	ns
	Total (%)			0.2	0.2	0.1	
	Aldehyde						
AL1	(*E*)-2-Hexenal	862	B[1]	8.2	7.0	14	ns
AL2	Octanal	1007	A	16[a]	5.1[b]	nd	**
	Total (%)			0.4	0.2	0.1	
	Monoterpene						
M1	alpha-Pinene	944	A	7.6	7.8	12	ns
M2	Sabinene	984	A	21	18	35	ns
M3	beta-Pinene	989	A	22[ab]	15[a]	34[b]	*
M4	beta-Myrcene	994	A	26[a]	24[a]	46[b]	*
M5	Limonene	1040	B[2]	29[ab]	16[a]	37[b]	*
M6	beta-Phellandrene	1042	B[3]	nd	3.1	4.6	ns
M7	Eucalyptol(1,8-Cineole)	1050	B[4]	1346[a]	996[a]	2168[b]	**
M8	(*Z*)-Sabinene hydrate	1083	A	12[ab]	10[a]	18[b]	*
M9	p-Cymenene	1099	A	33[a]	20[b]	23[ab]	*

M10	Linalool	1112	B[5]	623[a]	1113[a]	4944[b]	**
M11	Camphor	1171	B[6]	159[a]	50[b]	60[b]	*
M12	Borneol	1189	A	nd	nd	22	
M13	1-Terpinen-4-ol	1197	B[7]	2.8	nd	nd	
M14	alpha-Terpineol	1209	A	46[a]	39[a]	92[b]	***
M15	Bornyl acetate	1306	B[8]	22[a]	20[a]	176[b]	**
M16	Ocimene quintoxide	1052	A	38	59	nd	ns
	Total (%)			41	48	59	
	Other						
O1	Methional	1213	A	17	8.1	18	ns
O2	Coumarin	1470	A	nd	nd	8.6	
	Total (%)			0.3	0.2	0.2	
	Phenylpropanoid						
P1	Eugenol	1379	B[9]	828[a]	586[a]	2357[b]	**
P2	Methyl eugenol	1418	B[10]	1750	1392	1378	ns
	Total (%)			43	39	29	
	Sesquiterpene						
S1	alpha-Cubebene	1371	B[11]	nd	nd	4.9	
S2	alpha-Copaene	1404	B[12]	13[ab]	8.6[a]	23[b]	*
S3	alpha-Farnesene	1465	B[13]	243	129	278	ns
S4	alpha-Humulene	1491	B[14]	26[a]	15[a]	122[b]	**
S5	alpha-Muurolene	1505	B[15]	nd	nd	23	
S6	Germacrene D	1517	B[16]	21[a]	14[a]	111[b]	**
S7	(*Z*)-Nerolidol	1537	A	6.9[a]	11[a]	56[b]	***
S8	(*E*)-Nerolidol	1576	A	nd	nd	3.3	
	Total (%)			5.2	3.5	4.9	
	Unkowns						
U25	unkown	1186		7.2[a]	6.4[a]	15[b]	**
U26	unkown	1360		nd	nd	21	
U27	unkown	1458		456	317	479	ns
U13	unkown	1477		nd	nd	38	
U15	unkown	1498		9.1[a]	11[a]	36[b]	**
U17	unkown	1510		27	19	33	ns
U19	unkown	1527		11	6.0	nd	ns
U20	unkown	1533		nd	nd	93	
U21	unkown	1546		28[a]	24[a]	160[b]	*
U22	unkown	1551		4.5	2.4	9.9	ns
U28	unkown	1568		nd	nd	3.7	
U24	unkown	1597		nd	nd	14	
U29	unkown	1595		nd	14[a]	67[b]	*
	Total (%)			9.2	8.3	7.5	

[a] Linear retention index (LRI) on a DB-5 column. [b] A, mass spectrum and LRI agree with those of authentic compounds; B, mass spectrum (spectral quality value >80 was used) and LRI agrees with reference spectrum in the NIST/EPA/NIH mass spectra database and LRI agree with those in the literature cited [1]

(Yu et al 2004); [2] (Buchin et al 2002); [3] (Mesa-Arango et al 2010); [4] (Baranauskiene et al 2003); [5] (Merle et al 2004); [6] (Jürgens and Dötterl 2004); [7] (Bylaite and Meyer 2005); [8] (Asfaw et al 2005); [9] (Edris and Farrag 2003); [10] (Krauze-Baranowska et al 2002); [11] (Hongratanaworakit and Buchbauer 2007); [12] (Miyazaki et al 2011); [13] (G Flamini et al 2002); [14] (Sun and Petracek 1999); [15] (Sylvestre et al 2006); [16] (Sarikurkcu et al 2008). [c] Estimated abundance collected in the headspace of basil samples containing 2.8 mL of saturated calcium chloride, calculated by comparison with of 100 ppm propyl propanoate used as internal standard; internal standard was used to normalise chromatograms; means of three replicate samples are shown; means not labelled with the same letters are significantly different (p<0.05) and Tukey's HSD multiple pairwise comparison; nd - not detected; ns - not significant probability obtained by ANOVA, * significant at the 5 % level; ** significant at the 1 % level; *** significant at 0.1 % level.

Several studies have reported that monoterpenes and phenylpropanoids are the chemical compound groups that contribute to the aroma of basil (Bernhardt et al 2015, Calín-Sánchez et al 2012, Díaz-Maroto et al 2004, Klimánková et al 2008, Lee et al 2005, Patel et al 2021). The three basil samples in this study were mostly composed of monoterpenes and phenylpropanoids (80 - 90 %). Sample produced in hydroponic system (B3) displayed a composition of 59 % monoterpenes, 29 % phenylpropanoids and 5 % sesquiterpenes, whereas pot produced from West Sussex (B1) samples had 40 %, 43 % and 5 %, for monoterpenes, phenylpropanoids and sesquiterpenes, respectively, compared to 47 % monoterpenes, 39 % phenylpropanoids and 4 % sesquiterpenes in pots from Lincolnshire (B2). Monoterpenes comprised the majority of the aroma profile for all basil samples, with eucalyptol and linalool exhibiting the highest ratio (84-93 %) of monoterpenes (Calín-Sánchez et al 2012, Díaz-Maroto et al 2004, Lee et al 2005, Simon et al 1999). These two compounds have been reported to contribute to a floral, sweet and eucalyptus like aroma to basil and to display a high correlation with these attributes when assessed in sensory testing (Bernhardt et al 2015, Patel et al 2021).

Phenylpropanoids have also been described as the main contributors to the basil aroma, particularly eugenol and methyl eugenol (Calín-Sánchez et al 2012, Miele et al 2001, Patel et al 2021, Simon et al 1999) responsible for the cloves aroma (Patel et al 2021). The results in this study showed that methyl eugenol was the most abundant compound in pot samples, however no significant differences were detected between type of production. Growing basil

using a hydroponic system produced significantly higher ($p < 0.01$) amounts of eugenol compared to pot produced, this production (hydroponics) allows better control of available nutrients and water and no influence of the soil (Putra and Yuliando 2015), additionally this basil was produced at recommended temperature ranges (20-25 ˚C) and under LED lighting, and these conditions have been described to increase the volatile content of basil plants (Chang et al 2007, Litvin et al 2020). Conversely, the average percentage of phenylpropanoids was higher in basil produced in pots (~ 40 %) in comparison to hydroponics (~ 30 %). The higher percentage in composition of these phenylpropanoids compounds would contribute to a higher intensity of the cloves aroma.

Sesquiterpenes displayed a different pattern than monoterpenes and phenylpropanoids, where similar percentage in composition (~ 5 %) was observed between samples. Alpha-humulene and alpha-copaene were some of the most abundant sesquiterpenes with significant differences across samples. Sesquiterpenes have not been described as major contributors to the aroma of basil in literature, which is in agreement with the results of the present study.

3.3.1.2 Sensory evaluation of basil

The sensory profile of three basil samples was created by a trained panel who reached a consensus of 27 terms for the quantitative evaluation of samples grown in UK during the summer season of 2019. Means of panel scores were calculated (Table 3.3) and out of the 27 attributes that were profiled, 10 of these were found to be significantly different between the three basil samples.

Table 3.3: Mean panel scores for sensory attributes of the three basil samples.

Attribute	Score[A]			*p*-value[B]
	B1	B2	B3	
Appearance				
Colour of leaf	57.0[a]	49.9[b]	58.4[a]	**
Leaf size	58.1	54.7	61.9	ns
Stem thickness	53.1[ab]	48.1[b]	56.3[a]	*
Leaf damage	5.2[a]	16.4[b]	10.5[ab]	**
Freshness	74.3[a]	55.4[b]	60.6[b]	***
Odour				
Odour Intensity	57.6	53.1	54.6	ns
Fresh cut grass aroma	16.5[a]	20.6[b]	18.7[ab]	*
Tomato vine aroma	17.7[b]	12.8[b]	23.9[a]	***
Cloves aroma	44.2[a]	33.8[b]	41.1[ab]	*
Sweet aroma	26.3	26.2	23.2	ns
Taste/Flavour				
Bitter taste	33.0	33.3	34.1	ns
Sweet taste	19.0	19.0	17.4	ns
Salty taste	13.7	12.8	13.3	ns
Fresh cut grass flavour	23.3	23.1	19.9	ns
Soapy flavour	20.1	23.2	19.1	ns
Cloves flavour	31.2	29.1	36.8	ns
Menthol flavour	4.1[b]	3.4[b]	7.7[a]	*
Metallic flavour	4.2	7.9	6.3	ns
Mouthfeel				
Cooling mouthfeel	8.5	10.7	10.9	ns
Chewy mouthfeel	44.0	40.8	42.9	ns
Moisture mouthfeel	32.2[a]	37.0[ab]	39.0[b]	*
Aftereffects				
Cloves aftereffect	26.3[ab]	21.5[a]	30.8[b]	**
Soapy aftereffect	14.5	15.7	13.2	ns
Cooling aftereffect	10.0	10.5	9.8	ns
Numbing aftereffect	21.1	20.8	23.5	ns
Drying aftereffect	34.9	29.9	31.4	ns
Bitter aftereffect	21.6	23.0	22.4	ns

[A] Means are from two replicate samples, measured on an unstructured line scale (0-100); differing small letters represent sample significance from multiple comparisons and means not labelled with the same letters are significantly different ($p < 0.05$). [B] Probability obtained by ANOVA that there is a difference between means; ns, no significant difference between means ($p > 0.05$); * significant at the 5 % level; ** significant at the 1 % level; *** significant at 0.1 % level.

Appearance attributes displayed significant differences between samples and similarities were observed for leaf size attribute (Figure 3.1). A significant difference for freshness ($p < 0.001$) and for leaf damage ($p < 0.01$) was observed with pot sample B1 which scored the

highest for freshness and the least for leaf damage. Present results show a negative correlation between these two attributes and a similar relation was also observed for the other samples.

Figure 3.1: Images of the leaves of the three basil samples used in this study.

Similarities for odour intensity and sweet aroma were detected, however significant differences ($p < 0.05$) were observed between pot samples (B1 and B2) with opposite scorings for each attribute, with B1 presenting a higher clove aroma and B2 a higher grassy green aroma, this indicates that other growing factors such as growth temperature and type of soil (Table 3.1) influence the aroma of basil. Eugenol, which is characterised by its aroma of cloves, was reported to increase when basil was grown at 25 ˚C (Chang et al 2005, 2007). Apart from menthol flavour, that was significantly higher ($p < 0.05$) from plants grown in hydroponics (B3), all taste and flavour attributes displayed similarities between samples. Significant differences for mouthfeel ($p < 0.05$) and aftereffects ($p < 0.01$) were observed in moisture and cloves aftereffect, with sample B3 scored higher for both attributes. This sample also scored the highest for clove flavour and bitter taste, however not significantly.

3.3.1.3 Multiple factor analysis (MFA) of volatile compounds and sensory attributes

MFA was used to visualise the sensory and chemical differences observed across the different basil productions (Figure 3.2), with volatile compounds identified that expressed

significant differences between samples (Table 3.2) and the odour and flavour sensory attributes (Table 3.3). Multiple factor analysis dimension one (F1) and two (F2) explained 100 % of the total variation within the data. The first axis separated the samples B1 and B2 from B3 sample, whilst the second axis separated the samples B1 from B2 and B3.

Basil produced under hydroponics was highly associated with cloves and menthol flavour, and bitter taste (Figure 3.2), whereas pot produced samples were highly associated with sweetness attributes and fresh cut grass flavour. These results agree what has been described in literature, where hydroponic production was described as a system with higher essential oil yield and higher abundance of volatiles responsible for the aroma of the crop, and this was attributed to better nutrient control and seasonal adjustment possible with this production method (Ciriello et al 2021, Sharma et al 2018). Samples produced in Lincolnshire were highly associated with soapy and metallic flavour and fresh cut grass aroma, whilst basil produced in West Sussex was more associated with cloves and tomato vine aroma, salty taste and more intense aroma, differences between these samples could be due to the temperature during growth as West Sussex samples were produced at higher temperatures (20-25 ˚C) and included samples (B1 and B3) from different production methods (pot and hydroponic, respectively), soil type (peat substrate and no soil, respectively) and light source (natural/HPS and LED, respectively). Most monoterpenes and phenylpropanoids were positively correlated with first dimension (F1), whereas camphor (M11), *p* -cymenene (M9) and octanal (AL2) were negatively correlated with F1. Monoterpenes and phenylpropanoids were positioned in the outer rim of the biplot, with compounds like linalool (M10), eucalyptol (M7) and eugenol (P1) expressing a positive association to cloves attributes, menthol flavour and bitter taste, whereas octanal (AL2) expressed positive correlation with fresh cut grass flavour and sweetness attributes. West Sussex location showed a positive correlation with the second dimension (F2),

additionally odour intensity, cloves attributes and sweetness attributes were positively correlated with dimension F2, conversely soapy and metallic flavour, fresh cut grass aroma and bitter taste were negatively correlated with this dimension. Bitter taste showed a high negative correlation with sweetness attributes and was strongly associated with most compounds and hydroponic production.

Clear differences were expressed between samples influenced by the type of production and growing conditions. Pot production resulted in a more green and sweet basil whilst hydroponics resulted in a more clove and menthol basil. These differences were also reflected in the volatile composition of these samples (Table 3.2). Results from present study suggest that growing basil at optimal temperature range (20-25 ˚C), under LED lighting and with high nutrient availability will result in higher volatiles abundances and higher association with flavour attributes characteristic of basil. However, higher abundance of volatile compounds and more intense flavour attributes might not result in liking of certain attributes and higher preference by the consumer.

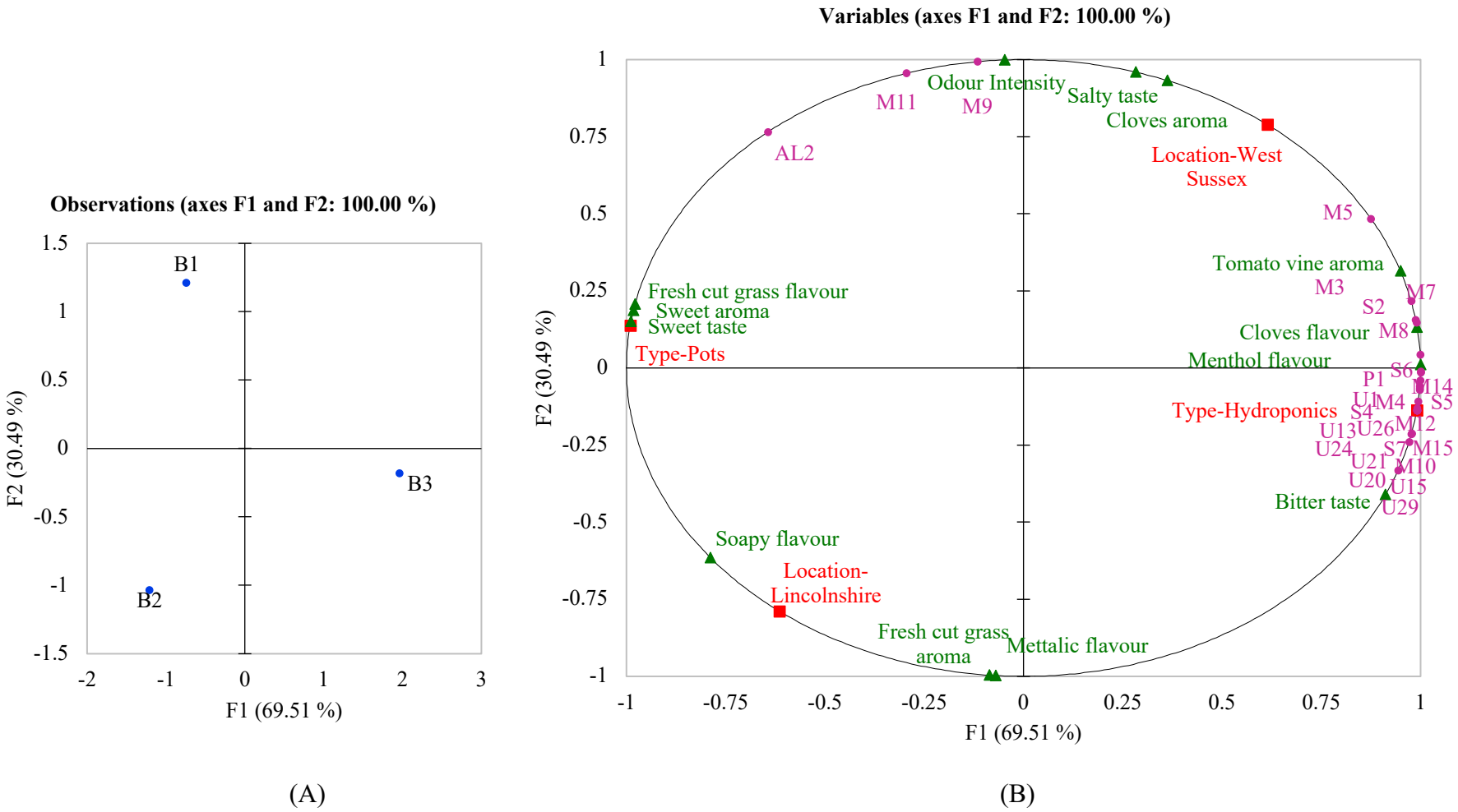


Figure 3.2: Multiple factor analysis of three basil samples showing correlations with volatile compounds and sensory attributes. (A) Projection of basil samples (B1- pots; B2- pots; B3- hydroponics); (B) Distribution of variables: red squares-growing conditions; green triangle-sensory attributes; pink circle-volatile compounds.

3.3.2 Coriander

3.3.2.1 Volatile composition

In total, 70 compounds were detected in the headspace of four different coriander samples (Table 3.4). The compounds detected included 12 aldehydes, 12 other, six alkanes, four alcohol and 33 unidentified compounds. Significant quantitative differences in the aroma profiles were observed between coriander samples in this study, confirmed by one-way ANOVA. Open field grown material produced samples of coriander containing the highest amounts of volatile compounds, with location further influencing the aroma volatile profile as samples grown in York (C3) had higher content of aldehydes whereas samples produced in West Sussex had higher content of alcohols. These differences might be due to differences in soil type or water amounts since both open field samples were produced at similar temperature ranges (11-15 ˚C) and similar photoperiod (14-15 h), however soil in York was a Loamy/clay type whilst in West Sussex was a sandy/loamy type, the latter is characterised by high drainage and acidic properties. No significant differences between methods of production were found in the relative amount of 29 compounds, including dodecanal and (*Z*)-3-hexenal.

Table 3.4: Relative abundance of aroma compounds identified in the headspace of fresh coriander samples.

Code	Compound name	LRI[a]	ID[b]	Relative abundance[b]				*p*-value
				C1	C2	C3	C4	
	Alcohol							
A1	(*Z*)-3-Hexen-1-ol	857	A	33[a]	28[a]	6.6[b]	37[a]	**
A2	(*E*)-2-Hexen-1-ol	866	A	7.2[a]	5.0[a]	4.4[a]	25[b]	**
A3	1-Nonanol	1171	A	nd	nd	13	11	ns
A4	1-Decanol	1272	A	2.7[a]	0.7[a]	88[a]	1019[b]	***
	Total (%)			4.4	5.6	5.1	31	
	Aldehyde							
AL1	(*Z*)-3-Hexenal	799	A	0.8	0.6	nd	nd	ns
AL2	(*E*)-2-Hexenal	854	A	4.5	2.9	nd	2.6	ns

AL3	Octanal	1004	A	1.1[a]	0.9[a]	1.6[a]	6.9[b]	***
AL4	Nonanal	1103	A	1.7[a]	1.2[a]	40[b]	43[b]	**
AL5	(*E*,*Z*)-2,6-Nonadienal	1162	A	nd	nd	1.2[a]	7.4[b]	***
AL6	(*E*)-4-Decenal	1197	B[1]	nd	nd	5.9[a]	40[b]	***
AL7	Decanal	1210	A	149[a]	65[a]	570[b]	671[b]	***
AL8	(*E*)-2-Decenal	1266	A	2.9[a]	1.1[a]	251[b]	nd	*
AL9	(*E*)-2-Undecenal	1370	A	8.4[a]	3.1[a]	109[b]	316[c]	***
AL10	Dodecanal	1413	A	145	84.6	163	65	ns
AL11	(*E*)-2-Dodecenal	1475	B[2]	188[a]	87[a]	323[b]	432[c]	***
AL12	Tetradecanal	1605	B[3]	19	13	17	21	ns
	Total (%)			53	43	68	46	
	Alkane							
AK1	Nonane	900	A	61[a]	54[a]	28[ab]	10[b]	**
AK2	Decane	1000	A	5.8	6.1	4.1	3.2	ns
AK3	Undecane	1098	A	14[a]	6.1[b]	7.3[ab]	8.2[ab]	*
AK4	Dodecane	1200	A	nd	nd	11a	75b	***
AK5	Tridecane	1300	A	nd	nd	8.3[a]	36[b]	***
AK6	Tetradecane	1401	A	21[a]	6.6[b]	34[c]	44[d]	***
	Total (%)			11	12	4.2	5.0	
	Ester							
E1	Methyl decanoate	1324	A	nd	nd	2.0[a]	5.2[b]	***
E2	Ethyl decanoate	1397	A	nd	3.4[ab]	6.2[b]	nd	**
E3	Methyl dodecanoate	1525	B[4]	nd	nd	0.3	nd	ns
	Total (%)			0.0	0.6	0.4	0.1	
	Monoterpene		A					
M1	Limonene	1036	A	nd	20	nd	nd	***
M2	gamma-Terpinene	1064	A	1.9[a]	0.9b	1.3[ab]	nd	*
M3	Terpinolene	1094	A	0.5	nd	nd	nd	ns
M4	Linalool	1099	A	nd	2.7	4.1	nd	ns
	Total (%)			0.3	4.1	0.3	0.0	
	Other							
O1	4-Methylthiazole	821	A	3.1[a]	2.6[a]	4.7[b]	6.3[b]	***
O2	2,3-Dimethylthiophene	892	A	1.8[ab]	1.4[a]	2.8[bc]	4.3[c]	**
O3	Styrene	895	A	nd	nd	1.3	nd	
O4	6-Methyl-3-hepten-2-one	995	A	nd	nd	0.7	nd	
O5	Methional	1028	A	1.0[a]	0.8[a]	0.6[b]	nd	**
O6	1-Tridecene	1292	B[5]	nd	0.2	1.2	nd	ns
O7	Carvacrol	1311	A	64[a]	35[a]	137[ab]	144[b]	*

O8	n-Decanoic acid	1359	B[6]	nd	nd	0.2	0.4	ns
	Total (%)			7.2	6.8	6.9	4.4	
	Unkowns							
U36	unknown	992		1.1	1.4	2.1	1.8	ns
U4	unknown	1090		nd	nd	2.1	nd	
U5	unknown	1146		1.4	1.2	1.8	2.6	ns
U6	unknown	1167		0.8	nd	22	14	ns
U10	unknown	1252		nd	nd	9.2[a]	56[b]	***
U11	unknown	1282		1.7[a]	0.9[a]	1.6[a]	3.4[b]	***
U13	unknown	1320		1.3	nd	1.8	2.3	ns
U14	unknown	1348		nd	nd	nd	1.3	
U15	unknown	1354		nd	nd	4.0[a]	17[b]	***
U17	unknown	1374		2.9[ab]	1.2[a]	12[b]	nd	*
U19	unknown	1435		0.9	nd	0.9	nd	ns
U21	unknown	1457		8.7[ab]	3.3[a]	14[b]	21[c]	***
U22	unknown	1488		1.0	0.8	0.8	nd	**
U23	unknown	1504		3.4[a]	2.7[a]	9.3[b]	11[b]	**
U37	unknown	1514		4.8	4.1	5.1	6.5	ns
U38	unknown	1519		nd	nd	1.1	nd	ns
U24	unknown	1531		0.9	nd	1.2	1.3	ns
U25	unknown	1544		1.8	1.0	3.1	2.8	ns
U26	unknown	1554		nd	nd	0.2	0.4	ns
U27	unknown	1559		0.8a	1.0ab	1.3[ab]	1.6[b]	*
U39	unknown	1575		26[a]	20[a]	40[ab]	51[b]	*
U40	unknown	1617		5.7	3.6	4.3	4.4	ns
U29	unknown	1662		6.7	4.6	6.3	8.0	ns
U30	unknown	1680		135[ab]	96[a]	149[ab]	200[b]	*
U31	unknown	1707		3.0	2.8	3.3	4.8	ns
U41	unknown	1715		0.7	0.6	2.0	1.6	ns
U42	unknown	1740		0.4	0.2	nd	nd	ns
U32	unknown	1760		nd	nd	nd	4.2	
U43	unknown	1765		0.9	0.8	1.2	1.8	ns
U33	unknown	1782		20[ab]	20[a]	27[ab]	44[b]	*
U34	unknown	1842		1.0[a]	1.3[a]	3.9[ab]	4.9[b]	*
U35	unknown	1884		2.6[a]	2.2[a]	3.8[ab]	6.4[b]	*
U20	unknown	1453		4.1[a]	1.0[b]	2.2[ab]	1.9[ab]	*
	Total (%)			24	28	15	14	

[a] Linear retention index (LRI) on a DB-5 column. [b] A, mass spectrum and LRI agree with those of authentic compounds; B, mass spectrum (spectral quality value >80 was used) and LRI agrees with reference spectrum in the NIST/EPA/NIH mass spectra database and LRI agree with those in the literature cited [1] (Morteza-Semnani et al 2006); [2] (Morteza-Semnani et al 2007); [3] (Senatore et al 2005); [4] (Rostad and Pereira 1986); [5] (Song et al 2003); [6] (Smelcerovic et al 2007). [c] Estimated abundance collected in the headspace of coriander samples containing 2.8 mL of saturated calcium chloride, calculated by comparison with of 100 ppm propyl propanoate used as internal standard; internal standard was used to normalise chromatograms; means of three replicate samples are shown; means not labelled with the same letters are significantly different (p<0.05) and Tukey's HSD multiple pairwise comparison; nd - not

detected; ns - not significant probability obtained by ANOVA, * significant at the 5 % level; ** significant at the 1 % level; *** significant at 0.1 % level.

Different studies have reported that aldehydes are the main chemical group responsible for the coriander aroma (Eyres et al 2005, Nurzyńska-Wierdak 2013, Potter and Fagerson 1990, Shahwar et al 2012). All the coriander samples in this study were mostly composed of aldehydes and unidentified compounds. However, differences were observed for a given production type depending on the location the plants were grown. As such, for open field conditions, the sample from York (C3) was comprised of 68 % aldehydes, 5 % alcohols, 4 % alkanes and 15 % unknown compounds, whereas the sample from West Sussex (C4) had an overall composition of 46 %, 31 %, 5 % and 14% for aldehydes, alcohols, alkanes and unknown compounds, respectively, these samples were grown at similar temperature, similar photoperiod and combination of irrigation and rainfall (Table 3.1), however no data could be collected on the amount of irrigation, with different soil types (loamy and sandy, respectively) indicating a significant influence of soil on volatile composition. Additionally, pot grown sample from West Sussex (C1) was composed of 53 %, 4 %, 10 % and 24% for aldehydes, alcohols, alkanes and unknown compounds, conversely coriander from Lincolnshire (C2) had a composition of 43 % aldehydes, 6 % alcohols, 12 % alkanes and 28 % unknown compounds, both pot samples were produced at similar temperatures but using different soil substrates, water sources and light which have been reported to influence the volatile content of the crop (El-Zaeddi et al 2016, Nadjafi et al 2009, Neffati and Marzouk 2008).

As previously mentioned, aldehydes were the predominant chemical group for all coriander samples, with decanal, (*E*)-2-undecenal, dodecanal and (*E*)-2-dodecenal exhibiting the highest ratio of aldehydes (79-94 % of aldehydes abundance) and were significantly ($p < 0.001$) higher in open field production than pot produced, except for dodecanal, this could be

due lower temperature range in open field samples but also due to higher plant density experienced in pots results in lower nutrient and water availability (Ciriello et al 2021). Several studies have identified (*E*)-2-decenal and (*E*)-2-dodecenal as some of the main compounds in coriander aroma, as well as decanal, dodecanal and octane, however different abundances have been reported, which could be due to different varieties and growing conditions which were not reported (El-Zaeddi et al 2016, Eyres et al 2005, Neffati and Marzouk 2008, Nurzyńska-Wierdak 2013). Conversely, the study by Tamura and co-workers (2013) reported a slightly different chemical profile of the oil of fresh coriander leaves with (*E*)-2-tetradecenal as the main volatile compound (40.6 %), followed by (*E*)-2-pentadecenal (7.8 %), (*E*)-2-dodecenal (6.6 %), hexacosane (5.9 %) and (*E*)-2-undecenal (5.6 %) (Tamura et al 2013). McAusland (2020) identified (*E*)-2-decenal as the defining compound for coriander aroma, additionally Cadwaller reported (*E*)-2-alkenals responsible for the typical coriander aroma, however no singular compound was identified (Cadwallader et al 1999, McAusland et al 2020). Sample grown in West Sussex from open field production (C4) expressed a higher composition of alcohol compounds (31 %), which was mainly comprised of 1-decanol, which has not been reported previously, this sample also showed higher volatiles abundance due to the presence of this compound, indicating influence of other growing conditions such as soil type (Table 3.1) suggesting that sandy/loamy soils, with high draining properties and acidic properties, will result in higher alcohol content in coriander plants.

3.3.2.2 Sensory evaluation of coriander

The sensory profile of four coriander samples was generated by a trained panel reaching a consensus of 31 terms for the quantitative evaluation of samples grown in UK during the summer season of 2019. Means of panel scores were calculated (Table 3.5) and out of all

attributes that were profiled, 17 of these were significantly different between the four coriander samples.

Table 3.5: Mean panel scores for sensory attributes of the four coriander samples.

Attribute	Score[A]				*p*-value[B]
	C1	C2	C3	C4	
Appearance					
Colour of leaf	56.2^{a}	53.9^{a}	63.1^{b}	62.4^{b}	***
Leaf size	59.9^{a}	44.9^{b}	45.9^{b}	49.1^{b}	**
Stem thickness	36.2^{ab}	19.6^{a}	33.5^{b}	40.5^{c}	***
Leaf damage	4.2^{a}	5.6^{a}	30.8^{b}	19.8^{c}	***
Freshness	49.7^{a}	49.6^{a}	30.5^{b}	41.3^{c}	***
Odour					
Odour Intensity	36.0^{a}	36.2^{a}	39.7^{a}	47.6^{b}	**
Fresh cut grass aroma	21.2	20.9	21.4	22.3	ns
Celery aroma	13.0	13.3	16.2	16.7	ns
Soapy aroma	16.8^{ab}	13.6^{a}	17.7^{ab}	20.6^{b}	*
Sweet aroma	21.0	20.4	22.9	20.3	ns
Taste/Flavour					
Bitter taste	28.6^{a}	26.1^{a}	37.8^{b}	31.5^{ab}	**
Sweet taste	15.3	13.6	17.0	13.8	ns
Salty taste	12.1^{ab}	9.7^{a}	14.7^{b}	11.8^{ab}	**
Umami taste	5.1^{a}	6.2^{a}	9.3^{ab}	13.9^{b}	**
Fresh cut grass flavour	21.0	18.8	23.1	20.1	ns
Soapy flavour	20.2	17.6	25.5	22.4	ns
Mouthfeel					
Cooling mouthfeel	2.1	1.8	0.8	1.3	ns
Chewy mouthfeel	34.9^{ab}	29.3^{a}	39^{b}	34.3^{ab}	**
Numbing mouthfeel	7.5	7.5	10.5	7.5	ns
Crunch mouthfeel	14.6^{ab}	11.7^{a}	12.6^{ab}	18.4^{b}	*
Mouth adhesion	25.1^{ab}	23.7^{a}	30.8^{c}	28.1^{bc}	**
Warming mouthfeel	1.2	0.0	2.4	0.6	ns
Aftereffects					
Celery aftereffect	7.6	9.1	10.7	11.0	ns
Soapy aftereffect	16.4	13.0	17.7	18.4	ns
Bitter aftereffect	18.6^{ab}	16.1^{a}	22.8^{b}	21.1^{ab}	*
Umami aftereffect	3.1^{a}	4.3^{ab}	5.6^{ab}	7.8^{b}	*
Fresh cut grass aftereffect	11.4	11.2	14.2	13.9	ns
Aniseed aftereffect	1.8^{ab}	1.3^{a}	5.9^{b}	3.0^{ab}	*
Numbing aftereffect	8.9	8.9	9.5	8.9	ns
Drying aftereffect	22.9	24.2	27.6	23.9	ns
Mouth residue aftereffect	22.4^{ab}	16.6^{a}	26^{b}	27.7^{b}	***

[A] Means are from two replicate samples, measured on an unstructured line scale (0-100); differing small letters represent sample significance from multiple comparisons and means not labelled with the same letters are significantly different ($p < 0.05$). [B] Probability obtained by ANOVA that there is a difference between means; ns, no significant difference between means ($p > 0.05$); * significant at the 5 % level; ** significant at the 1 % level; *** significant at 0.1 % level.

Appearance attributes displayed significant differences between samples in all attributes (Figure 3.3). Samples produced in an open field setting (C3 and C4) were scored significantly ($p < 0.001$) darker in colour, thicker stems and presented more leaf damage. These differences can be attributed to coriander grown in open field being exposed to more adverse conditions, such as lower temperatures and rainfall (Table 3.1).

Figure 3.3: Images of the leaves of the four coriander samples used in this study.

Open field samples produced in the south of England (C4) showed significantly higher odour intensity ($p < 0.01$) and soapy aroma ($p < 0.05$). The same differences were detected for umami taste. Bitter taste intensity was influenced by type of production, samples produced in open field (C3 and C4) were significantly ($p < 0.01$) more bitter than pot produced (C1 and C2). Although not significantly different, soapy flavour was scored higher for open field sample

from York (C3). However, the perception of soapiness aroma and flavour in coriander is associated with human genetics, where olfactory receptor gene OR6A2 which affects the perception of several aldehyde compounds as imparting soapy notes (Eriksson et al 2012). Pot produced samples (C1 and C2) scored significantly lower for mouthfeel attributes of chewy ($p < 0.01$), crunch ($p < 0.05$) and mouth adhesion ($p < 0.01$). Samples C3 and C4 were significantly higher ($p < 0.05$) in bitter, umami and aniseed aftereffects as well as mouth residue ($p < 0.001$). Samples C3 and C4 (open field production) scored higher than pot produced coriander for most flavour/odour attributes including adverse ones like bitterness and soapiness.

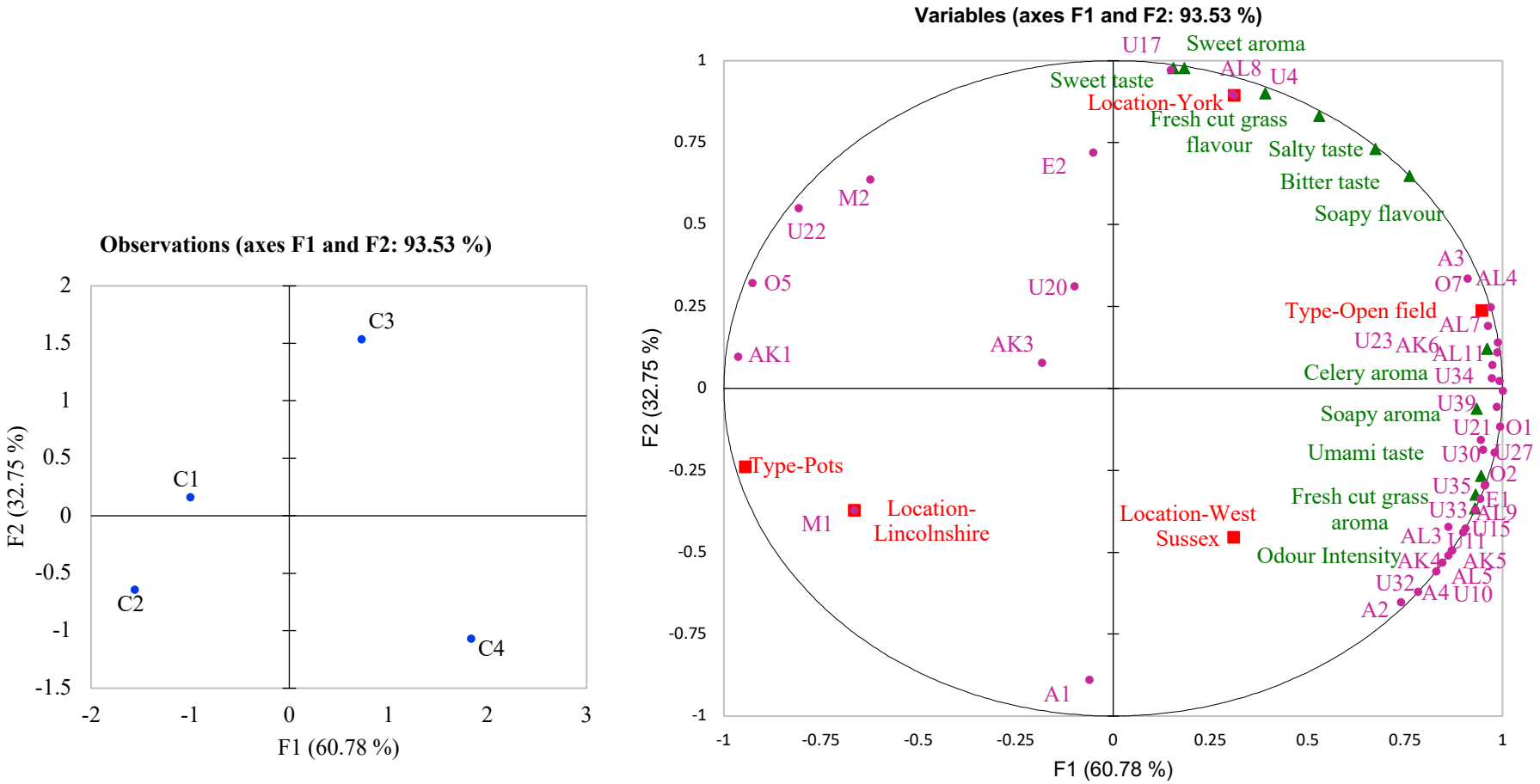
3.3.2.3 Multiple factor analysis (MFA) of volatile compounds and sensory attributes

MFA was used to visualise the sensory and chemical differences observed across the different coriander samples (Figure 3.4), with volatile compounds identified that expressed significant differences between samples (Table 3.4) and the sensory attributes related to odour and flavour (Table 3.5). Dimensions one (F1) and two (F2) from multiple factor analysis explained 93.53 % of the total variation within the data. The first axis separated the samples C1 and C2 from C3 and C4 samples, whilst the second axis separated the samples C1 and C3 from C2 and C4.

Open field produced coriander was highly associated with most aroma attributes except sweet taste (Figure 3.4), whereas pot produced and Lincolnshire location were negatively associated with sensory attributes. Samples produced in West Sussex were more associated with fresh cut grass aroma and odour intensity, whilst coriander produced in York was more associated with sweetness attributes, fresh cut grass flavour and soapy and bitter taste. Most alcohol, aldehydes and alkanes were positively correlated with the first dimension (F1), and

most of these were negatively correlated with the second dimension. Most compounds were positioned in the outer rim of the biplot, including aldehydes, with compounds like (*E*)-2-dodecenal (AL11) and decanal (AL7) displaying a positive association to soapy attributes and celery aroma, whereas (*E*)-2-decenal (AL8) expressed positive correlation with fresh cut grass and sweetness attributes. West Sussex and Lincolnshire locations showed negative correlation with second dimension whereas York location was positively correlated with the second dimension (F2), additionally sweetness attributes, fresh cut grass and soapy flavour, bitter and salty taste attributes were positively correlated with dimension F2, conversely odour intensity, fresh cut grass and soapy aroma were negatively correlated with this dimension. These results suggest that growing coriander at lower temperatures, but still within the range of 11-15 ˚C, using a loamy type soil and 14-15 h of sunlight would result in a crop with higher volatile abundance and more intense aroma and flavour attributes (Telci and Hisil 2008).

According to the results presented, the method of production greatly influences the aroma flavour of coriander, where open field produced plants displayed higher abundances of volatiles compounds (Table 3.4) and these were perceived in sensory tasting (Table 3.5), as these plants were exposed to lower temperatures (11-15 ˚C), water amounts of ~ 2 mm day^{-1}, 14-15 h of sunlight and lower plant density allowing more nutrient availability (Akbarinia et al 2007, Telci and Hisil 2008). This indicates that coriander grown under similar conditions to the ones described will result in more flavour compounds present in the plant but it will also result in higher bitterness which could trigger an adverse response by the consumer.



(A) (B)

Figure 3.4: Multiple factor analysis of four coriander samples showing correlations with volatile compounds and sensory attributes. (A)Projection of coriander samples (C1- pots; C2- pots; C3- open field; C4- open field); (B) Distribution of variables: red squares-growing conditions; green triangle-sensory attributes; pink circle-volatile compounds.

3.3.3 Rosemary

3.3.3.1 Volatile composition

In total, 112 compounds were detected in the headspace of three rosemary samples (Table 3.6). Detected compounds included 32 monoterpenes, 11 sesquiterpenes, four alcohols, two aldehydes and 42 unidentified compounds. Significant quantitative differences in the aroma profiles were observed between the three rosemary samples, confirmed by one-way ANOVA. Samples from York (R2 and R3) contained the highest amounts of volatile compounds and higher contents of monoterpenes compared to pot sample from West Sussex (R1). Furthermore, similar composition was observed between the two samples produced in this location (R2 and R3). No significant differences in the relative amount of aroma compounds were found in 34 compounds, however the majority of these compounds have been previously mentioned as relevant to the aroma of rosemary apart from verbenone.

Table 3.6: Relative abundance of aroma compounds identified in the headspace of fresh rosemary samples.

Code	Compound name	LRI[a]	ID[b]	Relative abundance [c]			*p*-value
				R1	R2	R3	
	Alcohol						
A1	(*Z*)-3-Hexen-1-ol	856	A	10[a]	15[ab]	34[b]	*
A2	1-Octen-3-ol	984	A	nd	35	32	ns
A3	3-Octanol	999	A	12	57	31	ns
A4	1-Nonanol	1176	A	35	nd	nd	**
	Total (%)			0.8	0.5	0.6	
	Aldehyde						
AL1	(*Z*)-3-Hexenal	798	A	12	13	18	ns
AL2	(*E*)-2-Hexenal	854	A	65	45	73	ns
	Total (%)			1.0	0.3	0.5	
	Ester						
E1	Butyl propanoate	906	A	nd	1.6	2.8	ns
E2	Hexyl 2-methylbutanoate	1236	A	5.8[a]	74[b]	24[a]	**
E3	Allyl octanoate	1277	A	18[a]	54[b]	31[a]	**

E4	Nonyl acetate	1312	A	14[a]	7.2[b]	7.0[b]	**
E5	(E)-3-Hexenyl 4-methylpentanoate	1344	A	14	nd	nd	
E6	Geranyl isovalerate	1613	A	3.4	5.2	6.9	ns
E7	Methyl jasmonate	1670	A	6.0[a]	8.2[ab]	12[b]	*
	Total (%)			0.8	0.7	0.5	
	Monoterpene						
M1	Tricyclene	930	B[1]	8.7[a]	81[b]	50[c]	***
M2	alpha-Pinene	942	A	350[a]	2050[b]	1477[b]	**
M3	2,4-Thujadiene	952	B[2]	nd	nd	2.2	
M4	Camphene	959	A	197[a]	1470[b]	1152[c]	***
M5	beta-Thujene	963	B[3]	74[a]	20[b]	23[b]	***
M6	Sabinene	981	A	50	59	50	ns
M7	beta-Pinene	987	A	154[a]	1587[b]	1341[b]	***
M8	beta-Myrcene	995	A	184[a]	1450[b]	1100[c]	***
M9	alpha-Phellandrene	1012	A	112[a]	93[a]	55[b]	**
M10	Limonene	1038	A	419	nd	nd	
M11	Eucalyptol (1,8-cineole)	1041	A	480[a]	3152[b]	2663[b]	**
M12	beta-Phellandrene	1061	B[4]	3.5	nd	nd	
M13	gamma- Terpinene	1065	A	67[a]	582[b]	307[c]	***
M14	Terpinolene	1095	A	265[a]	682[b]	376[a]	***
M15	Linalool	1102	A	418[ab]	490[a]	300[b]	*
M16	Fenchol	1126	A	5.5	10	9.1	ns
M17	cis-Verbenol	1154	B[5]	72[a]	32[b]	42[b]	*
M18	Camphor	1163	A	627[a]	2296[b]	1838[b]	**
M19	Pinocarvone	1178	B[6]	39	nd	50	***
M20	Borneol	1183	A	535[a]	1038[b]	1045[b]	*
M21	1-Terpinen-4-ol	1192	A	287[a]	523[b]	338[a]	*
M22	alpha-Terpineol	1204	A	127[a]	836[b]	574[c]	***
M23	Myrtenol	1211	B[7]	nd	40	27	*
M24	Verbenone	1229	B[8]	587	707	729	ns
M25	Neral	1248	A	3.5[a]	40[b]	22[c]	***
M26	Carvone	1258	A	17[a]	21[a]	10[b]	*
M27	Geranial	1261	B[9]	102[a]	nd	5.9[b]	***
M28	Bornyl acetate	1301	A	653[a]	1278[b]	1190[ab]	*
M29	Myrtenyl acetate	1338	B[10]	13	nd	nd	
M30	Geranyl acetate	1387	A	98	nd	nd	
M31	*p*-Cymene	1032	A	61	nd	nd	
M32	*cis*-Thujone	1115	B[11]	0.7	nd	nd	
M33	Ocimene quintoxide	1050	A	8.3	15	8.9	ns
	Total (%)			80	84	85	
	Other						
O1	Undecane	1097	A	nd	23[a]	14[b]	***

O2	4-Methylthiazole	821	A	4.5[a]	10[b]	8.3[b]	*
O3	Methyl propyl disulfide	933	A	12[a]	510[b]	417[b]	***
O4	*m*-Cymene	1028	A	1.8	nd	nd	
O5	(Z)-Sabinene hydrate	1074	A	62[a]	371[b]	335[b]	**
O6	p-Cymenene	1087	B[12]	0.6	nd	nd	
O7	Filifolone	1111	B[13]	9.4[ab]	18[a]	7.2[b]	*
O8	Verbenyl acetate	1297	B[14]	256[a]	nd	11[b]	***
	Total (%)			4.4	4.3	4.6	
	Phenylpropanoid						
P1	Estragole	1208	A	9.4	12	6.5	ns
P2	Methyleugenol	1409	A	34	34	16	ns
	Total (%)			0.6	0.2	0.1	
	Sesquiterpene						
S1	alpha-Cubebene	1368	B[15]	5.5	6.8	5.8	ns
S2	Ylangene	1394	B[16]	3.8[a]	62[b]	53[b]	**
S3	alpha-Copaene	1398	B[17]	7.1[a]	nd	1.7[b]	***
S4	Caryophyllene	1449	B[18]	206[a]	571[b]	451[c]	***
S5	alpha-Muurolene	1475	B[19]	nd	3.8	3.0	ns
S6	alpha-Humulene	1483	B[20]	45[a]	117[b]	98[b]	**
S7	Valencene	1516	A	1.8	nd	nd	
S8	beta-Bisabolene	1523	A	7.8[a]	26[b]	16[ab]	*
S9	beta-Sesquiphellandrene	1540	B[21]	7.1	12	8.5	ns
S10	trans-Calamenene	1549	B[22]	nd	9.1	10	ns
S11	(*Z*)-Nerolidol	1570	A	nd	12	13	**
	Total (%)			3.9	3.7	3.9	
	Terpenes						
T1	Chrysanthenone	1135	B[23]	65[a]	160[b]	71[a]	**
T2	Ipsdienol	1151	B[24]	nd	nd	21	
	Total (%)			0.9	0.7	0.5	
	Unkowns						
U37	unknown	846		1.3	nd	nd	
U38	unknown	945		nd	81	nd	
U39	unknown	1016		191[a]	24[b]	17[b]	***
U40	unknown	1024		38[a]	305[b]	147[a]	***
U41	unknown	1106		nd	163	121	***
U5	unknown	1117		5.8	9.5	nd	ns
U6	unknown	1132		3.6	nd	5.4	*
U7	unknown	1139		7.2	nd	nd	
U8	unknown	1147		6.3	8.5	6	ns
U11	unknown	1173		16	nd	nd	
U12	unknown	1186		nd	10	12	ns
U13	unknown	1195		10	21	17	ns

U14	unknown	1213	27[a]	14[b]	11[b]	*
U17	unknown	1242	nd	8.3	nd	***
U18	unknown	1254	44	nd	nd	
U19	unknown	1268	15[a]	12[ab]	6.9[b]	*
U20	unknown	1287	14[ab]	25[a]	9.0[b]	*
U21	unknown	1310	4.5	nd	nd	
U22	unknown	1323	9.5[a]	24[b]	6.4[a]	**
U23	unknown	1335	7.6	nd	nd	
U42	unknown	1358	3.2	4.4	7.4	ns
U24	unknown	1379	30	nd	nd	
U25	unknown	1402	29[ab]	48[a]	20[b]	*
U26	unknown	1431	9[ab]	13[a]	7[b]	*
U43	unknown	1456	3.7	3.9	4	ns
U27	unknown	1467	2.6[a]	32[b]	32[b]	*
U44	unknown	1499	7.9	6.1	7.6	ns
U45	unknown	1503	nd	33	28	*
U46	unknown	1510	nd	4.2	5.6	ns
U29	unknown	1519	1.7[a]	20[b]	20[b]	**
U47	unknown	1531	nd	45	39	**
U30	unknown	1545	12	8.2	8.8	ns
U48	unknown	1558	1.0	nd	nd	
U49	unknown	1580	nd	nd	0.9	
U50	unknown	1602	nd	7.2	11	***
U51	unknown	1619	21[a]	40[a]	57[b]	**
U52	unknown	1623	0.7	nd	2.7	ns
U32	unknown	1649	1.8[a]	nd	10[b]	***
U33	unknown	1663	2.1[a]	nd	5.1[b]	**
U34	unknown	1688	7.2[a]	14[b]	20[b]	**
U36	unknown	1702	2.3	nd	4.2	ns
U53	unknown	1216	71	nd	nd	
	Total (%)		8.2	5.3	3.8	

[a] Linear retention index (LRI) on a DB-5 column. [b] A, mass spectrum and LRI agree with those of authentic compounds; B, mass spectrum (spectral quality value >80 was used) and LRI agrees with reference spectrum in the NIST/EPA/NIH mass spectra database and LRI agree with those in the literature cited [1] (Lucero et al 2006); [2] (Adams and Nguyen 2005); [3] (El-Ghorab et al 2002); [4] (Bylaite and Meyer 2005); [5] (Flamini e t al 2006); [6] (Gohari et al 2006); [7] (Wang et al 2005); [8] (Hamm et al 2005); [9] (Ho et al 2009); [10] (Mosayebi et al 2008); [11] (Adams et al 2006); [12] (Angioni et al 2006); [13] (Morteza-Semnani et al 2005); [14] (Isidorov et al 1998); [15] (Della Porta et al 1999); [16] (Damon 2002); [17] (Gazim et al 2008); [18] (Buchin et al 2002); [19] (Moreno et al 2007); [20] (Dittmann and Nitz 2000); [21] (Silva et al 2012); [22] (Loayza et al 1995); [23] (Morteza-Semnani et al 2005); [24] (Javidnia et al 2002). [c] Estimated abundance collected in the headspace of rosemary samples containing 2.8 mL of saturated calcium chloride, calculated by comparison with of 100 ppm propyl propanoate used as internal standard; internal standard was used to normalise chromatograms; means of three replicate samples are shown; means not labelled with the same letters are significantly different ($p<0.05$) and Tukey's HSD multiple pairwise comparison; nd - not detected; ns - not significant probability obtained by ANOVA, * significant at the 5 % level; ** significant at the 1 % level; *** significant at 0.1 % level.

Studies have reported that monoterpenes are the main chemical group to contribute to the rosemary aroma (Díaz-Maroto et al 2007, Hcini et al 2013, Lakušić et al 2012). The three rosemary samples were mostly composed of monoterpenes (79 - 85 %). Samples produced in York (R2 and R3) displayed an average composition of 85 % monoterpenes, 4 % sesquiterpenes, 4 % unknown and 5 % other compounds, whereas the pot produced sample (R1) had a composition of 79 %, 4 %, 7 % and 6 %, respectively. These compositional differences could be due to different varieties between production sites.

The main monoterpenes identified were eucalyptol, camphor, borneol, alpha-pinene and camphene which exhibited the highest ratio of compounds accounting for over 60 % of rosemary's aroma composition (Hcini et al 2013, Lakušić et al 2012, Salido et al 2003, Szumny et al 2010, Zawirska-Wojtasiak and Wąsowicz 2009). Growing rosemary samples in York produced a higher abundance of volatiles compounds and higher monoterpene composition in these samples, particularly in eucalyptol, borneol, camphor, alpha-pinene and camphene, comprising an average percentage of 14 %, 5 %, 10 %, 9% and 7 % (respectively), contributing to aroma notes of camphoreous, herbal, woody, pine and eucalyptus. Samples from York were the same variety (var. Miss Jessops), grown in the same soil type (loamy/clay) and using natural light, however at different temperatures, and with sample R3 exposed to rainfall, confirming that crop's genotype will determine the main differences in the aroma of the plant. Bornyl acetate, with a characteristic woody and pine odour, was detected in higher abundance, displaying significantly higher amounts for field produced samples, however this compound has not been reported before as one of the main compounds contributing to the rosemary aroma (Hcini et al 2013, Salido et al 2003, Szumny et al 2010, Zawirska-Wojtasiak and Wąsowicz 2009). Samples grown under soil produced under protected conditions (R2), expressed higher abundance of total volatiles compared to open field, this could be due to higher temperature

range (20-25 ˚C) and longer photoperiod (14 h day^{-1} of sunlight) (Table 3.1), however no significant differences ($p < 0.05$) in composition and volatiles relative abundances were detected between both samples grown in York (R2 and R3), with both of these samples being from the same variety, Miss Jessops.

Sesquiterpenes, alcohols, aldehydes, unknown and other types of compounds accounted for 15 - 20 % of the aroma composition of rosemary samples. The majority of compounds within these groups were present at very low levels and have not previously been described as relevant to the aroma of rosemary, however significant differences were observed for some of the compounds (Lakušić et al 2012, Salido et al 2003, Socaci et al 2008). Similarly to what was displayed for monoterpenes composition, relative abundance of alcohols, sesquiterpenes, unknown and other compounds was significantly higher ($p < 0.05$) in field produce rosemary (R2 and R3) than pot produced (R1). Conversely, percentage composition of unknown and other compounds was higher in pots 7 % and 6 %, respectively, than field (5 % and 4 %, respectively), this could be due to higher plant density experienced in pots and lower nutrient availability (Ciriello et al 2021).

3.3.3.2 Sensory evaluation of rosemary

Three samples of rosemary were analysed, and the sensory profile was created by a trained panel who reached a consensus of 30 attributes for the quantitative evaluation of samples grown in UK during the summer season of 2019. Panel score means were calculated (Table 3.7) and all the attributes were profiled, out of these 24 were found to be significantly different between the three rosemary samples.

Table 3.7: Mean panel scores for sensory attributes of the three rosemary samples.

Attribute	Score [A]			*p*-value [B]
	R1	R2	R3	
Appearance				
Colour of leaf	63.6	64.3	61.3	ns
Leaf size	67.5[a]	38.4[b]	39.1[b]	***
Leaf thickness	50.8[a]	33.5[b]	35.0[b]	***
Stem thickness	45.0	40.7	39.7	ns
Colour of stem	40.9[a]	31.3[b]	34.2[b]	***
Freshness	74.8[a]	65.3[ab]	58.6[b]	*
Odour				
Odour intensity	44.1[a]	56.1[b]	55.7[b]	***
Fresh cut grass aroma	21.6[a]	13.3[b]	13.4[b]	*
Menthol aroma	11.7[a]	34.7[b]	30.3[b]	***
Pine aroma	37.2[a]	41.4[ab]	44.4[b]	*
Floral aroma	15.3	16.0	18.1	ns
Sweet aroma	20.5[a]	16.6[ab]	14.6[b]	*
Taste/Flavour				
Bitter taste	32.3[a]	52.2[b]	56.1[b]	***
Sweet taste	12.0[a]	8.4[b]	6.1[b]	**
Fresh cut grass flavour	27.6[a]	9.7[b]	10.7[b]	***
Pine flavour	33.2[a]	45.2[b]	48.2[b]	***
Soapy flavour	18.2[a]	38.8[b]	38.9[b]	***
Peppery flavour	5.3[a]	11.0[ab]	12.3[b]	*
Mouthfeel				
Cooling mouthfeel	1.4[a]	10.6[b]	7.3[ab]	**
Numbing mouthfeel	14.0[a]	26.0[b]	23.8[b]	**
Warming mouthfeel	5.4	6.2	7.1	ns
Chewy mouthfeel	49.3[a]	58.3[b]	62.6[b]	**
Leaf firmness mouthfeel	46.8[a]	55.5[b]	60.2[b]	***
Aftereffects				
Pine aftereffect	27.8[a]	37.5[b]	37.0[b]	**
Soapy aftereffect	17.5[a]	33.2[b]	30.9[b]	***
Bitter aftereffect	21.2[a]	38.8[b]	37.8[b]	***
Cooling aftereffect	1.7[a]	7.7[b]	6.9[ab]	*
Numbing aftereffect	15.5[a]	21.0[b]	19.4[ab]	*
Warming aftereffect	6.8	9.0	7.8	ns
Mouth residue	26.3	27.4	24.9	ns

[A] Means are from two replicate samples, measured on an unstructured line scale (0-100); differing small letters represent sample significance from multiple comparisons and means not labelled with the same letters are significantly different ($p < 0.05$). [B] Probability obtained by ANOVA that there is a difference between means; ns, no significant difference between means ($p > 0.05$); * significant at the 5 % level; ** significant at the 1 % level; *** significant at 0.1 % level.

Appearance attributes exhibited significant differences between samples, and similarities were observed between scoring for leaf colour and stem thickness (Figure 3.5). Samples R2 and R3 were significantly higher ($p < 0.001$) for leaf size and thickness and darker in stem colour, this might be due to these samples being produced in field, exposed to the sunlight and also for longer period of production. The opposite was observed for freshness attribute ($p < 0.05$), with the pot sample scoring higher, which could be due to the light green colour and thinner leaves associated with these sample.

Figure 3.5: Images of the leaves of the three rosemary samples used in this study.

Aroma attributes for rosemary samples displayed significant differences apart from floral aroma. The Pot produced sample (R1) scored significantly ($p < 0.05$) higher for grassy green and sweet aroma. Whereas York samples had significantly ($p < 0.001$) higher odour intensity and were significantly higher in pine ($p < 0.05$) and menthol ($p < 0.001$) aroma. Taste/flavour attributes displayed significant differences, where samples from York were scored significantly ($p < 0.001$) higher for all attributes apart from sweet taste and grassy green flavour that were significantly higher ($p < 0.05$) in pot sample from West Sussex. Similarities were observed between samples for warming mouthfeel and aftereffect and mouth residue. Additionally, samples from York (R2 and R3) scores significantly higher ($p < 0.05$) for mouthfeel and aftereffect attributes. Most differences for rosemary attributes were caused by location (West

Sussex vs York), however these differences could be attributed to the variety of the herbs, since each location is associated with the growth of a different variety.

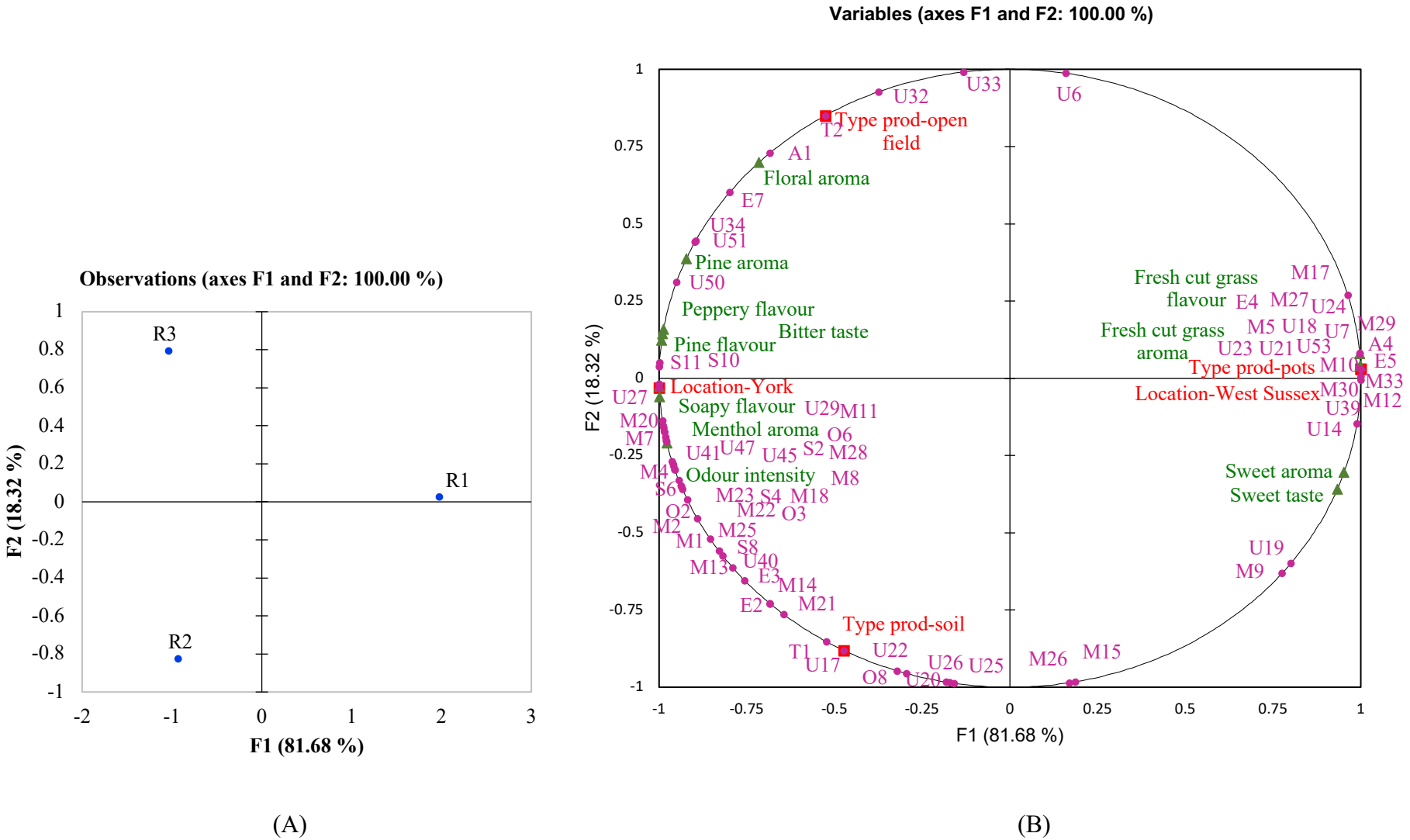
3.3.3.3 Multiple factor analysis (MFA) of volatile compounds and sensory attributes

MFA was used to visualise the sensory and chemical differences observed across the different rosemary productions (Figure 3.6), with volatile compounds identified that expressed significant differences between samples (Table 3.6) and the sensory attributes related to odour and flavour (**Error! Reference source not found.**). Multiple factor analysis dimension one (F1) and two (F2) explained 100 % of the total variation within data. The first axis separated the samples R2 and R3 from R1 sample, whilst the second axis separated the samples R1 and R3 from R2.

Rosemary produced in York was highly associated with most aroma attributes (Figure 3.6) except fresh cut grass and sweet attributes, which were highly associated with sample pot produced in West Sussex. Samples produced in York in open field setting were more associated with floral and pine attributes, whilst York rosemary produced under polytunnel were more associated with menthol and soapy attributes, but also higher odour intensity, these differences could be attributed to higher growth temperature (Salido et al 2003) in protected conditions and no exposure to rain (Table 3.1). Most monoterpenes and sesquiterpenes were negatively correlated with first dimension (F1), and other types of compounds and unidentified compounds were negatively correlated with F1. Monoterpenes were positioned in the outer rim of the biplot, with compounds like camphor (M18), camphene (M4), eucalyptol (M11), bornyl acetate (M28) and alpha-pinene (M32) displaying a positive association to pine attributes, menthol aroma and bitter taste, whereas limonene (M10) expressed positive correlation with fresh cut grass and

sweetness attributes. Location of production showed no correlation with the second dimension (F2), additionally pine and floral attributes were positively correlated with dimension F2, conversely odour intensity was negatively correlated with this dimension. Additionally, the majority of volatile compounds were negatively correlated with the second dimension (Figure 3.6). Bitter taste was negatively correlated with sweet taste and highly associated with York samples.

Although rosemary samples displayed clear differences between locations and type of production, this could be due to differences in variety. Field produced samples (protected and unprotected) expressed higher abundances of volatiles compounds (Table 3.6) and these were perceived in sensory analysis (Table 3.7), this suggest that differences detected between types of production are due to plant genotype (variety) since both samples from the same grower displayed similarities, and no significant differences in aroma composition could be attributed to differences in growing conditions such as growth temperature and photoperiod (Table 3.1).



(A) (B)

Figure 3.6 : Multiple factor analysis of three rosemary samples showing correlations with volatile compounds and sensory attributes. (A)Projection of rosemary samples (R1- pots; R2- protected soil; R3- open field); (B) Distribution of variables: red squares-growing conditions; green triangle-sensory attributes; pink circle-volatile compounds.

3.4 Conclusions

Production type displayed a strong influence over the aroma composition of three basil samples and three rosemary samples, with significant differences observed amongst them. Differences in production led to differences in the aroma volatile profile and consequently identification of differences in the sensory profile. Completing volatile analysis and sensory profiling of three basil samples and three rosemary samples demonstrated that samples produced in pots were perceived with stronger sweet and grassy green aroma and weaker 'basil' and 'rosemary' aroma than field and hydroponic produced samples. Lamiaceae herbs produced in field (rosemary) and hydroponics (basil), displayed higher composition of relevant compounds which was reflected in the sensory profiling of the same samples.

Similar findings were observed for coriander samples, where methods of production resulted in significant differences in volatile abundance and sensory evaluation, however similar volatile composition were observed between samples. Completing volatile analysis and sensory evaluation, demonstrated that field produced samples result in higher abundances of main compounds of herbs, due to the different environmental conditions experienced by these samples, and are perceived and reflected in the sensory profiling of the corresponding samples.

Differences in aroma and sensory profile were identified, as well as influence of growing variables, however, is impossible to identify which will be the most appealing without consumer preference studies. Combining data collected from this study and studies of growing conditions with consumer preference tests would help identify which attributes the consumer finds most important when consuming basil, coriander and rosemary. The findings of the present study could be provided to fresh herb growers to guide the production of herbs with flavour characteristics in mind. Additionally, providing information on growing conditions like

production type, environment, and location, will result in a better understanding on how these factors influence the aroma profile and, therefore sensory perception of herbs. Furthermore, combining this information will help the selection and delivery of a more consistent and better quality product.

Chapter 4: Investigating the relationship of growing conditions on the volatile profile of three culinary herbs: basil (*Ocimum basilicum* var. Sweet Genovese), coriander (*Coriandrum sativum* var. Cruiser) and rosemary (*Rosmarinus officinallis*)

4.1 Introduction

Culinary herbs are primarily grown for their flavour properties, hence their high use as seasoning in foods from different cuisines. Basil (*Ocimum basilicum*) and rosemary (*Rosmarinus officinallis*) are aromatic crops from the Lamiaceae family, used in culinary, perfumery and cosmetic industry due to their unique flavour. The aromatic profile of these two herbs is mainly comprised of monoterpenes, although they differ in their composition, but also phenylpropanoids in the case of basil (Bernhardt et al 2015, Hcini et al 2013). Key monoterpenes contributing to the aromatic profile of basil include eucalyptol (1,8-cineole), linalool, estragole (methyl chavicol) and eugenol whereas camphor, eucalyptol (1,8-cineole), borneol, verbenone, alpha-pinene and camphene are the main monoterpenes detected in rosemary (Hcini et al 2013, Klimánková et al 2008, Lakušić et al 2012, Lee et al 2005, Patel et al 2021, Salido et al 2003). Another culinary herb of high relevance is coriander (*Coriandrum sativum*) which belongs to the Apiaceae family and for which aldehydes and alcohols, namely (*E*)-2-decenal, (*E*)-2-dodecenal, decan-1-ol, dodecan-1-ol and decanal, have been identified as the main compounds imparting the characteristic aroma of coriander (Anjum et al 2011, Łyczko et al 2021, Neffati and Marzouk 2008, Nurzyńska-Wierdak 2013).

Environmental growing conditions including temperature, rainfall and relative humidity, have been reported to have an impact on the flavour profile of aromatic crops, as such, differences were detected in the volatile composition and sensory evaluation of celery (Turner et al 2021a). Limited research has been done on the impact of the environmental

conditions such as temperature, irrigation and lighting on the volatile composition of culinary herbs and how this might be perceived during consumption. The influence of temperature during growth on crop yield and essential oil yield has been described in the literature, a few studies concluded that growth temperatures between 20-30 ˚C will result in higher crop yield and essential oil yield (Hälvä et al 1993, Kosakowska et al 2019). Furthermore, basil grown at higher temperatures (25 ˚C) resulted in higher volatile content (Chang et al 2005, 2007). Similarly, coriander and rosemary, produced during the summer season, resulted in higher production of essential oils, with a reported increase of compounds such as (*E*)-2-decenal, linalool and 1,8-cineole (Lakušić et al 2012, Salido et al 2003, Telci and Hisil 2008).

Herb producers resort to different lighting conditions in order to be able to produce high value crops despite season or geographical location, however, light has been reported to affect the flavour profile of herbs, due to the influence on photosynthesis. Similar results were reported for basil and rosemary, where red lighting increased oil production and increase of volatiles like alpha-pinene and camphene in rosemary (Loughrin and Kasperbauer 2003, Mulas et al 2006). Additionally, basil grown under light-emitting diodes (LED) using blue, red, yellow or blue, red, green wavelengths resulted in shorter plants, higher photosynthesis and higher content of monoterpenes, sesquiterpenes, eugenol and estragole (Carvalho et al 2016, Litvin et al 2020). Additionally, coriander grown under red supplemented LED lighting produced higher contents of aldehydes described as relevant to the coriander aroma and 5-10 cm taller plants compared to blue, red-blue and red-blue-green wavelengths (McAusland et al 2020).

Exposing basil plants to different levels of water stress (50-125 % field water capacity) was reported to affect the essential oil yield, where 100 and 75 % field water capacity resulted in double essential oil yield in comparison with 125 % field water capacity, additionally field water capacity of 125 % and 75 % increased amounts of linalool, eucalyptol and eugenol,

methyl eugenol, respectively (Khalid 2006). Conversely, Ekren et al (2012) reported higher essential oil yield and abundance of compounds such eugenol and linalool at 125 % field water capacity (Ekren et al 2012). Gharib et al (2016), reported higher percentage of essential oi in rosemary plants, when these were irrigated once per week in comparison to twice per week irrigation (Gharib et al 2016). Conversely water stress decreased essential oil yield in coriander, however improvement in essential oil quality was reported due to an increase in linalool and penthadecanone (Nadjafi et al 2009). Additionally, water composition can vary depending on the geographical location and source of water, water with high concentration of salts used in coriander production was reported to decrease the essential oil yield of the crop and reduce the abundance of fatty acids (Neffati and Marzouk 2008).

The application of fertilizers is a common practice to optimise crop quality, however the use of these has been described to have an influence on the flavour profile of plants. Growers commonly add nitrogen, iron and potassium in order to increase crop yield and quality. Several studies confirmed this effect in aromatic crops like basil and rosemary, and also reported an increase in the essential oils and an increase of some monoterpenes constituents in the herbs (Moretti et al 1998, Nurzynska-Wierdak et al 2013, Sifola and Barbieri 2006). Furthermore, the application of sprays of zinc and iron led to an increase of essential oil in coriander and of compounds described as relevant to the aroma of coriander (Telci and Hisil 2008)

Limited research has been done to investigate the influence of soil type on the flavour of aromatic crops, with main focus on crop yield. Sahleh and co-workers (2019), studied dill and parsley and reported higher crop yield and quality when these plants were grown in Stender basis substrate (40 % white peat, 60 % black peat) when compared to peat moss and sandy soil (Saleh et al 2019).

Furthermore, the variety of herbs can influence the flavour profile of a particular herb, however this information is usually not reported. Differences in the volatile composition and the influence of genotype on this has been studied with differences in volatile composition reported in different cultivars of aromatic crops, where major components were detected in all samples however at different relative abundances (Hegmann et al 2020, Klimánková et al 2008, Muráriková et al 2017, Turner et al 2021b). It can be assumed that a similar effect would be detected in different varieties (Hegmann et al 2020, Klimánková et al 2008, Muráriková et al 2017, Turner et al 2021b).

The aim of this study was to investigate the relationship between method of production and environmental conditions on the volatile composition of fresh samples of basil, coriander and rosemary, grown in England across two seasons (Summer and Autumn) over multiple years (2018, 2019, 2020, 2021). Additionally, interactions between chemical groups and growing condition data were identified. This information will help growers to select and grow cultivars with a focus on flavour outcome from the growing conditions and tailor their product according to consumer preference.

4.2 Materials and methods

4.2.1 Plant material

Fresh herbs were sourced from different growers across the United Kingdom (UK) (Table 4.1) during summer and autumn season for the years of 2018, 2019, 2020 and 2021 (Table 4.2). Due to the current pandemic, no analysis of summer season of 2020 was carried out, for this reason herbs grown during summer 2021 were analysed. This material was harvested at commercial maturity in order to mimic products delivered to commercial chains and consumers.

Samples of Rosemary (*Rosmarinus officinallis*), Coriander (*Coriandrum sativum* var. Cruiser) and Basil (*Ocimum basilicum* var. Sweet Genovese*),* were sourced from growers involved in the study that are part of UK's fresh culinary herb production sector.

Herb samples provided (Table 4.1) were grown in pots under protected conditions (Pot), produced in soil protected under glass (Soil), grown in open field subject to weather conditions (Field) and using the hydroponics system (Hydroponics). This study defines the differences in the aroma profile of each herb influenced by seasonal variation for UK production.

Table 4.1: GPS coordinates, location and type of production for each sample for the three herbs.

Location	**GPS coordinates of production site**	**Type of production**		
		Basil	**Coriander**	**Rosemary**
Lincolnshire	52.7442˚N, 0.3779˚W	Pot (B2)	Pot (C2)	
Norwich	52.3927˚N, 1.2648˚E			Field (R6)
Reading	51.3697˚N, 0.9556˚W			Field (R4)
West Sussex	50.4848˚N, 0.4413˚W 50.4914˚N, 0.4445˚W 50.8198˚N, 0.7807˚W	Pot (B1) Hydroponics (B3)	Pot (C1) Field (C4)	Pot (R1)
Worcester	52.0736˚N, 2.0345˚W	Soil (B4)	Field (C5)	Field (R5)
York	54.1345˚N, 1.2430˚W		Field (C3)	Field (R3) Soil (R2)

Samples harvest conditions and storage after sample collection followed the same protocol as described in Chapter 2, with sample replication as in study from previous chapter (Chapter 2:. Preparation of samples across all seasons followed the method described in in Chapter 2: in subsection 2.2.2.

4.2.2 Growing conditions

The herb samples were supplied by herb businesses located in England and grown in commercial conditions and harvested in summer and autumn of 2018, 2019, 2020 and 2021. Samples were produced under commercial conditions in order to analyse characteristics of herbs available to the general consumer. Samples were produced in conditions determined by the grower and these were recorded in a submission form which was submitted with each sample. Environmental conditions were recorded, including temperature, water and light source, use of fertilisers, maturity at time of harvest and soil type, additionally variety of samples and type of production were also recorded (Table 4.2). Growing conditions were supplied by growers when records were available, with some information not shared due to commercial confidentiality

Table 4.2: Location, variety, type of production and growing conditions of all samples for the herbs in each growing season.

Sample	Location	Type of Production	Variety	Year	Season	Temperature[A] °C				Light supply[B]	Water supply[C]	Soil type[D]	Nutrition	Crop maturity
						Av	1 week	harvest	transport	(h day^{-1})	(mm day^{-1})			
B1	West Sussex	Pots	Sweet Genovese	2018	Summer	20-25	20-25	20-25	11-15	15 h-Sl	Ir	Peat	None	17cm
				2018	Autumn	16-20	11--16	11--16	6-10	11 h-Sl	Ir	Peat	None	17cm
				2019	Summer	20-25	11--16	20-25	11-15	16 h-Sl HPS	Ir	Peat	Yes	17cm
				2019	Autumn	20-25	11--16	11--16	6-10	11 h-Sl 9 h- HPS	Ir	Peat	Yes	17cm
				2020	Autumn	16-20	0-5	6--10	6-10	HPS	Ir	Peat	Yes	17cm
				2021	Summer	20-25	16-20	16-20	11-15	16 h-Sl	Ir	Peat	None	17cm
B2	Lincolnshire	Pots	Sweet Genovese	2018	Summer	20-25	20-25	16-20	11-15	16 h-Sl	Ir	Mixture	None	29cm
				2018	Autumn	16-20	6--10	6--10	11-15	11 h-Sl	Ir	Mixture	None	29cm
				2019	Summer	16-20	11--16	16-20	11-15	16 h-HPS	6.4 mm-Rf	Mixture	None	29cm
				2019	Autumn	16-20	6--10	0-5	>15	9 h-Sl 8 h- HPS	Ir	Peat	None	29cm
				2020	Autumn	16-20	11--16	11--16	11-15	HPS	Ir	Peat	Yes	29cm
				2021	Summer	16-20	11--16	16-20	11-15	16 h-Sl	Ir	Peat	None	29cm
B3	West Sussex	Hydroponics	Sweet Genovese	2018	Summer	20-25	11--16	16-20	11-15	12 h-LED	Ir	None	None	Fully matured
				2018	Autumn	20-25	6--10	11--16	11-15	12 h-LED	Ir	None	None	Fully matured
				2019	Summer	20-25	20-25	16-20	11-15	12 h-LED	Ir	None	None	Fully matured
			Grace	2019	Autumn	20-25	16-20	11--16	11-15	14 h-Sl	Ir	None	Yes	Fully matured

			Sweet Genovese	2020	Autumn	20-25	0-5	6--10	11-15	12 h-LED	Ir	None	None	Fully matured
B4	Worcs	Soil Protected	Sweet Genovese	2018	Summer	16-20	11--16	20-25	11-15	14 h-Sl	Ir	Loamy	Yes	First cut
C1	West Sussex	Pots	Cruiser	2018	Summer	20-25	20-25	16-20	6-10	16 h-Sl	Ir	Peat	None	17
				2018	Autumn	20-25	11-15	6-10	6-10	12 h-Sl 0.5h-HPS	Ir	Peat	None	17
				2019	Summer	16-20	16-20	11-15	6-10	16 h-Sl HPS	Ir	Peat	None	17
				2019	Autumn	16-20	20-25	16-20	6-10	8 h-Sl 2 h-HPS	Ir	Peat	None	17
				2020	Autumn	20-25	20-25	11-15	6-10	13 h-Sl	Ir	Peat	None	17
				2021	Summer	16-20	16-20	11-15	6-10	16 h-Sl	Ir	Peat	None	17
C2	Lincolnshire	Pots	Cruiser	2018	Summer	20-25	16-20	16-20	11-15	16 h-Sl	Ir	Mixture	None	25
				2018	Autumn	16-20	11-15	16-20	11-15	12 h-Sl	Ir	Mixture	None	25
				2019	Summer	16-20	11-15	11-15	6-10	HPS	4.5mm-Rf Ir	Mixture	None	25
				2019	Autumn	20-25	20-25	16-20	11-15	14 h-Sl	3.2mm-Rf	Mixture	Yes	25
				2020	Autumn	16-20	11-15	11-15	11-15	HPS	Ir	Peat	None	Fully matured
				2021	Summer	20-25	11-15	11-15	6-10	17 h-Sl	Ir	Peat	None	25
C3	York	Open field	Cruiser	2018	Summer	16-20	16-20	16-20	0-5	16 h-Sl	2.0mm-Rf 0.7mm-Ir	Loamy /clay	Yes	Fully matured
				2018	Autumn	11-15	6-10	11-15	11-15	13 h-Sl	2.0mm-Rf 2.0mm- Ir	Loamy /clay	Yes	Fully matured
				2019	Summer	11-15	16-20	11-15	6-10	15 h-Sl	1.3mm-Rf 0.7mm- Ir	Loamy /clay	Yes	Fully matured
				2019	Autumn	11-15	11-15	16-20	11-15	15 h-Sl	2.6mm-Rf	Loamy /clay	Yes	First cut

				2020	Autumn	16-20	11-15	11-15	0-5	14 h-Sl	4.8mm-Rf	Loamy /clay	Yes	First cut
				2021	Summer	11-15	11-15	16-20	11-15	16 h-Sl	2.8mm-Rf Ir	Loamy /clay	Yes	First cut
C4	West Sussex	Open field	Cruiser	2018	Summer	16-20	20-25	20-25	11-15	16 h-Sl	2.5mm-Rf Ir	Sandy	Yes	Fully matured
				2018	Autumn	11-15	11-15	16-20	6-10	13 h-Sl	2.5mm-Rf Ir	Sandy	Yes	Fully matured
				2019	Summer	11-15	11-15	11-15	unk	14 h-Sl	1.4mm-Rf Ir	Sandy	Yes	First cut
				2019	Autumn	16-20	20-25	11-15	0-5	15 h-Sl	2.5mm-Rf Ir	Sandy	Yes	Fully matured
				2020	Autumn	20-25	20-25	11-15	0-5	14 h-Sl	2.6mm-Rf Ir	Sandy	Yes	Second cut
				2021	Summer	11-15	11-15	16-20	0-5	15 h-Sl	2.3mm-Rf Ir	Sandy	Yes	First cut
C5	Worcester	Open field	Cruiser	2018	Summer	20-25	20-25	20-25	>15	16 h-Sl	2.3mm-Rf Ir	Loamy	Yes	Fully matured
				2019	Autumn	16-20	16-20	20-25	11-15	15 h-Sl	1.2mm-Rf Ir	Loamy	Yes	Second cut
				2020	Autumn	16-20	16-20	16-20	11-15	14 h-Sl	2.4mm-Rf	Loamy	Yes	First cut
R1	West Sussex	Pots	Perigord	2018	Summer	20-25	20-25	20-25	>15	16h-Sl	Ir	Peat	Yes	17cm
				2018	Autumn	20-25	6-10	11-15	6-10	13h-Sl 0.9h-HPS	Ir	Peat	Yes	17cm
				2019	Summer	20-25	11-15	11-15	6-10	15h-Sl HPS	Ir	Peat	Yes	17cm
				2019	Autumn	20-25	11-15	11-15	6-10	14h-Sl 2h-HPS	Ir	Peat	Yes	17cm
				2020	Autumn	20-25	11-15	11-15	6-10	13h-Sl	Ir	Peat	Yes	17cm
				2021	Summer	16-20	20-25	11-15	6-10	15h-Sl	Ir	Peat	Yes	17cm
R2	York	Soil Protected	Miss Jessops	2018	Summer	11-15	16-20	16-20	0-5	15h-Sl	1.7mm-Ir	Loam/Clay	Yes	Fully matured
				2018	Autumn	16-20	11-15	6-10	0-5	13h-Sl	1.4mm-Ir	Loam/Clay	Yes	Fully matured

				2019	Summer	20-25	11-15	11-15	6-10	14h-Sl	2.8mm-Ir	Loam/Clay	Yes	Fully matured
				2020	Autumn	16-20	11-15	11-15	6-10	12h-Sl	1.4mm-Ir	Loam/Clay	Yes	Second cut
				2021	Summer	16-20	11-15	11-15	6-10	15h-Sl	1.4mm-Ir	Loam/Clay	Yes	First cut
R3	York	Open field	Miss Jessops	2018	Summer	11-15	16-20	16-20	6-10	12h-Sl	1.8mm-Rf 0.4mm-Ir	Loam/Clay	Yes	First cut
				2018	Autumn	16-20	11-15	6-10	0-5	12h-Sl	2.2mm-Rf 0.2mm-Ir	Loam/Clay	Yes	Fully matured
				2019	Summer	16-20	11-15	16-20	6-10	12h-Sl	2.3mm-Rf 0.2mm-Ir	Loam/Clay	Yes	Fully matured
				2019	Autumn	11-15	11-15	11-15	6-10	12h-Sl	1.8mm-Rf	Loam/Clay	Yes	First cut
				2020	Autumn	11-15	16-20	11-15	6-10	12h-Sl	2.2mm-Rf	Loam/Clay	Yes	Second cut
				2021	Summer	11-15	11-15	11-15	6-10	12h-Sl	1.9mm-Rf	Loam/Clay	Yes	Fully matured
R4	Reading	Open field	Unknown	2018	Summer	16-20	20-25	20-25	>15	12h-Sl	5.1mm-Ir	Loam/Clay	Yes	Fully matured
				2018	Autumn	11-15	6-10	20-25	6-10	12h-Sl	2.0mm-Rf Ir	Loam/Clay	Yes	Fully matured
				2019	Autumn	16-20	16-20	11-15	11-15	12h-Sl	2.1mm-Rf Ir	Loam/Clay	Yes	Fully matured
				2020	Autumn	16-20	6-10	11-15	11-15	12h-Sl	2.3mm-Rf Ir	Loam/Clay	Yes	Fully matured
				2021	Summer	16-20	16-20	11-15	11-15	12h-Sl	1.9mm-Rf	Loam/Clay	Yes	Fully matured
R5	Worcester	Open field	Unknown	2018	Summer	16-20	20-25	20-25	>15	12h-Sl	1.9mm-Rf	Loam	Yes	Fully matured
			Unknown	2018	Autumn	11-15	16-20	11-15	6-10	12h-Sl	2.1mm-Rf	Loam	Yes	Fully matured
			Barbeque	2019	Autumn	11-15	20-25	16-20	11-15	12h-Sl	2.2mm-Rf	Loam	Yes	Second cut
R6	Norwich	Open field	Unknown	2018	Summer	16-20	16-20	16-20	11-15	12h-Sl	1.2mm-Rf 1.8mm-Ir	Loam	None	Fully matured
				2018	Autumn	11-15	11-15	11-15	6-10	12h-Sl	2.1mm-Rf Ir	Loam	None	Second cut

	2019	Autumn	11-15	11-15	16-20	11-15	12h-Sl	Ir	Loam	None	Second cut
	2020	Autumn	11-15	16-20	11-15	6-10	12h-Sl	2.2mm-Rf Ir	Loam	None	First cut
	2021	Summer	11-15	20-25	11-15	6-10	12h-Sl	1.9mm-Rf	Loam	None	First cut

[A] Average temperature over 24 h; [B] Average photoperiod and light source used: Sl- sunlight, HPS-high pressure sodium and LED- light emitting diode (Philips Toplights-DRW/LB); [C] Average water amount and water source used: I- irrigation and Rf- rainfall; [D] Type of soil used:mixture- composed of 90 % peat substrate and 10 % perlite.

4.2.3 Chemical reagents

Chemical reagents used for preparation and analysis of samples of present study are described in Chapter 2: in subsection 2.2.3.

4.2.4 Solid Phase Microextraction (SPME) followed by GC-MS

Preparation and analysis of herb samples were carried out using the method described in Chapter 2:,subsection 2.2.4 (page 58).

4.2.5 Statistical analysis

Quantitative data of each volatile compound identified in each sample that were obtained from the GC-MS analysis, were analysed by one-way analysis of variance (ANOVA) and multiple factor analysis (MFA) using XLSTAT version 2020.5.1 (Addinsoft, Paris, France). Statistical analysis was done as described in Chapter 2 (2.2.5). Only compounds with significant differences were included in the multiple factor analysis with the growing variables.

4.3 Results and Discussion

4.3.1 Basil

In total, 109 compounds were detected in the headspace of basil samples across two growing seasons during four years of harvest (Table 4.3). The compounds detected included 23 monoterpenes, 13 other compounds, 15 sesquiterpenes, four phenylpropanoids, nine aldehydes and 38 unidentified compounds. Quantitative differences in the aroma profiles were observed between production type, season and year of production of four basil samples confirmed by one-way ANOVA. Basil produced hydroponically contained the highest amounts of volatile

compounds when comparing to pot produced basil from the same harvest. Additionally, samples produced during the summer season resulted in higher amounts of volatile compounds in comparison to autumn season of the same year. For both methods of production, basil harvested in the autumn season of 2020 had the highest amount of aroma volatile compounds. Overall pot produced displayed higher percentage composition of monoterpenes and phenylpropanoids, conversely hydroponically produced basil displayed overall higher contents of other compounds and unidentified compounds. Eleven compounds showed no significant differences in relative amount between type of production, season or year of production although the majority were minor compounds.

Monoterpenes and phenylpropanoids have been identified as the main compound groups to contribute to the aroma of basil (Bernhardt et al 2015, Calín-Sánchez et al 2012, Díaz-Maroto et al 2004, Klimánková et al 2008, Lee et al 2005, Patel et al 2021). The three basil samples were mostly composed of monoterpenes and phenylpropanoids with 1-57 % and 4-65 %, respectively. According to previous reports in the literature, the key compounds responsible for the aroma of basil include eucalyptol, linalool, eugenol and methyl eugenol (Calín-Sánchez et al 2012, Díaz-Maroto et al 2004, Lee et al 2005, Miele et al 2001, Simon et al 1999),however some studies reported linalool as the major compound and others reported methyleugenol, and these were followed by eugenol. All of the compounds reported as main compounds for the aroma of basil were detected at higher abundances in present study, however the most abundant compound varied with type of production, season and year. This suggests that the presence of these compounds are determined by the variety of the basil, since most samples were from the same variety, however growing environment will further determine the relative abundances of these, as basil samples were from the same variety but differences in the volatile composition (Table 4.3) were detected and these were influenced by differences in growing conditions (Table 4.2) observed .

Table 4.3: Relative abundance of aroma compounds identified in the headspace of fresh basil samples.

Code	Compound name	LRI [a]	ID [b]	Relative abundance [c]																	*p*-value
				B1						B2						B3				B4	
				S18	A18	S19	A19	A20	S21	S18	A18	S19	A19	A20	S21	S18	A18	S19	A19	A20	S18	
	Alcohol																					
A1	(*Z*)-3-Hexen-1-ol	856	A	3.5^{a}	nd	nd	nd	12^{a}	14^{a}	5.3^{a}	nd	nd	32^{a}	455^{b}	nd	10^{a}	nd	nd	nd	35^{a}	17^{a}	***
	Total (%)			0.1	0.0	0.0	0.0	0.1	0.2	0.2	0.0	0.0	0.7	1.4	0.0	0.2	0.0	0.0	0.0	0.1	0.5	
	Aldehyde																					
AL1	(*Z*)-3-Hexenal	799	A	nd	nd	nd	nd	32^{a}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	59^{b}	nd	***
AL2	Hexanal	800	A	nd	nd	nd	nd	34^{a}	nd	nd	nd	nd	nd	160^{b}	nd	nd	nd	nd	nd	65^{a}	nd	***
AL3	(*E*)-2-Hexenal	853	A	6.3^{a}	6.0^{a}	nd	nd	368^{b}	210^{c}	10^{a}	10^{a}	nd	nd	nd	271^{c}	12^{a}	10^{a}	nd	nd	399^{b}	4.1^{a}	***
AL4	4-Methyl-(*E*)-2-hexenal	915	A	nd	nd	8.2	nd	13	5.4	nd	nd	7.0	nd	13	6.0	nd	nd	14	nd	nd	nd	ns
AL5	Octanal	1004	A	26^{a}	6.0^{a}	7.6^{a}	3.7^{a}	84^{b}	10^{a}	6.8^{a}	4.5^{a}	7.8^{a}	11^{a}	128^{c}	12^{a}	7.4^{a}	6.1^{a}	12^{a}	1.9^{a}	22^{a}	10^{a}	***
AL6	Nonanal	1108	A	2.1	nd	29	nd	39	4.4	nd	nd	9.2	95	nd	1.7	nd	nd	14	nd	nd	nd	ns
AL7	(*E*)-2-Nonenal	1135	B[1]	3.4^{a}	3.3^{a}	nd	nd	31^{a}	11^{a}	2.4^{a}	0.8^{a}	nd	nd	13^{a}	14^{a}	3.2^{a}	1.0^{a}	nd	1305^{b}	nd	1.1^{a}	***
AL8	Undecanal	1295	B[2]	nd	nd	17	nd	60	nd	nd	nd	8.1	nd	nd	nd	nd	nd	18	nd	nd	nd	ns
AL9	Tetradecanal	1615	B[3]	nd	nd	21^{a}	nd	22^{a}	nd	nd	nd	14^{a}	nd	nd	nd	nd	nd	111^{b}	nd	nd	nd	***
	Total (%)			0.8	0.5	1.4	0.1	6.1	3.8	0.6	0.4	0.9	2.4	0.9	4.7	0.4	0.5	1.3	21.7	1.4	0.5	
	Ester																					
E1	2-Methylbutyl propanoate	971	A	nd	24^{a}	nd	nd	nd	5.5^{b}	nd	50^{c}	nd	nd	nd	145^{d}	nd	54^{c}	nd	nd	nd	nd	***
E2	Hexyl hexanoate	1384	A	6.6^{ab}	9.5^{abcd}	nd	7.8^{abd}	nd	19^{c}	2.1^{b}	3.6^{b}	nd	nd	nd	15^{acd}	3.6^{b}	18^{cd}	nd	6.7^{ab}	18^{cd}	1.3^{b}	***
E3	Citronellyl isobutyrate	1484	A	3.3^{a}	12^{a}	nd	nd	nd	25^{ab}	8.9^{a}	0.8^{a}	nd	14^{a}	65^{b}	nd	36^{ab}	29^{ab}	nd	5.1^{a}	137^{c}	18^{a}	***

E4	Cinnamyl butyrate	1643	A	nd	nd	28 [a]	nd	nd	nd	nd	nd	nd	nd	29 [a]	1.1 [a]	18 [a]	4.4 [a]	160[b]	nd	154[b]	27 [a]	***
E5	Methyl octanoate	1121	A	nd	nd	113[a]	nd	12[a]	3.5[a]	nd	0.4[a]	88[a]	nd	22[a]	4.8[a]	nd	0.6[a]	2168[b]	nd	nd	1.8[a]	***
	Total (%)			0.2	1.4	1.9	0.2	0.1	0.8	0.4	1.5	1.8	0.3	0.3	2.6	0.7	2.7	16.6	0.2	0.4	0.7	
	Fatty acid																					
F1	Octanoic acid (caprylic acid)	1149	B[4]	nd	nd	12[a]	nd	30[a]	73[bc]	nd	nd	8.7[a]	nd	23[a]	104[b]	nd	79[b]	14[a]	nd	nd	37[ac]	***
	Total (%)			0.0	0.0	0.2	0.0	0.2	1.2	0.0	0.0	0.2	0.0	0.1	1.6	0.0	2.1	0.1	0.0	0.0	1.2	
	Monoterpene																					
M1	alpha-Thujene	929	B[5]	14[a]	7.8[ab]	nd	nd	7.3[b]	11[ab]	9.5[ab]	10[ab]	nd	nd	65[c]	14[a]	12[ab]	11[ab]	nd	nd	nd	11[ab]	***
M2	alpha-Pinene	938	A	34[ab]	7.9[b]	nd	nd	60[a]	39[ab]	18[b]	11[b]	nd	nd	194[c]	45[ab]	19[b]	19[b]	nd	nd	122[d]	16[b]	***
M3	Camphene	955	A	9.2[ab][c]	nd	nd	nd	27[a]	14[abc]	5.1[bc]	3.0[b]	nd	nd	113[d]	25[ac]	5.5[abc]	6.2[abc]	nd	nd	60[e]	3.9[bc]	***
M4	Sabinene	979	A	69[ab]	19[bc]	nd	nd	204[d]	113[a]	50[bc]	31[bc]	nd	9.9[c]	224[d]	109[a]	45[bc]	nd	nd	nd	563[e]	35[bc]	***
M5	beta-Pinene	983	A	82[ab]	nd	nd	nd	177[c]	107[a]	48[bd]	51[bd]	nd	nd	nd	nd	53[bd]	45[bd]	nd	nd	364e	43[d]	***
M6	beta-Myrcene	990	A	82[ab]	50[bc]	nd	nd	123[ad]	161[d]	61[abc]	0.6[c]	nd	7.4[c]	331[e]	114[abd]	97[abd]	57[abc]	nd	nd	nd	87[ab]	***
M7	alpha-Phellandrene	1010	A	7.8[ab]	3.4[b]	nd	17[ab]	15[ab]	11[ab]	4.6[ab]	2.0[b]	nd	nd	79[c]	26[a]	nd	2.6[b]	nd	2.1[b]	nd	6.0[ab]	***
M8	delta-3-Carene	1016	A	11	9.8	nd	53	49	11	3.6	8.2	nd	1.5	59	12	nd	9.0	nd	18	82	2.0	**
M9	alpha-Terpinene	1021	A	nd	nd	nd	nd	8.3	nd	nd	nd	nd	nd	133	nd	12	nd	nd	22	1727	9.5	ns
M10	Limonene	1040	B[6]	nd	101[a]	nd	6.9[a]	126[a]	415[b]	139[a]	108[a]	nd	nd	458[b]	501[b]	nd	148[a]	nd	nd	248[a]	1.6[a]	***
M11	Eucalyptol (1,8-cineole)	1056	B[7]	578[a]	564[a]	1346[ab][c]	14[a]	1839[ab][c]	1230[ab][c]	455[a]	613[a]	996[ac]	732[a]	3053[b]	1226[ab][c]	540[a]	609[a]	nd	15[a]	4288[b]	424[a]	***
M12	gamma-Terpinene	1061	A	13[abc]	12[abc]	26[a]	8.9[abc]	17[abc]	15[abc]	7.0[bc]	5.9[bc]	24[ab]	nd	2.6[c]	18[abc]	13[abc]	11[abc]	46[d]	nd	18[abc]	11[abc]	***
M13	Sabinene hydrate	1070	B[8]	42[ab]	nd	16[b]	74[ac]	38[ab]	16[b]	7.0[b]	nd	5.1[b]	nd	0.0	13[b]	27[a]	15[b]	nd	1038[d]	111[c]	16[b]	***
M14	Terpinolene	1092	A	74[a]	nd	nd	nd	265[b]	nd	36[a]	nd	nd	15[a]	476[c]	nd	nd	nd	nd	6.2[a]	243[b]	nd	***
M15	Linalool	1106	A	672[a][b]	656[ab]	623[ab]	2358[a][c]	650[ab]	1214[ab]	562[a][b]	694[ab]	16[b]	6.1[b]	2613[a][c]	587[ab]	1047[a][b]	581[ab]	37[b]	7.1[b]	4202[c]	697[ab]	***
M16	allo-ocimene	1141	B[9]	4.9	nd	nd	nd	nd	nd	3.0	nd	nd	nd	25	nd	5.3	2.2	nd	nd	24	4.3	ns
M17	Camphor	1157	A	169[a][b]	130[b]	12[b]	nd	256[abc]	nd	101[b]	82[b]	10[b]	81[b]	469[ac]	nd	31[b]	0.9[b]	18[b]	nd	529[c]	1.7[b]	***

M18	1-Terpinen-4-ol	1191	A	15^{ab}	nd	nd	16^{ab}	104^{c}	46^{abc}	1.2^{b}	nd	nd	nd	29^{ab}	41^{abc}	70^{ac}	56^{abc}	nd	99^{c}	307^{d}	45^{abc}	***
M19	alpha-Terpineol	1199	A	93^{ab}	37^{ab}	nd	nd	79^{ab}	6.8^{b}	58^{ab}	56^{ab}	nd	17^{ab}	173^{a}	25^{ab}	2.1^{b}	2.1^{b}	nd	9.1^{b}	66^{ab}	1.0^{b}	*
M20	Nerol	1228	A	1.8^{a}	1.9^{a}	nd	45^{b}	nd	nd	1.3^{a}	0.6^{a}	nd	nd	nd	nd	3.3^{a}	9.4^{a}	nd	48^{b}	26^{c}	3.1^{a}	***
M21	Bornyl acetate	1289	B[10]	54^{ab}	49^{ab}	46^{ab}	107^{ab}	106^{ab}	149^{ab}	44^{ab}	30^{ab}	39^{ab}	14^{ab}	1088^{c}	58^{ab}	78^{ab}	80^{ab}	92^{ab}	7.2^{a}	468^{b}	116^{ab}	***
M22	Carvacrol	1316	A	nd	0.9^{a}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.7^{a}	nd	29^{b}	nd	1.1^{a}	***
M23	Ocimene quintoxide	1048	A	nd	nd	21^{a}	5.2^{a}	525^{b}	nd	nd	nd	18^{a}	nd	nd	13^{a}	181^{c}	nd	35^{a}	nd	1246^{d}	125^{a}	***
	Total (%)			42	50	35	57	29	56	53	47	22	20	29	44	38	44	1.5	21	35	49	
	Other																					
O1	4-Methylthiazole	823	A	nd	nd	nd	nd	24^{a}	nd	nd	nd	nd	nd	71^{b}	nd	nd	nd	nd	nd	nd	nd	***
O2	4-Methylpyridine	865	A	nd	nd	nd	nd	8.2	0.7	nd	nd	nd	5.9	nd	1.4	nd	nd	nd	nd	nd	1.1	
O3	Cyclohexanone	896	A	nd	1.2^{a}	nd	nd	14^{a}	2.1^{a}	nd	0.9^{a}	nd	nd	33^{b}	2.2^{a}	nd	0.5^{a}	nd	5.6^{a}	18^{a}	7.5^{a}	***
O4	Dehydrocineole	995	B[11]	2.3^{a}	nd	nd	nd	nd	11^{a}	2.7^{a}	9.4^{a}	nd	8.1^{a}	685^{b}	8.0^{a}	3.4^{a}	3.1^{a}	nd	nd	270^{c}	4.2^{a}	***
O5	Furaneol	1066	A	nd	6.7^{a}	nd	856^{b}	86^{a}	5.2^{a}	nd	15^{a}	nd	1.7^{a}	156^{a}	22^{a}	nd	4.7^{a}	nd	nd	101^{a}	nd	***
O6	Octyl acetate	1208	A	8.2	4.1	nd	11	nd	nd	5.5	nd	nd	nd	4398	nd	nd	2.3	nd	nd	nd	nd	ns
O7	Coumarin	1469	A	5.3^{a}	3.0^{a}	nd	nd	20^{a}	75^{a}	2.4^{a}	27^{a}	nd	nd	78^{a}	4.0^{a}	29^{a}	65^{a}	2357^{b}	691^{c}	54^{a}	3.1^{a}	***
O8	delta-Decalactone	1507	A	11^{a}	25^{a}	nd	6.3^{a}	27^{a}	68^{a}	5.2^{a}	11^{a}	nd	9.4^{a}	167^{b}	11^{a}	45^{a}	66^{a}	nd	4.0^{a}	67^{a}	25^{a}	***
O9	Eugenol acetate	1535	A	25^{a}	3.4^{a}	nd	27^{a}	12^{a}	9.8^{a}	11^{a}	23^{a}	nd	nd	616^{b}	18^{a}	85^{a}	6.7^{a}	nd	23^{a}	80^{a}	49^{a}	***
O10	Geranyl butyrate	1561	A	1.9^{a}	nd	243^{bc}	64^{ad}	nd	nd	nd	nd	129^{cd}	nd	138^{cd}	nd	1.9^{a}	nd	278^{b}	8.0^{a}	23^{a}	1.3^{a}	***
O11	Propyl cinnamate	1586	A	nd	nd	26^{a}	4.3^{a}	nd	nd	nd	nd	15^{a}	nd	nd	1.1^{a}	1.6^{a}	nd	122^{b}	1.4^{a}	nd	nd	***
O12	Phthalol	1607	B[12]	nd	nd	27^{ab}	nd	20^{a}	nd	nd	nd	19^{a}	nd	nd	nd	nd	nd	33^{b}	nd	nd	nd	***
O13	Methyl jasmonate	1668	A	nd	nd	nd	3.2^{a}	20^{b}	21^{b}	13^{ab}	nd	7.2^{a}	nd	nd	nd	4.0^{a}	nd	11^{a}	10^{a}	nd	nd	***
	Total (%)			1.1	1.3	5.8	21	5.3	3.0	1.3	2.4	3.7	0.6	19	1.3	6.8	4.1	23	13	5.2	7.8	
	Phenylpropanoid																					
P1	Estragole	1204	A	4.4	5.5	nd	7.4	54	2.2	3.5	2.5	nd	11	127	1.1	2.0	4.4	nd	8.5	29	9.8	ns

P2	Eugenol	1388	B[13]	704[a]	371[a]	828[a]	481[a]	630[a]	255[a]	459[a]	625[a]	586[a]	2619[b]	1205[ab]	1323[ab]	2.3[a]	1.7[a]	176[a]	1043[a]	5558[c]	1.9[a]	***
P3	Methyleugenol	1433	B[14]	469[a]	690[a]	1750[a]	225[a]	3570[ab]	1.8[a]	208[a]	427[a]	1392[a]	217[a]	5902[b]	294[a]	367[a]	205[a]	1378[a]	559[a]	3697[ab]	142[a]	***
P4	Isoeugenol	1418	A	1.9[a]	0.7[a]	nd	12[b]	nd	3.1[a]	1.4[a]	3.1[a]	nd	nd	nd	3.1[a]	2.8[a]	0.7[a]	nd	1.6[a]	nd	4.2[a]	***
	Total (%)			24	33	43	15	30	4.1	22	29	39	65	22	25	6.9	5.6	12	27	24	5.0	
	Sesquiterpene																					
S1	alpha-Copaene	1391	A	19[a]	0.7[a]	22[a]	nd	68[b]	7.0[a]	8.9[a]	14[a]	20[a]	nd	33[ab]	7.3[a]	36[ab]	7.8[a]	nd	nd	143[c]	2.4[a]	***
S2	Sesquithujene	1396	B[15]	8.6[a]	nd	nd	nd	13[a]	828[b]	4.4[a]	3.9[a]	nd	5.1[a]	194[a]	nd	12[a]	107[a]	nd	nd	74[a]	24[a]	***
S3	beta-Caryophyllene	1441	A	16[ab]	85[ab]	nd	nd	35[ab]	369[abc]	5.2[ab]	214[abc]	nd	nd	140[ab]	294[abc]	8.1[ab]	416[bc]	nd	559[c]	134[ab]	11[ab]	***
S4	Bergamotene	1447	B[16]	368[a]	8.5[a]	nd	nd	1530[b]	31[a]	239[a]	5.7[a]	nd	nd	1375[b]	15[a]	28[a]	5.3[a]	21[a]	nd	4750[c]	22[a]	***
S5	(+)-Aromadendrene	1459	A	10[a]	3.9[a]	nd	nd	986[b]	15[a]	4.9[a]	4.0[a]	nd	nd	1714[c]	11[a]	225[a]	11[a]	nd	nd	39[a]	26[a]	***
S6	alpha-Humulene	1475	A	77[a]	3.9[a]	nd	16[a]	189[a]	17[a]	26[a]	1.1[a]	nd	nd	36[a]	123[a]	6.2[a]	11[a]	nd	12[a]	703[b]	49[a]	***
S7	beta-Guaiene	1491	B[17]	18[ab]	9.2[bc]	nd	6.3[c]	16[abc]	32[abd]	8.1[bc]	16[abc]	nd	nd	36[ad]	23[ac]	56[d]	41[ad]	nd	nd	90[c]	32[abd]	***
S8	trans-Murrola-4(14),5-diene	1496	B[18]	50[a]	12[a]	13[a]	nd	144[a]	65[a]	25[a]	11[a]	nd	2.3[a]	351[b]	33[a]	34[a]	62[a]	23[a]	nd	492[b]	8.3[a]	***
S9	Germacrene D	1501	A	106[ab]	8.6[b]	nd	20[b]	246[a]	15[b]	39[b]	38[b]	8.6[b]	11[b]	144[ab]	42[b]	131[ab]	17[b]	nd	28[b]	1650[c]	61[ab]	***
S10	alpha-Farnesene	1515	B[19]	75[a]	16[a]	nd	15[a]	86[a]	46[a]	31[a]	38[a]	nd	5.9[a]	306[b]	49[a]	149[ab]	41[a]	nd	15[a]	640[c]	82[a]	***
S11	beta-Sesquiphellandrene	1523	B[20]	37[a]	25[a]	nd	1.3[a]	58[a]	63[a]	18[a]	25[a]	nd	3.5[a]	801[b]	14[a]	114[ac]	78	nd	23[a]	515[bc]	78[a]	***
S12	Calamenene	1540	B[21]	5.8[a]	2.4[a]	nd	nd	nd	3.9[a]	2.8[a]	2.6[a]	nd	2.6[a]	539[b]	8.7[a]	12[a]	6.3[a]	nd	nd	49[a]	8.1[a]	***
S13	(E)-Nerolidol	1565	A	nd	nd	nd	6.2[a]	nd	nd	nd	nd	nd	nd	42[b]	nd	4.3[a]	nd	13[a]	1.8[a]	nd	1.6[a]	***
S14	diepi-Cubenol	1634	B[22]	5.3[a]	3.4[a]	nd	nd	11[a]	nd	nd	nd	nd	nd	nd	nd	nd	nd	93[b]	nd	nd	nd	***
S15	Cadinol	1655	B[23]	21[a]	17[a]	6.8[a]	nd	37[a]	nd	3.5[a]	5.0[a]	24[a]	nd	157[b]	12[a]	82[ab]	16[a]	15[a]	1.4[a]	23[a]	1.7[a]	***
	Total (%)			17	6.0	0.7	1.4	24	23	14	11	1.1	0.7	18	10	17	22	1.3	11	24	13	
	Unkown																					
U30	unknown	848		nd	5.8	nd	nd	18	12	nd	11	nd	nd	13	13	nd	6.9	nd	nd	nd	nd	ns
U31	unknown	886		nd	nd	nd	6.2[a]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	19[b]	nd	nd	***

U32	unknown	999	nd	14[a]	nd	nd	nd	38[b]	0.9[c]	nd	nd	nd	nd	44[d]	2.3[c]	1.3[c]	nd	nd	nd	0.9[c]	***
U33	unknown	1025	8.1[a]	nd	nd	nd	39[b]	5.3[a]	5.3[a]	nd	nd	nd	4.4[a]	8.6[a]	nd	nd	nd	nd	19[a]	nd	ns
U34	unknown	1054	204[a]	nd	22[a]	nd	15[a]	13[a]	nd	10[a]	15[a]	57[a]	1269[b]	2.7[a]	nd	nd	34[a]	nd	31[a]	nd	***
U35	unknown	1084	nd	31.5[ab]	nd	7.2[cd]	15[bcd]	88[e]	26[abc]	24[abc]	nd	nd	nd	130[f]	42[a]	38[a]	nd	89[e]	20[cd]	37[a]	***
U36	unknown	1128	1.5[a]	1.6[a]	nd	nd	nd	7.0[ab]	1.2[a]	1.3[a]	nd	nd	11[b]	8.5[ab]	2.4[ab]	3.5[ab]	nd	nd	nd	2.8[ab]	**
U4	unknown	1170	nd	7.7[ab]	nd	nd	nd	9.8[ab]	nd	9.0[ab]	nd	nd	nd	2.8[b]	nd	11[a]	nd	nd	nd	9.2[ab]	***
U37	unknown	1173	20[a]	3.5[a]	33[a]	nd	19[a]	26[a]	12[a]	26[a]	20[a]	nd	24[a]	39[ab]	73[b]	21[a]	23[a]	nd	57[ab]	16[a]	***
U25	unknown	1180	14[a]	13[a]	nd	nd	24[a]	1.5[a]	21[a]	2.3[a]	1113[b]	2.1[a]	55[a]	2.3[a]	2.1[a]	2.7[a]	4944[c]	nd	172[ab]	1.4[a]	***
U38	unknown	1236	nd	nd	nd	2.4[ab]	nd	nd	nd	nd	nd	nd	nd	nd	4.2[a]	nd	nd	nd	nd	1.1[b]	***
U5	unknown	1250	6.5[a]	5.6[a]	159[b]	nd	136[b]	1.2[a]	3.8[a]	5.0[a]	50[a]	nd	39[a]	nd	14[a]	6.2[a]	60[a]	nd	76[a]	7.2[a]	***
U39	unknown	1264	nd	nd	7.2[ab]	nd	nd	nd	nd	nd	6.4[a]	nd	7.8[ab]	nd	nd	nd	15[b]	nd	nd	nd	***
U40	unknown	1270	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	22[a]	nd	28[b]	nd	***
U41	unknown	1276	nd	nd	8.5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
U42	unknown	1335	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	4.7[a]	2.3[b]	nd	nd	nd	2.8[b]	***
U6	unknown	1345	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	8.2[a]	nd	nd	nd	11[b]	***
U7	unknown	1347	10[ab]	4.0[b]	nd	nd	15[ab]	4.6[b]	4.4[b]	0.5[b]	nd	nd	27[a]	3.3[b]	23[a]	0.6[b]	nd	nd	59[c]	2.0[b]	***
U26	unknown	1358	nd	nd	nd	nd	57[a]	1.0[a]	nd	4.7[a]	nd	198[abc]	56[a]	62[ac]	735[d]	371[b]	nd	nd	nd	296[bc]	***
U9	unknown	1410	29	nd	nd	1.4	nd	nd	2.0	0.4	nd	nd	nd	nd	8.0	9.1	nd	8.4	21	5.8	ns
U43	unknown	1428	9.3[ab]	nd	nd	26[a]	18[ab]	3.0[b]	4.6[b]	3.7[b]	nd	nd	20[ab]	nd	6.1[b]	4.1[b]	nd	26[a]	81[c]	nd	***
U10	unknown	1435	5.2[ab]	3.5[b]	nd	81[c]	nd	13[a]	2.3[b]	3.6[b]	nd	nd	nd	23[ab]	2.5[b]	13[ab]	nd	nd	42[a]	4.9[ab]	***
U11	unknown	1451	nd	123[ab]	nd	nd	nd	219[acd]	nd	151[abcd]	nd	nd	nd	282[cd]	297[c]	132[abd]	nd	nd	320[a]	50[b]	***
U27	unknown	1456	272[a]	4.7[a]	nd	nd	45[a]	11[a]	125[a]	2.4[a]	nd	nd	176[a]	5.8[a]	73[a]	16[a]	nd	5.3[a]	1732[b]	119[a]	***
U12	unknown	1464	17[a]	19[a]	nd	1.1[a]	26[a]	3.9[a]	7.4[a]	1.7[a]	nd	197[b]	172[bc]	nd	42[a]	5.1[a]	15[a]	3.2[a]	85[ab]	14[a]	***

U14	unknown	1480	20[a]	nd	nd	87[a]	23[a]	nd	nd	7.1[a]	nd	nd	720[b]	13[a]	130[a]	1.4[a]	nd	151[a]	nd	10[a]	***
U19	unknown	1530	98[a]	15[a]	nd	21[a]	164[ab]	24[a]	48[a]	48[a]	nd	12[a]	376[b]	49[a]	188[ab]	36[a]	nd	24[a]	801[c]	97[a]	***
U21	unknown	1547	4.0[a]	3.7[a]	nd	40[a]	14[a]	4.8[a]	1.9[a]	3.4[a]	nd	nd	99[b]	3.6[a]	10[a]	8.8[a]	nd	41[a]	24[a]	6.0[a]	***
U22	unknown	1553	6.3[a]	nd	456[b]	nd	nd	nd	2.8[a]	5.0[a]	317[b]	nd	105[a]	4.5[a]	19[a]	0.4[a]	479[b]	50[a]	32[a]	10[a]	***
U28	unknown	1575	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	38	nd	nd	nd	
U29	unknown	1597	nd	nd	9.1[a]	nd	nd	nd	nd	nd	11[a]	nd	nd	nd	nd	nd	36[b]	nd	nd	nd	***
U44	unknown	1605	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	23	nd	nd	nd	
U45	unknown	1628	nd	nd	17[a]	nd	nd	4.1[b]	nd	nd	18[a]	nd	nd	nd	nd	nd	nd	nd	nd	nd	*
U46	unknown	1637	nd	nd	6.9[a]	nd	nd	nd	nd	nd	11[a]	nd	nd	nd	nd	nd	56[b]	nd	35[c]	7.0[a]	***
U25	unknown	1688	nd	nd	nd	18[a]	20[a]	nd	nd	nd	nd	nd	nd	nd	nd	nd	9.8[b]	nd	nd	nd	***
U47	unknown	1766	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	14	nd	nd	nd	
U48	unknown	1797	nd	nd	nd	nd	14[a]	nd	nd	nd	14[a]	nd	nd	nd	nd	nd	67[b]	nd	nd	nd	***
U49	unknown	1870	nd	nd	nd	nd	31	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
	Total (%)		15	7.8	12	6.1	4.9	7.7	8.8	8.8	31	11	9.6	11	31	19	45	6.9	9.5	23	

[a] Linear retention index (LRI) on a DB-5 column. [b] A, mass spectrum and LRI agree with those of authentic compounds; B, mass spectrum (spectral quality value >80 was used) and LRI agrees with reference spectrum in the NIST/EPA/NIH mass spectra database and LRI agree with those in the literature cited [1] (Peterson and Reineccius 2003); [2] (Berdague et al 1991); [3] (Ramarathnam et al 1993b); [4] (Miyazaki et al 2011); [5] (Adams et al 2006); [6] (Merle et al 2004); [7] (El-Ghorab et al 2002); [8] (Cao et al 2011); [9] (V Isidorov et al 1997); [10] (Adams et al 2006); [11] (Baccouri et al 2007); [12] (Ramarathnam et al 1993a); [13] (Pessoa et al 2002); [14] (Nivinskienė et al 2007); [15] (Courtois et al 2009); [16] (Limberger et al 2003); [17] (Tellez et al 2001); [18] (Adams et al 2005); [19] (Batista-Pereira et al 2006); [20] (Viña and Murillo 2003); [21] (Cui et al 2011); [22] (Smelcerovic et al 2007); [23] (Angioni et al 2006). [c] Estimated abundance collected in the headspace of basil samples containing 2.8 mL of saturated calcium chloride, calculated by comparison with of 100 ppm propyl propanoate used as internal standard; internal standard was used to normalise chromatograms; means of three replicate samples are shown; means not labelled with the same letters are significantly different (p<0.05) and Tukey's HSD multiple pairwise comparison; nd - not detected; ns - not significant probability obtained by ANOVA, * significant at the 5 % level; ** significant at the 1 % level; *** significant at 0.1 % level.

Multiple factor analysis

Multiple factor analysis was used to visualise the differences in environmental factors and the chemical composition (Table 4.3) observed for the season of autumn (A) and summer (S), for the years of 2018, 2019, 2020 and 2021 (Figure 4.1). Abiotic stresses like temperature, water, nutrition and light have been reported to influence the synthesis of secondary metabolites in plants (Akula and Ravishankar 2011, Arbona et al 2013, Miller et al 2008).

As it can be seen in Figure 4.1, basil grown across the studied seasons and years, expressed variation between samples, where first (F1) and second (F2) dimension explained 49.29 % of the total variation within the data. The first axis separated basil grown in the autumn of 2020 from the other seasons, whilst the second axis separated summer and autumn seasons of 2019 and 2020 respectively. The majority of basil samples were from the same variety (var. Sweet Genovese), apart from one sample, however these factors were grouped in the middle of the observation plot with no strong associations with the majority of the volatile compounds (Table 4.2). Basil was produced in different locations, with Lincolnshire displaying a high association with nerol (M20) and sesquithujene (S2) (sweet and citrus aroma), whereas Worcs was highly associated with sabinene hydrate (M13) and (*E*)-2-nonenal (AL7) (minty, eucalyptus and fatty, green aroma), these are minor volatiles not reported as relevant to the aroma of basil. Similar associations were observed for samples from West Sussex, however no strong associations with the main volatile compounds could be established. Previously in this project, differences in volatile profile were observed between type of production, and this was further confirmed in the biplot (Figure 4.1), where hydroponically produced plant material was highly associated with main compounds such as eucalyptol (M11), linalool (M15) and eugenol (P2), whereas pots and soil under protected basil were closely associated with some minor

monoterpenes and sesquiterpenes such as sabinene hydrate (M13), sesquithujene (S2) and beta-caryophyllene (S3) which impart a eucalyptus, minty, sweet and woody odour.

The maturity of crop at time of harvested showed some association with the composition of basil samples, with samples harvested when 'fully matured' or at a taller target (29 cm) being highly associated with compounds including eugenol (P2), linalool (M15) and eucalyptol (M11). These results indicate that leaves from more mature plants express higher abundance of volatiles compounds which might be due to the synthesis of secondary metabolites for a longer period of time.

Different types of soil will result in different soil properties like water holding capacity and mineral composition thus affecting the production of primary and secondary compounds. As it can be seen in Figure 4.1, basil produced in loamy soil, mixture or peat was highly associated with compounds including isoeugenol (P4), sabinene hydrate (M13), (*E*)-2-nonenal (AL7), whereas samples produced with no soil (hydroponic production) were associated with some of the main compounds such as eugenol (P2), this could be because nutrients and water have higher availability and there is no influence by soil characteristics (Putra and Yuliando 2015). Application of fertilisers will increase the soil nutrient content which will lead to the availability for crop intake of elements like nitrogen, zinc and sulphur, that are involved in the synthesis of primary and secondary compounds (Broadley et al 2012, Mousavi et al 2012, Waterman and Mole 1989). In this study, the influence of application or absence of fertilizers on the aroma profile was examined (Figure 4.1). Absence of fertilisers was negatively associated with compounds in basil including eucalyptol (M11), linalool (M15) and eugenol (P2), whereas the use of fertiliser was highly correlated with compounds such as nerol (M20), 2-methylpyridine (O2) and furaneol (O4) (sweet and astringent aroma). Furthermore, these

factors were positioned in the middle of the variables plot meaning these play a less significant role in determining the differences between basil samples.

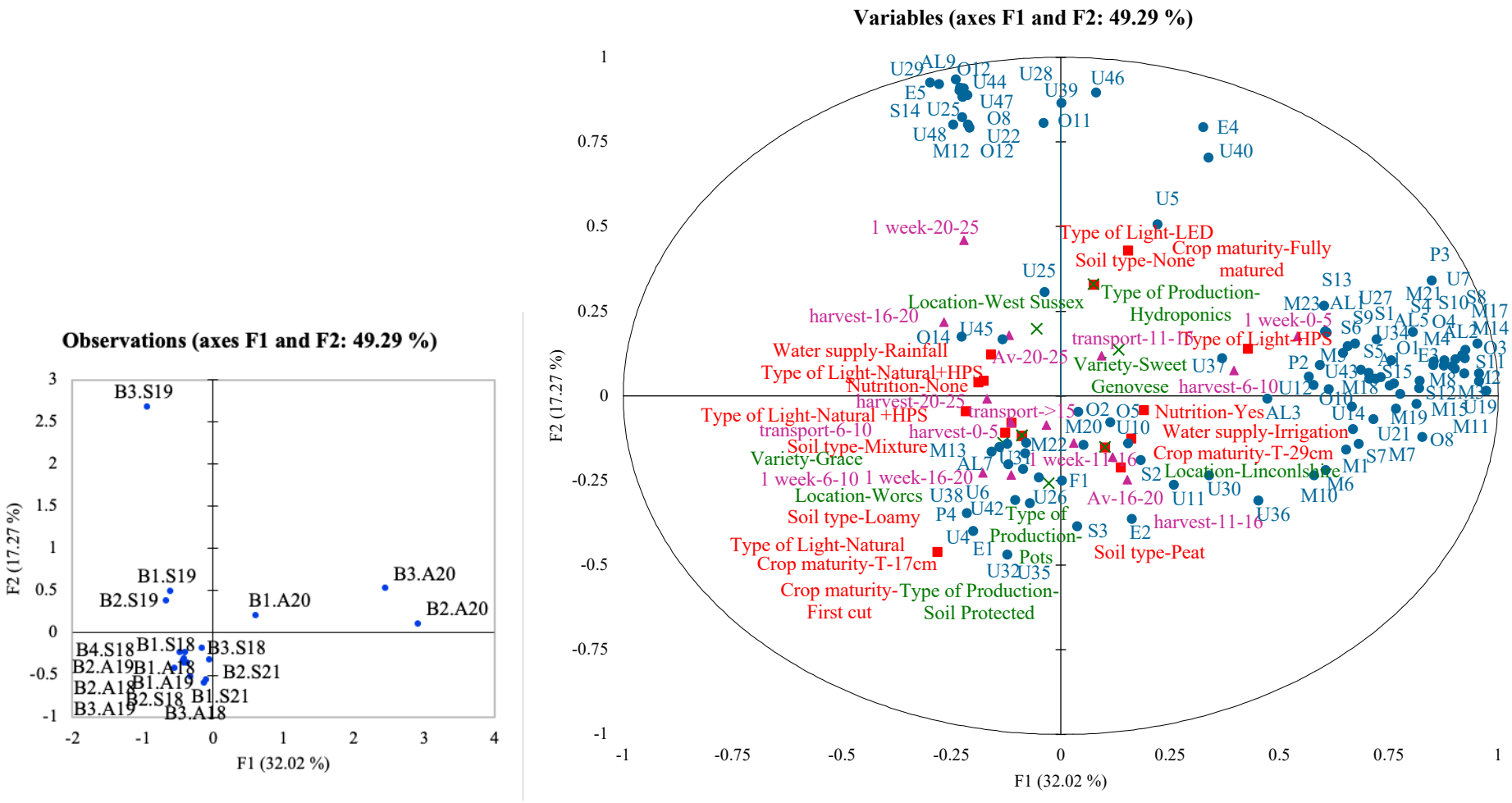
Water composition can vary significantly, which means different mineral composition, and this will lead to variances in the soil's mineral uptake. Rainfall water is considered a soft water as it has low amounts of minerals, salts and chemicals with a more acidic pH (5-7), whereas irrigation water will be more alkaline and with various minerals and salts, which will further influence the mineral uptake of the plants. Irrigation water displayed a positive correlation with most volatile compounds and rainfall being positively associated with nerol (M20), sesquithujene (S2), methylpyridine (O2) and furaneol (O6) and negatively associated with main compounds like eugenol (P2) and linalool (M15).

Light is another environmental factor that will influence secondary metabolites composition as it is a determining factor in the photosynthesis process in plants and consequently the plant metabolism. Light quality, quantity and photoperiod have been described to affect the volatile composition of plants (Akula and Ravishankar 2011, Carvalho et al 2016, Mulas et al 2006). Present study results showed that high pressure sodium (HPS) lighting was highly associated with most of the main compounds such as eucalyptol (M11), linalool (M15) and eugenol (P2), and positively correlated with the first dimension (Figure 4.1). Conversely, light emitting diode (LED) displayed a low correlation with main compounds and higher association with some unidentified compounds, this could be due to the wavelength used by the grower or photoperiod (12 h day^{-1}) and due to the lack of heat that is associated with the use of HPS lights. Additionally, sun lighting or its combination with HPS were highly associated and expressed a negative correlation with compounds such as eucalyptol (M11), linalool (M15) and eugenol (P2), sunlight photoperiod varied significantly between samples (11-16 h day^{-1}) which might have influenced this association. LED lighting has been reported

to increase the volatile content in comparison to HPS, however no information on light intensity was displayed which has been described to affect the essential oil content (Fernandes et al 2013, Litvin et al 2020).

Temperature has been reported to influence the aroma profile of plants, Turner et al. (2021) identified differences in volatile composition between celery grown at different average temperatures, with higher abundances of alcohol, aldehyde, sesquiterpene and phtalide when grown at higher temperatures (Lucy Turner et al 2021b). Basil produced at an average growth temperature (over a 24 h period) of 16-20 ˚C, expressed positive association with main compounds such as eucalyptol (M11) and most minor compounds such as sesquithujene (S2), conversely basil grown at 20-25 ˚C displayed a negative correlation with main volatiles and high association with some unknown compounds (Figure 4.1), contrary to what has been reported in literature (Chang et al 2005, 2007). Temperature exposure of plants one week before harvest was also correlated with composition, where stress temperatures of 0-5 ˚C expressed high association with main volatiles, conversely temperatures of 6-10; 16-20; 20-25 ˚C were negatively correlated with eugenol (P2), linalool (M15) and eucalyptol (M11), but were highly correlated with minor compounds including gamma-terpinene (M12), sesquithujene (S2) and isoeugenol (P4). Additionally, temperature on day of harvest of 6-10 ˚C was highly associated with most compounds including main compounds such as eugenol (P2), eucalyptol (M11) and linalool (M15) responsible for a spicy, cloves, eucalyptus and herbal odour (Figure 4.1) temperature of 11-16 ˚C showed a lower correlation with these compounds. Both lower and higher temperatures (<6 ˚C; >16 ˚C), were negatively associated with main compounds and positively associated with some minor compounds. Transport temperatures were also analysed, however these were grouped in the middle of the biplot meaning low association with most compounds, showing low influence on the composition of basil. This could be due to the short length of transport, as all samples were received in less than a day of being shipped.

Aroma compounds production is a classical protection and adaptative crop response to stresses in the growth environment. It is clear that basil produced under different environmental conditions will result in differences in abundances of principal compounds (eugenol, estragole, linalool and eucalyptol). However, differences are not caused by one individual growing factor, but the combination of optimal conditions in the production environment. Variety of the plant will also play a significant role on the composition and protection capabilities of each plant.



(A) (B)

Figure 4.1: Multiple factor analysis of four basil samples harvested in the summer and autumn for the years of 2018, 2019, 2020 and 2021 showing correlations with volatile compounds and growing conditions. (A)Projection of samples; (B) Distribution of variables: green cross-grower information; pink triangles-temperature intervals; red squares-environment conditions; blue circle-volatile compounds.

4.3.2 Coriander

In total, 92 compounds were detected in the headspace of coriander samples for two growing seasons and three years of harvest (Table 4.4). The compounds detected included 16 aldehydes, seven other, seven alcohol, six alkanes and 38 unidentified compounds. Quantitative differences in the aroma profiles were observed between coriander samples of this study, confirmed by one-way ANOVA. Open field produced (C3, C4 and C5) coriander expressed the highest amounts of volatile compounds when comparing to pot produced (C1 and C2) during the same season and same year. Additionally, coriander produced in pots produced higher amounts of compounds during the autumn production, conversely open field composition varied with year of production. Year of 2018 expressed highest abundance of compounds during the summer whilst autumn produced higher abundance in 2019, furthermore, when comparing seasons highest volatile content was found in 2019 (autumn season) and in 2021 (summer season). Pot produced samples displayed higher percentage of aldehydes and alkanes, conversely open field coriander showed higher percentage of alcohol compounds and unidentified compounds. Three compounds showed no significant differences in relative amount between type of production, season or year of production and were unidentified compounds.

It has been reported in the literature that aldehydes are the main compounds to contribute to the coriander aroma (Eyres et al 2005, Nurzyńska-Wierdak 2013, Potter and Fagerson 1990, Shahwar et al 2012). All the coriander samples were mostly composed of aldehydes, alcohol and unidentified compounds. Aldehydes varied between 0-65 %, alcohol compounds between 1-22 % and unidentified between 15-68 %. Aldehydes have been identified to be essential to the aroma including Decanal, (*E*)-2-Undecenal, (*E*)-2-Dodecenal and (*E*)-2-Decenal, these were detected in the coriander samples, however these were not detected in all the samples and

different proportions were observed (El-Zaeddi et al 2016, Eyres et al 2005, Neffati and Marzouk 2008, Nurzyńska-Wierdak 2013). (*E*)-2-alkenals have been reported to be responsible for the typical coriander aroma, however no individual compound was identified (Cadwallader et al 1999, McAusland et al 2020). This suggests that the combination of these compounds makes the coriander aroma. Samples grown in West Sussex from open field production (C4) expressed a higher composition in alcohol compounds (25-40 %), which has not been reported previously. Coriander samples were from the same variety, so differences in aroma profile can be attributed to differences in production factors.

Table 4.4: Relative abundance of aroma compounds identified in the headspace of fresh coriander samples.

Code	Compound name	LRI [a]	ID [b]	Relative Abundance [c]																										p-value
				C1						C2						C3						C4						C5			
				S18	A18	S19	A19	A20	S21	S18	A18	S19	A19	A20	S21	S18	A18	S19	A19	A20	S21	S18	A18	S19	A19	A20	S21	S18	A19	A20	
	Alcohol																														
A1	(*E*)-3-Hexen-1-ol	846	A	13^{b}	3.3^{b}	4.5^{b}	2.5^{b}	0.6^{b}	188^{b}	5.4^{b}	4.8^{b}	2.9^{b}	nd	5.5^{b}	269^{b}	2.4^{b}	2.4^{b}	nd	33^{b}	4.0^{b}	1262^{ab}	2.7^{b}	0.3^{b}	2.6^{b}	nd	nd	1991^{a}	1.8^{b}	nd	3.2^{b}	***
A2	(*Z*)-3-Hexen-1-ol	849	A	12^{b}	32^{b}	33^{b}	30^{b}	9.6^{b}	95^{b}	45^{b}	60^{b}	30^{b}	32^{b}	60^{b}	181^{b}	27^{b}	nd	7.6^{b}	29^{b}	62^{b}	729^{a}	27^{b}	4.1^{b}	29^{b}	31^{b}	30^{b}	412^{ab}	29^{b}	27^{b}	0.7^{b}	***
A3	1-Octanol	1076	A	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.4^{b}	nd	nd	nd	nd	nd	0.5^{b}	133^{a}	1.8^{b}	nd	nd	nd	0.6^{b}	187^{a}	nd	nd	nd	***
A4	(*Z*)-5-Octen-1-ol	1081	A	0.7^{b}	1.8^{b}	nd	nd	nd	12^{b}	nd	nd	nd	nd	nd	18^{b}	nd	0.4^{b}	nd	nd	nd	219^{a}	24^{b}	0.6^{b}	nd	nd	nd	61^{b}	65^{b}	nd	3.2^{b}	***
A5	2-Nonanol	1098	A	nd	nd	13^{c}	0.8^{c}	nd	nd	nd	nd	6.7^{c}	nd	nd	nd	nd	nd	4.5^{c}	nd	nd	14^{c}	nd	nd	45^{b}	91^{a}	nd	nd	nd	nd	44^{b}	***
A6	1-Nonanol	1151	B[1]	nd	nd	1.2^{d}	nd	2.1^{d}	7.5^{bcd}	nd	0.4^{d}	1.2^{d}	nd	nd	nd	39^{b}	nd	2.0^{d}	nd	5.3^{bcd}	38^{bc}	4.4^{cd}	0.5^{d}	8.1^{bcd}	94^{a}	7.9^{bcd}	3.5^{d}	2.3^{d}	6.4^{bcd}	3.1^{d}	***
A7	1-Decanol	1271	A	nd	nd	2.7^{c}	nd	7.1^{c}	nd	nd	nd	0.5^{c}	0.9^{c}	1.5^{c}	nd	5.9^{c}	nd	87^{b}	nd	1.2^{c}	nd	2.7^{c}	nd	1026^{a}	nd	5.3^{c}	nd	8.1^{c}	nd	3.9^{c}	***
	Total (%)			8.2	4.4	6.2	2.4	0.7	22	10	6.9	6.3	3.5	8.8	21	1.4	0.7	5.2	0.8	2.6	6.4	1.5	0.7	32	3.1	1.2	9.7	1.9	0.3	1.2	
	Aldehyde																														
AL1	(*Z*)-3-Hexenal	808	A	nd	nd	0.8^{b}	nd	nd	nd	nd	nd	0.6^{b}	nd	3.0^{a}	1.0^{b}	nd	nd	nd	nd	4.3^{a}	nd	nd	nd	nd	nd	4.5^{a}	nd	nd	nd	nd	***
AL2	(*E*)-2-Hexenal	858	A	1.6^{b}	8.6^{b}	7.6^{b}	4.9^{b}	69^{b}	110^{b}	12^{b}	12^{b}	5.6^{b}	0.5^{b}	8.8^{b}	192^{b}	15^{b}	0.4^{b}	4.8^{b}	nd	37^{b}	1819^{a}	19^{b}	1.6^{b}	20^{b}	27^{b}	19^{b}	1747^{a}	15^{b}	28^{b}	44^{b}	***
AL3	(*E*)-2-Heptenal	964	A	0.9^{b}	11^{b}	nd	nd	nd	nd	nd	nd	nd	nd	nd	55^{a}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	***
AL4	Octanal	1008	A	nd	nd	0.8^{b}	nd	1.3^{b}	0.9^{b}	nd	nd	0.9^{b}	nd	2.0^{b}	7.5^{b}	nd	nd	0.9^{b}	nd	0.6^{b}	780^{a}	nd	nd	6.4^{b}	nd	nd	180^{b}	11^{b}	nd	8.5^{b}	***
AL5	Nonanal	1112	A	nd	nd	1.6^{a}	nd	nd	6.5^{a}	nd	nd	1.2^{a}	nd	nd	1.2^{a}	nd	0.4^{a}	34^{a}	nd	1.5^{a}	44^{a}	20^{a}	nd	2.1^{a}	nd	3.0^{a}	nd	38^{a}	56^{a}	50^{a}	**
AL6	(*Z*)-2-Nonenal	1134	B[2]	1.1^{b}	101^{a}	nd	nd	2.4^{b}	2.4^{b}	1.5^{b}	1.9^{b}	nd	nd	1.6^{b}	2.0^{b}	3.4^{b}	1.3^{b}	nd	nd	1.8^{b}	50^{ab}	7.3^{b}	1.5^{b}	nd	nd	11^{b}	98^{a}	12^{b}	7.3^{b}	21^{b}	***
AL7	(*E,E*)-2,6-Nonadienal	1157	A	nd	0.3^{b}	nd	nd	2.5^{b}	0.8^{b}	nd	1.3^{b}	nd	nd	nd	nd	18^{ab}	nd	0.4^{b}	17^{b}	3.6^{b}	50^{a}	30^{ab}	1.1^{b}	14^{b}	19^{ab}	3.9^{b}	nd	26^{ab}	15^{b}	7.7^{b}	***
AL8	(*E*)-2-Nonenal	1163	A	nd	nd	0.6^{b}	nd	nd	nd	nd	nd	nd	nd	nd	nd	13^{b}	nd	22^{b}	5.0^{b}	nd	177^{a}	13^{b}	nd	11^{b}	nd	nd	78^{b}	13^{b}	4.1^{b}	12^{b}	***
AL9	(*E*)-4-Decenal	1195	B[3]	nd	nd	nd	nd	nd	nd	nd	215^{b}	nd	nd	65^{b}	nd	831^{b}	nd	3.9^{b}	nd	6.3^{b}	9095^{a}	735^{b}	185^{b}	73^{b}	nd	nd	nd	1249^{b}	nd	20^{b}	***

AL10	Decanal	1222	B[4]	nd	nd	128[b]	nd	1.3[b]	nd	nd	nd	74[b]	nd	nd	nd	nd	nd	515[b]	nd	nd	nd	nd	nd	nd	nd	nd	8029[a]	2.0[b]	66[b]	nd	***
AL11	(*E*)-2-Decenal	1263	A	0.6[d]	41[d]	1.7[d]	nd	16 [d]	nd	1.2[d]	3.0 [d]	0.4[d]	nd	nd	nd	nd	nd	nd	1825[bc]	11[d]	2081[b]	3.5[d]	0.7[d]	nd	1912[b]	10 [d]	nd	nd	4766[a]	673[cd]	***
AL12	Undecanal	1309	A	nd	nd	57 [b]	nd	1.2[b]	3.7[b]	nd	nd	39[b]	nd	1.0[b]	nd	nd	nd	124[b]	nd	225[b]	145[b]	6.6[b]	nd	40 [b]	nd	1.9[b]	599[a]	2.6[b]	nd	nd	***
AL13	(*E*)-2-Undecenal	1365	A	nd	nd	7.4[d]	nd	3.3[d]	nd	nd	nd	3.6[d]	nd	nd	nd	nd	nd	95[c]	nd	1.3[d]	nd	nd	nd	45[cd]	21[cd]	nd	nd	3.4[d]	646[a]	243[b]	***
AL14	Dodecanal	1413	A	nd	6.7[de]	134[ab]	nd	2.1[e]	107[ab] [c]	nd	1.0[e]	93[bc]	nd	nd	nd	2.0[e]	nd	163[a]	nd	nd	nd	nd	nd	65[cd]	nd	nd	nd	2.2[e]	nd	nd	***
AL15	(*E*)-2-Dodecenal	1472	B[5]	nd	nd	175[b]	nd	2.2[b]	nd	nd	2.2[b]	98[b]	nd	nd	nd	nd	nd	291[b]	nd	nd	nd	nd	nd	21[b]	nd	nd	1412[a]	nd	nd	nd	***
AL16	Tetradecanal	1602	A	nd	nd	6.3[bc]	nd	nd	nd	nd	nd	3.6[c]	nd	3.7[c]	nd	nd	1.8[c]	4.5[bc]	nd	2.5[c]	nd	7.1[bc]	4.1[c]	20 [bc]	nd	5.4 [bc]	59 [a]	10[bc]	4.2[c]	34[ab]	***
	Total (%)			1.3	20	59	0.3	3.7	17	3.1	25	49	0.1	11	12	16	0.9	65	23	11	38	20	25	9.1	28	1.6	45	25	47	24		
	Alkane																														
AK1	Nonane	900	A	nd	0.4[c]	nd	nd	nd	7.9[c]	nd	nd	55[a]	nd	nd	nd	12[c]	nd	32[b]	nd	nd	4.6[c]	nd	nd	nd	nd	nd	nd	6.5[c]	nd	nd	***
AK2	Decane	1000	A	nd	nd	5.3[b]	nd	0.6[b]	nd	nd	nd	6.4[b]	nd	1.9[b]	nd	nd	nd	4.5[b]	nd	1.2[b]	440[a]	1.9[b]	nd	3.3[b]	nd	nd	nd	47[b]	nd	4.3[b]	***
AK3	Undecane	1100	A	nd	nd	nd	nd	nd	nd	nd	nd	2.7[b]	nd	nd	nd	nd	nd	4.1[b]	nd	nd	60[b]	nd	1.0[b]	nd	nd	nd	159[a]	nd	nd	3.7[b]	***
AK4	Dodecane	1200	A	nd	nd	nd	346[cd]	781[c]	nd	nd	nd	nd	223[cd]	nd	nd	nd	nd	5.5[d]	nd	610[cd]	nd	2.5[d]	nd	nd	2149[b]	24[d]	nd	nd	3211[a]	3.7[d]	***
AK5	Tridecane	1300	A	nd	nd	nd	53[cd]	166[b]	nd	nd	nd	nd	23[d]	1.3[d]	1.3[d]	217[ab]	nd	7.7[d]	12 [d]	0.5[d]	nd	nd	nd	148[bc]	169[b]	214[ab]	nd	5.2[d]	274[a]	10[d]	***
AK6	Tetradecane	1400	A	nd	nd	19[e]	133[cd] [e]	249[abc]	nd	nd	63[de]	7.2[e]	80[de]	67[de]	nd	nd	nd	31[e]	193[abc] [d]	300[ab]	nd	nd	nd	331[a]	301[ab]	147[bcd] [e]	nd	nd	139 [cde]	31[e]	***
	Total (%)			0.0	0.05	2.8	38	43	0.6	0.0	6.6	11	36	9.1	0.1	4.2	0.0	4.3	2.6	33	1.3	0.1	0.1	14	37	11	0.6	1.1	30	1.1	
	Alkene																														
AKE 1	1-Octene	791	A	2.9[a]	nd	nd	nd	nd	nd	2.7[a]	nd	nd	nd	0.9[ab]	nd	nd	nd	nd	nd	0.9[ab]	nd	nd	nd	nd	nd	nd	nd	nd	nd	3.0[a]	***
AKE 2	1-Nonene	887	A	82[ab] [c]	111[a]	1.8[d]	nd	23[bcd]	4.7[d]	nd	nd	1.4[d]	nd	1.0[d]	6.1[d]	4.3[d]	1.2[d]	2.9[d]	52[abcd]	nd	36 [bcd]	2.4[d]	nd	3.7[d]	11 [d]	12[cd]	86[ab]	4.3 [d]	25[bcd]	33 [bcd]	***
AKE 3	(*E*)-2-Nonene	921	B[6]	1.0[b]	0.4[b]	nd	nd	nd	26[b]	1.1[b]	nd	nd	nd	nd	6.5[b]	14[b]	nd	nd	nd	nd	182[a]	nd	nd	nd	nd	nd	51[b]	70 [b]	nd	79[b]	***

AKE 4	1-Dodecene	1190	A	9.6^{b}	2.0^{b}	nd	nd	6.5^{b}	75 ^{b}	47^{b}	0.4^{b}	nd	nd	1.1^{b}	nd	69b	24^{b}	nd	15^{b}	nd	152^{b}	62^{b}	0.7^{b}	664^{a}	18 ^{b}	830^{a}	111^{b}	67^{b}	50^{b}	21^{b}	***
	Total (%)			30	13	0.2	0.0	1.1	7.7	10	0.05	0.2	0.0	0.4	0.6	1.6	6.0	0.1	0.8	0.03	1.0	1.5	0.1	19	0.4	23	0.9	2.6	0.6	2.9	
	Ester																														
E1	(*E*)-2-Hexenyl acetate	1018	A	nd	2.5^{b}	nd	nd	1.8^{b}	3.5^{b}	nd	1.6^{b}	nd	nd	nd	0.3^{b}	6.8^{b}	nd	nd	nd	1.9^{b}	377^{a}	28^{b}	2.0^{b}	nd	nd	nd	340^{a}	122^{ab}	nd	1.8^{b}	***
E2	Hexyl 2-methylbutanoate	1236	A	nd	1.3^{b}	nd	nd	5.8^{b}	nd	nd	nd	nd	nd	nd	221^{a}	nd	nd	nd	nd	4.2^{b}	71^{b}	30^{b}	4.6^{b}	nd	27^{b}	27^{b}	2168 ^{a}	90^{ab}	nd	0.6^{b}	***
E3	Methyl decanoate	1320	A	nd	nd	0.4^{b}	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.4^{b}	nd	1.2^{b}	304^{a}	nd	nd	1.1^{b}	nd	0.7^{b}	nd	nd	nd	8.0 ^{b}	nd	nd	***
E4	Ethyl decanoate	1392	A	30 ^{b}	3.0^{b}	nd	8.8^{b}	nd	nd	30^{b}	0.4^{b}	2.2^{b}	6.2^{b}	8.9^{b}	203^{b}	182^{b}	63^{b}	4.8^{b}	22^{b}	22^{b}	447^{a}	61 ^{b}	128^{b}	nd	120^{b}	nd	74^{b}	127^{b}	28 ^{b}	5.0^{b}	***
E5	Methyl dodecanoate	1526	B[7]	nd	1.1^{cd}	nd	nd	nd	nd	nd	2.7^{bc}	nd	nd	nd	nd	nd	nd	0.3^{d}	nd	nd	nd	3.7^{b}	nd	nd	nd	nd	nd	6.1^{a}	nd	1.7 ^{bcd}	***
	Total (%)			9.3	0.9	0.05	0.6	0.2	0.2	6.2	0.5	0.3	0.7	1.2	19	3.5	15	0.3	4.1	1.0	2.4	2.9	17	0.02	2.1	0.7	2.3	6.4	0.2	0.2	
	Fatty acid																														
FA1	Decanoic acid	1375	A	nd	nd	3.0^{b}	nd	nd	nd	0.8^{b}	nd	1.1^{b}	nd	nd	nd	nd	nd	12^{a}	nd	nd	nd	2.0^{b}	nd	nd	nd	nd	nd	nd	nd	nd	***
	Total (%)			0.0	0.0	0.3	0.0	0.0	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.05	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	Monoterpene																														
M1	alpha-Pinene	944	A	nd	nd	nd	nd	nd	11^{b}	nd	nd	nd	nd	nd	nd	5.3^{b}	nd	nd	nd	nd	92^{a}	nd	nd	nd	nd	nd	nd	33^{b}	nd	nd	***
M2	Sabinene	981	A	0.9^{b}	1.8^{b}	nd	nd	nd	3.9^{b}	1.2b	3.8^{b}	nd	nd	nd	0.6^{b}	28^{b}	1.1^{b}	nd	nd	nd	354^{a}	5.2^{b}	0.7^{b}	nd	nd	nd	92^{b}	27^{b}	nd	1.7^{b}	***
M3	beta-Myrcene	988	A	5.4^{c}	2.9^{c}	nd	nd	nd	38^{bc}	4.7^{c}	nd	1.5^{c}	nd	5.3^{c}	6.6^{c}	13^{c}	21^{c}	nd	nd	14^{c}	456^{a}	3.7^{c}	9.1^{c}	nd	nd	7.8^{c}	210^{b}	3.9^{c}	nd	6.9^{c}	***
M4	Limonene	1032	A	1.1^{b}	nd	nd	nd	nd	nd	nd	nd	21 ^{b}	nd	nd	nd	nd	nd	nd	nd	nd	115^{a}	nd	7.6^{b}	nd	nd	0.6^{b}	nd	nd	nd	nd	***

M5	gamma-Terpinene	1065	A	nd	nd	1.5^{a}	nd	nd	nd	nd	nd	0.9^{a}	nd	1.3^{a}	nd	nd	nd	0.9^{a}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	***
M6	Terpinolene	1093	A	nd	nd	0.5^{f}	8.4 ^{ef}	10^{ef}	nd	nd	nd	nd	8.1^{ef}	1.2^{f}	nd	78^{a}	nd	nd	31^{cde}	15^{def}	nd	55^{ab}	nd	8.6^{ef}	11 ^{ef}	37^{bcd}	nd	52 ^{bc}	49^{bc}	24 ^{def}	***
M7	Carvone	1256	A	nd	0.3^{c}	nd	nd	nd	nd	nd	4.5^{c}	nd	3.0^{c}	1.1^{c}	38^{c}	1515$^{ab}_{c}$	2.1^{c}	175^{bc}	5.7 ^{c}	nd	2646^{a}	1241$^{ab}_{c}$	3.2^{c}	nd	nd	692^{bc}	1779$^{}_{ab}$	1122$^{ab}_{c}$	nd	23^{c}	***
M8	Ocimene quintoxide	1051	A	2.2^{b}	27^{b}	nd	nd	nd	4.1^{b}	1.7^{b}	1.3^{b}	nd	nd	0.4^{b}	5.0^{b}	6.1^{b}	0.8^{b}	nd	nd	nd	504^{a}	2.6^{b}	0.8^{b}	nd	2.0 ^{b}	nd	32^{b}	59^{b}	nd	1.2^{b}	***
	Total (%)			2.3	0.6	0.2	0.6	0.4	3.9	1.2	0.9	3.6	1.2	1.2	2.1	30	5.9	9.0	0.5	1.1	9.8	31	2.6	0.2	0.2	20	7.6	22	0.4	1.2	
	Other																														
O1	Styrene	892	A	0.7 ^{b}	1.1^{b}	54^{b}	55^{b}	20 ^{b}	331^{b}	69^{b}	54^{b}	nd	71 ^{b}	47^{b}	456^{b}	43^{b}	94 ^{b}	1.3^{b}	nd	90 ^{b}	1093^{a}	14^{b}	76^{b}	10^{b}	69^{b}	47^{b}	473^{b}	29 ^{b}	36^{b}	13^{b}	***
O2	6-Methyl-3-hepten-2-one	995	A	nd	nd	nd	nd	2.1^{bc}	nd	0.7$^{b}_{c}$	nd	nd	nd	1.3^{bc}	nd	2.7^{bc}	nd	0.7^{bc}	nd	nd	84^{a}	2.5^{bc}	0.9^{bc}	nd	4.7^{bc}	1.7^{bc}	41^{b}	3.1^{bc}	4.3^{bc}	20^{bc}	***
O3	m-Cymene	1023	A	0.9^{b}	1.6^{b}	nd	nd	nd	26^{b}	1.2^{b}	nd	nd	nd	nd	6.8^{b}	27^{b}	0.2^{b}	nd	nd	nd	363^{a}	48^{b}	nd	nd	nd	nd	185^{ab}	107^{b}	11^{b}	75^{b}	***
O4	PG dimer #1	1027	A	0.9^{b}	nd	0.9^{b}	nd	nd	nd	nd	nd	0.9^{b}	nd	nd	nd	12^{b}	nd	0.2^{b}	11^{b}	4.7^{b}	4.2^{b}	9.8^{b}	nd	nd	13^{b}	nd	nd	117^{a}	14^{b}	46^{b}	***
O5	1-Tridecene	1281	B^{8}	nd	7.3^{b}	1.4^{b}	nd	nd	12^{b}	nd	nd	1.0^{b}	nd	nd	52^{b}	33^{b}	0.1^{b}	1.5^{b}	nd	nd	185^{b}	19^{b}	nd	3.6^{b}	nd	nd	1607^{a}	19^{b}	nd	nd	***
O6	Cinnamic acid	1451	A	52^{b}	2.4^{b}	7.7^{b}	nd	nd	66^{b}	nd	172^{b}	3.9^{b}	nd	nd	141^{b}	782^{b}	80^{b}	1.4^{b}	19^{b}	15^{b}	2242^{a}	31^{b}	127^{b}	1.2^{b}	21 ^{b}	22^{b}	16^{b}	nd	29^{b}	8.8^{b}	***
O7	4-Methylthiazole	829	A	0.8^{ef}	nd	3.0$^{bc}_{d}$	nd	4.4^{abc}	1.2^{def}	nd	nd	2.6$^{cd}_{e}$	nd	2.1^{de}	0.7^{ef}	nd	nd	4.7^{ab}	nd	nd	nd	nd	nd	5.8^{a}	nd	1.5^{def}	nd	nd	nd	nd	***
	Total (%)			18	4.6	7.5	3.9	1.0	32	15	24	1.3	7.6	6.6	30	17	42	0.5	0.4	3.9	12	3.0	26	0.6	1.6	2.0	8.6	6.0	0.8	3.5	
	Unknown																														
U44	unknown	973		nd	nd	nd	nd	nd	7.7^{b}	nd	nd	nd	nd	nd	0.4^{b}	6.2^{b}	nd	nd	nd	nd	117^{a}	nd	nd	nd	nd	nd	nd	30^{b}	nd	nd	***
U36	unknown	992		nd	0.5^{b}	1.0^{b}	4.5^{b}	3.3^{b}	7.1^{b}	nd	1.4^{b}	nd	2.8^{b}	nd	13^{b}	14^{b}	nd	2.3^{b}	4.1^{b}	nd	39^{a}	7.4^{b}	nd	1.7^{b}	9.6^{b}	3.9^{b}	2.7^{b}	4.4^{b}	8.5^{b}	nd	***
U45	unknown	1086		nd	1.5	nd	nd	3.7	17	7.7	17	nd	nd	6.7	19	nd	12	nd	8.6	9.2	64	nd	11	nd	nd	1.9	66	8.0	30	165	ns
U4	unknown	1089		7.7 ^{b}	0.5^{b}	nd	6.2^{b}	12 ^{b}	nd	1.3^{b}	2.8^{b}	nd	5.1^{b}	5.4^{b}	nd	16^{b}	1.0 ^{b}	1.9^{b}	9.8^{b}	12^{b}	182^{a}	9.3^{b}	2.2^{b}	nd	8.0 ^{b}	16^{b}	nd	nd	19 ^{b}	6.0^{b}	***
U7	unknown	1182		nd	nd	nd	nd	nd	nd	1.3^{b}	nd	nd	nd	nd	nd	26^{ab}	nd	11^{b}	nd	nd	68^{a}	29^{ab}	nd	26^{ab}	nd	1.2^{b}	65^{a}	nd	nd	5.5^{b}	***

U9	unknown	1232	nd	nd	nd	nd	1.7^{c}	nd	nd	1.2^{c}	nd	nd	0.3^{c}	nd	117^{c}	nd	nd	3046^{a}	nd	133^{c}	110^{c}	nd	nd	nd	1.8^{c}	98^{c}	nd	nd	923^{b}	***
U10	unknown	1251	nd	2.3^{b}	nd	10^{b}	236^{b}	13^{b}	2.4^{b}	44^{b}	nd	nd	0.7^{b}	1.7^{b}	nd	nd	6.6^{b}	20^{b}	194^{b}	5439^{a}	nd	2.5^{b}	60 ^{b}	8.7 ^{b}	nd	4045^{a}	nd	nd	nd	***
U12	unknown	1292	5.3^{b}	2.9^{b}	nd	nd	2.4^{b}	24^{b}	14^{b}	29^{b}	0.2^{b}	nd	26^{b}	8.1^{b}	6.6^{b}	22^{b}	1.2^{b}	nd	1.3^{b}	981^{a}	92^{b}	51^{b}	nd	nd	9.0 ^{b}	61^{b}	117^{b}	17^{b}	2.7^{b}	***
U13	unknown	1330	nd	nd	nd	nd	nd	nd	0.7^{b}	nd	nd	nd	nd	nd	2.3^{b}	nd	1.6^{b}	nd	nd	4.1^{b}	1.9^{b}	nd	3.4^{b}	nd	nd	nd	16a	nd	nd	***
U14	unknown	1344	nd	8.1^{b}	nd	nd	1.4^{b}	0.7^{b}	1.3^{b}	16^{b}	nd	nd	nd	2.6^{b}	32^{b}	1.4 ^{b}	nd	2.9^{b}	0.8^{b}	1445^{a}	35^{b}	nd	0.9^{b}	2.7^{b}	nd	nd	3.1^{b}	18^{b}	293^{b}	***
U15	unknown	1351	1.7^{b}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	5.5^{b}	3.9^{b}	2.4^{b}	nd	74^{b}	300^{a}	367^{a}	3.8^{b}	17 ^{b}	nd	7.1^{b}	114^{b}	nd	nd	nd	***
U16	unknown	1357	nd	101$^{bc}_{d}$	nd	18 ^{cd}	81^{bcd}	nd	8.7^{d}	0.6^{d}	nd	8.3^{d}	9.5^{d}	nd	568^{a}	2.0^{d}	0.3^{d}	386^{ab}	1.0^{d}	nd	2.9^{d}	1.6^{d}	0.5^{d}	9.1^{d}	234^{bcd}	656^{a}	340^{abc}	nd	7.5^{d}	***
U17	unknown	1384	2.2^{b}	1.1^{b}	nd	0.6^{b}	28^{ab}	1.9^{b}	4.1^{b}	8.7^{b}	nd	nd	nd	3.2^{b}	44^{ab}	3.8^{b}	nd	nd	nd	121^{a}	33^{ab}	6.4^{b}	nd	nd	29^{ab}	120^{a}	51^{ab}	nd	1.5^{b}	***
U19	unknown	1432	1.3^{b}	158^{a}	0.3^{b}	nd	nd	1.2^{b}	2.1^{b}	nd	nd	nd	nd	0.3^{b}	4.3^{b}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	3.6^{b}	nd	nd	***
U46	unknown	1437	1.6^{b}	1.6^{b}	nd	1.8^{b}	21 ^{b}	nd	3.1^{b}	7.4^{b}	nd	nd	5.6^{b}	nd	9.0^{b}	3.5^{b}	nd	nd	nd	39b	5.6^{b}	3.0 ^{b}	nd	nd	2.0^{b}	302^{a}	4.6^{b}	nd	278^{a}	***
U20	unknown	1442	nd	nd	3.6^{d}	7.9^{cd}	nd	nd	nd	nd	0.3^{d}	5.2^{d}	nd	nd	41^{bc}	2.7^{d}	0.3^{d}	nd	1.0^{d}	nd	548^{a}	5.1^{d}	nd	2.3^{d}	nd	nd	50^{b}	nd	nd	***
U47	unknown	1462	0.6^{e}	nd	nd	478$^{cd}_{e}$	510 ^{cde}	nd	97 ^{de}	1.1^{e}	nd	274$^{cd}_{e}$	178$^{d}_{e}$	nd	nd	nd	12^{e}	1330^{a}	452 ^{cde}	nd	nd	nd	435^{cde}	1234^{ab}	548^{cd}	nd	772^{bc}	1601^{a}	25^{e}	***
U22	unknown	1488	1.1^{b}	4.7^{b}	1.0^{b}	nd	5.5^{b}	0.5^{b}	1.3^{b}	nd	0.9^{b}	nd	2.3^{b}	4.5^{b}	11^{b}	1.7^{b}	0.3^{b}	nd	6.5^{b}	3.3^{b}	4.5^{b}	1.2^{b}	nd	nd	7.2^{b}	7.7^{b}	4.5^{b}	3.6^{b}	598^{a}	***
U23	unknown	1499	nd	3.1^{e}	3.0^{e}	2.5^{ef}	7.4^{bcde}	nd	2.1^{e}	3.3^{e}	3.1^{e}	nd	3.5^{e}	nd	13^{ab}	2.1^{e}	9.9$^{bc}_{d}$	12^{bc}	18^{a}	nd	6.2^{cde}	4.3 $_{de}$	11 ^{bc}	6.4^{cde}	10^{bcd}	nd	6.8^{bcde}	12^{bc}	10^{bcd}	***
U37	unknown	1513	nd	nd	4.7^{a}	nd	nd	nd	nd	nd	4.4^{ab}	nd	nd	nd	2.7^{b}	nd	3.1^{ab}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.6^{b}	nd	nd	***
U38	unknown	1517	nd	nd	nd	nd	1.2^{c}	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.4^{c}	nd	nd	nd	1.9^{c}	nd	6.3^{b}	nd	1.2^{c}	nd	nd	nd	16^{a}	***
U24	unknown	1530	1.6$^{b}_{c}$	nd	nd	1.8^{bc}	4.6^{ab}	nd	1.9$^{b}_{c}$	nd	nd	1.9^{bcd}	nd	nd	5.5^{a}	nd	0.9^{c}	nd	2.1^{bc}	nd	nd	0.4^{c}	0.9^{c}	nd	3.2^{abc}	nd	3.5^{abc}	nd	1.9^{bc}	***
U48	unknown	1540	nd	1.0^{bc}	1.7$^{ab}_{c}$	nd	1.5^{abc}	nd	nd	0.4^{c}	1.1^{bc}	nd	2.8$^{ab}_{c}$	nd	4.2^{a}	nd	3.7^{ab}	nd	nd	nd	2.9^{abc}	nd	nd	nd	2.9^{abc}	nd	nd	nd	nd	***
U25	unknown	1546	6.4^{b}	23^{a}	nd	nd	nd	nd	nd	nd	nd	nd	0.5^{de}	nd	2.4^{cde}	1.6$^{cd}_{e}$	0.2^{e}	nd	3.0^{bcde}	nd	1.6^{cde}	0.9^{de}	3.0 $_{bcde}$	nd	4.0^{bcd}	nd	2.0 ^{cde}	nd	4.7^{bc}	***
U27	unknown	1556	nd	8.7^{cd}	0.8^{d}	19^{cd}	78^{bcd}	9.2^{cd}	13 $_{cd}$	32$^{bc}_{d}$	0.6^{d}	14^{cd}	0.5^{d}	21^{cd}	96^{bc}	1.3^{d}	1.2^{d}	121^{b}	2.3^{cd}	228^{a}	nd	1.2^{d}	0.4^{d}	nd	0.6^{d}	2.0^{cd}	nd	nd	3.5^{cd}	***
U39	unknown	1573	3.1^{e}	4.8^{e}	25de	6.5^{e}	20^{e}	5.5^{e}	4.0^{e}	9.0^{e}	23^{e}	5.4^{e}	27 ^{de}	11 ^{e}	12^{e}	6.3^{e}	37 $_{cde}$	nd	110 $_{abc}$	nd	75^{bcde}	22 ^{e}	1.6^{e}	104 $_{abcd}$	115^{abc}	158^{a}	79^{abcde}	135^{ab}	2.4^{e}	***
U28	unknown	1593	2.8^{d}	5.2^{d}	19^{cd}	3.7^{d}	6.6^{d}	3.1^{d}	3.8^{d}	4.2^{d}	14^{cd}	2.9^{d}	12^{cd}	3.4^{d}	11 ^{cd}	2.3^{d}	16^{cd}	11 ^{cd}	20 ^{cd}	75 ^{b}	15 ^{cd}	5.1^{d}	50^{bc}	13 ^{cd}	19^{cd}	7.9^{d}	17^{cd}	14 ^{cd}	136^{a}	***
U49	unknown	1642	1.6^{b}	125^{a}	nd	4.0^{b}	9.9^{b}	0.8^{b}	2.1^{b}	5.5^{b}	nd	3.3^{b}	5.5^{b}	0.5^{b}	8.2^{b}	1.2^{b}	nd	4.5^{b}	6.2^{b}	3.7^{b}	10^{b}	2.9^{b}	4.2^{b}	8.6^{b}	9.5^{b}	1.5^{b}	12^{b}	10^{b}	7.0^{b}	***
U29	unknown	1661	60^{b}	1.7^{b}	6.7^{b}	203^{b}	284^{b}	105^{b}	74 ^{b}	130^{b}	5.0 ^{b}	149^{b}	159^{b}	198^{b}	287^{b}	41^{b}	6.1^{b}	373^{b}	10^{b}	1301^{a}	284^{b}	84^{b}	7.8^{b}	415^{b}	295^{b}	2.3^{b}	315^{b}	470^{b}	17^{b}	***
U30	unknown	1685	nd	23^{b}	136^{b}	nd	4.0^{b}	nd	nd	1.9^{b}	102^{b}	nd	2.6^{b}	nd	2.2^{b}	nd	140^{b}	nd	299^{b}	nd	2.4^{b}	0.4^{b}	197^{b}	nd	6.5^{b}	1068^{a}	2.2^{b}	nd	412^{b}	***

U31	unknown	1704	nd	nd	3.1	nd	nd	nd	nd	nd	2.9	nd	nd	nd	nd	nd	3.0	nd	nd	nd	nd	nd	4.2	nd	nd	nd	nd	nd	13	ns
U41	unknown	1714	nd	nd	0.5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.7	nd	nd	19	nd	nd	0.5	nd	nd	nd	nd	nd	9.6	ns
U42	unknown	1740	nd	nd	0.5^{c}	nd	nd	nd	nd	nd	0.2^{c}	nd	nd	nd	nd	nd	nd	nd	7.6^{a}	nd	nd	nd	nd	nd	nd	nd	3.9^{b}	nd	1.1^{c}	***
U32	unknown	1755	nd	nd	nd	nd	nd	21 ^{b}	14^{b}	28^{b}	nd	nd	nd	36^{b}	44 ^{b}	nd	nd	nd	3.6^{b}	315^{a}	nd	nd	4.2^{b}	nd	2.5^{b}	nd	nd	nd	1.9^{b}	***
U43	unknown	1764	nd	nd	nd	nd	nd	nd	nd	nd	0.6^{f}	10^{ef}	28 ^{cde}	nd	nd	nd	0.8^{f}	nd	2.8^{ef}	nd	49 ^{bc}	18 ^{def}	1.1^{f}	71 ^{ab}	91^{a}	5.7 ^{ef}	41^{cd}	81^{a}	4.1^{ef}	***
U33	unknown	1775	nd	nd	0.9^{b}	nd	nd	nd	nd	nd	21 ^{b}	nd	nd	nd	nd	7.2^{b}	23^{b}	61^{b}	100 ^{ab}	nd	nd	nd	43^{b}	nd	nd	202^{a}	nd	nd	nd	***
U50	unknown	1824	0.7^{b}	1.1^{b}	0.6^{b}	nd	nd	3.7^{b}	1.4^{b}	0.7^{b}	1.1^{b}	nd	0.9^{b}	5.4^{b}	2.7^{b}	1.8^{b}	3.9^{b}	nd	2.6^{b}	25^{b}	5.2^{b}	1.5^{b}	4.7^{b}	nd	3.2^{b}	3.2^{b}	3.3^{b}	nd	129^{a}	***
U51	unknown	1867	1.1^{b}	3.5^{b}	2.7^{b}	nd	48 ^{a}	nd	1.7^{b}	3.9^{b}	nd	nd	3.3^{b}	nd	6.8^{b}	1.0 ^{b}	3.4^{b}	4.1^{b}	8.8^{b}	nd	8.0^{b}	2.2^{b}	6.1^{b}	7.2^{b}	8.1^{b}	41 ^{a}	6.9^{b}	9.1^{b}	16^{b}	***
	Total (%)		31	56	24	54	50	16	54	37	28	51	62	15	26	29	15	68	48	29	40	29	25	27	40	26	34	20	66	

[a] Linear retention index (LRI) on a DB-5 column. [b] A, mass spectrum and LRI agree with those of authentic compounds; B, mass spectrum (spectral quality value >80 was used) and LRI agrees with reference spectrum in the NIST/EPA/NIH mass spectra database and LRI agree with those in the literature cited [1] (Rahimi-Nasrabadi et al 2009); [2] (Peterson and Reineccius 2003); [3] (Morteza-Semnani et al 2006); [4] (Whetstine et al 2005); [5] (Marques et al 2000); [6] (Macku and Shibamoto 1991); [7] (Rostad and Pereira 1986); [8] (Luo and Agnew 2001). [c] Estimated abundance collected in the headspace of coriander samples containing 2.8 mL of saturated calcium chloride, calculated by comparison with of 100 ppm propyl propanoate used as internal standard; internal standard was used to normalise chromatograms; means of three replicate samples are shown; means not labelled with the same letters are significantly different (p<0.05) and Tukey's HSD multiple pairwise comparison; nd - not detected; ns - not significant probability obtained by ANOVA, * significant at the 5 % level; ** significant at the 1 % level; *** significant at 0.1 % level.

Multiple factor analysis

Multiple factor analysis was used to visualise the differences in environmental factors and the chemical composition (Table 4.4) observed for the autumn (A) and summer (S) seasons, and for the years of 2018, 2019, 2020 and 2021 (Figure 4.2). Coriander grown across the studied seasons and years, expressed variation between samples, where first (F1) and second (F2) dimension explain 42.36 % of the total variation within data. The first axis separated coriander grown in open field during the autumn of 2019 and 2020, and summer of 2019 from the other samples, whilst the second axis separated open field samples from summer 2018 and from the rest of the samples. All of the coriander samples were from the same variety (var. Cruiser), so differences seen between samples were caused by factors other than genotype, for this reason variety was not included in the analysis. Coriander was produced in four different locations, with York displaying an high association with (*E*)-2-dodecenal (AL15), undecanal (AL12) and decanal (AL10) and was positively correlated with most compounds. West Sussex, Worcester and Lincolnshire locations expressed low correlation with most compounds apart from dodecanal (AL14), nonane (AK1) and (*E*)-2-undecenal (Figure 4.2). In previous experiments, differences in volatile profile were observed between type of production, and this was observed in the biplot, where open field production was highly associated with main compounds and pot produced highly associated with dodecanal (AL14) and (*E*)-2-heptenal (AL3) and associated with some minor compounds.

Crop maturity at point of harvest showed some association with the composition of coriander samples apart from fully matured that showed no association. Coriander harvested at targeted stages (17 or 25 cm in height) were highly associated with dodecanal (AL14) and (*E*)-2-heptenal (AL3) however low association with most compounds. First cut of coriander sample expressed high association with most compounds including tetradecanal (AL16), whereas

second cut was highly associated with most alkane compounds which have been described as contributing to unpleasant aromas.

Soil type will affect water capacity and mineral availability, and the results from this study showed that coriander produced in loamy/clay soil was positively correlated with most compounds including (*E*)-2-dodecenal (AL15), decanal (AL10) and undecanal (AL12), whereas loamy soil was highly associated with most alkane compounds, peat was associated with some minor compounds and sandy expressed a low positive correlation with main compounds.

Contrary to the basil results, the application of fertilizers to coriander was positively correlated with most compounds and highly associated with (*E*)-2-decenal (AL11), tetradecanal (AL16) and nonanal (AL5), whereas no application resulted in high association with some minor compounds (Figure 4.2). This confirms what has been described in literature where the application of fertilisers will lead higher availability for crop intake of elements like carbon, nitrogen, zinc and sulphur, and promote the synthesis of primary and secondary compounds (Broadley et al 2012, Mousavi et al 2012, Waterman and Mole 1989).

As previously mentioned, source of irrigation will also have an effect of mineral availability, coriander main compounds expressed high association with a combination of rainfall water and irrigation, however the use of only one type (rainfall or irrigation) displayed a negative correlation with main compounds and high association with minor compounds including dodecanal (AL14) and gamma-terpinene (M5). Neffati and Marzouk (2008) described a negative influence on the essential oil of coriander with the use of irrigation water high in salts and minerals, with positive effects of medium levels of salts and minerals, this suggests that combining tap water and rain water would have a positive effect on the essential

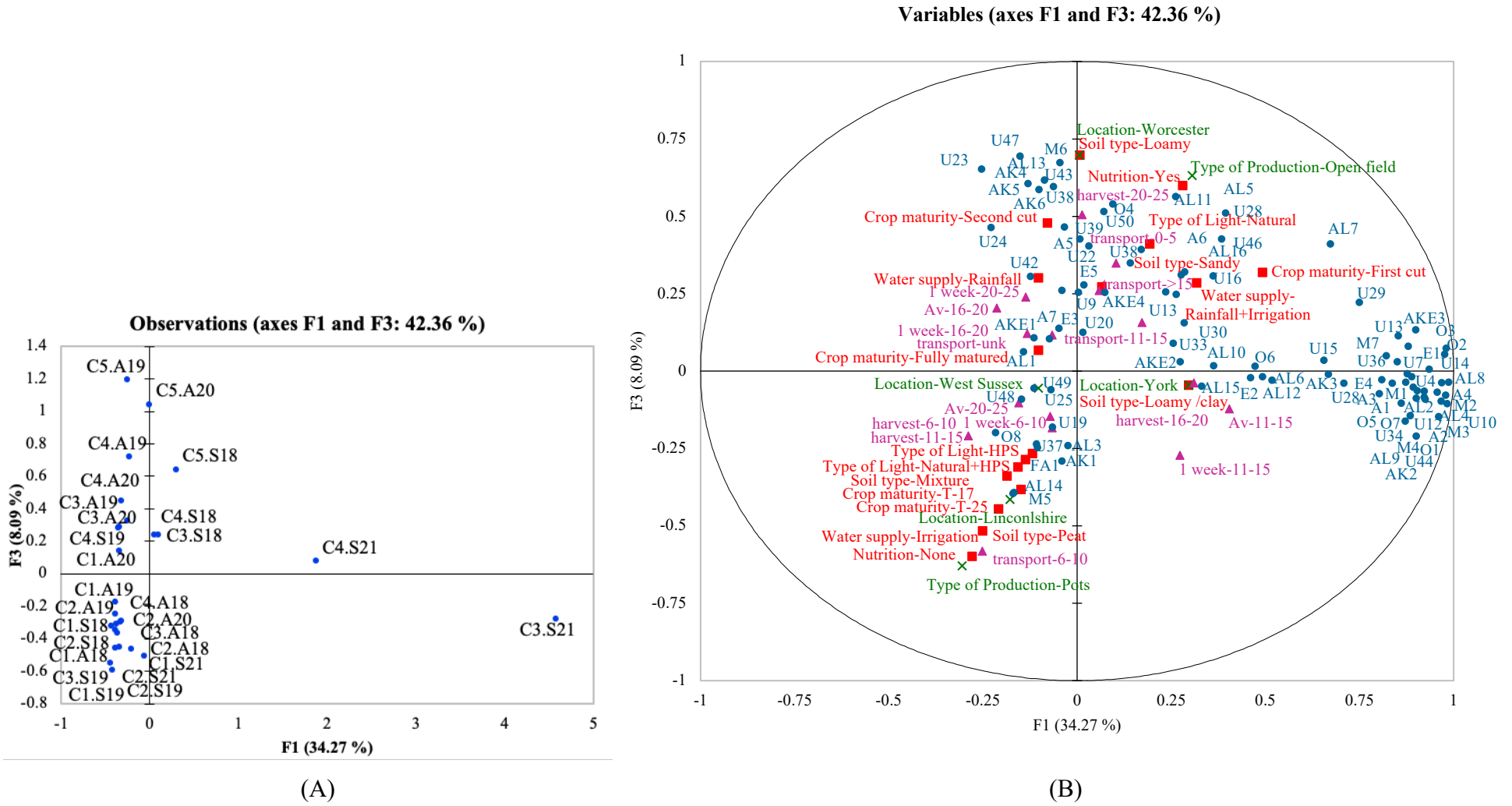
oil since the tap water is rich in minerals and salts and rain water is soft (Neffati and Marzouk 2008). Similar rainfall amounts were experienced by open field samples (Table 4.2), which might have been in low levels so the combination with irrigation would result in appropriate amounts for coriander, this suggests that deficit of water in coriander would result in lower relative abundances of volatile compounds. Coriander results demonstrate that for this herb, the use of both water from rain and irrigation will lead to higher abundances of volatiles and result in more aromatic herb.

Light plays a key role in the photosynthesis which will influence secondary metabolites production and further influence its composition. Influence of the light source was analysed, results showed that high pressure sodium (HPS) lighting and its combination with sunlight (due to shorter photoperiod, <13 h) was highly associated and highly associated with compounds like dodecanal (AL14) and (*E*)-2-heptenal (AL3), whereas sunlight experienced by open field samples, which had similar photoperiods (14-16 h day^{-1}) was positively associated with most compounds (Figure 4.2), and highly associated with (*E*)-2-decenal (AL11) (waxy, fatty and coriander odour notes), which has been described as a main contributor to the aroma of coriander.

Temperature has been identified as one factor that influences the aroma profile of plants. Coriander produced at an average growth temperature of 11-15 ˚C, expressed positive association with main compounds including (*E*)-2-dodecenal (AL15), undecanal (AL12) and (*Z*)-2-nonenal (AL6) (impart citrus, waxy, soapy and floral aroma notes), contrary to what has been described in literature where higher abundance of volatile compounds were detected when coriander was grown between 15-22 ˚C (Telci and Hisil 2008). Additionally, coriander grown at 16-20 ˚C displayed a high correlation with most alkane compounds and growth temperatures of 20-25 ˚C resulted in high correlation with some minor compounds including dodecanal

(AL14) (Figure 4.2). Air temperature one week before harvest can also influence the composition, results showed that keeping similar temperature to the average growth temperature (11-15 ˚C) expressed higher association with main compounds such as (*E*)-2-dodecenal (AL15) and undecanal (AL12), whereas exposing coriander to higher temperatures (>16 ˚C) were associated with alkanes compounds such as tridecane (AK4) and dodecane (AK5) (Figure 4.2). Temperatures of 6-10 ˚C were displayed in the centre of the biplot, so no high association was detected with majority of compounds. Temperature at the time of harvest have been mentioned to influence the composition of the crop, harvest at temperatures between 6-15 ˚C expressed a high association with alkane compounds, whilst temperatures of 16-20 ˚C showed higher association with some minor compounds and dodecanal (AL14). Additionally, warmer temperatures of 20-25 ˚C displayed a positive correlation with main compounds for the coriander aroma. Transport temperatures (0-5 ˚C; 6-10 ˚C; >11 ˚C) displayed a low correlation with main compounds from coriander leaves, however temperatures of 6-10 ˚C were highly associated with dodecanal (AL14) which imparts a citrus, floral and soapy aroma, this indicates a small influence of transport temperatures on the volatile composition of coriander leaves (Figure 4.2).

The synthesis of aroma compounds is part of the crop's response to abiotic and biotic stresses as a protective and adaptative mechanism to the growing environment. Coriander results confirm what was previously hypothesised, whereby different production factors will result in differences in the aromatic profiles of this herb even with samples analysed being from the same variety. However, differences detected between samples are not caused by one individual growing factor, but the combination of environmental conditions for the production of desired compounds.



(A) (B)

Figure 4.2: Multiple factor analysis of five coriander samples harvested in the summer and autumn for the years of 2018, 2019, 2020 and 2021 showing correlations with volatile compounds and growing conditions. (A)Projection of samples; (B) Distribution of variables: green cross-grower information; pink triangles-temperature; red squares-environment conditions; blue circle-volatile compounds.

4.3.3 Rosemary

In total, 125 compounds were detected in the headspace of rosemary samples for two growing seasons over three years (Table 4.5). Detected compounds included 45 monoterpenes, eight sesquiterpenes, three alcohol, three aldehydes and 46 unidentified compounds. Quantitative differences in the aroma profiles were observed between the rosemary samples, confirmed by one-way ANOVA. Rosemary produced in field (protected or unprotected) expressed the highest abundance of volatile compounds. and higher contents of monoterpenes, furthermore similar composition was observed between the two samples produced in this location. Additionally, pot plants produced during the summer of 2021 expressed the highest contents out of this method of production. Furthermore, some rosemary field samples displayed higher contents for the summer season and others during the autumn season, this could be due to different varieties of the samples. Due to the rosemary's different genotypes limited conclusions that can be draw from results presented in this study, further analysis would be required comparing samples from the same variety. Majority of rosemary expressed over 50 % of composition in monoterpene compounds, apart from open field sample R4 in the summer of 2019. All detected compounds were found significantly different between samples, growing seasons and years.

Studies have reported that monoterpenes are the main compounds group to contribute to the rosemary aroma (Hcini et al 2013, Lakušić et al 2012). All of the rosemary samples were associated with the production of monoterpenes (50 - 91 %). Monoterpenes comprised most of composition of the aroma profile of all rosemary samples, with eucalyptol, camphor, borneol, alpha-pinene and camphene exhibiting the highest ratio of monoterpenes (Hcini et al 2013, Lakušić et al 2012, Salido et al 2003, Szumny et al 2010, Zawirska-Wojtasiak and Wąsowicz 2009). These compounds were detected in rosemary samples however not all the samples had

all of these compounds in the composition, this could be due the differences in variety of the samples. When comparing the samples produced in the same year, autumn produced rosemary expressed a higher percentage of monoterpenes compounds than summer production, this would indicate that the growing conditions to which rosemary is exposed for the autumn harvest would result in higher monoterpene content which has been described to be determinant to the aroma profile of this herb.

Sesquiterpenes, alcohols, aldehydes, unknown and other types of compounds accounted for 9-69 % of the aroma composition of rosemary samples. Majority of compounds within these groups were detected at minor abundances, however significant differences were observed. Samples with lower monoterpene content, displayed higher abundances of other compounds. However, these compounds have not been reported previously as relevant to the aroma of rosemary plants and were not detected in high abundances.

Table 4.5:Relative abundance of aroma compounds identified in the headspace of fresh rosemary samples.

Code	Compound name	LRI	ID	Relative abundance																														p-value
				R1						R2						R3						R4						R5						R6		
				S18	A18	S19	A19	A20	S21	S18	A18	S19	A20	S21	S18	A18	S19	A19	A20	S21	S18	A18	A19	A20	S21	S18	A18	A19	S18	A18	A19	A20	S21	
	Alcohol																																	
A1	1-Hepten-3-ol	840	A	nd	nd	3.0^{b}	nd	nd	9.4^{b}	nd	nd	9.6^{b}	nd	6.6 ^{b}	nd	nd	8.3^{b}	20 ^{b}	nd	3.9^{b}	nd	nd	nd	nd	14^{b}	nd	nd	45^{a}	nd	nd	nd	nd	21^{b}	***
A2	(*Z*)-3-Hexen-1-ol	858	A	4.7^{b}	12^{b}	74^{b}	24^{b}	21^{b}	5.5^{b}	nd	34^{b}	38^{b}	16^{b}	nd	11^{b}	58^{b}	31^{b}	nd	26^{b}	nd	47^{b}	64^{b}	83^{b}	19^{b}	nd	62^{b}	83^{b}	nd	64^{b}	48^{b}	31^{b}	41 ^{b}	867^{a}	***
A3	(*Z*)-2-Hexen-1-ol	872	A	nd	nd	8.4^{de}	nd	nd	nd	nd	nd	15^{cde}	nd	8.6^{de}	nd	nd	nd	13de	nd	nd	nd	nd	52^{b}	2.1^{e}	40^{bc}	7.9^{de}	nd	79^{a}	nd	nd	nd	nd	32^{bcd}	***
	Total (%)			0.1	0.3	1.1	0.3	0.3	0.2	0.0	0.2	0.3	0.1	0.02	0.04	0.2	0.3	0.1	0.1	0.01	0.2	0.3	0.4	0.1	0.1	0.5	0.3	0.3	0.3	0.2	0.1	0.3	1.0	
	Aldehyde																																	
AL1	Hexanal	802	A	nd	nd	12^{c}	nd	7.4^{cd}	nd	nd	nd	14^{c}	24^{ab}	nd	nd	nd	15^{bc}	nd	24^{a}	nd	nd	nd	nd	17^{abc}	nd	nd	nd	nd	nd	nd	17^{abc}	17^{abc}	nd	***
AL2	(*E*)-2-Hexenal	854	A	31^{d}	19^{d}	1.3^{d}	34^{d}	42^{d}	242^{cd}	82^{d}	27^{d}	nd	27^{d}	568^{ab}	65^{d}	86^{d}	72^{d}	nd	13 ^{d}	355^{bc}	145^{cd}	85 ^{d}	nd	45^{d}	557^{ab}	94^{d}	101^{d}	nd	145^{cd}	63^{d}	49^{d}	13^{d}	656^{a}	***
AL3	(*E*)-2-Heptenal	960	A	63^{def}	61^{def}	nd	64^{def}	121^{def}	95^{def}	1713^{b}	nd	nd	36^{ef}	nd	126^{def}	23^{f}	2.2^{f}	nd	1436^{bc}	9.0^{f}	79 ^{def}	71^{def}	882^{cd}	1049^{bc}	nd	852^{ce}	nd	nd	42^{ef}	18 ^{f}	1108^{bc}	655^{cdef}	8006^{a}	***
	Total (%)			2.0	1.9	0.2	1.4	2.7	3.3	7.0	0.2	0.1	0.5	0.9	0.7	0.3	0.6	0.0	6.6	0.8	0.9	0.6	2.7	4.7	1.1	6.4	0.3	0.0	1.0	0.3	4.5	5.1	9.2	
	Alkane																																	
AK1	Decane	1000	A	nd	nd	187^{c}	6.9^{c}	25^{c}	9.0^{c}	46^{c}	63^{c}	1626^{a}	25^{c}	6.9^{c}	122^{c}	57^{c}	1212^{b}	nd	76^{c}	9.1^{c}	91^{c}	87^{c}	43^{c}	136^{c}	nd	12^{c}	nd	17^{c}	nd	nd	nd	17^{c}	nd	***
AK2	Heptadecane	1710	A	nd	nd	2.4^{b}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	4.3^{b}	nd	nd	nd	nd	nd	nd	nd	nd	nd	28^{a}	nd	nd	nd	nd	nd	***
	Total (%)			0.0	0.0	2.5	0.1	0.4	0.1	0.2	0.3	7.4	0.2	0.01	0.4	0.2	7.8	0.0	0.3	0.02	0.4	0.3	0.1	0.6	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.0	

	Alkene																															
AKE 1	1-Decene	995	A	8.3^{e}	10^{e}	160^{e}	114^{e}	131^{e}	16^{e}	1579^{cd}	1042^{cde}	12^{e}	442de	5800^{a}	96^{e}	2191^{c}	32^{e}	nd	1403^{c}	4320^{b}	666de	nd	371de	540de	75^{e}	8.9^{e}	24^{e}	nd	14^{e}	31^{e}	410de	nd	38^{e}	***
	Total (%)			0.2	0.3	2.1	1.6	2.1	0.2	6.2	5.6	0.1	2.6	9.5	0.3	6.3	0.2	0.0	6.3	10	2.6	0.0	1.1	2.3	0.2	0.1	0.1	0.0	0.1	0.1	1.6	0.0	0.04	
	Ketone																															
K1	3-Octanone	991	A	125^{e} fg	nd	nd	27^{g}	35^{fg}	348de fg	631^{cd} e	160^{ef} g	60^{fg}	nd	nd	165 6^{b}	798^{cd}	46^{fg}	nd	958^{c}	nd	590^{cd} ef	539^{cd} efg	654^{cd} e	nd	1542 b	474^{cd} efg	744^{cd}	nd	592^{cd} ef	3.3^{g}	nd	309^{defg}	2728^{a}	***
K2	Filifolone	1104	B[1]	7.3^{g}	4.0^{g}	nd	554^{c} d	29^{g}	9.8^{g}	94^{fg}	25^{g}	693^{bc}	357de	nd	9.8^{g}	27^{g}	322de f	72^{fg}	25^{g}	nd	nd	nd	1093 a	890^{ab}	nd	60^{g}	14^{g}	nd	78^{fg}	15^{g}	224^{efg}	nd	nd	***
K3	Geranyl acetone	1450	B[2]	4.9^{c}	4.3^{c}	nd	nd	2.8^{c}	5.6^{c}	nd	nd	nd	9.4^{c}	nd	nd	nd	nd	nd	25^{b}	99^{a}	nd	nd	nd	5.6^{c}	nd	nd	nd	nd	nd	nd	nd	nd	nd	***
	Total (%)			2.8	0.2	0.0	8.0	1.0	3.6	2.8	1.0	3.4	2.1	0.0	5.8	2.4	2.4	0.2	4.5	0.2	2.3	2.1	5.3	3.7	3.1	3.6	2.7	0.0	3.5	0.1	0.9	2.3	2.9	
	Monoterpene																															
M1	Tricyclene	925	B[3]	5.5^{c}	5.1^{c}	nd	nd	4.3^{c}	18^{c}	87^{bc}	31^{bc}	2.8^{c}	nd	72^{bc}	103^{b} c	24^{c}	1.6^{c}	nd	4.6^{c}	45^{bc}	73^{bc}	56^{bc}	nd	6.7^{c}	141^{b}	37^{bc}	101 bc	nd	56^{bc}	97^{bc}	nd	nd	409^{a}	***
M2	alpha-Thujene	931	B[4]	8.1^{g}	16^{g}	nd	2.6^{g}	20^{g}	18^{g}	570 cde	478 def	nd	33^{g}	1923 a	931 bc	128 fg	nd	nd	47^{fg}	1180 b	208 efg	187 efg	13^{g}	35^{g}	332de fg	112^{fg}	239 efg	nd	161 efg	264 efg	15^{g}	24^{g}	722 cd	***
M3	alpha-Pinene	938	A	257 gh	247 gh	9.5^{h}	nd	nd	865 efgh	nd	2107 cde	81^{h}	96^{h}	nd	294 3^{bc}	1150 efgh	49^{h}	nd	351 fgh	6191 a	1411 efg	1121 efgh	58^{h}	139 gh	3410 b	1160 efgh	2856 bc	nd	1554 def	2741 bcd	60^{h}	107^{h}	nd	***
M4	Camphene	957	A	131 ef	nd	nd	nd	nd	nd	nd	1197 cdef	81^{ef}	841 def	5536 a	44^{ef}	2637 bc	nd	306^{ef}	14^{ef}	3780 b	1450 ^{c}de	1268 cdef	nd	nd	3487 b	nd	1948 cd	97^{ef}	1163 def	1918 cd	nd	nd	nd	***
M5	4-thujene	965	B[5]	nd	nd	210^{c}	52^{c}	117^{c}	nd	34^{c}	nd	1493 b	nd	nd	nd	nd	1027 bc	4268 a	nd	nd	nd	nd	26^{c}	71^{c}	nd	29^{c}	nd	2199 b	nd	7.3^{c}	18^{c}	42^{c}	nd	***
M6	Sabinene	979	A	31^{b}	23^{b}	nd	3.1^{b}	73^{b}	21^{b}	75^{b}	80^{b}	nd	17^{b}	95^{b}	171 5^{a}	131^{b}	nd	nd	61^{b}	66^{b}	67^{b}	79^{b}	nd	nd	33^{b}	45^{b}	76^{b}	nd	56^{b}	84^{b}	nd	nd	nd	***
M7	beta-Pinene	983	A	72^{e}	84^{e}	nd	40^{e}	nd	199 de	nd	nd	nd	76^{e}	5489 a	nd	nd	nd	nd	60^{e}	3893 b	nd	1558 c	nd	63^{e}	4088 b	699 cde	1351 cd	1497 c	nd	1336 cd	24^{e}	40^{e}	59^{e}	***
M8	beta-Myrcene	987	A	nd	99^{ef}	51^{ef}	44^{ef}	72^{ef}	nd	1560 bcd	1274 bcde	nd	629 cdef	nd	859 bcdef	1940 b	nd	1653 bcd	1067 bcdef	nd	1745 bcd	118^{ef}	1229 bcdef	1869 bc	nd	nd	nd	nd	980 bcdef	740 bcdef	817 bcdef	539^{def}	6483 a	***
M9	alpha-Phellandrene	1008	A	69^{e}	73^{e}	13^{e}	nd	nd	149^{e}	126^{e}	83^{e}	1455 a	1.7^{e}	161^{e}	36^{e}	142^{e}	977 bcd	17^{e}	nd	104^{e}	783 cd	680^{d}	nd	692^{d}	1325 ab	792 cd	1319 ab	1086 abc	948 bcd	1381 a	nd	nd	nd	***
M10	delta-3-Carene	1012	A	111 de	107 de	nd	56de	103 de	395 cde	22^{e}	27de	38de	806 bc	13^{e}	379 cde	52de	30de	1313 b	176 de	9.4^{e}	16^{e}	25^{e}	226 de	nd	40de	7.5^{e}	35de	nd	19^{e}	36de	832 bc	579^{cd}	3930 a	***
M11	delta-2-Carene	1017	B[6]	43^{c}	33^{c}	119^{c}	91^{c}	99^{c}	58^{c}	nd	279^{b}	nd	4.6^{c}	414^{b}	nd	nd	nd	991^{a}	23^{c}	nd	15^{c}	nd	nd	nd	nd	16^{c}	nd	351^{b}	20^{c}	nd	nd	7.6^{c}	129^{c}	***
M12	*m*-Cymene	1022	A	2.3^{d}	35^{d}	201^{d}	22^{d}	58^{d}	1.8^{d}	502 cd	nd	94^{d}	24^{d}	nd	462 0^{a}	440 cd	50^{d}	1893 b	472 cd	249^{d}	279^{d}	256^{d}	nd	nd	339 cd	247^{d}	432 cd	nd	303^{d}	492 cd	3.8^{d}	11^{d}	1011 c	***

M13	p-Cymene	1028	A	60 ef	223 cd	39 f	33 f	72 ef	150 de	nd	nd	23 f	474 a	nd	nd	nd	15 f	11 f	nd	nd	nd	nd	89 ef	335 b	nd	nd	nd	nd	nd	nd	146 de	287 bc	nd	***
M14	Limonene	1036	A	296 e	nd	1.8 e	279 e	288 e	856 e	nd	nd	314 e	nd	9969 b	nd	438 e	135 e	nd	4303 d	8560 bc	4240 d	6455 cd	nd	nd	11179 b	nd	nd	nd	nd	nd	nd	nd	20755 a	***
M15	Eucalyptol (1,8-cineole)	1040	A	317 h	372 gh	64 h	793 efgh	512 fgh	684 efgh	3713 bcdef	4060 bcd	nd	3813 bcde	1765 cdefgh	17 h	6680 ab	nd	74 h	nd	nd	nd	nd	nd	5016 bc	nd	2672 cdefgh	6612 ab	894 defgh	3582 bcdefg	6762 ab	8592 a	2860 cdefgh	nd	***
M16	alpha-Ocimene	1049	B7	nd	nd	487 cd	nd	18 d	1.7 d	22 d	nd	3196 a	24 d	nd	648 c	nd	2464 b	159 cd	641 c	nd	40 d	nd	nd	nd	nd	36 d	15 d	174 cd	13 d	5.8 d	nd	nd	nd	***
M17	gamma-Terpinene	1061	A	68 e	47 e	nd	nd	80 e	86 e	nd	440 d	14 e	nd	906 ab	412 d	703 bc	7.9 e	nd	nd	540 cd	nd	nd	nd	nd	984 a	nd	nd	nd	nd	nd	nd	nd	nd	***
M18	Sabinene hydrate	1073	A	nd	nd	70 b	39 b	16 b	1.4 b	182 b	193 b	597 b	94 b	nd	730 b	285 b	269 b	10695 a	627 b	nd	214 b	247 b	237 b	121 b	253 b	182 b	249 b	12480 a	263 b	295 b	273 b	98 b	431 b	***
M19	Terpinolene	1090	A	202 efgh	153 fgh	64 gh	nd	nd	452 cdefg	nd	nd	374 cdefgh	nd	1533 a	612 bcd	730 bc	307 defgh	360 cdefgh	14 h	895 b	481 cdef	355 cdefgh	nd	nd	716 bcd	390 cdefgh	595 bcde	667 bcd	454 cdefg	614 bcd	nd	306 defgh	17964 a	***
M20	*p*-Cymenene	1096	A	nd	nd	0.6 h	156 gh	189 gh	312 efg	898 ab	422 def	nd	512 cde	709 bc	182 gh	376 defg	nd	nd	337 defg	488 cdef	14 h	7.0 h	152 gh	375 defg	1079 a	8.1 h	nd	nd	9.7 h	nd	283 fg	5.2 h	537 cd	***
M21	Linalool	1100	A	315 bcd	203 cdefg	273 cdef	nd	377 bc	nd	541 b	350 bc	nd	3.2 g	78 defg	33 fg	73 defg	nd	nd	17 g	51 efg	1074 a	848 a	nd	nd	nd	284 cde	266 cdef	85 defg	287 cde	367 bc	nd	201 cdefg	nd	***
M22	alpha-Thujone	1109	A	4.0 c	nd	430 a	8.4 c	nd	1.8 c	11 c	13 c	7.7 c	23 bc	24 bc	nd	8.9 c	14 c	nd	15 c	17 bc	nd	nd	nd	16 c	nd	nd	nd	nd	nd	9.5 c	64 b	17 bc	nd	***
M23	beta-Thujone	1119	A	4.7 c	3.2 c	8.8 c	nd	nd	nd	12 c	11 c	163 b	nd	nd	nd	nd	116 b	336 a	nd	nd	nd	nd	nd	nd	nd	nd	nd	271 a	nd	nd	nd	nd	nd	***
M24	Fenchol	1124	A	nd	nd	0.7 f	nd	4.5 ef	33 b	9.9 def	nd	18 cde	nd	nd	15 cdef	19 bcde	4.8 ef	5.0 ef	65 a	nd	24 bcd	68 a	nd	nd	26 bc	nd	nd	10 def	nd	nd	nd	nd	nd	***
M25	cis-Verbenol	1153	B8	28e	16e	8.3e	nd	32e	807e	20e	nd	nd	3.6 e	4132 bc	2824 d	nd	nd	nd	2494d	3171c d	nd	nd	nd	nd	5216 b	nd	nd	nd	nd	nd	nd	nd	8339a	***
M26	Camphor	1158	A	509d e	472d e	6.8 e	43e	710d	nd	2466 b	2237 bc	8.2e	nd	nd	1603c	3817 a	6.0 e	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	***
M27	(Z)-beta-Terpineol	1160	A	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	20 e	nd	nd	nd	2993 bc	4102a b	nd	nd	nd	1805d	4213 a	nd	2337 cd	3886 ab	4623a	nd	nd	***
M28	p-Menth-1,5-dien-8-ol	1164	B9	6.3 d	6.2d	77d	1265 c	nd	33d	nd	nd	32d	2377b c	nd	nd	5.1d	41d	nd	4.0d	nd	nd	nd	7404 a	3202b	nd	nd	nd	nd	nd	nd	nd	1727c	nd	***
M29	Pinocarvone	1173	B10	25	22	nd	17	24	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
M30	Isoborneol	1173	A	395b	226c	629a	22e	59de	29e	nd	nd	nd	nd	nd	nd	nd	nd	nd	14e	7.7e	nd	nd	nd	nd	121d	nd	nd	nd	nd	nd	nd	nd	nd	***
M31	Borneol	1179	A	nd	150e f	37f	585c def	556c def	287ef	25f	1124 bc	nd	973cd	399de f	666c de	1680 ab	1627 ab	nd	458def	299ef	1574 ab	2007a	115ef	188ef	1789 a	41f	nd	nd	64f	23f	nd	nd	nd	***

M32	alpha-Terpinol	1198	B[1]	82[b]	53[b]	nd	nd	146[b]	20[b]	773[b]	475[b]	13[b]	750[b]	4.6[b]	28[b]	654[b]	11[b]	nd	nd	nd	nd	nd	nd	nd	795[b]	553[b]	594[b]	5389[a]	646[b]	687[b]	228[b]	241[b]	962[b]	***
M33	Myrtenol	1206	B[2]	19[c]	31[c]	10[c]	93[c]	24[c]	nd	31[c]	31[c]	14[c]	56[c]	nd	19[c]	50[c]	15[c]	nd	31[c]	nd	36[c]	47[c]	639[b]	830[a]	43[c]	22[c]	9.8[c]	nd	34[c]	16[c]	nd	4.5[c]	75[c]	***
M34	gamma-Terpineol	1211	A	50[c]	nd	128[c]	17[c]	68[c]	nd	13[c]	nd	850[a]	10[c]	nd	1068[a]	14[c]	542[b]	1113[a]	10[c]	nd	17[c]	6.4[c]	19[c]	66[c]	nd	11[c]	nd	nd	13[c]	nd	26[c]	29[c]	nd	***
M35	Verbenone	1225	B[3]	400[cd]	331[d]	27[e]	nd	569[bc]	nd	939[a]	nd	9.4[e]	666[b]	27[e]	nd	nd	10[e]	nd	nd	nd	nd	nd	nd	422[cd]	nd	468[cd]	nd	nd	nd	nd	nd	nd	20[e]	***
M36	Carveol 2	1238	A	nd	nd	596[ab]	nd	nd	26[d]	15[d]	nd	743[a]	nd	36[d]	nd	nd	637[ab]	486[c]	nd	16d	7.3[d]	nd	nd	nd	nd	5.3[d]	nd	504[bc]	nd	nd	nd	nd	nd	***
M37	Neral	1245	A	33[bcd]	20[defg]	5.8[gh]	nd	2.1[gh]	9.7[efgh]	48[b]	26[cdef]	75[a]	3.0[gh]	43[bc]	28[cde]	34[bcd]	26[cdef]	nd	26[cdef]	nd	8.7[fgh]	14[efgh]	nd	11[efgh]	4.0[gh]	6.2[gh]	17[defgh]	nd	12[efgh]	17[defgh]	nd	7.9[fgh]	nd	***
M38	Geraniol	1251	A	14[cdef]	2.1[ef]	nd	23[bcde]	38[b]	64[a]	26[bcd]	5.8[def]	8.5[def]	7.6[def]	nd	nd	nd	nd	nd	nd	25[bcd]	14[cdef]	nd	nd	nd	nd	nd	nd	nd	nd	3[bc]	5.4[def]	nd	25[bcd]	***
M39	Carvone	1254	A	71[b]	45[b]	2.3[b]	10[b]	17[b]	9.9[b]	nd	nd	nd	10[b]	nd	16[b]	nd	nd	569[a]	3.9[b]	nd	15[b]	20[b]	nd	13[b]	12[b]	37[b]	26[b]	516[a]	37[b]	nd	nd	nd	nd	***
M40	Piperitone	1271	A	9.9[fghi]	4.8[hi]	107[a]	nd	17[efghi]	5.6[ghi]	68[b]	27[defg]	18[efghi]	31[cdef]	49[bc]	31[cdef]	46[cd]	3.9[hi]	10[fghi]	18[efghi]	nd	18[efghi]	13[efghi]	nd	15[efghi]	3.2[hi]	13[efghi]	28[cdef]	nd	24[efgh]	28[cdef]	nd	14[efghi]	32[cde]	***
M41	Geranial	1280	A	6.4[b]	3.3[b]	16[b]	8.7[b]	19[b]	82[a]	12[b]	14[b]	12[b]	9.3[b]	nd	nd	23[b]	6.5[b]	nd	nd	nd	7.6[b]	11[b]	nd	13[b]	nd	nd	nd	nd	nd	16[b]	11[b]	9.9[b]	nd	***
M42	Bornyl acetate	1294	A	324[ghi]	398[ghi]	14[i]	92[hi]	453[ghi]	nd	1844[def]	nd	25[i]	4.9[i]	3978[c]	nd	nd	30[i]	nd	921[fghi]	2959[cd]	nd	nd	nd	15[i]	5923[b]	nd	1439[efg]	nd	nd	1667[ef]	2551[de]	1195[fgh]	7657[a]	***
M43	Thymol	1299	A	nd	6.3[e]	171[e]	788[d]	1.0[e]	1.5[e]	nd	nd	nd	1089[cd]	nd	8.2[e]	1206[cd]	6.4[e]	nd	6.4[e]	nd	2577[a]	2352[a]	nd	2005[ab]	33[e]	1224[cd]	nd	nd	1429[bc]	2.6[e]	nd	nd	nd	***
M44	Myrtenyl acetate	1330	B[4]	5.2[b]	4.9[b]	nd	nd	4.6[b]	13[b]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	12[b]	nd	nd	nd	20[b]	nd	nd	6731[a]	nd	1.7[b]	nd	nd	nd	***
M45	Geranyl acetate	1382	A	41[bcd]	0.8[e]	5.9[de]	46[bc]	51[b]	4.1[e]	nd	52[b]	nd	nd	136[a]	nd	nd	nd	nd	nd	126[a]	7.9[de]	nd	nd	nd	11[cde]	nd	nd	nd	nd	nd	nd	nd	nd	***
M46	Ocimene quintoxide	1046	A	10[b]	5.7[b]	428[b]	nd	3.2[b]	14[b]	343[b]	18[b]	nd	nd	92[b]	nd	30[b]	nd	nd	26[b]	68[b]	nd	7.6[b]	10530[a]	nd	15[b]	nd	nd	nd	nd	nd	nd	nd	70[b]	***
	Total (%)			83	85	50	64	77	56	57	81	44	78	62	70	67	53	73	55	75	76	85	31	66	83	74	80	91	75	82	71	62	57	
	Other																																	
O1	Styrene	902	A	nd	nd	nd	nd	2.7[c]	nd	25[abc]	1.9[c]	nd	49[a]	13[bc]	nd	nd	nd	nd	30[ab]	7.7[bc]	nd	nd	nd	1.8[c]	11[bc]	nd	17[bc]	nd	nd	nd	nd	32[ab]	33[ab]	***

O2	Propyl 2-methylbutanoate	943	A	nd	nd	13^{d}	223^{d}	283^{d}	nd	2563^{bc}	nd	484^{cd}	1167^{c}	9386^{a}	9.5^{d}	4574^{b}	380^{cd}	nd	2085^{c}	nd	nd	nd	780^{cd}	1012^{c}	nd	nd	nd	nd	nd	nd	1898^{c}	830^{cd}	1085^{a}	***
O3	2-Methylphenol	1055	A	2.2^{d}	2.3^{d}	9.0^{bcd}	nd	nd	3.8^{cd}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	15^{b}	49^{a}	nd	nd	nd	nd	nd	nd	nd	12^{bc}	nd	***
O4	1-Terpinen-4-ol	1183	A	192^{fg}	5.1^{g}	40^{g}	nd	256^{fg}	2.3^{g}	570^{ef}	375^{fg}	nd	nd	nd	30^{g}	526^{ef}	17^{g}	nd	nd	nd	341^{fg}	320^{fg}	3000^{a}	2027^{b}	311^{fg}	816de	1116^{cd}	nd	1071^{cd}	1335^{c}	12^{g}	36^{g}	2149^{b}	***
O5	gamma-Octalactone	1263	A	nd	nd	18^{def}	nd	12^{defg}	10^{defg}	13^{defg}	8.7^{defg}	39^{bc}	8.9^{defg}	nd	76^{a}	8.5^{defg}	9.8^{defg}	nd	44^{b}	24^{cd}	13^{defg}	3.9^{fg}	nd	25^{cd}	nd	9.5^{defg}	18^{defg}	5.9^{efg}	10^{defg}	20^{def}	24^{cde}	9.0^{defg}	nd	***
O6	Methyl decanoate	1320	A	nd	3.2^{b}	15^{b}	nd	nd	7.8^{b}	nd	nd	7.1^{b}	9.3^{b}	nd	nd	nd	7.0^{b}	3692^{b}	6.0^{b}	nd	6.2^{b}	nd	nd	3.0^{b}	nd	nd	7.5^{b}	nd	6.4^{b}	7.3^{b}	nd	2.7^{b}	nd	***
O7	Neryl acetate	1359	A	nd	3.2^{d}	16^{cd}	nd	3.7^{d}	1.5^{d}	nd	5.6^{d}	nd	nd	nd	84^{a}	17^{cd}	nd	nd	nd	nd	nd	5.6^{d}	nd	nd	nd	nd	38^{bc}	nd	nd	46^{b}	nd	nd	8.8^{d}	***
O8	(*Z*)-Jasmone	1406	A	nd	nd	29^{cdefg}	7.9^{fg}	nd	nd	nd	nd	48^{abc}	nd	nd	nd	nd	13^{efg}	58^{abc}	13^{defg}	nd	nd	2.4^{fg}	nd	9.5^{fg}	nd	31^{cdef}	67^{ab}	nd	42^{bcde}	75^{a}	31^{cdef}	43^{bcd}	nd	***
O9	Caryophyllene oxide	1550	B[15]	nd	nd	7.7^{b}	nd	nd	nd	nd	nd	12^{a}	nd	nd	nd	nd	7.9^{b}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	***
	Total (%)			4.2	0.5	7.5	3.2	8.8	0.4	14	2.2	2.7	7.1	16	0.7	15	2.8	11	9.9	0.2	1.4	1.3	43	13	0.7	5.8	4.5	0.02	5.9	5.2	7.5	7.2	14	
	Phenylpropenoid																																		
P1	Estragole	1203	A	nd	15^{d}	292^{c}	nd	6.0^{d}	35^{d}	14^{d}	nd	534^{b}	3.6^{d}	36^{d}	49^{d}	9.4^{d}	309^{c}	nd	14^{d}	26^{d}	802^{a}	667^{ab}	nd	nd	nd	9.2^{d}	66^{d}	36^{d}	11^{d}	62^{d}	649^{ab}	515^{b}	31^{d}	***
P2	Safrole	1307	A	nd	nd	707^{c}	nd	10^{d}	3.9^{d}	nd	nd	1296^{b}	6.5^{d}	nd	nd	nd	3.6^{d}	15^{d}	nd	nd	24^{d}	18^{d}	4001^{a}	16^{d}	nd	nd	nd	nd	nd	nd	nd	nd	nd	***
P3	Eugenol	1366	A	5.2^{de}	13^{cde}	2.2^{de}	nd	1.6^{de}	23^{bc}	15^{bcde}	nd	3.0^{de}	17^{bcd}	nd	nd	nd	2.5^{de}	nd	54^{a}	nd	nd	nd	nd	7.2^{cde}	nd	9.7^{cde}	nd	nd	30^{b}	nd	16^{bcde}	17^{bcd}	nd	***
P4	Methyl eugenol	1397	B[16]	20^{bcdef}	4.5^{ef}	2.5^{f}	24^{bcdef}	28^{bcdef}	10^{bcdef}	18^{bcdef}	20^{bcdef}	nd	16^{a}	13^{a}	34^{a}	51^{a}	51^{a}	nd	11^{def}	9.7^{def}	13^{def}	24^{bcde}	nd	nd	nd	nd	34^{abc}	nd	nd	38^{ab}	nd	nd	nd	***
	Total (%)			0.5	0.8	13	0.3	0.7	0.7	0.2	0.1	8.3	0.3	0.1	0.3	0.2	2.3	0.04	0.4	0.1	3.3	2.7	12	0.1	0.0	0.1	0.4	0.1	0.2	0.4	2.6	4.0	0.03	
	Sesquiterpene																																		
S1	alpha-Copaene	1387	B[17]	5.1^{d}	7.2^{d}	33bc^{d}	nd	nd	22^{bcd}	61^{bc}	nd	nd	nd	8.7^{d}	63^{b}	131^{a}	nd	nd	nd	9.9^{cd}	7.7^{d}	nd	nd	nd	7.3^{d}	nd	nd	nd	nd	nd	nd	nd	nd	***

S2	beta-Caryophyllene	1439	A	75^{c}	119^{c}	8.7^{c}	nd	90^{c}	667^{c}	521^{c}	226^{c}	nd	nd	2548^{b}	nd	471^{c}	4.6^{c}	9.2^{c}	nd	2154^{b}	486^{c}	246^{c}	nd	nd	1739^{b}	nd	786^{c}	33^{c}	589^{c}	592^{c}	nd	nd	8355^{a}	***
S3	Aromadendrene	1457	B[18]	nd	0.8^{f}	212^{c}	6.8^{f}	8.2^{f}	7.5^{f}	29^{ef}	26^{ef}	580^{a}	nd	104^{d}	36^{ef}	68de	448^{b}	nd	5.2^{f}	26^{ef}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	***
S4	alpha-Humulene	1477	A	13^{d}	nd	1.7^{d}	nd	19^{d}	nd	113^{cd}	nd	32^{d}	55^{d}	nd	73^{cd}	nd	30^{d}	nd	30^{d}	nd	926^{a}	510^{b}	266^{c}	620^{b}	nd	nd	nd	nd	nd	nd	nd	71^{cd}	nd	***
S5	alpha-Muurolene	1493	B[19]	3.3^{ef}	5.5^{ef}	47^{cd}	nd	5.1^{ef}	4.0^{ef}	35de	29^{def}	121^{a}	nd	85^{b}	nd	68^{bc}	98^{ab}	nd	29^{def}	75^{bc}	7.5^{ef}	10^{ef}	nd	18^{def}	nd	nd	nd	nd	nd	nd	nd	nd	nd	***
S6	Valencene	1514	A	nd	nd	nd	nd	5.5^{de}	nd	17^{cde}	14^{cde}	33^{abc}	nd	41^{ab}	23^{bcd}	34^{abc}	25^{abcd}	nd	25^{abcd}	44^{a}	nd	nd	nd	9.9^{de}	nd	nd	nd	nd	6.3^{de}	nd	nd	1.2^{e}	nd	***
S7	Bisabolene	1521	B[20]	nd	nd	1.8^{ef}	nd	nd	18^{def}	51^{ab}	32^{bcde}	4.3^{ef}	4.1^{ef}	49^{abc}	19^{cdef}	63^{a}	5.6^{ef}	30^{bcde}	30^{bcde}	16^{def}	9.1^{def}	nd	nd	nd	14^{def}	nd	nd	nd	nd	nd	nd	nd	37^{abcd}	***
S8	Calacorene	1540	B[21]	5.4^{c}	nd	nd	nd	9.4^{c}	nd	9.9^{c}	nd	46^{a}	nd	nd	nd	15^{bc}	36^{ab}	46^{a}	18^{bc}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	***
	Total (%)			2.1	3.2	3.9	0.1	2.2	7.1	3.3	1.8	3.7	0.3	4.7	0.7	2.5	4.1	0.3	0.6	5.4	5.7	3.0	0.8	2.7	3.6	0.0	2.8	0.1	3.1	2.1	0.0	0.5	9.0	
	Unknowns																																	
U38	unknown	952		nd	106^{c}	358^{c}	nd	nd	342^{c}	nd	6.4^{c}	2052^{a}	nd	nd	1897^{a}	38^{c}	1376^{b}	nd	151^{c}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	***
U2	unknown	974		13^{c}	14^{c}	78^{a}	nd	nd	41^{b}	nd	nd	13^{c}	nd	nd	nd	nd	21^{c}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	***
U4	unknown	1066		9.4^{h}	24^{h}	3.8^{h}	35^{h}	nd	26^{h}	858^{b}	nd	nd	571^{bcdef}	304^{efgh}	nd	nd	nd	nd	55^{h}	221^{gh}	684^{bcd}	498^{cdefg}	270^{fgh}	579^{bcdef}	nd	447^{defg}	791^{bc}	nd	623^{bcde}	893^{b}	489^{cdefg}	428^{defg}	2659^{a}	***
U5	unknown	1113		nd	nd	nd	nd	3.8^{c}	3.8^{c}	nd	1.8^{c}	494^{a}	nd	nd	11^{c}	nd	289^{b}	nd	nd	5.4^{c}	nd	nd	nd	nd	nd	nd	3.3^{c}	nd	nd	4.3^{c}	nd	nd	19^{c}	***
U6	unknown	1130		29^{efgh}	16^{fgh}	5.8^{gh}	nd	0.9^{gh}	1.1^{gh}	72^{defg}	67^{defgh}	9.5^{gh}	7.8^{gh}	55de^{fgh}	179^{b}	143^{bc}	nd	255^{a}	16^{fgh}	38^{efgh}	46^{defgh}	nd	43^{defgh}	57^{defgh}	30^{efgh}	52^{defgh}	94^{cde}	171^{b}	87^{cdef}	110^{bcd}	nd	nd	82^{cdef}	***
U7	unknown	1135		24^{bc}	1.5^{d}	5.8^{cd}	40^{ab}	50^{a}	nd	nd	nd	9.6^{cd}	48^{a}	nd	51^{a}	nd	3.0^{d}	nd	nd	8.5^{cd}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	48^{a}	38^{ab}	nd	***
U54	unknown	1142		nd	nd	2.4^{b}	nd	nd	2.6^{b}	12^{ab}	2.8^{b}	nd	nd	24^{a}	nd	5.6^{b}	5.4^{b}	nd	3.0^{b}	2.9^{b}	nd	nd	nd	nd	nd	nd	7.5^{b}	nd	nd	4.4^{b}	nd	nd	8.0^{b}	***
U8	unknown	1147		4.2^{c}	nd	64^{b}	nd	7.6^{c}	28^{bc}	23^{c}	13^{c}	158^{a}	12^{c}	17^{c}	63^{b}	29^{bc}	64^{b}	nd	3.9^{c}	11^{c}	12^{c}	7.8^{c}	nd	18^{c}	nd	8.4^{c}	nd	nd	10^{c}	2.0^{c}	nd	7.7^{c}	nd	***
U55	unknown	1170		27^{a}	18^{ab}	nd	nd	nd	nd	nd	nd	nd	nd	11^{bc}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	***
U11	unknown	1175		nd	nd	17^{e}	18^{e}	nd	405^{d}	1056^{c}	nd	2341^{a}	25^{e}	1700^{b}	nd	25^{e}	nd	nd	1237^{c}	1298^{c}	201de	198de	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	***

U12	unknown	1190	7.2^{g}	nd	557^{cd} efg	331^{ef} g	13^{g}	57^{g}	24^{g}	nd	1061 bc	426^{cd} efg	942^{bc} de	998^{b} cd	nd	947^{bc} de	3754^{a}	638^{cd} efg	615^{cd} efg	23^{g}	8.0^{g}	253^{fg}	378^{def} g	4.8^{g}	280^{fg}	317^{ef} g	nd	306^{ef} g	360de fg	1382^{b}	906bc de^{f}	464^{cd} efg	***
U14	unknown	1216	nd	nd	10^{e}	42^{e}	nd	518^{cd}	nd	nd	7.7^{e}	nd	884^{b}	nd	nd	4.3^{e}	nd	1036^{b}	619^{c}	nd	nd	nd	nd	351^{d}	nd	nd	1427 a	nd	nd	nd	7.9^{e}	nd	***
U56	unknown	1222	nd	nd	nd	nd	nd	nd	nd	463^{cd} e	39^{gh}	nd	nd	68^{fgh}	1151 a	25^{gh}	274^{def} gh	nd	nd	411^{cd} ef	364^{def} g	nd	nd	6.3^{h}	nd	1086 a	219^{ef} gh	710^{bc}	934^{ab}	577^{cd}	522^{cde}	1174^{a}	***
U15	unknown	1227	nd	nd	74^{c}	890^{a}	nd	nd	nd	17^{c}	nd	nd	nd	10^{c}	44^{c}	nd	nd	15^{c}	20^{c}	58^{c}	16^{c}	313^{b}	nd	4.2^{c}	nd	21^{c}	nd	nd	22^{c}	nd	nd	nd	***
U16	unknown	1232	11^{e}	nd	nd	nd	nd	nd	126^{a}	nd	nd	11^{e}	nd	56^{b}	nd	nd	nd	26^{cde}	nd	70^{b}	nd	nd	23de	nd	45^{bcd}	nd	nd	52^{bc}	nd	8.7^{e}	6.1^{e}	nd	***
U19	unknown	1260	11^{cde} f	7.0^{de} f	46^{b}	58^{b}	88^{a}	nd	nd	nd	nd	nd	nd	nd	nd	22^{c}	nd	nd	2.7^{ef}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	16^{cde}	18^{cd}	***
U20	unknown	1286	51^{d}	43 ^{d}	19 ^{d}	8.9^{d}	36 ^{d}	1146 b	nd	620^{c}	53 ^{d}	14 ^{d}	nd	1917 ^{a}	nd	nd	nd	2.2 ^{d}	nd	nd	3.6 ^{d}	nd	7.7 ^{d}	nd	7.6 ^{d}	18 ^{d}	nd	14 ^{d}	8.9 ^{d}	nd	1.3 ^{d}	nd	***
U21	unknown	1316	3.5^{b}	4.4^{b}	4.5^{b}	nd	3.9^{b}	5.8^{b}	15 ^{b}	3.7^{b}	nd	nd	nd	12 ^{b}	2.8^{b}	1066 ^{a}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	***
U22	unknown	1335	13^{b}	4.8^{cd} e	11 ^{bc}	4.4^{de}	7.2^{bc} d	nd	nd	nd	23 ^{a}	nd	nd	nd	nd	2.1 ^{de}	nd	nd	nd	nd	5.3 cde	nd	2.3 ^{de}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	***
U23	unknown	1343	nd	nd	8.1^{a}	nd	7.0^{a}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	10^{a}	nd	nd	nd	nd	nd	nd	nd	nd	***
U42	unknown	1349	nd	nd	14^{a}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	16^{a}	nd	nd	5.8^{b}	nd	nd	nd	nd	nd	nd	nd	nd	nd	***
U24	unknown	1376	17^{c}	40^{b}	nd	15^{cd}	16^{c}	63^{a}	nd	nd	2.1^{cd}	nd	nd	nd	nd	3.9^{cd}	nd	nd	nd	nd	nd	nd	nd	12^{cd}	nd	nd	nd	nd	nd	nd	nd	10^{cd}	***
U57	unknown	1392	15^{b}	5.8^{b}	110^{a}	nd	7.7^{b}	11^{b}	nd	2.4^{b}	nd	nd	nd	nd	6.5^{b}	nd	nd	18^{b}	nd	nd	nd	nd	nd	nd	nd	nd	11^{b}	nd	nd	nd	nd	nd	***
U25	unknown	1401	nd	nd	7.1^{efg}	nd	27^{cdef}	nd	29^{cde}	23^{def} g	64^{ab}	71^{a}	nd	21^{def} g	45^{bcd}	1.7^{fg}	nd	16^{efg}	nd	nd	nd	nd	5.5^{efg}	nd	13^{efg}	nd	nd	29^{cde}	nd	6.2^{efg}	14^{efg}	50^{abc}	***
U26	unknown	1422	4.5^{b}	4.9^{b}	41^{b}	6.6^{b}	25^{b}	8.1^{b}	nd	11^{b}	31^{b}	14^{b}	14^{b}	nd	27^{b}	15^{b}	nd	250^{a}	7.6^{b}	nd	12^{b}	nd	11^{b}	6.6^{b}	nd	21^{b}	nd	9.7^{b}	22^{b}	nd	14^{b}	42^{b}	***
U43	unknown	1444	nd	3.2^{c}	nd	78^{c}	nd	14^{c}	nd	2.2^{c}	13^{c}	318^{a}	29^{c}	417^{a}	3.0^{c}	nd	nd	6.4^{c}	24^{c}	nd	nd	117^{bc}	280^{ab}	16^{c}	415^{a}	10^{c}	nd	nd	5.1^{c}	422^{a}	377^{a}	nd	***
U27	unknown	1464	nd	0.8^{c}	3.9^{c}	nd	nd	4.0^{c}	nd	2.9^{c}	1.3^{c}	nd	30^{c}	nd	11^{c}	2.7^{c}	nd	nd	312^{b}	nd	13^{c}	nd	nd	23^{c}	nd	9.7^{c}	1094 a	nd	2.5^{c}	nd	11^{c}	63^{c}	***
U28	unknown	1471	nd	25^{d}	5.6^{d}	nd	nd	115^{cd}	nd	43^{d}	6.7^{d}	8.4^{d}	375^{c}	nd	83^{cd}	nd	314^{cd}	48^{d}	nd	11^{d}	nd	nd	20^{d}	3060 a	68^{cd}	146^{cd}	nd	94^{cd}	125^{cd}	nd	9.5^{d}	1435^{b}	***
U44	unknown	1485	nd	nd	nd	15^{c}	nd	25^{abc}	9.6^{c}	nd	3.0^{c}	nd	9.6^{c}	nd	10^{c}	3.9^{c}	45^{ab}	nd	8.1^{c}	nd	nd	nd	nd	22^{bc}	nd	nd	nd	nd	nd	52^{a}	nd	nd	***
U45	unknown	1500	nd	nd	nd	nd	nd	1.4^{e}	nd	2.2^{de}	nd	nd	7.2^{cde}	36^{bc}	2.9^{de}	nd	59^{b}	7.5^{cde}	35^{bcd}	nd	nd	nd	2.4^{de}	21^{cde}	nd	nd	135^{a}	nd	nd	nd	nd	nd	***

U46	unknown	1510	nd	3.8^{ef}	8.3^{def}	nd	nd	19^{bcdef}	23^{abcde}	15^{cdef}	4.1^{ef}	nd	40^{a}	nd	38^{ab}	4.8^{def}	nd	16^{cdef}	35^{abc}	nd	4.2^{ef}	nd	nd	25^{abcd}	nd	5.1^{def}	nd	nd	9.8^{def}	nd	nd	41^{a}	***
U47	unknown	1529	nd	4.5^{cde}	0.6^{e}	nd	nd	nd	8.7^{bcde}	3.6^{de}	20^{bc}	nd	18^{bcd}	43^{a}	16^{bcde}	18^{bcd}	nd	2.2^{de}	21^{b}	nd	nd	nd	nd	nd	nd	5.1^{bcde}	nd	nd	9.3^{bcde}	nd	nd	nd	***
U58	unknown	1534	2.6^{e}	8.9^{de}	8.7^{de}	nd	3.6^{e}	35^{a}	9.3^{cde}	14^{bcde}	25^{abc}	6.7^{de}	30^{ab}	9.7^{cde}	21^{abcd}	14^{bcde}	nd	14^{bcde}	5.2^{de}	nd	nd	nd	15^{bcde}	nd	nd	nd	nd	nd	nd	7.5^{de}	nd	***
U48	unknown	1555	nd	nd	13^{b}	nd	nd	nd	nd	nd	8.3^{c}	nd	23^{a}	nd	nd	8.3^{c}	nd	nd	22^{a}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	***
U31	unknown	1560	nd	nd	nd	nd	nd	nd	11^{b}	13^{b}	9.4^{bc}	nd	nd	12^{b}	28^{a}	8.8^{bc}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	***
U49	unknown	1572	nd	nd	1.0^{b}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	5.4^{ab}	2.4^{ab}	nd	nd	nd	13^{a}	7.9^{ab}	nd	2.3^{ab}	nd	nd	4.7^{ab}	nd	8.3^{ab}	9.4^{ab}	***
U59	unknown	1592	nd	nd	nd	nd	nd	nd	nd	nd	12^{c}	2.7^{d}	nd	nd	nd	11^{c}	nd	nd	nd	nd	nd	nd	2.1^{d}	20^{b}	nd	9.9^{c}	nd	nd	12^{c}	nd	nd	32^{a}	***
U50	unknown	1602	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	3.8^{d}	nd	nd	0.9^{d}	nd	nd	5.2^{d}	9.7^{d}	12^{d}	nd	12^{d}	109^{b}	nd	47^{c}	nd	48^{c}	54^{c}	nd	7.7^{d}	200^{a}	***
U60	unknown	1611	4.2^{g}	3.8^{g}	nd	7.0^{fg}	0.8^{g}	8.4^{efg}	19^{defg}	12^{efg}	7.1^{fg}	3.4^{g}	59^{ab}	26cdef	21defg	10^{efg}	nd	nd	55^{ab}	64^{a}	42^{bc}	33^{cd}	26^{cdef}	nd	29^{cde}	3.2^{g}	nd	16^{defg}	3.2^{g}	nd	13^{defg}	nd	***
U51	unknown	1619	nd	nd	2.3^{d}	nd	nd	nd	nd	nd	3.5^{bcd}	6.7^{ab}	nd	nd	nd	6.4^{abc}	nd	nd	nd	nd	nd	nd	2.7^{cd}	nd	nd	nd	nd	nd	nd	nd	7.5^{a}	nd	***
U52	unknown	1627	nd	nd	23^{c}	nd	nd	nd	nd	nd	39^{b}	3.6^{d}	nd	nd	nd	56^{a}	nd	nd	nd	nd	nd	nd	1.5^{d}	43^{b}	nd	nd	nd	nd	nd	nd	nd	nd	***
U32	unknown	1639	nd	nd	0.7^{f}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.7^{ef}	nd	nd	nd	39^{b}	27^{bcd}	21^{cd}	17de	19^{d}	nd	nd	31^{bcd}	nd	nd	36^{bc}	1.8^{f}	54^{a}	***
U33	unknown	1659	2.5^{h}	3.7^{gh}	1.2^{h}	nd	1.7^{h}	1.9^{h}	nd	5.7^{fgh}	nd	8.6^{fgh}	17^{cdef}	7.9^{fgh}	12^{efgh}	9.6^{fgh}	nd	nd	14^{defg}	22^{bcde}	22^{bcde}	nd	30^{ab}	34^{a}	9.4^{fgh}	24^{abcd}	nd	nd	28^{abc}	4.1^{gh}	12^{efgh}	nd	***
U61	unknown	1673	nd	6.4^{defg}	1.4^{fg}	nd	1.1^{fg}	3.9efg	8.0^{cdefg}	nd	nd	nd	22^{c}	14^{cdef}	22^{c}	5.1^{efg}	nd	nd	20^{cd}	41^{b}	40^{b}	nd	21^{c}	13^{cdefg}	17^{cde}	39^{b}	nd	nd	nd	nd	nd	55^{a}	***
U34	unknown	1681	3.5^{gh}	nd	6.8^{efgh}	nd	5.2^{fgh}	nd	15de	11^{efg}	8.2^{efgh}	15de	nd	nd	nd	12^{efg}	nd	nd	nd	14^{def}	8.7^{efg}	nd	31^{b}	32^{b}	nd	nd	nd	26^{bc}	43^{a}	4.7^{gh}	21^{cd}	nd	***
U35	unknown	1693	nd	nd	7.8^{de}	nd	nd	nd	nd	nd	14^{cd}	nd	nd	nd	nd	19^{bc}	nd	nd	nd	32^{a}	27^{ab}	nd	24^{ab}	nd	nd	3.0^{e}	22^{bc}	nd	6.4^{de}	nd	nd	nd	***
	Total (%)		17	15	50	36	23	44	43	19	56	22	38	29	32	46	26	45	25	24	15	69	34	17	26	20	9	25	18	29	38	43	

[a] Linear retention index (LRI) on a DB-5 column. [b] A, mass spectrum and LRI agree with those of authentic compounds; B, mass spectrum (spectral quality value >80 was used) and LRI agrees with reference spectrum in the NIST/EPA/NIH mass spectra database and LRI agree with those in the literature cited [1] (Morteza-Semnani et al 2005); [2] (Mevy et al 2006); [3] (Adams et al 2006); [4] (Adams et al 2006); [5] (Angioni et al 2006); [6] (Lucero et al 2006); [7] (Özel et al 2006); [8] (G Flamini et al 2006); [9] (Adams et al 2005); [10] (Gohari et al 2006); [11] (Cho et al 2007); [12] (Patil et al 2009); [13] (Hamm et al 2005); [14] (Blázquez et al 2003); [15] (Marongiu et al 2003); [16] (Adams et al 2006); [17] (Angioni et al 2006); [18] (Buchin et al 2002); [19] (Adams et al 2006);[20] (Bell 2004); [21] (Schwob et al 2002). [c] Estimated abundance collected in the headspace of rosemary samples containing 2.8 mL of saturated calcium chloride, calculated by comparison with of 100 ppm propyl propanoate used as internal standard; internal standard was used to normalise chromatograms; means of three replicate samples are shown; means not labelled with the same letters are significantly different (0.05) and Tukey's HSD multiple pairwise comparison; nd - not detected; ns - not significant probability obtained by ANOVA, * significant at the 5 % level; ** significant at the 1 % level; *** significant at 0.1 % level.

Multiple factor analysis

Multiple factor analysis was used to visualise the differences in environmental factors and the chemical composition (table) observed for the season of autumn (A) and summer (S), for the years of 2018, 2019, 2020 and 2021 (Figure 4.3). Environmental conditions including temperature, water supply, nutrition and light are known to influence the synthesis and accumulation of plant secondary metabolites (Akula and Ravishankar 2011, Arbona et al 2013, Miller et al 2008). Rosemary was grown during different seasons and years, variation of results were detected between samples, where first (F1) and second (F2) dimension explain 25.33 % of the total variation within data. The first axis separated rosemary grown in the autumn and summer of 2018 and summer of 2021 from the other seasons, whilst the second axis separated summer 2018, 2019 and 2021 from the other seasons of production. Rosemary samples were from different varieties and some unknown ones, where varieties Barbeque and Perigord correlate and highly associated with some monoterpenes including linalool (M21) (citrus and floral aroma notes) and some other compounds, whereas Miss Jessops was highly associated with compounds including gamma-terpineol (M34) and neral (M37) (pine, floral, sweet and citrus aroma notes), additionally rosemary from unknown variety was highly associated with eucalyptol (M15) and geraniol (M38) (eucalyptus, herbal, sweet and floral odour notes). Rosemary was produced in sites in England, with Reading and Norwich positively correlated and associated with compounds eucalyptol (M15), (*E*)-2-heptenal (AL3) and geraniol (M38). Furthermore, West Sussex and Worcester were highly associated with linalool (M21) and thymol (M43), whereas rosemary from York was associated with some minor monoterpenes including gamma-terpineol (M34) and fenchol (M24). Rosemary produced in pots was associated with mostly monoterpenes including linalool (M21) and isoborneol (M30), when produced in field under protected conditions resulted in high association with minor compounds

like neral (M37), conversely open field production was associated with come some main compounds like eucalyptol (M15) and geraniol (M38) (Figure 4.3).

Maturity of crop at time of harvest showed opposite correlations in volatile composition of rosemary, samples harvested when 'fully matured' or as first cut were negatively correlated with other maturities (Figure 4.3) and highly associated with main compounds like borneol (M31), alpha-pinene (M3), camphene (M4) and camphor (M26), conversely second cut and target of 17 cm were highly associated with isoborneol (M30), linalool (M21) and thymol (M43). These results show that older plants (fully matured and first cut) have higher abundance of main volatiles, however further cuts lead to synthesis of other compounds, this agrees with Zigene et al (2012) findings where older plants (harvested 11 months after transplanting) started displaying adverse effects on the aroma (Zigene et al 2012).

The type of soil influences water and nutrition intake by the plant, rosemary produced in loamy soil was associated with some of the main compounds like eucalyptol (M15), peat was highly associated with some minor compounds and linalool (M21), whereas samples produced in loamy/clay soil were associated with compounds including camphor (M26) and borneol (M31).

The influence of application or non-application of fertilizers on the aroma profile was examined (Figure 4.3), where the application of fertilisers was negatively correlated with main compounds and highly associated with minor compounds including gamma-terpineol (M34) and neral (M37), conversely not using fertilisers was associated with compounds including eucalyptol (M15) and geraniol (M38).

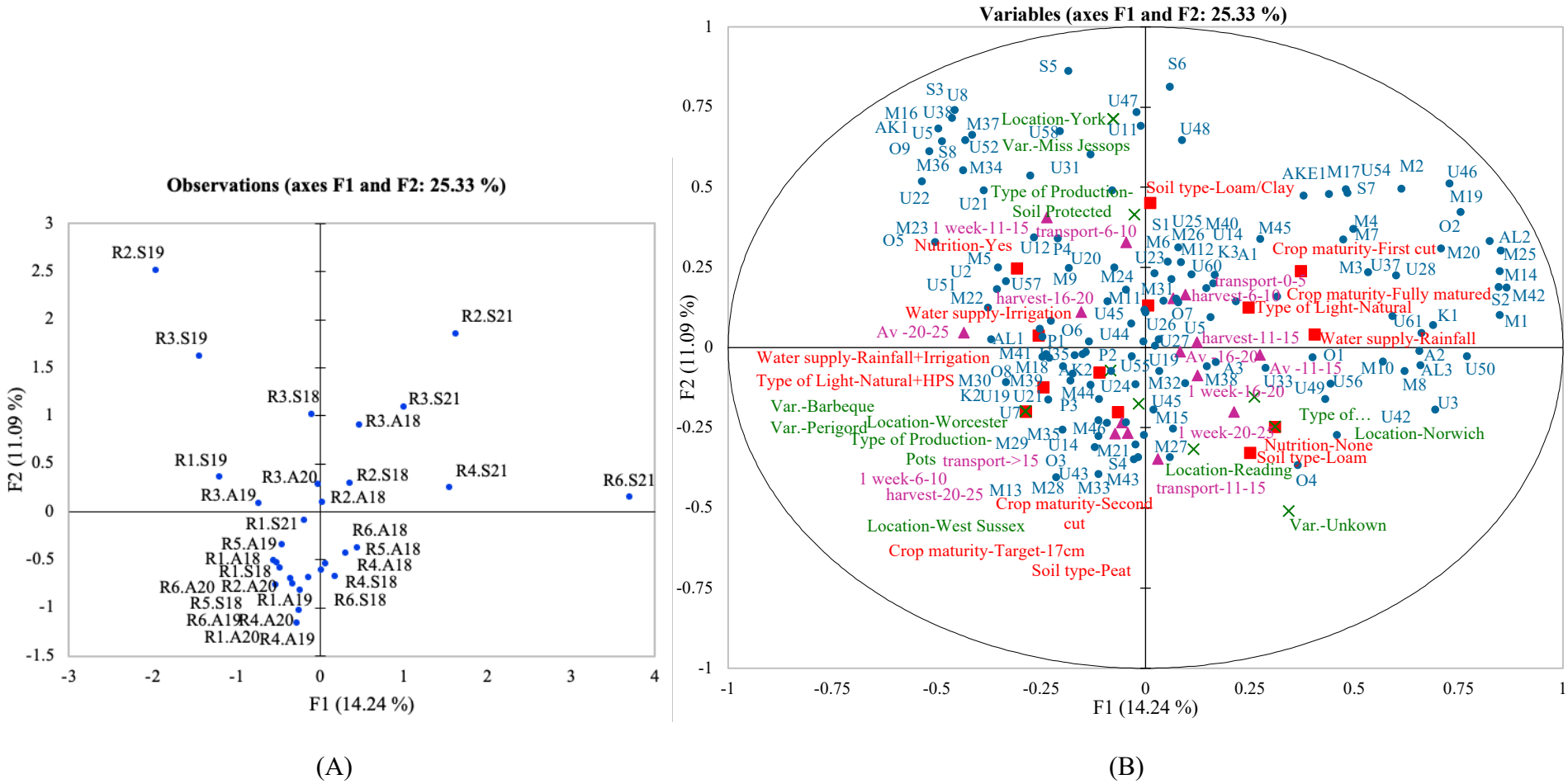
The source of water and amounts can affect the volatile composition of plants, present results show irrigation associated with minor compounds including gamma-terpineol, whereas the combination of rainfall and irrigation was associated with some minor compounds and linalool (M21). The use of rainfall was highly correlated with principal compounds including camphor (M26), alpha-pinene (M3) and camphene (M4). Similar rainfall amounts (1.8-2.2 mm day^{-1}), whilst the combination of rainfall and irrigation resulted in higher amounts (>2.2 mm day^{-1}) and the use of irrigation resulted in lower amounts (<1.7 mm day^{-1}), suggesting more aromatic rosemary plants when produced using amounts of water between 1.8-2.2 mm day^{-1}, and lower or higher differences will result in adverse effects, however further research would need to carried out since other growing factor and plant's genotypes could be affecting these associations.

Light source showed associations with composition, where sunlight was negatively correlated with combination of natural and high pressure sodium (HPS) and was highly associated with main compounds including camphor (M26), camphene (M4) and alpha-pinene (M3), whereas the combination of both types of lighting was highly associated with some minor compounds and linalool (M21). Similar sunlight photoperiods were experienced in all open field samples (12 h day^{-1}) and protected field (12-15 h day^{-1}), however this was also similar to pot's sunlight photoperiod (13-16 h day^{-1}), suggesting that the use of HPS lighting leads to lower abundances of rosemary's volatile compounds, however other growing effects might be influencing these associations.

Temperature has been identified as a factor that affects the aroma composition of plants, rosemary produced at an average growth temperature of 20-25 ˚C, expressed negative association with main compounds, conversely rosemary grown at 11-15 ˚C and 16-20 ˚C displayed a positive correlation with main volatiles (Figure 4.3) and high association with

compounds like borneol (M31). Literature has described higher volatile content and higher abundances of main compounds such as eucalyptol and camphor when rosemary was produced at higher temperatures during the summer season, however no indication of temperature range or variety of samples which could explain the differences with present study results (Lakušić et al 2012, Salido et al 2003). Temperature exposure of plants one week before harvest was also correlated with composition, where temperatures of 16-20 ˚C and 20-25 ˚C expressed a small positive association with some compounds including eucalyptol (M15) and geraniol (M38), temperatures of 6-10 ˚C were negatively correlated with main compounds but highly associated with minor compounds and linalool (M21), conversely temperatures of 11-15 ˚C were negatively correlated with main compounds and associated with minor compounds. Additionally, associations between temperature on day of harvest and the composition were observed (Figure 4.3), whereby 6-10 ˚C and 11-15 ˚C were highly associated with main compounds, conversely temperatures of 16-20 ˚C and 20-25 ˚C showed a positive correlation with minor compounds and a negative correlation with main volatiles. Transport temperatures with higher correlation with main compounds were of 0-5 ˚C, this confirms literature findings where post-harvest temperatures of 0-5 ˚C in rosemary allowed for the preservation of its quality (Cantwell and Reid 1993, Chadwick 2018). Transport at 6-10 ˚C showed high correlation with some unknown compounds and higher temperatures (> 10 ˚C) expressed low correlation with most compounds apart from linalool (M21) and eucalyptol (M15).

Rosemary results demonstrate that variety of the plant plays a fundamental role in determining the aroma composition of the herbs, however differences in environment during growth will further determine the aroma profile of the crop. All growing factors affected the composition of rosemary plant, making it difficult to identify individual conditions and their effect on profile. It's necessary to compare rosemary from the same variety to fully understand what conditions are affecting significantly the aroma profile of the plant.



(A) (B)

Figure 4.3:Multiple factor analysis of six rosemary samples harvested in the summer and autumn for the years of 2018, 2019, 2020 and 2021 showing correlations with volatile compounds and growing conditions. (A)Projection of samples; (B) Distribution of variables: green cross-grower information; pink triangles-temperature; red squares-environment conditions; blue circle-volatile compounds.

4.4 Conclusion

Varieties of herbs play a predetermining role in defining the compounds responsible for a herb's aroma, with major differences in the profile observed due to the different genotypes. However, completing the aroma profile it was observed significant differences influenced by the type of production, where pot produced result in low compounds abundances for every herb.

Maturity of the plant at point of harvest was observed to play a role in the composition, with mature plants from first cut displaying higher abundances of volatiles and determining compounds to the aroma, additionally second cut rosemary expressed higher amounts of other compounds and unidentified ones, which might be due to induced reactions after first cut. Furthermore, type of soil, nutrition, light source, photoperiod and water supply exhibited influence in the volatile composition, where growth under hydroponic conditions resulted in higher amounts of volatile compounds for basil, similarly with loam/clay soil for coriander and rosemary. Adding fertilisers was beneficial for coriander, however no significant effect was observed for Lamiaceae herbs. Water supply was dependent on the herb, with amounts of rainfall displaying an effect on the aroma profile.

The influence of temperatures on the aroma composition was also evident in this study, with different volatiles composition observed when herbs were grown at different average temperatures, different temperature at time of harvest and during transport. Basil was not affected by transport due to short transport durations and positively affected when exposed to low temperature stresses a week before harvest. Average growth temperature was specific to each herb, with temperatures between 10-20 ˚C associated with most compounds, however low and high extremes resulted in a negative correlation with main compounds. This suggests that

temperatures must be selected for each herb, and exposing to stress temperatures before harvest could have a beneficial effect on the aroma profile.

Apparent differences were detected in the flavour profiles of the three herbs influenced by growing conditions, however, it is impossible to conclude which would be the most appealing to the consumer without carrying out consumer preference trials on these herbs. Combining data collected from this study and information collected from other experiments with consumer preference tests will help understand which attributes consumers find the most important in culinary herbs, including preferences for bitter, sweet and flavour intensity. The findings from this study will provide the herb growers community with a better understanding of the flavour variation of their crops. Additionally, growers will benefit by elucidating how environmental factors including temperature, light source, water source and type of production influence the flavour profile of herbs and a better understanding on how this will affect the sensory perception. Merging all these findings will help with crop selection and production, leading to higher quality and more consistent products.

Chapter 5: Investigating the relationship of volatile composition and sensory profile with rosemary (Rosmarinus officinallis var. Miss Jessops) plant and leaf maturity

5.1 Introduction

Rosmarinus officinallis, commonly known as rosemary, is an aromatic herb with needle like leaves that belongs to the Lamiaceae family. Due to its strong aroma, rosemary essential oil is widely used in the cosmetic and pharmaceutical industries, and its leaves are also used in culinary applications in both fresh and dried form (Hcini et al 2013, Socaci et al 2008, Zigene et al 2012). Compounds that constitute the rosemary aroma profile are mainly from the monoterpenes class, with main constituents being camphor; 1,8-cineole; alpha-pinene; borneol; limonene. These compounds have been identified as responsible for odour characteristic of rosemary, described as 'lemon', 'lavender', 'eucalyptus' and 'musk' aroma (van der Walt et al 2013). The main differences in chemical profile found between rosemary samples is typically attributed to the impact arising from different plant varieties (Lakušić et al 2012, Pintore et al 2002, Salido et al 2003, Socaci et al 2008).

Rosemary is a perennial herb, which means it has the ability to generate leaves every year and live during multiple years. Therefore, perennial plants get exposed to multiple external stresses but also internal factors including plant growth and photosynthesis rate (Munné-Bosch 2007). It has been reported that mature plants develop an adaptation to abiotic stresses when these have a history of exposure to previous abiotic stresses (Oñate et al 2011). The ageing of the plant and increase in size, will lead to reduction of photosynthesis due to stomatal closure to help maintain water homeostasis, resulting in lower production of primary and secondary metabolites (Munné-Bosch 2007). Changes in the shoot meristems can happen during repeated

cell division and become permanent during plant developments, affecting stomatal conductance and photosynthesis rate in leaves originated from these meristems (Munné-Bosch 2007). These changes in photosynthesis rate will affect the production of organic compounds such as carbohydrates and proteins, further affecting the production of secondary metabolites which use these as precursors (Cruickshank 2012, Rosenthal and Berenbaum 2012). Secondary metabolites are necessary for plant survival in the ecosystem due to their interactions with the environment, by conferring resistance to environmental stresses including certain temperature and light regimes, and also by communicating with other organisms such as attracting pollinators or as anti-herbivore activity (Dudareva et al 2004, Rosenthal and Berenbaum 2012, Verpoorte 2000).

Time of harvest and harvesting stage have an influence on the yield and the essential oil composition of aromatic crops. A study analysing sweet marjoram (*Majorana hortensis*) essential oil reported higher essential oil yield from at flowering stage that vegetative stage, however the main volatile compound (cis-sabinene) was at lowest abundance in this stage (Verma et al 2010). Similarly, *Tagetes minuta* aerial parts showed the highest essential oil yield at budding stage, followed by the flowering stage (Moghaddam et al 2007). Conversely, a study on rosemary plants detected a highest essential oil content in samples harvested 10 months after transplanting, however a decrease in essential oil yield was detected in samples 11 months after transplanting (Zigene et al 2012). A study using sage (*Salvia officinalis*) detected the highest essential oil yield after the flowering stage followed by vegetative stage, and lowest essential oil yield prior and during flowering stage (Maric et al 2006). Rosemary grown in Portugal was reported to be affected by the harvest stage, where first cut samples exhibited higher essential oil yield and higher abundance of alpha and beta-pinene than subsequent cuts, however no significant effect was detected on the oil composition (Serrano et al 2002).

The aim of the present study was to investigate the relationship between plant and leaf maturity of rosemary on the volatile composition of the herb. Maturity was considered both in terms of leaf age and plant age, enabling comparison between old leaves from young plants and vice versa. Sensory evaluation using a trained panel was completed to understand how chemical changes affect sensory perception. Finally, this information can be used to help growers get more out of the crop and understand what will significantly affect their product during consumption.

5.2 Materials and methods

5.2.1 Plant material

The variety of rosemary samples was selected from previously analysed samples in order to investigate if plant and leaf maturity would influence the flavour of the herb. The same variety of rosemary (*Rosmarinus officinalis* var. Miss Jessops) was sourced and delivered by two growers based in the United Kingdom (UK). This material was harvested at commercial maturity, around 15-20 cm, in order to mimic products delivered to commercial chains and consumers.

Samples of rosemary sown under protected field conditions (old plants) or rosemary produced in pots (young plants) were used for plant maturity comparison (Table 5.1). Leaf maturity analysis was carried out by separate assessment of top leaves (young) and bottom of the sprig leaves (older). Rosemary grown in an open field setting was cut a second time, nine weeks after first analysis. Samples were harvested the day before assessment and for each sample three independent replicates were used. However, rosemary from the present study was produced at two production sites, due to the industry growers involved in the project, resulting

in differences in growth temperature, soil type, irrigation volume and daylight length. This information was supplied by growers when records were available, with some information not shared due to commercial confidentiality

Table 5.1: Location, plant and leaf maturity and growth environment for each sample of rosemary.

Location	GPS coordinates	Sample	Plant age	Cut	Leaf age	Type of production	Av T[A] (˚C)	Soil	Water source[B]	Light source[C]
West Sussex	50.4848˚N 0.4413˚W	Rc	Young		Young	Pot	16-20	Peat	Ir	15h-Sl
		Rd			Old					
York	54.1345˚N 1.2430˚W	Ra	Old	First	Young	Field protected	16-20	Loam Clay	1.4mm-Ir	15h-Sl
		Rb			Old					
		Re		Second	Young				1.4mm-Ir	14h-Sl
		Rf			Old					

[A] Average temperature over 24 h; [B] Average water amount and water source used: I- irrigation and Rf- rainfall; [C] Average photoperiod and light source used: Sl- sunlight

First week of analysis was carried during the summer season of 2021, at commercial maturity and sent by a courier in boxes with cooling packs. Second week of analysis was carried out at the end of the summer season (second week of September) to allow nine weeks between plant cuts. Samples were received within a day of harvest and were washed and cut to separate leaves of different maturities. Samples were placed in small bags and kept at 5 ºC until sensory and laboratory analysis was undertaken.

5.2.2 Chemical reagents

Chemical reagents used for preparation and analysis of samples of present study are described in Chapter 2: in subsection 2.2.3 (page 57).

5.2.3 Solid Phase Microextraction (SPME) followed by GC-MS

Herb samples were prepared as described in Chapter 2:,subsection 2.2.2 and analysis of samples on the SPME GC-MS was carried out as described in subsection 2.2.4

5.2.4 Sensory evaluation of fresh rosemary samples

Sensory evaluation was carried out using quantitative descriptive analysis (QDA) to determine sensory characteristics of six rosemary samples and estimated quantitatively. The trained sensory panel at the Sensory Science Centre (University of Reading, n=11; 10 female and 1 male), was used to assess samples using previously developed consensus vocabulary for fresh rosemary. The consensus vocabulary consisted of 24 attributes describing appearance, aroma, taste, flavour, mouthfeel and aftereffects, which were refreshed before sample's scoring sessions. Samples were assessed in assessors' home due to limitations for assessment on site as consequence of the pandemic. Members of the panel collected rosemary samples on the day of first assessment that were previously prepared. The panellists scored a total of six rosemary samples with each sample being in duplicate, in separate sessions, and the data was collected using Compusense Cloud Software. Samples were presented using a random three-digit number, which were provided in a monadic balanced order, with samples sets allocated randomly to panellists. The panellists were asked to assess appearance first, break the leaves to assess the aroma, and to eat some leaf material to assess the flavour and mouthfeel; this was followed by a 30 s delay to assess the aftereffects. The intensity of each attribute was scored on a 100 point unstructured line scale. Between each sample panellists were asked to cleanse their palate using water and plain yogurt. Assessment of second cut samples was done nine weeks after the assessment of first cut and young plant samples.

5.2.5 Statistical analysis

Statistical analysis of relative abundance of each compound was carried out as described in Chapter 3: (subsection 3.2.5), additionally analysis of the data from the sensory panel followed the method described in Chapter 3: subsection 3.2.5.

5.3 Results and Discussion

5.3.1 Volatile composition

In total, 84 compounds were detected in the headspace of the rosemary from six different stages with each analysed in triplicate (Table 5.2). The compounds detected included 33 monoterpenes, ten sesquiterpenes, three aldehydes, two alcohol and 25 unidentified compounds. Quantitative differences in the aroma profiles were observed between six maturity samples of this study, confirmed by one-way ANOVA. Older plants of rosemary expressed the highest amounts of volatile compounds, furthermore older leaves of older plants displayed the higher contents of monoterpenes, sesquiterpenes, other and unidentified compounds. No significant differences in relative amount were found in 27 compounds.

Table 5.2: Relative abundance of aroma compounds identified in the headspace of fresh rosemary samples.

Code	Compound name	LRI [a]	ID [b]	Relative abundance [c]						*p*-value
				Ra	Rb	Rc	Rd	Re	Rf	
	Alcohol									
A1	*(Z)*-3-Hexen-1-ol	857	A	nd	nd	nd	nd	57	nd	
A2	Ipsdienol	1153	B[1]	44[a]	44[a]	17[a]	19[a]	29[a]	nd	*
	Total (%)			0.07	0.04	0.04	0.05	0.12	0.0	
	Aldehyde									
AL1	(*Z*)-3-Hexenal	800	A	68[a]	134[b]	75[a]	nd	nd	nd	***
AL2	(*E*)-2-Hexenal	855	A	466[ab]	901[a]	335[b]	328[b]	215[b]	347[b]	**
AL3	Heptanal	903	A	nd	nd	nd	nd	18	nd	

	Total (%)			0.8	0.9	0.9	0.8	0.3	0.3	
	Monoterpene									
M1	Tricyclene	930	B[2]	100[a]	316[b]	94[a]	89[a]	146[ab]	224[ab]	**
M2	Camphene	960	A	4997[ab]	9094[a]	3363[b]	3313[b]	5608[ab]	7899[ab]	**
M3	Sabinene	982	A	135	175	136	77	183	223	ns
M4	beta-Pinene	988	A	5870[ab]	8803[a]	3861[b]	3449[b]	4882[b]	5904[ab]	**
M5	Myrcene	997	B[3]	5544[ab]	10116[a]	3878[b]	4103[b]	5717[ab]	8700[ab]	**
M6	alpha-Phellandrene	1012		163[ab]	295[ab]	125[a]	139[ab]	232[ab]	340[b]	*
M7	(1-Methylpropyl)benzene	1016	A	39	51	32	32	51	51	ns
M8	delta-3-Carene	1025	B[4]	451[ab]	1128[a]	334[b]	484[ab]	645[ab]	1087[a]	**
M9	Limonene	1044	B[5]	11734	17362	9927	7160	12272	17260	ns
M10	Eucalyptol (1,8-cineole)	1045	B[6]	nd	5872	nd	nd	nd	nd	*
M11	beta-Ocimene	1051	B[7]	55	66	28	26	77	92	*
M12	gamma-Terpinene	1067	A	841[ab]	2213[c]	669[b]	1037[abc]	1178[abc]	1975[ab]	**
M13	(Z)-Sabinene hydrate	1076	A	393[ab]	606[a]	258[bc]	268[bc]	266[bc]	168[c]	***
M14	Terpinolene	1097	A	1496[abc]	3105[a]	1077[b]	1286[bc]	1834[abc]	2834[ac]	**
M15	Linalool	1104	A	962[ab]	1421[a]	580[b]	596[b]	806[b]	876[ab]	**
M16	alpha-Thujone	1108	A	131[a]	254[b]	85[a]	111[a]	95[a]	nd	***
M17	Fenchol	1127	A	nd	24[ab]	nd	8.8[b]	nd	34	**
M18	beta-cis-Ocimene	1136	B[8]	89[ab]	241[c]	52[b]	60[b]	111[ab]	164[ac]	***
M19	Camphor	1163	A	6012[a]	11682[b]	3881[a]	3720[a]	6469[ab]	8736[ab]	**
M20	Pinocarvone	1177	B[9]	23	nd	nd	nd	nd	nd	
M21	Borneol	1182	A	2916	2766	1478	1114	2265	2313	ns
M22	1-Terpinen-4-ol	1190	A	646[a]	1264[b]	397[a]	472[a]	690[ab]	978[ab]	**
M23	alpha-Terpineol	1203	A	1385[ab]	2189[a]	877[b]	827[b]	1260[ab]	1635[ab]	*
M24	Myrtenol	1210	B[10]	53[ab]	91[a]	19[b]	33[ab]	40[ab]	69[ab]	*
M25	Verbenone	1226	B[11]	1201[ab]	2582[a]	558[b]	898[b]	1571[ab]	2537[a]	**
M26	Neral	1248	A	42[a]	134[b]	28[a]	45[a]	54[a]	80[ab]	**
M27	Geraniol	1258	A	37	63	85	23	38	47	ns
M28	Piperitone	1269	A	16	76	nd	15	20	33	ns
M29	Geranial	1277	A	68[a]	184[b]	46[a]	57[a]	92[ab]	134[ab]	**

M30	Bornyl acetate	1301	B[12]	6875	5572	5413	3160	5945	4748	ns
M31	Geranyl acetone	1459	B[13]	nd	27a	10b	10b	nd	nd	***
M32	alpha-Pinene	944	A	8369[ab]	16515[ab]	5618[a]	6519[ab]	10924[ab]	18130[b]	*
M33	Thuja-2,4(10)-diene	963	B[14]	41[ab]	80[ab]	28[a]	20[a]	71[ab]	100[b]	*
	Total (%)			90.4	87.5	90.1	90.7	88.7	85.1	
	Other									
O1	1-Ethyl-3-methylpyrrole	911	A	nd	24[a]	18[a]	nd	26[a]	nd	ns
O2	Methyl propyl disulfide	934	A	1119[a]	2002[b]	1061[a]	1186[a]	1319[ab]	1536[ab]	*
O3	3-Octanone	989	A	nd	5313	nd	nd	1107	2848	ns
O4	p-Cymen-8-ol	1195	B[15]	27[a]	51[b]	nd	15[a]	nd	nd	***
O5	Ethyl decanoate	1392	A	158[ab]	386[a]	64[b]	72[b]	183[ab]	282[ab]	*
O6	Methyleugenol	1409	A	41	56	37	28	44	95	ns
O7	2-Methylbutyl octanoate	1449	A	2751	3552	1972	1584	2837	3358	ns
O8	delta-Decalactone	1508	A	35[a]	nd	25[ab]	12[ab]	20[ab]	nd	*
O9	6-Methylcoumarin	1589	A	21[ab]	27[a]	11[b]	9.0[b]	nd	nd	***
O10	delta-Undecalactone	1616	A	114[a]	106[ab]	84[abc]	40[bc]	29[c]	28[c]	**
O11	Methyl jasmonate	1668	A	33	29	23	10	15	nd	**
	Total (%)			6.4	9.7	6.9	6.8	7.8	7.9	
	Sesquiterpene									
S1	alpha-Copaene	1397	B[16]	nd	29	nd	nd	15	nd	***
S2	Caryophyllene	1432	B[17]	16	33	nd	nd	43	70	*
S3	alpha-Humulene	1474	A	43	50	24	18	70	102	ns
S4	Germacrene D	1499	A	47[a]	64[a]	nd	nd	44[a]	187[b]	***
S5	Valencene	1516	A	42	54	29	31	39	nd	**
S6	Bisabolene	1523	B[18]	75	127	43	45	80	109	ns
S7	beta-Sesquiphellandrene	1539	B[19]	40	56	25	21	43	56	ns
S8	Calamenene	1547	B[20]	nd	59	23	16	45	72	*
S9	(*E*) or (*Z*)-Nerolidol	1569	A	62	90	24	23	61	88	*
S10	Caryophyllene oxide	1607	A	16	nd	17	nd	10	nd	ns
	Total (%)			0.5	0.5	0.4	0.4	0.6	0.7	
	Unknown									
U62	unknown	790		nd	nd	nd	85[a]	51[b]	nd	***
U37	unknown	848		nd	nd	12[a]	12[a]	nd	nd	ns

U63	unknown	870	nd	nd	3.5[a]	nd	30[a]	18[a]	ns
U64	unknown	892	nd	nd	nd	nd	12	nd	
U40	unknown	1034	nd	nd	178	nd	524	1093	ns
U65	unknown	1040	nd	nd	nd	nd	nd	3800	
U5	unknown	1112	nd	31[a]	nd	nd	17[a]	49[b]	**
U6	unknown	1121	26	52	17	16	57	33	ns
U8	unknown	1148	29	58	26	18	36	50	ns
U12	unknown	1185	nd	49[a]	nd	nd	22[a]	nd	ns
U14	unknown	1214	nd	37	nd	nd	nd	nd	
U16	unknown	1235	20[ab]	29[a]	nd	8.8[b]	22[ab]	nd	***
U20	unknown	1285	nd	28	nd	nd	14	67	ns
U21	unknown	1312	nd	nd	11	nd	nd	nd	
U25	unknown	1401	nd	39	nd	nd	22	46	ns
U26	unknown	1417	nd	18	nd	nd	nd	nd	
U43	unknown	1454	38[ab]	42[ab]	25[ab]	16[a]	74[ab]	96[b]	*
U27	unknown	1465	159	202	85	63	145	171	ns
U28	unknown	1482	550	598	294	212	406	481	ns
U45	unknown	1502	149[ab]	241[a]	66[b]	64[b]	141[ab]	nd	**
U29	unknown	1517	77	103	nd	nd	55	72	ns
U47	unknown	1530	90	111	43	27	63	75	ns
U30	unknown	1544	64	43	20	19	50	34	ns
U32	unknown	1649	16	nd	19	nd	nd	nd	ns
U34	unknown	1685	43	42	33	20	32	35	ns
	Total (%)		1.9	1.4	1.7	1.3	2.5	6.0	

[a] Linear retention index on a DB-5 column. [b] A, mass spectrum and LRI agree with those of authentic compounds; B, mass spectrum (spectral quality value >80 was used) and LRI agrees with reference spectrum in the NIST/EPA/NIH mass spectra database and LRI agree with those in the literature cited [1] (Javidnia et al 2002); [2] (Lucero et al 2006); [3] (Buchin et al 2002); [4] (Buchin et al 2002); [5] (Buchin et al 2002); [6] (Baranauskiene et al 2003); [7] (Reverchon et al 1997); [8] (Ana Paula Santos et al 2004); [9] (Gohari et al 2006); [10] (Cui et al 2011); [11] (Hamm et al 2005); [12] (Asfaw et al 2005); [13] (Adams et al 2005); [14] (El-Ghorab et al 2002); [15] (Jardim et al 2008); [16] (Högnadóttir 2003); [17] (Kant et al 2004); [18] (Bell 2004); [19] (Silva et al 2012); [20] (Zunino et al 1997).[c] Estimated abundance collected in the headspace of basil samples containing 2.8 mL of saturated calcium chloride, calculated by comparison with of 100 ppm propyl propanoate used as internal standard; internal standard was used to normalise chromatograms; means of three replicate samples are shown; means not labelled with the same letters are significantly different (0.05) and Tukey's HSD multiple pairwise comparison; nd - not detected; ns - not significant probability obtained by ANOVA, * significant at the 5 % level; ** significant at the 1 % level; *** significant at 0.1 % level.

Previous research has shown that most of the rosemary essential oil is constituted of monoterpenes. In this study, monoterpenes represented 85-91 % of the aroma composition for the rosemary analysed. A study of rosemary grown in Portugal reported an aroma profile mainly composed of monoterpenes, where the main compounds were myrcene (~ 23 %), 1,8-cineole (~ 16 %), limonene (~ 16 %) and camphor (~ 19 %), similar main volatiles were detected in samples from the present study however at different proportions at ~8.5 %, ~0.8 %, ~17.2 % and ~8.8 % , respectively (Serrano et al 2002). Similarly, Polish rosemary was reported as having over 80 % of monoterpenes in the aroma profile, however main compound identified was alpha-pinene, followed by bornyl acetate, camphene and 1,8-cineole (Szumny et al 2010). Another study conducted in the Balkan Peninsula, showed 1,8-cineole as the major compound with camphor, alpha-pinene and borneol described amongst the most abundant (Lakušić et al 2012). Contrary to what was reported by these authors, results from the present study found alpha-pinene as the second highest compound, with limonene being the most abundant compound, these were followed by camphor, myrcene, camphene and bornyl acetate. These differences could be due to differences in variety, as no information in regards to this can be found in most literature, and differences in growing conditions and in maturity of the samples between the experiments. Rosemary has been classified into different chemotypes according to main aroma compounds, however no study has reported a limonene chemotype, with 1,8-cineole and alpha-pinene being the most reported ones (Hcini et al 2013, Salido et al 2003).

Regarding other compounds, smaller contributions to the aroma profile were detected: alcohols ~0.1 %, aldehydes 0.3-0.9 %, sesquiterpenes ~0.5 % and unidentified compounds 2.5-6.0 %. Limited research has reported the presence and relevance of these compounds to the

aroma profile of rosemary plant. This study observed that these compounds accounted for 10-15 % of the aroma composition of rosemary.

Young plants showed a composition of 90 % monoterpenes, with limonene (17-21 %) the most abundant compound, these also presented a composition of 0.9 % of aldehydes, leading to a more citrus and green aroma. Small differences were observed between leaf maturity from the same plant with no significant differences detected. These plants were produced in pots, so under controlled environment and less exposed to environmental stresses, and this resulted in lower abundance of volatile compounds, with similar proportions of main compounds were detected in young plants (Ra, Rb) and first cut of older plants (Rc, Rd), however these samples were produced under difference conditions (pot vs protected field). Higher photosynthesis rate has been reported in younger plants which would lead to elevated synthesis of organic compounds, which would serve as precursors for the synthesis of volatiles, explaining the high proportion of monoterpenes found in younger plants (Cruickshank 2012, Hcini et al 2013, Munné-Bosch 2007).

Older rosemary plants showed an aroma composition constituted by monoterpenes (85-90 %), other compounds (6-8 %), unidentified (1.4-6 %) and sesquiterpenes (0.5-0.7 %). Compounds previously described as most abundant were detected in higher amounts in leaves from the first cut (Ra, Rb) compared to leaves from second cut (Re, Rf), apart from camphene which was found in similar quantities across all samples, and this was observed in majority of rosemary compounds. This confirms what has been described in literature, where reduction in photosynthesis rate is observed as plants age, resulting in lower secondary metabolites, which was observed in second cut of older plant (Munné-Bosch 2007). Sesquiterpenes were detected at higher abundances in second cut of old plant than first cut, whilst other compounds were

detected at higher proportion in first cut of old plants than the second cut, these compounds have been described as imparting a woody odour. This indicates that other environmental factors during growth will affect the synthesis of secondary compounds due to continuous exposure of the plant.

5.3.2 Sensory evaluation of fresh rosemary samples

The sensory profile of six rosemary samples was generated by a trained panel, with a consensus vocabulary generated previously of 24 terms. Mean panel scores for these attributes are presented in Table 5.3. From the 24 attributes that were profiled, only nine of these were significantly different between maturities.

Appearance attributes exhibited significant differences between cuts of the older plants and between leaf ages, conversely similarities between leaf age of the young plant were observed. A significant difference ($p < 0.05$) was apparent for colour of leaf, leaf size and leaf thickness, where young leaves from the first cut of older (Ra) plants were scored lower than older leaves from the same plant and leaves from second cut of older plant, furthermore young leaves were scored as being thinner in comparison with older leaves from the same plant. Additionally, samples Re and Rf (second cut of older plant) were scored as being darker in colour.

Half of the aroma attributes showed significant differences between samples, with similarities in odour intensity, pine and floral aroma. Grassy green aroma was significantly higher ($p < 0.01$) in the second cut sample of the older rosemary for both top and bottom leaves than first cut of older plants and young plant, however other environmental factors such as type of production and soil could have influenced these differences apart from plant maturity, additionally no differences between leaf maturity of the same sample were detected. Menthol

aroma differences were only detected between sample Ra (protected field) and Rc (pots), where the second was scored significantly ($p < 0.05$) higher, however due to uncontrolled growing conditions no conclusions can be drawn. Samples from second cut of older rosemary were scored as higher in sweet odour, but were not significantly different from first cut of older plants.

Cooling mouthfeel was significantly higher ($p < 0.05$) in the second cut of older samples (Re and Rf) than leaves from the first cut (Ra, Rb), and expressed a positive correlation with menthol aroma. Mouthfeel related to the texture of the leaves (chewiness and firmness) was scored significantly higher ($p < 0.05$) in older leaves compared to younger leaves of the same plant, additionally leaf firmness was score higher in samples from the first cut compared to second cut of the older plant.

Table 5.3: Mean panel scores for sensory attributes of the six rosemary samples.

Attribute	**Score** [A]						***p*-value** [B]
	Ra	**Rb**	**Rc**	**Rd**	**Re**	**Rf**	
Appearance							
Colour of Leaf	51.4^{c}	60.9^{b}	62.0^{b}	61.3^{b}	73.8^{a}	68.2^{ab}	***
Leaf Size	43.4^{d}	54.3^{abc}	60.2^{a}	57.8^{ab}	52.7^{bc}	50.6^{c}	***
Leaf thickness	37.4^{c}	46.1^{ab}	47.5^{a}	50.4^{a}	39.8^{bc}	47.2^{a}	**
Odour							
Odour Intensity	52.8	57.5	55.4	54.7	55.8	57.6	ns
Grassy/green	12.5^{c}	17.8^{bc}	11.8^{c}	17.0^{bc}	29.1^{a}	24^{ab}	**
Menthol	16.0^{b}	18.5^{ab}	29.0^{a}	22.8^{ab}	23.5^{ab}	23.1^{ab}	*
Sweet	22.5^{a}b	24.2^{ab}	20.5^{b}	22.5^{ab}	30^{a}	27.5^{ab}	*
Pine	42.4	43.9	42.3	43.6	39.4	41.0	ns
Floral	12.0	14.5	13.7	10.0	13.7	9.6	ns
Taste/Flavour							
Bitter	44.9	46.5	48.4	45.5	47.8	52.6	ns
Sweet	9.6	7.2	7.8	9.1	13.0	9.1	ns
Pine	48.6	50.7	44.1	44.9	47.6	51.7	ns
Grassy/green	12.6	12.6	12.6	12.7	16.2	14.4	ns
Peppery	9.2	9.7	8.6	11.0	10.9	10.5	ns

Soapy Mouthfeel	36.1	36.4	34.8	33.5	38.6	39.9	ns
Cooling	19.5[b]	23.3[ab]	21.9[ab]	18.4[b]	28.2[a]	28.2[a]	*
Numbing	23.9	27.4	24.6	21.5	21.4	27.2	ns
Chewy	47.6[ab]	57.0[a]	43.7[b]	49.1[ab]	50.6[ab]	52.3[ab]	*
Leaf Firmness	46.5[b]	56.6[a]	46.1[b]	46.3[b]	45.5[b]	52.2[ab]	*
Aftereffects							
Pine	39.1	41.1	35.7	36.8	36.7	43.4	ns
Soapy	30.0	33.0	26.6	27.6	31.7	33.7	ns
Cooling	16.0	19.3	18.9	16.8	20.3	19.4	ns
Numbing	19.2	23.1	23.6	21.1	18.4	24.2	ns
Bitter	36.2	43.1	40.4	37.8	34.0	41.7	ns

[A] Means are from two replicate samples, measured on an unstructured line scale (0-100); differing small letters represent sample significance from multiple comparisons and means not labelled with the same letters are significantly different ($p < 0.05$). [B] Probability obtained by ANOVA that there is a difference between means; ns, no significant difference between means ($p > 0.05$); * significant at the 5 % level; ** significant at the 1 % level; *** significant at 0.1 % level.

Although, no significant differences were detected for attributes of taste, flavour and aftereffects, most of the flavour attributes were scored as elevated in samples from the second cut of the older plants (sample Re and Rf) than samples from the first cut (Ra and Rb). Additionally, attributes including bitter taste, pine flavour and soapy flavour were scored as being higher in older leaves in comparison to younger leaves of the same plant. Furthermore, leaves from young plants (Rc and Rd) were scored lower for most of the attributes with no significant differences, however this difference could be attributed to the differences in growing conditions such as type of production (pot vs protected field) and soil (peat vs loamy/clay).

5.3.3 Multiple Factor Analysis of flavour attributes and volatile compounds

MFA was used to visualise the sensory and chemical differences observed across the different plant and leaf maturity (Figure 5.1), with volatile compounds identified that expressed significant differences between samples (Table 5.2) and the sensory attributes related to odour

and flavour (Table 5.3). Multiple factor analysis dimension one (F1) and two (F2) explained 71.63 % of the total variation within data. The first axis separated the first cut of older samples (Ra and Rb) and young leaves of young plant (Rc) from the other samples, whilst the second axis separated young leaves from first cut (Ra) and leaves from young plant (Rc and Rd) from the rest of the samples. Young leaves from first cut (Ra) was displayed closer to the centre of the biplot accompanied by floral aroma attribute displayed no strong association with most flavour/aroma attributes and compounds. Young maturity (plant and leaves) was highly associated with most aroma attributes except fresh cut grass (Figure 5.1), whereas older maturity was associated flavour and taste attributes, including adverse attributes like bitterness. Cut of the herbs showed the opposite association, where first cut was highly correlated with fresh aroma of pine, whereas the second cut was correlated with sweet and fresh cut grass attributes, cut of herbs produces a similar reaction to plant wounding causing membrane and cell wall damage, this initiates the oxidation of polyunsaturated fatty acids through enzymatic reactions leading to the production of aldehydes and alcohols responsible for the green leaf aroma (Brilli et al 2011).

Most monoterpenes and sesquiterpenes were positively correlated with first dimension, (F1), and other types of compounds and unidentified compounds were negatively correlated with F1. Monoterpenes were positioned in the outer rim of the biplot, with compounds like camphor (M19), camphene (M2) and alpha-pinene (M32) displaying a positive association to pine flavour and aftereffect, whereas eucalyptol (M10) expressed positive correlation with bitter aftereffect. Sweetness attributes and fresh cut grass attributes were negatively correlated with the second dimension (F2), conversely pine attributes, floral aroma and odour intensity were positively correlated with dimension F2. Additionally, majority of volatile compounds

were positively correlated with the second dimension (Figure 5.1). Unidentified compounds were distributed around the plot, with no drivers of certain attributes identified.

Perennial herbs like rosemary are subject to harvest at different plant maturities and multiple cuts, and it is clear these produce differences in composition and sensory perception. Samples from this study were produced at similar temperature ranges (16-20 ˚C), similar photoperiod (14-15 h day^{-1} of sunlight), however different production methods (pot and protected field), different soil (peat and loamy/clay) in addition to different maturities (Table 5.1) Comparing samples from York (Ra, Rb, Re and Rf), suggests that older plants are exposed for longer to more environmental stresses, including multiple cuts, since they grow for several years they get exposed seasonal weather variation, in order for the plant to survive these conditions, secondary metabolites will be synthesised resulting in higher abundances (Table 5.2) of volatile compounds (Akula and Ravishankar 2011, Sato 2014, Verpoorte 2000) and these are perceived when tasted by the trained sensory panel (Table 5.3).

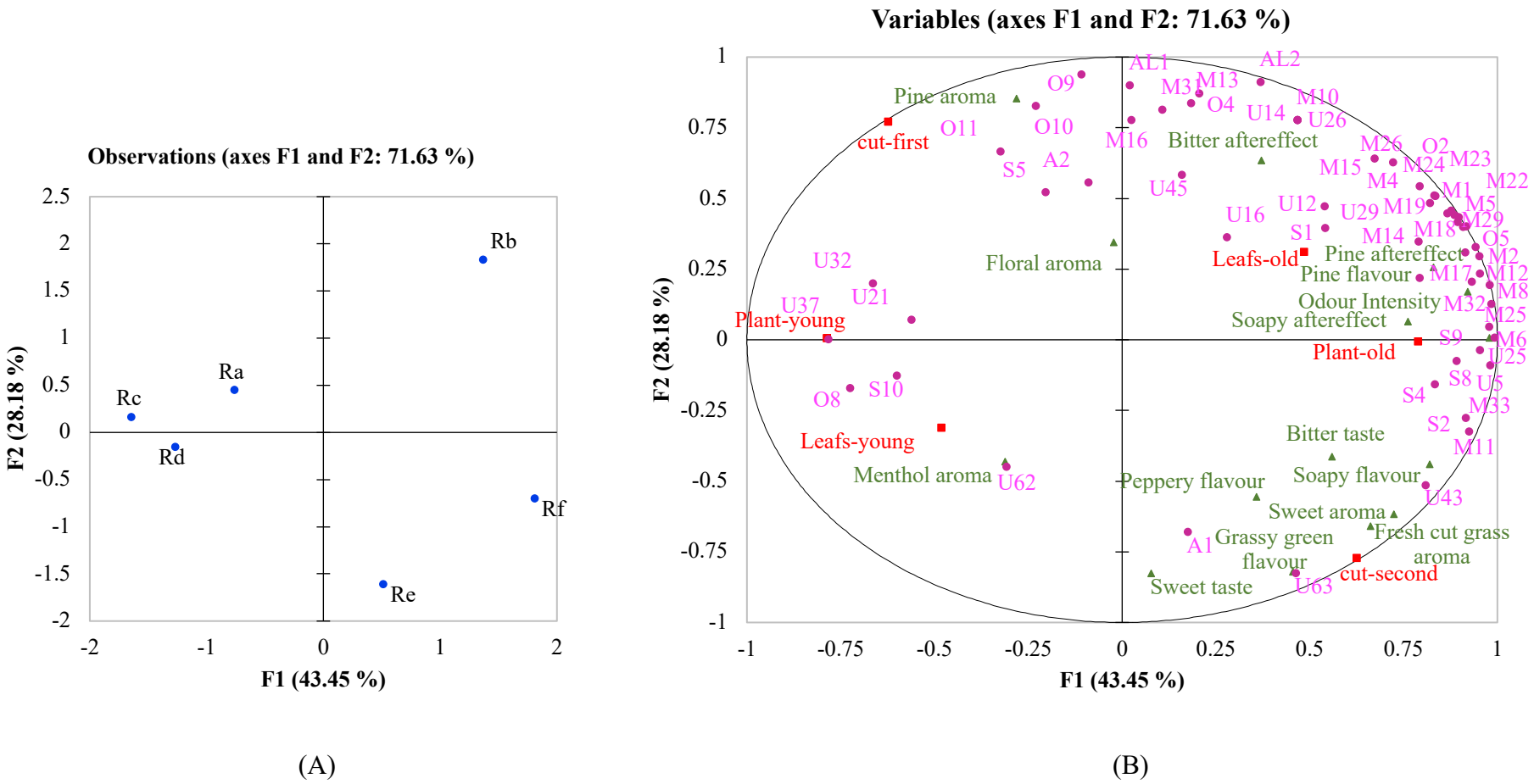


Figure 5.1:Multiple factor analysis of six rosemary samples with different maturities showing correlations with volatile compounds and sensory attributes. (A) Projection of samples (Ra- young leaves, old plant, first cut; Rb-old leaves, old plant, first cut; Rc-young leaves, young plant; Rd- old leaves, young plant; Re- young leaves, old plant, second cut; Rf- old leaves, old plant, second cut); (B) Distribution of variables: red squares-maturity; green triangle-sensory attributes; pink circle-volatile compounds.

5.4 Conclusions

Rosemary maturity displayed an influence on the aroma composition of rosemary and changes in the sensory perception. Completing volatile analysis and sensory evaluation of the rosemary samples demonstrated that older plants develop higher amounts of volatiles and present a wider range of volatiles, specially second cut samples, however young and old plants of rosemary were produced under different conditions that could be affecting the aroma composition. These were also described as imparting a sweeter aroma and bitter taste. Younger plants seem to produce a greener and menthol aroma with a lower amount of volatiles than older plants, additionally these were produced in pots where there is a high plant density and using a different soil substrate. Combining these findings, the first cut of older plant is likely to provide rosemary with the highest amounts of main volatiles, but consumers may prefer the second cut as this has lower levels of bitterness than the first cut.

Leaf age was also found to influence the aroma and sensory attributes, however not always in a significant way. Most of the main rosemary compounds were found in higher amounts for older leaves when compared to older leaves of the same plant, and this was also observed for odour and flavour attributes. Additionally, young leaves of the young plants showed lower abundance for most compounds and were described as the least for most sensory attributes, however this could be due to different soil types or plant density from pots. Equally to plant maturity, mature leaves led to higher abundance of volatiles and higher intensity of aroma attributes and bitter intensity.

Currently there is limited studies to support the impact of plant and leaf maturity on the volatile composition and sensory profile of rosemary, and in order to confirm current findings,

further work using herb maturity with chemical and sensory analysis needs to be carried out to provide a better understanding of this effect. In order to confirm the finding from this study, young and old plants of rosemary should be grown in the same controlled environment using a block design randomized setting, controlling the emergence of new leaves. Providing clarification on causes of aroma composition variation in rosemary and other culinary herbs, will help growers select what to produce and know what results to expect in response to different variables. This information combined with crop quality control will result in better quality products.

Chapter 6: Consumer acceptability of fresh culinary herbs: basil (*Ocimum basilicum* var. Sweet Genovese) and coriander (*Coriandrum sativum* var. Cruiser)

6.1 Introduction

Herbs and spices have been widely used in medicine, culinary and pharmaceutical industry (Peter and Shylaja 2012). In culinary terms, these can be consumed fresh in salads, as garnish, and cooked in sauces, soup, stocks and can also be used dried (Pushpangadan and George 2012). Basil owes its aroma and flavour profile to the presence of terpenes (monoterpenes and sesquiterpenes) and phenylpropanoids, responsible for its herbal and spicy odour (Klimánková et al 2008, Lee et al 2005, Pushpangadan and George 2012). Coriander leaf aroma and flavour is due to the presence of aldehydes, conferring a green, waxy and floral odour (Łyczko et al 2021, Nurzyńska-Wierdak 2013).

Although basil and coriander are commonly grown and consumed, research investigating the perception of their flavour is limited, with only a few studies evaluating the sensory characteristics of dried basil and coriander (Bušić et al 2014, Łyczko et al 2021). Further to this, there has been no research conducted evaluating aroma profile of fresh herbs and aligning this to consumer perception and preferences. Previous research identified that external characteristics of the product, such as appearance, are the main influencers of consumer intent to purchase, whereas internal characteristics (aroma, taste, flavour, texture) influence consumer acceptability (Caracciolo et al 2020). Without understanding consumer acceptability and the characteristics that consumers find desirable in culinary herbs, growers are missing important information about their market.

Basil and coriander have been analysed in previous chapters of this thesis, where the aroma profile was determined and the influence of factors such as type of production, geographical location and climate were investigated. Studies investigating aroma composition and sensory profiling in other aromatic herbs concluded that samples with higher abundance of volatile compounds were scored higher for sensory attributes (Calín-Sánchez et al 2012, Sárosi et al 2013). However, no research about consumer preference has been carried out in herbs. The closest parallel is a study investigating consumer preference for different celery genotypes, which grouped consumers into three separate clusters by the differences in overall liking of samples, with most consumers preferring samples with hight intensity for most attributes (Turner et al 2021).

Providing culinary herb growers with information collected in this investigation will help them understand consumer preference when it comes to herbs and their consumption behaviour. The aim of this study was to conduct consumer evaluation to understand acceptability, liking and preference of different samples of basil and coriander and to associate this with biochemical composition. The aroma profile of the herbs was assessed in parallel using solid phase microextraction gas chromatography/mass spectrometry (SPME GC/MS) to identify differences and similarities in volatile profile.

6.2 Materials and methods

6.2.1 Plant material

Fresh herbs were produced and delivered by different growers across the United Kingdom (UK). This material was harvested at commercial maturity, in order to mimic products delivered to commercial chains and consumers, during Autumn season of 2021. Basil (*Ocimum basilicum*

var. Sweet Genovese) and Coriander (*Coriandrum sativum* var. Cruiser) were chosen due to their commercial value and easiness of consumption in a raw state. These samples were provided by growers involved in the study that are part of UK's fresh culinary herb production sector.

Samples were produced in different sites using different types of agronomic practice, to represent the industry. Location, type of production and environmental characteristics were registered (Table 6.1) in order to assess how different growing conditions were between types of production, where herbs produced in pots were grown under protected conditions and herbs grown in open field were subject to weather conditions (Field). This information was collected from the producers when records were available, however some information was not shared due to commercial confidentiality.

Table 6.1: Growing conditions and location for each sample for of basil and coriander.

Herb	Sample	Type of production	Location	GPS coordinates	Av Temp[A] (˚C)	Soil type[B]	Water supply[C] (mm day^{-1})	Light source[D] (h day^{-1})
Basil	B1	Pot	West Sussex	50.4848˚N, 0.4413˚W	16-20	Peat	Ir	12h-Sl HPS
	B2	Pot	Lincolnshire	52.7442˚N, 0.3779˚W	16-20	Mixture	Ir	12h-Sl
Coriander	C1	Pot	West Sussex	50.4848˚N, 0.4413˚W	16-20	Peat	Ir	12h-Sl 1h-HPS
	C2	Pot	Lincolnshire	52.7442˚N, 0.3779˚W	20-25	Mixture	Ir	12h-Sl

C3	Open field	York	54.1345˚N, 1.2430˚W	11-15	Loamy Clay	2.8mm-Rf	14h-Sl
C4	Open field	West Sussex	50.8198˚N, 0.7807˚W	16-20	Sandy	2.6mm-Rf Ir	14h-Sl

[A] Average temperature over 24 h; [B] Type of soil used:mixture- composed of 90 % peat substrate and 10 % perlite [C] Average water amount and water source used: I- irrigation and Rf- rainfall; [D] Average photoperiod and light source used: Sl- sunlight and HPS-high pressure sodium.

Samples were stored as described in Chapter 2 until time of analysis and collection by the consumer.

6.2.2 Chemical reagents

Chemical reagents used for preparation and analysis of samples of present study are described in Chapter 2: in subsection 2.2.3 (page 57).

6.2.3 Solid Phase Microextraction (SPME) followed by GC-MS

Basil and coriander samples were prepared, including replication, following the method described in Chapter 2:,subsection 2.2.2, and relative abundance of compounds was determined using SPME GC-MS with method described in Chapter 2 (2.2.4).

6.2.4 Consumer evaluation of fresh herbs samples

Study preparation was conducted at the Sensory Science Centre at the University of Reading (UK) and samples assessment was done at home due to limitations for assessment on site as consequence of the coronavirus pandemic. One hundred and six people were recruited for the coriander study and one hundred and seventeen people were recruited for the basil study

(male and female, aged 18 years and above, without allergies to wheat, gluten, coriander, basil and/or dairy). Participants collected their at-home test kit (Figure 6.1) from the University of Reading, which included the samples to be assessed, palate cleanser and instructions on how to take part in the study. Samples were assessed in a randomized order. Participants were asked to rate their liking of appearance, followed by liking of aroma after breaking the leaves and finally were asked to rate their liking of flavour, texture and overall. This was done using a 9-point hedonic scale (where 1: dislike extremely, 5: neither like nor dislike, 9: like extremely) and all sample were scored. Consumers were also asked to indicate appropriateness of attribute level on a 5-point Just-About-Right (JAR) scale for the attributes: aroma intensity, bitter intensity, sweetness intensity (basil), salty intensity (coriander), flavour intensity and mouthfeel (where 1: much too weak, 3:JAR and 5: much too strong). Finally, participants were asked to rank the samples according to their preference (ranking from most preferred to least preferred), rank the following attributes: appearance, strong aroma, strong flavour and appearance (ranking: most preferred to least preferred), if they liked the herb, whether they regularly consumed or purchased the herbs and how usually they consume them. Participants were given the opportunity to leave additional comments on each sample if they wanted to. The studies of basil and coriander were done in separate weeks, and in total two samples (basil) and four samples (coriander) were evaluated. Samples were scored in a monadic balanced order using Williams design, with sample sets randomly assigned to participants. The assessment took place at participant's homes, and they were asked to complete the test within three days of sample collection and to keep samples refrigerated until assessment. Data was collected using Compusense Cloud Software. The study was done in September 2021 (coriander) and October 2021 (basil) and approved by the School of Chemistry, Food and Pharmacy Research Ethics Committee, University of Reading (study number: 30/2021). Informed consent was obtained from all participants.

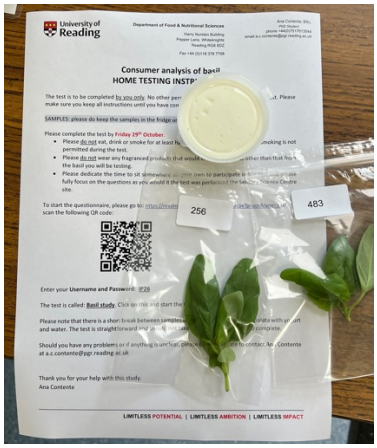


Figure 6.1: At-home test kit collected by participants.

6.2.5 Statistical analysis

Analysis of data for all compounds identified using the SPME GC-MS was carried out as described in Chapter 2: (2.2.5).

Consumer results were analysed using XLSTAT 2020.5.1 version as follows: (1) one-way ANOVA and Fisher's LSD test for consumer liking, (2) analysis of the preference (ranking) for coriander was done using Friedman's test, (3) analysis of the preference for basil was done using 2-AFC test, (4) agglomerative hierarchical clustering (AHC) for overall liking, (5) penalty analysis for JAR data for intensity and mouthfeel attributes. Agglomerative hierarchical clustering was carried out and dissimilarity of responses was determined by Euclidean distance, and agglomeration was done using Ward's method, set with automatic truncation. Penalty analysis was done to determine the influence of consumer perception of appropriateness of attribute level rating (JAR) on consumer liking by calculating mean drop in liking (scale 1-9) compared to mean liking of consumers that rated the attribute as JAR (JAR 3 on a 1-5 scale), and significance of drop in liking was determined.

6.3 Results and Discussion

6.3.1 Volatile composition of samples

6.3.1.1 Basil

In total, 72 compounds were identified in the headspace of the two pot-grown basil samples (Table 6.2) including 21 monoterpenes, 10 sesquiterpenes, four aldehydes, two phenylpropanoids, two alcohols and 16 unknown compounds. Quantitative differences were observed between the samples used in this study and one-way ANOVA revealed significant differences in the relative abundance of some minor aroma compounds. Compounds such as 1-octanol, octanal, methyl octanoate, hexyl 2-methylpropanoate, isoborneol and octyl acetate were significantly different between samples including some unknown compounds.

A high proportion of the aroma compounds was comprised of monoterpenes and phenylpropanoid with eucalyptol, linalool, eugenol and methyl eugenol exhibiting the highest relative abundance within each compound group. Monoterpenes have been shown to have a higher proportion of the aroma composition, with compounds like eucalyptol and linalool as the most abundant and part of the main contributors to the basil aroma (Calín-Sánchez et al 2012, Chang et al 2007, Klimánková et al 2008, Lee et al 2005). Sample B1 exhibited the highest proportion of phenylpropanoids whilst sample B2 the highest proportion of monoterpenes, both samples were produced in pots, with similar temperature ranges, similar sunlight photoperiod and same water source, however using different soil substrates and additional HPS lighting (B1), suggesting these factors will influence the compound composition. Furthermore, sample B2 from Lincolnshire, showed the highest relative abundance in eucalyptol, suggesting that the use of natural lighting and mixture substrate leads to higher abundance of monoterpenes including eucalyptol.

Sesquiterpenes have been described as constituents of basil aroma composition, however, they have not previously been described as key compounds. Analysis of basil essential oil was carried out and sesquiterpenes were detected, however no contribution to the flavour was reported (Hanif et al 2011). Furthermore, in the present study, no significant differences in sesquiterpenes relative abundance were detected between samples (Table 6.2), which was also consistent with the literature (Hanif et al 2011, Klimánková et al 2008, Lee et al 2005, Patel et al 2021).

Phenylpropanoid methyl eugenol has been shown as one of the main compounds contributing to basil flavour, being responsible for one of the main chemotypes that basil volatile profile can be classified into (Calín-Sánchez et al 2012, Lucchesi et al 2004, Pushpangadan and George 2012). Conversely, a study of four Italian basils reported a higher abundance of eugenol, being the main contributor for clove like aroma (Patel et al 2021). In the present study, sample B1 displayed the higher abundance of methyl eugenol (Table 6.2), this sample was produced in West Sussex, using peat substrate and high pressure sodium (HPS) in addition to sunlight, suggesting that these environmental factors affected the volatiles abundance of basil however not in a significantly (p<0.05). Peat substrate has good nutrient and water uptake, which are essential to on synthesis pathway, including secondary metabolites such as monoterpenes and phenylpropanoids, however growing basil under HPS has been reported to increase plants height but not the volatile composition (Litvin et al 2020, Waterman and Mole 1989). These results suggest that soil and light source could be used to change monoterpenes and phenylpropanoids abundance in order to achieve desirable basil flavour as these compounds are responsible for strong basil aroma (Calín-Sánchez et al 2012, Lucchesi et al 2004, Patel et al 2021, Pushpangadan and George 2012), however differences in other

growing factors such as type of production or growth temperatures may further influence this composition.

Table 6.2: Relative abundance of aroma compounds identified in the headspace of fresh basil samples.

Code	Compound name	LRI [a]	ID [b]	Relative abundance [c]		*p*-value
				B1	B2	
	Alcohol					
A1	(*Z*)-3-Hexen-1-ol	857	A	24	9.3	ns
A2	1-Octanol	1071	A	34	4.1	**
	Total (%)			0.3	0.1	
	Aldehyde					
AL1	(*Z*)-3-Hexenal	799	A	85	78	ns
AL2	(*E*)-2-Hexenal	855	A	256	333	ns
AL3	Octanal	1003	A	17	49	*
AL4	Perilla aldehyde PG acetal (isomer 2)	1555	A	51	21	ns
	Total (%)			2.0	5.1	
	Fatty acid					
F1	Methyl hexanoate	932	A	32	20	ns
F2	Methyl octanoate	1124	A	nd	5.0	
F3	Undecylenic acid	1468	A	67	22	ns
	Total (%)			0.5	0.5	
	Monoterpene					
M1	alpha-Pinene	941	A	90	65	ns
M2	Camphene	957	A	37	28	ns
M3	beta-Pinene	985	A	149	93	ns
M4	beta-Myrcene	993	A	451	284	ns
M5	alpha-Phellandrene	1011	A	36	23	ns
M6	delta-3-Carene	1017	A	18	13	ns
M7	alpha-Terpinene	1023	A	73	49	ns
M8	*p*-Cymene	1031	A	16	12	ns
M9	Limonene	1037	A	259	149	ns
M10	Eucalyptol/1,8-cineole	1042	A	2348	1714	ns
M11	gamma-Terpinene	1066	A	92	62	ns
M12	Terpinolene	1096	A	222	122	ns
M13	Linalool	1106	A	3510	1400	ns
M14	allo-ocimene	1133	B [1]	10	nd	
M15	Hexyl 2-methylpropanoate	1146	A	nd	7.4	*

M16	Camphor	1159	A	201	112	ns
M17	Isoborneol	1180	A	65	19	*
M18	1-Terpinen-4-ol	1189	A	20	12	ns
M19	alpha-Terpineol	1201	A	109	59	ns
M20	*cis*-Geraniol	1233	B [2]	13	nd	
M21	Bornyl acetate	1298	A	306	203	ns
M22	Ocimene quintoxide	1052	A	994	446	ns
	Total (%)			39	47	
	Other					
O1	Decane	1000	A	11	13	ns
O2	4-Methylthiazole	822	A	nd	7.1	
O3	2-Ethylthiophene	870	B [3]	5.5	4.0	ns
O4	Benzeneacetonitrile	1148	A	11	5.0	ns
O5	(*E*)-2-Hexenyl lactate	1206	A	44	16	ns
O6	Octyl acetate	1209	A	nd	7.9	
O7	Ethyl decanoate	1392	A	17	nd	ns
O8	Allyl cyclohexylpropionate	1438	A	16	nd	ns
O9	Cinnamic acid	1453	A	1209	426	ns
O10	Citronellyl isobutyrate	1484	A	51	15	ns
O11	Isopropyl cinnamate	1522	A	495	172	ns
O12	Irone2	1544	A	291	112	ns
O13	Vanillyl methyl ketone	1547	A	64	24	ns
O14	Geranyl butyrate	1560	A	82	30	ns
O15	Methyl jasmonate	1665	A	105	28	ns
	Total (%)			17	14	
	Phenylpropanoid					
P1	Eugenol	1370	A	1238	552	ns
P2	Methyl eugenol	1413	A	3648	1252	ns
	Total (%)			24	19	
	Sesquiterpene					
S1	Copaene	1396	B [4]	86	34	ns
S2	(*E*)-β-Bergamotene	1431	B [5]	16	37	ns
S3	Caryophyllene	1446	B [6]	73	18	ns
S4	alpha-Humulene	1482	A	427	95	ns
S5	alpha-Muurolene	1498	B [7]	210	79	ns
S6	alpha-Farnesene	1504	B [8]	91	32	ns
S7	Germacrene D	1508	B [9]	46	11	ns
S8	Valencene	1515		205	80	ns

S9	Nerolidol	1538	A	423	116	ns
S10	Calacorene	1568	B [10]	22	10	ns
	Total (%)			8	5	
	Unknowns					
U30	unknown	847		8.5	12	ns
U32	unknown	980		127	79	ns
U34	unknown	1057		nd	5.6	
U35	unknown	1090		13	nd	
U2	unknown	1136		13	7.5	ns
U37	unknown	1177		16	10	ns
U5	unknown	1257		30	11	ns
U6	unknown	1342		12	nd	
U7	unknown	1351		21	nd	
U27	unknown	1459		125	46	ns
U12	unknown	1464		1219	434	ns
U15	unknown	1491		25	8.8	ns
U19	unknown	1529		218	81	ns
U45	unknown	1642		23	nd	
U19	unknown	1529		218	81	ns
U46	unknown	1642		23	nd	
	Total (%)			10	8	

[a] Linear retention index (LRI) on a DB-5 column. [b] A, mass spectrum and LRI agree with those of authentic compounds; B, mass spectrum (spectral quality value >80 was used) and LRI agrees with reference spectrum in the NIST/EPA/NIH mass spectra database and LRI agree with those in the literature cited [1] (Sabulal et al 2007); [2] (Jantan et al 2005); [3] (Methven et al 2007); [4] (Högnadóttir and Rouseff 2003); [5] (de L. Nogueira et al 2001); [6] (Buchin et al 2002); [7] (Adams et al 2006); [8] (Zoghbi et al 1998); [9] (Bouzouita et al 2003); [10] (Lazari et al 2000). [c] Estimated abundance collected in the headspace of basil samples containing 2.8 mL of saturated calcium chloride, calculated by comparison with of 100 ppm propyl propanoate used as internal standard; internal standard was used to normalise chromatograms; means of three replicate samples are shown; nd - not detected; ns - not significant probability obtained by ANOVA, * significant at the 5% level; ** significant at the 1% level; *** significant at 0.1% level.

6.3.1.2 Coriander

In total, 72 compounds were identified in the headspace of the four coriander samples (Table 6.3) including 16 monoterpenes, 10 aldehydes, six alcohol, six esters and three alkanes, also 23 unknown compounds. Quantitative differences were observed between the samples of this study and one-way ANOVA showed significant differences in the relative abundance of some minor aroma compounds, that were not previously reported as relevant to the aroma of

coriander, however, (*E*)-2-Decenal , which has been reported as a principle compound in the aroma profile, expressed significant differences between samples, with sample C3 significantly higher ($p< 0.05$) (Cadwallader et al 1999, Neffati and Marzouk 2008). This sample was produced in open field at lower temperatures (11-15 ˚C) than the other samples (Table 6.1) and in a loamy/clay type of soil, with similar rainfall amounts to other open field sample (C4), aldehydes have been reported to affected by temperature where warmer climate resulted in higher amounts of aldehydes (Turner et al 2021), suggesting that this difference could result from the soil or lower total water amounts. Majority of significantly different compounds were detected in higher amounts in open field produced coriander, this is due to differences in crops growing conditions. Open field sample C3 was grown at same temperature range as pot sample C1, however open field produced were grown in different soils, longer photoperiods (14 h day^{-1} of sunlight) and exposed to rainfall (2.6-2.8 mm day^{-1}) which might be the driver of differences.

A large proportion of the aroma profile was made of aldehydes, alcohols and alkanes (> 60 %) for all coriander samples, followed by some monoterpenes and unidentified compounds. Compounds like (*E*)-2-Hexenal, (*E*)-2-Decenal, Decanal, Undecanal, Dodecanal and (*E*)-2-Dodecenal were the most abundant aldehydes, nonane and decane the most abundant alkanes detected in the samples from this study. Aldehydes have been shown to have the highest contribution to the aroma composition in several studies (Anjum et al 2011, Cadwallader et al 1999, Neffati and Marzouk 2008, Nurzyńska-Wierdak 2013, Potter and Fagerson 1990). Some of these compounds have been reported as the main contributors for the coriander aroma, including pot production, hydroponics and field grown, however (*E*)-2-Decenal was not present in one of the samples (C1) (Anjum et al 2011, Cadwallader et al 1999, Neffati and Marzouk 2008, Nurzyńska-Wierdak 2013). This absence could be due to substrate used in the sample

(peat) which influenced the nutrient and water availability or the use of supplementary HPS lighting, since these growing conditions (Table 6.1) were different than other samples (C2, C3 and C4).

Alkane compounds have not been described in literature as part of the most abundant compounds or main contributors for coriander aroma profile, in the present study alkanes accounted for 5-24 % of volatiles compositions for open field and pot produced samples (respectively), however it was reported in low abundance in coriander samples obtained from the distillation of fresh coriander leaves grown in a garden setting (Potter and Fagerson 1990). In pot produced coriander over 15 % of the aroma composition was represented by alkanes, this could be due to abiotic stresses experienced by these plants like higher competition for nutrients due to higher plant density (Akbarinia et al 2007), however this this was not measured in the present study or due to the higher temperature ranges experienced in the pot samples (Table 6.1). Additionally, nonane was the most abundant compound in the group of alkanes and major compound in pot produced samples (C1 and C2), and was responsible for 22 % of samples C2 composition, this could be due to higher average growth temperatures (20-25 ˚C), suggesting that higher temperatures will lead to higher amounts of alkanes, but it could also be due the use of mixture as a substrate for plant growth.

Monoterpenes are highly present in aromatic crops, including coriander, however these compounds have not been reported as main contributors to the coriander aroma (Cadwallader et al 1999, Nurzyńska-Wierdak 2013). A study analysing the oil content of coriander seeds reported linalool as the most abundant compound, contributing to a floral odour (Ravi et al 2007). Linalool was discovered in higher amounts for pot produced that in coriander produced in open field, however no significant differences between samples were detected.

Aldehydes are commonly reported as the most abundant type of compound in coriander aroma composition (Cadwallader et al 1999), with alcohol compounds present in the composition but in lower abundances than aldehydes. Conversely, a study conducted in Fiji analysed essential oil of coriander leaves (variety unknown), reported the most abundant compounds as alcohol compounds (*(E)*-2-decen-1-ol and decanol) (Eyres et al 2005). However, in samples analysed in the present study, *(E)*-2-decen-1-ol was not part of the profile and decan-1-ol was detected only in open field production, this compound uses as a precursor the aldehyde decanal which was detected at much higher abundances in open field samples (C3 and C4) resulting in the synthesis of this compound.

Table 6.3: Relative abundance of aroma compounds identified in the headspace of fresh coriander samples.

Code	Compound name	LRI[a]	ID[b]	Relative abundance [c]				*p*-value
				C1	C2	C3	C4	
	Alcohol							
A1	Prenol	772	A	nd	nd	681	nd	
A2	(*Z*)-3-Hexen-1-ol	858	A	9468	5745	9355	4785	ns
A3	(*E*)-2-Hexen-1-ol	868	A	12094	7755	32401	14332	ns
A4	2-Ethyl-1-hexanol	1037	A	nd	nd	nd	315	
A5	Decanol	1274	A	nd	nd	5164	7404	ns
A6	Undecanol	1374	B[1]	nd	nd	384	nd	
	Total (%)			21	12	19	18	
	Aldehyde							
AL1	(*Z*)-3-Hexenal	799	A	nd	36	nd	nd	
AL2	(*E*)-2-Hexenal	855	A	7637	6417	20620	9614	ns
AL3	5-Methyl-(E)-2-hexenal	925	A	685	296	760	234	ns
AL4	Nonanal	1105	A	nd	nd	397	nd	
AL5	Decanal	1208	A	6440	10315	52471	34237	ns
AL6	(*E*)-2-Decenal	1262	B[2]	nd[a]	224[ab]	3638[b]	1903[ab]	*
AL7	Undecanal	1307	A	2745	3520	9268	4161	ns
AL8	(*E*)-2-Undecenal	1368	A	216	179	1107	999	ns
AL9	Dodecanal	1412	A	8271	9862	10946	5521	ns
AL10	(*E*)-2-Dodecenal	1472	B[3]	6710	4863	12711	11058	ns
	Total (%)			25	28	40	37	

	Alkane							
AK1	Nonane	901	A	16744	23486	15752	6191	ns
AK2	Decane	1000	A	1919	2446	3284	1489	ns
AK3	Undecane	1100	A	448	581	839	417	ns
	Total (%)			18	24	8.0	5.3	
	Ester							
E1	Methyl hexanoate	932	A	146[a]	nd	319[b]	nd	*
E2	*cis*-3-Hexenyl Acetate	1006	B[4]	531	309	nd	nd	ns
E3	Methyl octanoate	1124	A	664	315	630	562	ns
E4	Methyl nonanoate	1224	A	101	nd	nd	nd	
E5	Methyl decanoate	1324	A	728	618	1025	1418	ns
E6	Methyl dodecanoate	1524	B[5]	532	262	300	429	ns
	Total (%)			2.6	1.4	0.9	1.6	
	Fatty acid							
F1	Nonanoic acid	1272	A	1499	2833	6809	3172	ns
F2	Dodecanoic acid	1574	A	1030	1313	3428	1995	ns
	Total (%)			2.4	3.8	4.1	3.4	
	Monoterpene							
M1	alpha-Pinene	941	A	2189	2126	4714	1524	ns
M2	Camphene	957	A	766	701	1811	522	ns
M3	beta-Pinene	985	A	349	351	1013	317	ns
M4	beta-Myrcene	993	A	283	281	293	292	ns
M5	alpha-Phellandrene	1010	A	nd	nd	nd	316	
M6	*p*-Cymene	1031	A	368[ab]	331[ab]	nd	662[b]	**
M7	Limonene	1036	A	230	377	308	515	ns
M8	Eucalyptol	1039	A	1492	3679	4144	1255	ns
M9	gamma-Terpinene	1065	A	271	230	289	280	ns
M10	*p*-Cymenene	1096	A	nd	nd	nd	253	
M11	Linalool	1101	A	1022	1199	483	531	ns
M12	Camphor	1159	A	571	750	1461	463	ns
M13	Isoborneol	1179	A	191	190	487	nd	ns
M14	Bornyl acetate	1298	A	702	627	1936	809	ns
	Total (%)			8.1	9.9	6.8	5.1	
	Other							
O1	3-Hexanone	779	A	nd	nd	234	nd	
O2	4-Methylthiazole	822	A	623	549	568	511	ns
O3	*p*-Xylene	873	B[6]	nd	242	nd	nd	
O4	Cyclohexanone	895	A	302	269	272	397	ns
O5	Pentylbenzene	1167	A	nd	nd	nd	282	
O6	Heptanal PG acetal 2	1197	A	nd	nd	nd	874	
O7	beta-Caryophyllene	1446	B[7]	185	215	478	294	ns

O8	3-Propylidene phthalide	1599	A	nd	nd	nd	248	
	Total (%)			1.1	1.2	0.6	1.7	
	Unknowns							
U52	unknown	801		554	447	917	466	ns
U1	unknown	845		155	nd	325	180	ns
U36	unknown	966		nd	nd	436	nd	
U3	unknown	1029		nd	204	nd	nd	
U5	unknown	1148		216	nd	nd	nd	
U7	unknown	1204		nd	nd	nd	1098	
U10	unknown	1265		nd	nd	nd	4382	
U18	unknown	1401		629	426	1037	968	ns
U19	unknown	1424		165	nd	135	nd	ns
U21	unknown	1455		206	nd	384	367	ns
U23	unknown	1501		365	346	728	641	ns
U37	unknown	1513		350	463	726	384	ns
U28	unknown	1604		1006	1018	2433	1691	ns
U40	unknown	1615		309	320	362	271	ns
U29	unknown	1659		266	295	759	552	ns
U30	unknown	1677		8622	9557	20998	14574	ns
U53	unknown	1698		nd	nd	nd	210	
U31	unknown	1704		238[ab]	nd	nd	537[b]	*
U54	unknown	1728		nd	nd	694[b]	361[a]	*
U32	unknown	1774		2215	2162	5310	4085	ns
U33	unknown	1792		nd	nd	nd	120	
U34	unknown	1834		490	502	1144	615	ns
U35	unknown	1875		244	nd	690	435	ns
	Total (%)			22	19	20	28	

[a] Linear retention index (LRI) on a DB-5 column. [b] A, mass spectrum and LRI agree with those of authentic compounds; B, mass spectrum (spectral quality value >80 was used) and LRI agrees with reference spectrum in the NIST/EPA/NIH mass spectra database and LRI agree with those in the literature cited 1 (Xu et al 2003); 2 (Lucero et al 2006); 3 (Morteza-Semnani et al 2007); 4 (Dickens 1999); 5 (Yuanyuan Wang et al 2005); 6 (Guido Flamini et al 2003); 7 (Buchin et al 2002). [c] Estimated abundance collected in the headspace of basil samples containing 2.8 mL of saturated calcium chloride, calculated by comparison with of 100 ppm propyl propanoate used as internal standard; internal standard was used to normalise chromatograms; means of three replicate samples are shown; means not labelled with the same letters are significantly different (p<0.05) and Tukey's HSD multiple pairwise comparison; nd - not detected; ns - not significant probability obtained by ANOVA, * significant at the 5 % level; ** significant at the 1 % level; *** significant at 0.1 % level.

6.3.2 Consumer evaluation of fresh herbs

6.3.2.1 Basil

One hundred and seventeen consumers evaluated the basil samples, and demographic data is in Table 6.4. More than half of the consumers were female (69.5 %), with mean and median ages of 34.5 and 30 respectively. More than half of the consumers were students (51.7 %) and 42.4 % were working. In total, 55.9 % of the participants are involved in food, nutrition or sensory sector. The largest ethnic group of the sample population was White (68.6 %). Most consumers participating in the study stated they liked basil (92.4 %) and the most common frequency of consumption were two to three times a month (26.3 %), once a month (24.6 %) and once a week (22.0 %).

Table 6.4: Consumer demographics and characteristics of the consumer pane for basil.

Consumers	Number	Percentage (%)
Total number of volunteers	117	
Age		
mean	34.5	
median	30	
min	18	
max	71	
Gender		
female	82	69.5
male	35	29.7
Working status		
working	50	42.4
unemployed	1	0.85
student	61	51.7
other	5	4.24
working in food/nutrition/sensory sector	66	55.9
Ethnic group		
White	81	68.6
Mixed or Multiple ethnic groups	4	3.39
Asian or Asian British	12	10.2
Black, African, Caribbean or Black British	6	5.08
other ethnic group	14	11.9
Basil liking		
Yes	109	92.4
No	8	6.78
Consumption Frequency		

never	4	3.39
less than once a month	21	17.8
once a month	29	24.6
2 to 3 times per month	31	26.3
once a week	26	22.0
2 to 4 time per week	6	5.08
once a day	0	0.00
Purchase Frequency		
once a month	58	49.2
2-3 per month	24	20.3
once a week	8	6.78
Twice or more per week	0	0.00
never	27	22.9
Method of consumption		
I do not eat basil	6	5.08
raw (on its own)	19	16.1
raw (in salads)	62	52.5
cooked (boiled, roasted, fried, on its own)	66	55.9
cooked (in soups, stocks or sauces)	79	66.9
dried	47	39.8
other (pesto)	5	4.24

The mean liking scores of the basil samples (Table 6.5) demonstrated significant differences in aroma, taste, texture and overall liking, with results ranging from dislike very much to like extremely. No significant differences were identified in appearance with and average score of 7: ‘like moderately’. Sample B1 was scored the lowest for overall liking, this was produced in West Sussex, and displayed the highest proportion of phenylpropanoid compounds. Consumers were asked to rank samples according to their preference from most (1) to least (2), significant differences were identified with 71.2 % of participants preferring the B2 sample (Lincolnshire) grown in mixture substrate and natural lighting. This sample exhibited the highest proportion of monoterpenes (Table 6.2), suggesting consumers prefer a basil with higher monoterpene content.

Table 6.5: Liking scores and preference ranking for basil (*Ocimum basilicum* var. Sweet Genovese) samples.

Sample	**Liking** [A]					**Ranking** [B]
	Appearance	Aroma	Taste	Texture	Overall	
B1	7.2	6.9	5.6	6.2	6.1	28.8 %
B2	7.3	7.6	6.6	6.9	7.1	71.2 %
p-value	0.819	<0.0001	<0.0001	0.0004	<0.0001	<0.0001

[A] Means not labelled with the same letter are significantly different (p<0.05); means are from 117 consumers on a 9-point hedonic scale (from dislike extremely to like extremely). [B] Percentage of consumers that selected sample as most preferred.

Consumers were also asked to rank a list of four attributes that they found important when consuming basil, the list included the main attributes when consuming herbs. The attribute 'strong aroma' was ranked the most important followed by strong taste, and texture of leaves was ranked as the least important attribute for basil (Table 6.6).

Table 6.6: Consumers' ranking for attributes when consuming basil.

Attributes	**Ranking** [A]
Strong aroma	1.9[a]
Strong flavour	2.1[a]
Appearance	2.8[b]
Texture	3.3[c]

[A] Mean rank (1: most important to 4: least important)

Agglomerative hierarchical cluster (AHC) analysis was completed to identify relatively homogeneous groups of consumers based on their overall liking scores (Table 6.7), with two clusters of consumers identified. Consumers in cluster 1 (76.9 %) liked moderately both samples with no significant differences (p< 0.05) detected. Conversely, consumers in cluster 2 (23.1 %) showed significant differences in liking of basil, the sample B1 (West Sussex) was disliked moderately and the sample B2 (Lincolnshire) was liked moderately. Cluster 2 participants consisted of consumers that had higher liking for basil with higher monoterpene content.

Participants from each cluster were classified as likers and non-likers, where 95.6 %, 85.2 % were likers in cluster 1 and cluster 2 respectively. Cluster 1 contained the highest proportion of likers, however no significant differences between samples were perceived by participants. On the other hand, cluster 2 had a lower proportion of likers and they liked the most samples from Lincolnshire, which had the lower abundance of the volatile compounds but highest proportion of monoterpenes and were classified as the most intense for aroma and flavour, suggesting that higher proportion of monoterpenes is favourable for consumer preference. Conversely, sample from West Sussex (B1), was described as having higher intensity in bitter taste, suggesting that higher amounts of aroma compounds result in higher bitterness, leading to smaller proportion of acceptance by the consumer.

Table 6.7: Overall liking of basil samples for the clusters of consumers obtained from agglomerative hierarchical clustering for basil samples.

Cluster/ Percentage of Consumers	**Samples**		*p*-value
	B1	B2	
1 (76.9 %)	6.8	7.0	0.280
2 (23.1 %)	3.8	7.4	<0.0001
Overall liking	6.1	7.1	<0.0001

[A] Means not labelled with the same letters are significantly different ($p<0.05$); means are from 90 consumers for cluster 1, 27 consumers for cluster 2, respectively. The mean for overall liking of 117 consumers.

Penalty analysis was used to relate Just-About-Right (JAR) data to liking scores and explain how aroma, bitterness, sweetness, flavour and texture intensity affect the overall liking of the samples (Table 6.8).

Table 6.8: Mean Just-About-Right ratings and influence on overall liking ratings for basil samples.

Sample	Overall	Significance of Sample (*p*-value)	Penalty Analysis			
			Too Little		Too Much	
			Mean Drop	Frequency (%)	Mean Drop	Frequency (%)
JAR Aroma Intensity						
B1	2.6	0.002	0.97*	43.6 %	1.08	12.0 %

B2	2.9		0.91*	22.2 %	1.90	9.4 %
JAR Bitter Intensity						
B1	3.3	0.002	0.76	15.4 %	1.50*	43.6 %
B2	2.9		0.43	20.5 %	0.60	17.9 %
JAR Sweetness Intensity						
B1	2.4	0.025	0.70*	55.6 %	1.52	1.7 %
B2	2.6		0.70*	35.9 %	1.01	2.6 %
JAR Flavour Intensity						
B1	2.7	0.381	0.33	44.4 %	1.88*	20.5 %
B2	2.8		1.18*	33.3 %	1.33	13.7 %
JAR Mouthfeel						
B1	3.1	0.277	0.38	14.5 %	0.60	17.9 %
B2	3.0		0.06	18.8 %	0.75	15.4 %

*Represents a significant difference ($p<0.05$) within a sample in overall liking compared with mean liking rating when the sample was considered Just-About-Right. Frequency is the percentage of consumers within each group.

When certain attributes are not at the correct intensity for consumers this may affect the overall liking. Aroma was ranked by the consumers as the most important characteristic, and this translated in Table 6.8, where for all samples there was a negative effect on overall liking when aroma intensity was considered too low. The same effect on overall liking was seen for sweetness intensity. Flavour was the second most important characteristic, however for sample B1, from West Sussex using peat substrate and added HPS lighting, a negative effect on overall liking was detected when the flavour intensity was too high but the opposite occurred with sample B2 (Lincolnshire) produced using mixture substrate and natural lighting, where too low flavour intensity caused a drop in overall liking. Texture of leaves was ranked the least important attribute of basil (Table 6.6), this was confirmed as no effect on the overall liking of samples was detected (Table 6.8).

Additional comments on samples provided by the participants contained both negative and positive points (Table 6.9).

Table 6.9: Examples of comments from participants highlighting some negative and positive points relating to the basil samples used in the study.

Sample	Comments and Participants Details
B1	The aroma is light and the whole taste not very strong (IP13). Initial taste is sweet and flavourful but aftertaste is quite bitter (IP15). Fresh, aromatic, slightly numbing/tingling aftertaste (IP103). I really liked how the basil looked. I could even smell the aroma. However, when I tasted the basil, there was almost no flavour possible to be detected (IP111).
B2	I think it smells more strong than sample B1. But this one smells like a mint and cinnamon combination (IP173). Smells and tastes quite strong. This sample had some very curled leaves and one with a couple of holes in it (IP96). Good overall taste. Appearance of leaves are smooth but a bit small size (IP101). I didn't like the basil much at first look. However, the taste was much better (IP111).

6.3.2.2 Coriander

One hundred and six consumers evaluated the coriander samples, and demographic data is in Table 6.10. Around half of the consumers were female (56.6 %), with mean and median ages of 35.6 and 34 respectively. More than half of the consumers were working (59.4 %) and 36.8 % were students. In total, 40.6 % of the participants are involved in food, nutrition or sensory sector. The largest ethnic group of the sample population was White (61.3 %). More than half of consumers participating in the study stated they liked coriander (81.1 %) and the most common frequency of consumption was two to three times a month (33.0 %).

Table 6.10: Consumer demographics and characteristics of the consumer panel for coriander.

Consumers	Number	Percentage (%)
Total number of volunteers	106	
Age		
mean	35.6	
median	34	
min	18	
max	67	
Gender		
female	60	56.6
male	46	43.4
Working status		
working	63	59.4

unemployed	3	2.8
student	39	36.8
other	1	0.9
working in food/nutrition/sensory sector	43	40.6
Ethnic group		
White	65	61.3
Mixed or Multiple ethnic groups	2	1.9
Asian or Asian British	13	12.3
Black, African, Caribbean or Black British	7	6.6
other ethnic group	19	17.9
Coriander liking		
Yes	86	81.1
No	20	18.9
Consumption Frequency		
never	6	5.7
less than once a month	17	16.0
once a month	15	14.2
2 to 3 times per month	35	33.0
once a week	19	17.9
2 to 4 time per week	12	11.3
once a day	2	1.9
Purchase Frequency		
once a month	37	34.9
2-3 per month	26	24.5
once a week	17	16.0
Twice or more per week	3	2.8
never	23	21.7
Method of consumption		
I do not eat coriander	5	4.7
raw (on its own)	9	8.5
raw (in salads)	45	42.5
cooked (boiled, roasted, fried, on its own)	60	56.6
cooked (in soups, curry,stocks or sauces)	76	71.7
dried	24	22.6
other	12	11.3

The mean liking scores of the coriander samples (Table 6.11) demonstrated significant differences in results ranging from dislike extremely to like extremely. No significant differences were identified in appearance, taste, texture and overall liking with and average score of 6: ‘like slightly’ for all attributes apart from taste which had an average score of 5: ‘neither like nor dislike’. Sample C2, produced in Lincolnshire in pots at the highest

temperature range (20-25 ˚C) was scored the highest for overall liking but no significant differences were detected. Consumers were asked to rank samples according to their preference from most (1) to least (4), significant differences were identified between sample C2 (pots, Lincolnshire) and sample C4 (open field, West Sussex), with the first scored as most preferred. This suggests the consumer prefers a coriander with higher proportion of alkanes and lower proportion of alcohol compounds, which resulted from production in pots, using mixture substrate (90 % peat and 10 % perlite), irrigation and natural light, and produces using a temperature range of 20-25 ˚C.

Table 6.11: Liking scores and preference ranking for coriander (*Coriandrum sativum* var. Cruiser) samples.

Sample	**Liking** [A]					**Ranking** [B]
	Appearance	Aroma	Taste	Texture	Overall	
C1	6.4	5.8^{b}	5.5	6.1	5.7	2.6^{ab}
C2	6.6	6.0^{ab}	5.9	6.3	6.0	2.2^{a}
C3	6.1	6.3^{ab}	5.5	5.8	5.7	2.6^{ab}
C4	6.4	6.3^{a}	5.6	6.0	5.7	2.7^{b}
p-value	0.207	0.025	0.384	0.101	0.475	0.029

[A] Means not labelled with the same letter are significantly different ($p<0.05$); means are from 117 consumers on a 9-point hedonic scale (from dislike extremely to like extremely). [B] Mean rank (1: most preferred to 4: least preferred).

Ranking of importance of attributes when consuming coriander was also asked, the list included the main attributes when consuming herbs. The attribute ‘strong taste’ was ranked the most important followed by strong aroma, with texture of leaves ranked as the least important attribute for coriander (Table 6.12).

Table 6.12: Consumers' ranking for attributes when consuming coriander.

Attributes	**Ranking**[A]
Strong flavour	1.8^{a}
Strong aroma	2.1^{a}
Appearance	3.0^{b}
Texture	3.1^{b}

[A] Mean rank (1: most important to 4: least important)

Agglomerative hierarchical cluster (AHC) analysis was also completed to identify relatively homogeneous groups of consumers based on their overall liking scores for coriander

(Table 6.13), with two clusters of consumers identified. Consumers in cluster 1 (60.4 %) neither liked nor disliked any of the coriander samples, however significant differences ($p< 0.05$) between sample C2, produced in pots, at higher temperature ranges, and open field samples (C4 and C3) were detected. This indicates that consumers in cluster 1, preferred a coriander with higher proportion of alkanes, which could due high temperature stress that coriander were exposed to. Consumers from cluster 2 (39.6 %) liked slightly pot samples (C1 and C2) and liked moderately open field samples (C4 and C3), with C4 sample being significantly ($p< 0.05$) most liked, this sample was produced in West Sussex, and grown in sandy soils allowing good water drainage, and using supplemented irrigation and medium range temperatures (16-20 ˚C). These conditions resulted in higher proportions of aldehydes (Table 6.3), as a result consumers in Cluster 2 demonstrated higher preference for coriander with higher proportion of aldehydes.

Participants from each cluster were classified as likers and non-likers, where 71.9 % and 95.2 % were likers in cluster 1 and cluster 2 respectively. Interestingly, cluster 2 contained the highest proportion of likers of coriander and they liked the most samples cultivated under open field production, which had the highest amount of volatile compounds, higher abundance of the main aroma contributors and were classified as the most intense for aroma and flavour by the participants. The liking of coriander leaves has been reported to have a connection to human genetics, with proportions of dislikers varying with ethnicity, with variances between 3-21 % reported (Mauer and El-Sohemy 2012). This aversion to coriander leaves has been associated with genetic variants in olfactory receptors associated with the *OR6A2* gene (Precone et al 2019). Further investigation needs to be done in order to understand the perceived flavour of coriander.

Table 6.13: Overall liking of coriander samples for the clusters of consumers obtained from agglomerative hierarchical clustering for coriander samples.

Cluster/ Percentage of Consumers	Samples				*p*-value
	C1	C2	C3	C4	
1 (60.4 %)	5.1^{ab}	5.6^{a}	4.8^{b}	4.6^{b}	0.003
2 (39.6 %)	6.5^{a}	6.6^{a}	7.1^{ab}	7.4^{b}	0.003
Overall liking	5.7	6.0	5.7	5.7	0.475

[A] Means not labelled with the same letters are significantly different (p<0.05); means are from 64 consumers for cluster 1, 42 consumers for cluster 2, respectively. The mean for overall liking of 106 consumers.

Penalty analysis was used to relate Just-About-Right (JAR) data to liking scores and explain how aroma, bitterness, sweetness, flavour and texture intensity affect the overall liking of the samples (Table 6.14).

Table 6.14: Mean Just-About-Right ratings and influence on overall liking ratings for coriander samples.

Sample	Overall[A]	Significance of Sample (p-value)	Penalty Analysis			
			Too Little		Too Much	
			Mean Drop	Frequency (%)	Mean Drop	Frequency (%)
JAR Aroma Intensity						
C1	2.4^{a}	<0.0001	0.59	53.8 %	1.05	6.6 %
C2	2.4^{a}		0.97*	52.8 %	2.84	4.7 %
C3	2.7^{b}		1.29*	35.8 %	1.61	15.1 %
C4	2.8^{b}		1.14*	30.2 %	1.89	17.9 %
JAR Bitter Intensity						
C1	3.0^{ab}	0.0002	0.30	23.6 %	1.02*	24.5 %
C2	2.9^{a}		0.61*	25.5 %	1.61	18.9 %
C3	3.2^{bc}		0.80	15.1 %	2.28*	34.0 %
C4	3.3^{c}		0.98	9.4 %	2.40*	37.7 %
JAR Salty Intensity						
C1	2.5	0.510	0.79*	40.6 %	0.67	2.8 %
C2	2.5		0.39	36.8 %	-0.36	1.9 %
C3	2.6		0.61	34.9 %	0.84	6.6 %
C4	2.7		-0.03	32.1 %	1.27	7.5 %
JAR Flavour Intensity						
C1	2.7^{a}	<0.0001	1.34*	44.3 %	1.63	19.8 %
C2	2.6^{a}		1.30*	41.5 %	2.93	10.4 %
C3	3.1^{b}		3.43*	25.5 %	5.49*	29.2 %
C4	3.2^{b}		1.31*	23.6 %	2.18*	34.9 %
JAR Mouthfeel						
C1	3.1^{ab}	0.025	0.52	9.4 %	1.12	18.9 %
C2	3.0^{b}		1.78	10.4 %	0.29	17.0 %
C3	3.2^{ab}		1.50	10.4 %	0.61	28.3 %

C4	3.3[a]		2.17	10.4 %	1.72*	36.8 %

[A] Means not labelled with the same letters are significantly different ($p<0.05$); *Represents a significant difference ($p<0.05$) within a sample in overall liking compared with mean liking rating when the sample was considered Just-About-Right. Frequency is the percentage of consumers within each group.

Flavour was scored by the consumers as the most important attribute for coriander, and this was detected in Table 6.14, where for all samples there was a negative effect on overall liking when flavour intensity was considered too low. Further to this, when flavour intensity was too high for the samples produced in the open field (C4 and C3), a negative effect on overall liking was perceived. On the other hand, when aroma intensity was too low it caused a drop on overall liking, except for sample C1 produced in pots in peat substrate using additional HPS lighting, this match results in Table 6.12, with aroma attribute ranked as second most important attribute. Bitter intensity, when too high, negatively affected the overall liking for most samples apart from C2 where the opposite was detected, this sample was produced in pots at a higher temperature range (20-25 ˚C), resulting in higher proportion of alkanes and lower proportion of alcohol, thus affecting the bitter intensity of coriander. No effect on the overall liking was detected with saltiness intensity, except in sample C1 (pot from West Sussex) which displayed the lowest abundance of volatile compounds, with too low saltiness causing a drop in overall liking.

Additional comments on samples provided by the participants contained both negative and positive points (Table 6.15).

Table 6.15: Examples of comments from participants highlighting some negative and positive points relating to the coriander samples used in the study.

Sample	Comments and Participants Details
C1	This sample looked good and had unexpected peppery taste. It is unlike the normal coriander I buy and have at home (IP8). Good appearance, not enough flavour (IP27). Appearance is good - looks like something you would find in the shops as opposed to growing out back. Smell leans towards underwhelming, but the taste is

	good (IP73). Couldn't detect much aroma, which is a shame, and the taste was nice but could have been a little stronger (IP101).
C2	Lacking aroma and flavour I was expecting. Looked good but taste and aroma did not match appearance (IP8). Besides the apparent I like this coriander. I find that the taste is well balanced (IP12). Aroma too low, taste way too bitter, how am I supposed to rate flavour (IP46). Low taste, slightly bitter, good appearance (IP118).
C3	Although it doesn't look very appetising, it has more flavour (IP23). Appearance was a disappointing, not a good spread of leaves and a bit too curly. The taste was nice and strong but a bit too bitter (IP39). Looks a bit too old, as the colour is darker green than previous samples. More chewy and stronger taste of all I tried so far (IP63). Good sample nice balance of spicy and herbal notes (IP113).
C4	Didn't taste as good as it smelled. Very bitter and faint curry taste in the background (IP39). Strong bitterness is noticed, and some burning feeling on the tip of tongue after chewing (IP68). The bitterness and chewy ness were the most striking characteristics of this sample (IP93). Big leaves, would be great in a curry very aromatic, would buy this happily (IP107).

6.4 Conclusions

The present study aimed to identify differences and similarities between fresh samples of basil and coriander and to evaluate consumer liking and perceptions of samples that have been previously analysed in the present thesis. Significant differences between fresh samples were observed in the aroma composition of basil and coriander and in consumer liking. Basil produced in Lincolnshire, using different soil and lighting, expressed higher contents of monoterpene compounds which was preferred by the consumer. Additionally, coriander with higher proportion of alkane compounds and lower proportion of alcohols was preferred by the consumer, and this profile resulted from production in pots in Lincolnshire and using lower temperature (16-20 ˚C). However, human genetics is predetermining factor of coriander liking, with a cluster of participants with a higher proportion of likers, preferring coriander with a higher proportion of aldehydes which resulted from open field production from West Sussex using sandy soil and medium range temperatures.

The participants from this study ranked flavour and aroma intensity as the most important attributes in aromatic herbs, however basil with highest abundance in aroma compounds was the least preferred by the participants but in the case of coriander the opposite was observed, with consumers' preference being for the samples with highest abundance of volatiles. Further consumer studies on coriander are needed in order to relate human genetics and the perception and preference of coriander flavour by the consumer. Additionally, results show that culinary herbs are consumed in different formats, understanding if the consumer looks for different attributes for different consumption could lead to the production of herbs driven by purpose.

The findings from this study combined with previous experiments completed in this thesis will contribute to further understanding of how changes in the aroma profile of herbs may influence consumer acceptability and preference, and relates this to the environmental conditions that plants were exposed to during their cultivation and harvesting. This work elucidates what attributes determine the consumer preference and what will cause differences in liking, allowing the fresh herb grower's community information to produce crops with the knowledge of consumer preferences.

Chapter 7: Overall discussion, future work and final remarks

7.1 Overall discussion and conclusions

Culinary herbs are grown and consumed worldwide, featuring in many countries' culinary dishes. This is due to their range, strength of flavour, and versatility to be used raw, cooked or dried. They are also a beneficial alternative to the addition of salt to foods. Their distinct flavours are due to their volatile composition. In basil this is mainly constituted of monoterpenes and phenylpropanoids, in rosemary mostly monoterpenes, and in coriander aldehydes. These have been identified several times in the literature as contributors to the respective herbs' aroma. Additionally, compound groups like alcohols, alkanes and sesquiterpenes have also been detected in samples, but in lower amounts. These compound groups were detected in all the seasons and samples, confirming their significant contribution to the flavour of herbs.

This thesis identified the influence of common cultivation variables of culinary herbs on the aroma composition and how the sensory perception is consequently affected. The study focused on three herbs (basil, coriander and rosemary) examining a single known variety for basil and coriander, however rosemary samples variety was undetermined due to the participation of commercial producers with no records of the variety of rosemary produced in open field setting, with different production systems and locations within the UK, across two different seasons and over a period of three years. Analysis of how the method of production, temperature regime, lighting conditions, soil composition and method of irrigation impacted on the synthesis of secondary metabolites was carried out, determining consequently the flavour of the herbs. Limited research has been done to evaluate the influence of these variables on the

aroma profile of herbs, with the main studies observing the influence of single factors or by hypothesising using examples of similar crops.

The results presented in this study demonstrate the significant influence of external factors on the aroma composition of the studied herbs. The variety of the herbs has been identified, where possible, as this will be an important determinate on the aroma profile of a plant. Consequently, choosing the variety of the crop will predetermine the flavour characteristics and response to the environment. However, for basil and coriander it was possible to use the same variety across all samples, but still differences in aroma composition were detected. From these results it is possible to conclude that variety predetermines the aroma composition, but equally environmental conditions during growth will influence the production of secondary metabolites and consequently the final aroma composition of the herbs. As discussed in Chapter 1, significant differences were observed between samples with pot grown material producing lower total amounts of volatile compounds as well as lower relative abundance of main compounds. The hypothesis is that this is due to the exposure of open field samples to environmental stresses. This suggests that open field production would be desirable for a more aromatic crop.

Additionally, sensory profiling showed differences in the scoring of the different samples of basil, coriander and rosemary. For the three herbs, samples produced using the pot system were scored significantly lower for most aroma, flavour and taste attributes apart from grassy green aroma and flavour and sweet taste. Hydroponically produced samples of basil were described as having a cloves and menthol aroma, open field samples of coriander with a soapy and celery like aroma and field samples of rosemary with a pine and floral aroma, furthermore these samples were also scored more highly for bitterness. It was hypothesised that growing

herbs in an open field setting exposes the plants to more environmental stresses which leads to higher production of secondary metabolites, resulting in higher abundance of volatile compounds. Differences between type of production observed in the aroma composition were expressed in the scoring of the sensory attributes. A negative correlation was observed between bitter taste and sweet taste and flavour and taste attributes showed a positive correlation with corresponding aroma attributes.

Furthermore, an influence of external factors on the aroma profile of the herbs in a multi-year experiment was observed. In Chapter 4 it was discussed how differences in environment including temperature regime, water availability, light quality and quantity, and soil composition affected sample composition. It was observed that open field production results in higher abundances of compounds, however other factors like temperature also influence the crop, where temperature ranges between 11-20 ˚C resulted in higher abundance of characteristic compounds for each herb. Soil high in nutrients and with good water holding capacity and the use of rainfall combined with irrigation resulted in higher abundances of volatiles, which result in more intense aroma for herbs. Additionally, the use of fertilisers was also observed to cause an increase in volatiles abundance for some of the herbs, this suggests that the use of fertilisers for crop health should be used moderately as the application of these might result in higher abundance of volatile compounds not associated with the aroma profile of the crop. Further investigation needs to be carried in order to fully understand the effect of the application of different fertilisers on the aroma composition of the crop. Finally, results presented demonstrated that growing a crop in open field will result in a more aromatic crop, additionally the application of supplemented lighting (such as LED), making nutrients more available to the plant and growing at optimal temperatures could result in a crop with volatile composition and consequently with high higher flavour and aroma intensity which are preferred by the

consumer. Pot produced plants were associated with lower abundances of compounds, this suggests that plant density leads to lower nutrient and water availability resulting in lower preference by the consumer. It was hypothesised that differences in the aroma are not attributed to one individual factor but the combination of optimal conditions that are specific to each crop.

Rosemary is a perennial herb, which means that several harvests can be made from one single plant. Different associations with compounds depending on the maturity of rosemary were observed, however these differences could be due to the different varieties. Therefore, it was analysed if the maturity of the plant and leaves would have a significant influence on the aroma composition and sensory profiling using one variety across all samples. This influence was confirmed as it was observed that older plants (first and second cut), additionally multiple harvests, due to cuts of the plant expressed higher relative abundance of chemical compounds and higher scoring for sensory attributes. However, it was also described by the sensory panel as imparting a bitter taste. Additionally, young plants produced in pots were associated with grass/green and menthol aroma and scored with lower intensity for other attributes. Leaf maturity was assessed by comparing top and bottom leaves of a rosemary sprig and this showed an influence on the aroma and sensory profile, with mature leaves (bottom) having higher abundance of volatiles and higher intensity of sensory attributes, including bitterness in comparison to young leaves (top) of the same plant. Finally, upon investigating the plant maturity influence on flavour, it was concluded that mature plants would be more desirable for the consumer, however repeated cuts (wounding) will increase bitter intensity which can lead to adverse response by the consumer. However, this study was limited due to the commercial nature of the samples, therefore further investigation should be carried in order to fully understand maturity influence on the aroma profile of crops.

By investigating consumer acceptance and preference of basil and coriander, it was possible to identify that for both basil and coriander, aroma and flavour intensity were drivers of liking and conversely bitter taste was a driver of disliking. This indicates consumers' preference for more flavour and aroma intense crops, however without increase of bitterness. Completing agglomerative hierarchical cluster (AHC) analysis, two clusters were identified for basil and coriander, cluster 1 (76.9 % and 60.4 %, respectively) and cluster 2 (23.1 % and 39.6 %, respectively), with oppositive preferences, where the second expressed higher preference for samples scored as most intense for aroma attribute. Additionally, human genetics contributes to liking and disliking of coriander's flavour, so preference drivers in this crop might be due to this factor instead of crop characteristics. Further research investigating the sensory profiling and consumer preference including various seasons will identify samples attributes that drive the preference of these samples.

7.2 Industrial Relevance, Application and Future Work

The herb samples used in this project were chosen by a steering group who comprised several UK herbs growers, and whom were brought together by the project sponsors AHDB via the British Herbs Trade Association. Basil and coriander were chosen due to their market importance to herb growers and rosemary was chosen to represent a perennial herb. Any decisions made during the project were discussed with the project steering group and regular meetings were held with the steering group. The information collected during this project educated growers involved on how growing variables could affect the aroma composition of herbs and their sensory profile. The information gathered during this project will be offered to the herb grower community to guide how growing factors can affect the aroma composition of their crops and updated guides will be shared by AHDB to the community. This will be

beneficial to open field growers considering the increasing changes in weather conditions due to phenomena such as climate change.

Future work should explore which of the volatile compounds detected in the samples would be odour active and responsible for the characteristic basil, coriander and rosemary aroma. This would be done by undertaking a gas chromatography-olfactometry (GC-O) analysis of fresh samples of the herbs so that the results could be compared to the ones observed in this study. Furthermore, it would be valuable to investigate how the variables studied in this project would affect non-volatile content of herbs like sugars and phenolic compounds. It would be expected that significant differences in growing environment would lead to differences in sugars and phenolic compounds, this has been previously observed by Jasper et al. (2020) where high temperatures resulted in higher concentration of glucosinolates in rocket salads. Consumer analysis identified sweetness and bitterness as drivers of liking for basil and coriander, so understanding how these attributes are affected by the growing variables would further assist growers to achieve a product with the characteristics the consumer desires.

Vertical farming is becoming more popular, as an alternative way of food production to satisfy population growth and decline in land agricultural land due to climate change. This is an alternative indoor farming that requires controlled environment, making it possible to manipulate growing conditions such as light quality, quantity and photoperiod and temperature in order to produce higher quality crops. It would be valuable to explore the differences in secondary product profiles of herbs produced in vertical farming set ups compared to conventionally grown herbs by analysing the volatile composition and sensory profiling, using the same methods as described in this thesis. Little research has been done comparing both growing techniques, however LED lighting manipulation has been reported to affect the flavour

profile of crops, but no sensory analysis was carried out (Carvalho et al 2016, Hammock et al 2021, McAusland et al 2020). Therefore, LED-mediated differences in light quality (wavelength) or quantity (intensity) will produce changes in the volatile compositions, however the impact of these differences on sensory characteristics needs further studying. Finally, consumer analysis could be done in order to assess if differences would be detected between herbs grown under different LED systems and vertical farming compared to conventional production.

7.3 Final Remarks

This thesis has shown that by manipulating the environment in which a herb is cultivated it is possible to influence the secondary product profile of coriander, basil, and rosemary and impact aroma development. Crop variety will have a big impact in the aroma composition, however growing environment including type of production, temperature and lighting will result in significant changes in the aroma composition. The conclusion of this project will provide knowledge to herb growers on how the growing variables influence herbs flavour and consumer perception, helping achieve optimal crop quality and consumer satisfaction.

Chapter 8: References

Adams, R.P. & Nguyen, S. (2005) Infra-specific variation in *Juniperus deppeana* and f. sperryi in the Davis mountains of Texas: variation in leaf essential oils and random amplified polymorphic DNAS (RAPDS). Phytologia, 87, 97–109

Adams, R.P., González Elizondo, M.S., Elizondo, M.G. & Slinkman, E. (2006) DNA fingerprinting and terpenoid analysis of *Juniperus blancoi* var. huehuentensis (Cupressaceae), a new subalpine variety from Durango, Mexico. Biochemical Systematics and Ecology, 34, 205–211

Adams, R.P., Morris, J.A., Pandey, R.N. & Schwarzbach, A.E. (2005) Cryptic speciation between *Juniperus deltoides* and *Juniperus oxycedrus* (Cupressaceae) in the Mediterranean. Biochemical Systematics and Ecology, 33, 771–787

Akbarinia, A., Daneshian, J. & Mohmmad Beygi, F. (2007) Effect of nitrogen fertilizer and plant density on seed yield, essential oil and oil content of *Coriandrum Sativum L*. Iranian Journal of Medicinal and Aromatic Plants, 22, 410–419

Akula, R. & Ravishankar, G.A. (2011) Influence of abiotic stress signals on secondary metabolites in plants. Plant signaling & behavior, 6, 1720–1731

Amoroso, L., Rizzo, V., Mazzaglia, A., Licciardello, F., Restuccia, C. & Muratore, G. (2017) Sous-vide packaging: sensory characteristics of potato slices treated with rosemary oil. Italian Journal of Food Science

Angioni, A., Barra, A., Coroneo, V., Dessi, S. & Cabras, P. (2006) Chemical composition, seasonal variability, and antifungal activity of *Lavandula stoechas L*. ssp. stoechas essential oils from stem/leaves and flowers. Journal of Agricultural and Food Chemistry, 54, 4364–4370

Anjum, F.M., Nadeem, M., Hussain, S., Butt, M.S., Shahwar, M.K. & El-Ghorab, A.H. (2011) Characterization of coriander (*Coriandrum sativum L.*) seeds and leaves: volatile and non volatile extracts . International Journal of Food Properties, 15, 736–747

Anon (2016) UK herb consumption forecast to grow. International Trade Centre. Available at: https://www.intracen.org/itc/blog/market-insider/UK-herb-consumption-forecast-to-grow/ (accessed 16/03/22).

Anon (2022) Spices & Culinary Herbs: United Kingdom. Statista. Available at: https://www.statista.com/outlook/cmo/food/sauces-spices/spices-culinary-herbs/united-kingdom (accessed 16/03/22).

Arbona, V., Manzi, M., Ollas, C. & Gómez-Cadenas, A. (2013) Metabolomics as a tool to investigate abiotic stress tolerance in plants. International journal of molecular sciences, 14, 4885–4911

Asfaw, N., Licence, P., Novitskii, A.A. & Poliakoff, M. (2005) Green chemistry in Ethiopia: the cleaner extraction of essential oils from *Artemisia afra*: a comparison of clean technology with conventional methodology. Green Chemistry, 7, 352–356

Avato, P., Raffo, F., Baxter, I.A. & Vartanian, S.T. (2004) Essential oils of *Varthemia iphionoides* from Jordan. Flavour and Fragrance Journal, 19, 559–561

Baccouri, B., Temime, S.B., Campeol, E., Cioni, P.L., Daoud, D. & Zarrouk, M. (2007) Application of solid-phase microextraction to the analysis of volatile compounds in virgin olive oils from five new cultivars. Food Chemistry, 102, 850–856

Baher, Z.F., Mirza, M., Ghorbanli, M. & Bagher Rezaii, M. (2002) The influence of water stress on plant height, herbal and essential oil yield and composition in *Satureja hortensis L.* Flavour and Fragrance Journal, 17, 275–277

Baranauskiene, R., Venskutonis, R.P. & Demyttenaere, J.C.R. (2003) Sensory and instrumental evaluation of catnip (*Nepeta cataria L.*) aroma. Journal of Agricultural and Food Chemistry, 51, 3840–3848

Batista-Pereira, L.G., Fernandes, J.B., Corrêa, A.G., Silva, M. & Vieira, P.C. (2006) Electrophysiological responses of eucalyptus brown looper *Thyrinteina arnobia* to essential oils of seven eucalyptus species. Journal of the Brazilian Chemical Society, 17, 555–561

Bell, W.A.-M. (2004) Examination of aroma volatiles formed from thermal processing of Florida reconstituted grapefruit juice. University of Florida

Berdague, J.L., Denoyer, C., Le Quere, J.L. & Semon, E. (1991) Volatile components of dry-cured ham. Journal of Agricultural and Food Chemistry, 39, 1257–1261

Bernhardt, B., Sipos, L., Kókai, Z., Gere, A., Szabó, K., Bernáth, J. & Sárosi, S. (2015) Comparison of different *Ocimum basilicum L.* gene bank accessions analyzed by GC–MS and sensory profile. Industrial Crops and Products, 67, 498–508

Biała, W. & Jasiński, M. (2018) The phenylpropanoid case – it is transport that matters. Frontiers in Plant Science, 1610

Blázquez, M.A., Pérez, I. & Boira, H. (2003) Essential oil analysis of *Teucrium libanitis* and *T. turredanum* by GC and GC–MS. Flavour and Fragrance Journal, 18, 497–501

Bouzouita, N., Kachouri, F., Hamdi, M. & Chaabouni, M.M. (2003) Antimicrobial activity of essential oils from Tunisian aromatic plants. Flavour and Fragrance Journal, 18, 380–383

Bower, A., Marquez, S. & de Mejia, E.G. (2016) The health benefits of selected culinary herbs and spices found in the traditional mediterranean diet. Critical Reviews in Food Science and Nutrition, 56, 2728–2746

Brilli, F., Ruuskanen, T.M., Schnitzhofer, R., Müller, M., Breitenlechner, M., Bittner, V., Wohlfahrt, G., Loreto, F. & Hansel, A. (2011) Detection of plant volatiles after leaf wounding

and darkening by proton transfer reaction “time-of-flight” mass spectrometry (PTR-TOF). Plos One, 6, e20419

Broadley, M., Brown, P., Cakmak, I., Rengel, Z. & Zhao, F. (2012) Function of nutrients: micronutrients. Marschner’s mineral nutrition of higher plants. Elsevier, 191–248

Buchin, S., Salmon, J.-C., Carnat, A.-P., Berger, T., Bugaud, C. & Bosset, J.-O. (2002) Identification de composés monoterpéniques, sesquiterpéniques et benzéniques dans un lait d’alpage trés riche en ces substances. Mitteilungen aus Lebensmitteluntersuchung und Hygiene, 93, 199–216

Bušić, A., Vojvodić, A., Komes, D., Akkermans, C., Belščak-Cvitanović, A., Stolk, M. & Hofland, G. (2014) Comparative evaluation of CO2 drying as an alternative drying technique of basil (*Ocimum basilicum L.*) -the effect on bioactive and sensory properties. Food Research International, 64, 34–42

Bylaite, E. & Meyer, A.S. (2005) Characterisation of volatile aroma compounds of orange juices by three dynamic and static headspace gas chromatography techniques. European Food Research and Technology, 222, 176

Cadwallader, K.R., Surakarnkul, R., Yang, S.-P. & Webb, T.E. (1999) Character-impact aroma components of coriander (*Coriandrum Sativum L.*) herb. Flavor Chemistry of Ethnic Foods, 77–84

Calín-Sánchez, Á., Lech, K., Szumny, A., Figiel, A. & Carbonell-Barrachina, Á.A. (2012) Volatile composition of sweet basil essential oil (*Ocimum basilicum L.*) as affected by drying method. Food Research International, 48, 217–225

Cantwell, M.I. & Reid, M.S. (1993) Postharvest physiology and handling of fresh culinary herbs. Journal of Herbs, Spices & Medicinal Plants, 1, 93–127

Cao, H., Li, Z. & Chen, X. (2011) QSRR Study of GC Retention indices of volatile compounds emitted from *Mosla chinensis Maxim* by multiple linear regression. Chinese Journal of Chemistry, 29, 2187–2196

Caracciolo, F., El-Nakhel, C., Raimondo, M., Kyriacou, M.C., Cembalo, L., De Pascale, S. & Rouphael, Y. (2020) Sensory attributes and consumer acceptability of 12 microgreens species. Agronomy, 10

Carvalho, S.D., Schwieterman, M.L., Abrahan, C.E., Colquhoun, T.A. & Folta, K.M. (2016) Light quality dependent changes in morphology, antioxidant capacity, and volatile production in sweet basil (*Ocimum basilicum*). Frontiers in Plant Science, 7, 1328

Chadwick, M. (2018) Optimising flavour in coriander. AHDB, 1–6

Chadwick, M. (2018) Optimising flavour in rosemary. AHDB Growers Guide, 1–6

Chang, X., Alderson, P. & Wright, C. (2005) Effect of temperature integration on the growth and volatile oil content of basil (*Ocimum basilicum L.*). The Journal of Horticultural Science and Biotechnology, 80, 593–598

Chang, X., Alderson, P.G., Hollowood, T.A., Hewson, L. & Wright, C.J. (2007) Flavour and aroma of fresh basil are affected by temperature. Journal of the Science of Food and Agriculture, 87, 1381–1385

Chaves, M.M., Pereira, J.S., Maroco, J., Rodrigues, M.L., Ricardo, C.P.P., Osório, M.L., Carvalho, I., Faria, T. & Pinheiro, C. (2002) How plants cope with water stress in the field? Photosynthesis and Growth, 89, 907–916

Chehab, E.W., Kaspi, R., Savchenko, T., Rowe, H., Negre-Zakharov, F., Kliebenstein, D. & Dehesh, K. (2008) Distinct roles of jasmonates and aldehydes in plant-defense responses. PLoS ONE, 3

Cho, I.H., Lee, S.M., Kim, S.Y., Choi, H.-K., Kim, K.-O. & Kim, Y.-S. (2007) Differentiation of aroma characteristics of pine-mushrooms (*Tricholoma matsutake Sing.*) of different grades

using gas chromatography−olfactometry and sensory analysis. Journal of Agricultural and Food Chemistry, 55, 2323–2328

Chohan, M., Forster-Wilkins, G. & Opara, E.I. (2008) Determination of the antioxidant capacity of culinary herbs subjected to various cooking and storage processes using the ABTS*+ radical cation assay. Plant Foods for Human Nutrition, 63, 47–52

Chyau, C.-C., Tsai, S.-Y., Yang, J.-H., Weng, C.-C., Han, C.-M., Shih, C.-C. & Mau, J.-L. (2007) The essential oil of *Glossogyne tenuifolia*. Food Chemistry, 100, 808–812

Ciriello, M., Formisano, L., El-Nakhel, C., Corrado, G., Pannico, A., De Pascale, S. & Rouphael, Y. (2021) Morpho-physiological responses and secondary metabolites modulation by preharvest factors of three hydroponically grown genovese basil cultivars. Frontiers in plant science, 12, 671026

Costa, A.S., Arrigoni-Blank, M.F., Silva, M.A.A.P., Alves, M.F., Santos, D.A., Alves, P.B. & Blank, A.F. (2014) The impact of hybridization on the volatile and sensorial profile of *Ocimum basilicum L*. The Scientific World Journal, 824594

Courtois, E.A., Paine, C.E.T., Blandinieres, P.-A., Stien, D., Bessiere, J.-M., Houel, E., Baraloto, C. & Chave, J. (2009) Diversity of the volatile organic compounds emitted by 55 species of tropical trees: a survey in French Guiana. Journal of Chemical Ecology, 35, 1349

Cruickshank, B. (2012) Improving the quality of cut herbs through optimisation of the growing environment. University of Reading

Cui,J., Yang, X., Dong, A., Cheng, D., Wang, J., Zhao, H., Xu, R., Wang, P. & Li, W. (2011) Chemical composition and antioxidant activity of *Euphorbia fischeriana* essential oil from China. Journal of Medicinal Plants Research, 5, 4794–4797

Damon, A. (2002) Analysis of the fragrance produced by the epiphytic orchid Anathallis (Pleurothallis) racemiflora (Orchidaceae) in the Soconusco region, Chiapas, Mexico. Lindleyana, 17, 93–97

Deng, Y. & Lu, S. (2017) Biosynthesis and regulation of phenylpropanoids in plants. Critical Reviews in Plant Sciences, 36, 257–290

Díaz-Maroto, M., Palomo, E., Castro, L., González Viñas, M.A. & Pérez-Coello, M.S. (2004) Changes produced in the aroma compounds and structural integrity of basil (*Ocimum basilicum L*) during drying. Journal of the Science of Food and Agriculture, 84, 2070–2076

Díaz-Maroto, M., Pérez-Coello, M., Sánchez-Palomo, E. & González Viñas, M. (2007) Impact of drying and storage time on sensory characteristics of rosemary (*Rosmarinus officinalis L.*). Journal of Sensory Studies, 22, 34–48

Dicke, M. & van Loon, J.J.A. (2000) Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. Entomologia Experimentalis et Applicata, 97, 237–249

Dicke, M. & Van Poecke, R. (2002) Signalling in plant-insect interactions: signal transduction in direct and indirect plant defence. Plant signal transduction, 289–316

Dickens, J.C. (1999) Predator–prey interactions: olfactory adaptations of generalist and specialist predators. Agricultural and Forest Entomology, 1, 47–54

Dudareva, N., Pichersky, E. & Gershenzon, J. (2004) Biochemistry of plant volatiles. Plant Physiology, 135, 1893–1902

Dziba, L.E. & Provenza, F.D. (2008) Dietary monoterpene concentrations influence feeding patterns of lambs. Applied Animal Behaviour Science, 109, 49–57

Edris, A.E. & Farrag, E.S. (2003) Antifungal activity of peppermint and sweet basil essential oils and their major aroma constituents on some plant pathogenic fungi from the vapor phase. Food Nahrung, 47, 117–121

Ekren, S., Sönmez, Ç., Özçakal, E., Kurttaş, Y.S.K., Bayram, E. & Gürgülü, H. (2012) The effect of different irrigation water levels on yield and quality characteristics of purple basil (*Ocimum basilicum L.*). Agricultural Water Management, 109, 155–161

El-Ghorab, A.H., Fadel, H.M. & El-Massry, K.F. (2002) The Egyptian *Eucalyptus camaldulensis* var. brevirostris: chemical compositions of the fruit volatile oil and antioxidant activity. Flavour and Fragrance Journal, 17, 306–312

El-Zaeddi, H., Martínez-Tomé, J., Calín-Sánchez, Á., Burló, F. & Carbonell-Barrachina, Á.A. (2016) Volatile composition of essential oils from different aromatic herbs grown in mediterranean regions of Spain. Foods, 5

Eriksson, N., Wu, S., Do, C.B., Kiefer, A.K., Tung, J.Y., Mountain, J.L., Hinds, D.A. & Francke, U. (2012) A genetic variant near olfactory receptor genes influences cilantro preference. Flavour, 1, 22

Everaerts, C., Roisin, Y., Le Quéré, J.-L., Bonnard, O. & Pasteels, J.M. (1993) Sesquiterpenes in the frontal gland secretions of nasute soldier termites from New Guinea. Journal of Chemical Ecology, 19, 2865–2879

Eyres, G., Dufour, J.-P., Hallifax, G., Sotheeswaran, S. & Marriott, P.J. (2005) Identification of character-impact odorants in coriander and wild coriander leaves using gas chromatography-olfactometry (GCO) and comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry (GC×GC–TOFMS). Journal of Separation Science, 28, 1061–1074

Fernandes, V.F., Almeida, L.B., Feijó, E.V.R.S., Silva, D.C., Oliveira, R.A., Mielke, M.S. & Costa, L.C.B. (2013) Light intensity on growth, leaf micromorphology and essential oil production of *Ocimum gratissimum*. Revista Brasileira de Farmacognosia, 23, 419–424

Flamini, G., Cioni, P.L. & Morelli, I. (2003) Volatiles from leaves, fruits, and virgin oil from *Olea europaea Cv. Olivastra Seggianese* from Italy. Journal of Agricultural and Food Chemistry, 51, 1382–1386

Flamini, G., Ertugrul, K., Cioni, P.L., Morelli, I., Dural, H. & Bagci, Y. (2002) Volatile constituents of two endemic Centaurea species from Turkey: *C. pseudoscabiosa* subsp. pseudoscabiosa and *C. hadimensis*. Biochemical Systematics and Ecology, 30, 953–959

Flamini, G., Tebano, M., Cioni, P.L., Bagci, Y., Dural, H., Ertugrul, K., Uysal, T. & Savran, A. (2006) A multivariate statistical approach to Centaurea classification using essential oil composition data of some species from Turkey. Plant Systematics and Evolution, 261, 217–228

Gang, D.R., Wang, J., Dudareva, N., Nam, K.H., Simon, J.E., Lewinsohn, E. & Pichersky, E. (2001) An investigation of the storage and biosynthesis of phenylpropenes in sweet basil. Plant Physiology, 125, 539–555

Geissman, T.A. & Crout, D.H.G. (1969) Compounds derived from shikimic acid. Organic Chemistry of Secondary Plant Metabolism, 136–166

Gharib, F., Ghazi, S., Aly, H., El-Araby, M. & Moustafa, S. (2016) Effect of soil type and water content on rosemary growth and essential oil yield. International Journal of Scientific and Engineering Research, 7,183-189

Gohari, A.R., Hadjiakhoondi, A., Sadat-Ebrahimi, E., Saeidnia, S. & Shafiee, A. (2006) Composition of the volatile oils of *Satureja spicigera* C. Koch Boiss. and *S. macrantha C. A. Mey* from Iran. Flavour and Fragrance Journal, 21, 510–512

Gurkan, H. & Hayaloglu, A.A. (2017) Volatiles and sensory characteristics of yogurt manufactured by incorporating basil (*Ocimum basilicum L.*). International Journal of Food Properties, 20, S779–S789

Hälvä, S., Craker, L.E., Simon, J.E. & Charles, D.J. (1993) Growth and essential oil in dill, *Anethum graveolens L.*, in response to temperature and photoperiod. Journal of Herbs, Spices & Medicinal Plants, 1, 47–56

Hamm, S., Bleton, J. & Tchapla, A. (2004) Headspace solid phase microextraction for screening for the presence of resins in Egyptian archaeological samples. Journal of Separation Science, 27, 235–243

Hamm, S., Bleton, J., Connan, J. & Tchapla, A. (2005) A chemical investigation by headspace SPME and GC–MS of volatile and semi-volatile terpenes in various olibanum samples. Phytochemistry, 66, 1499–1514

Hammock, H.A., Kopsell, D.A. & Sams, C.E. (2021) Narrowband blue and red LED supplements impact key flavor volatiles in hydroponically grown basil across growing seasons. Frontiers in Plant Science, 12

Hanif, M.A., Al-Maskari, M.Y., Al-Maskari, A., Al-Shukaili, A., Al-Maskari, A.Y. & Al-Sabahi, J.N. (2011) Essential oil composition, antimicrobial and antioxidant activities of unexplored Omani basil. Journal of Medicinal Plants Research, 5, 751–757

Hcini, K., Sotomayor, J.A., Jordan, M.J. & Bouzid, S. (2013) Chemical composition of the essential oil of rosemary (*Rosmarinus officinalis L.*) of Tunisian origin. Asian Journal of Chemistry, 25, 2601

Heck, R.T., Fagundes, M.B., Cichoski, A.J., de Menezes, C.R., Barin, J.S., Lorenzo, J.M., Wagner, R. & Campagnol, P.C.B. (2019) Volatile compounds and sensory profile of burgers with 50% fat replacement by microparticles of chia oil enriched with rosemary. Meat Science, 148, 164–170

Hegmann, E., Niether, W., Rohsius, C., Phillips, W. & Lieberei, R. (2020) Besides variety, also season and ripening stage have a major influence on fruit pulp aroma of cacao (*Theobroma cacao L.*). J. Appl. Bot. Food Qual, 93, 266–275

Högnadóttir, Á. & Rouseff, R.L. (2003) Identification of aroma active compounds in orange essence oil using gas chromatography–olfactometry and gas chromatography–mass spectrometry. Journal of Chromatography, 998, 201–211

Hongratanaworakit, T. & Buchbauer, G. (2007) Chemical composition and stimulating effect of *Citrus hystrix* oil on humans. Flavour and Fragrance Journal, 22, 443–449

Hossain, M.B., Barry-Ryan, C., Martin-Diana, A.B. & Brunton, N.P. (2010) Effect of drying method on the antioxidant capacity of six Lamiaceae herbs. Food Chemistry, 123,85–91

Isidorov, V., Sacharewicz, T. & Zenkevich, I. (1997) Calculation of gas chromatographic retention indices for monoterpenes and terpenoids from their physico-chemical constants. Chemia analityczna, 42, 627–634

Isidorov, V.A., Zenkevich, I.G., Dubis, E.N., Slowikowski, A. & Wojciuk, E. (1998) Group identification of essential oils components using partition coefficients in a hexane–acetonitrile system. Journal of Chromatography, 814, 253–260

Jantan, I. bin, Yalvema, M.F., Ayop, N. & Ahmad, A.S. (2005) Constituents of the essential oils of *Cinnamomum sintoc Blume* from a mountain forest of Peninsular Malaysia. Flavour and Fragrance Journal, 20, 601–604

Jardim, C.M., Jham, G.N., Dhingra, O.D. & Freire, M.M. (2008) Composition and antifungal activity of the essential oil of the brazilian *Chenopodium ambrosioides L*. Journal of Chemical Ecology, 34, 1213–1218

Jasper, J., Wagstaff, C. & Bell, L. (2020) Growth temperature influences postharvest glucosinolate concentrations and hydrolysis product formation in first and second cuts of rocket salad. Postharvest Biology and Technology, 163, 111157

Javidnia, K., Miri, R., Kamalinejad, M. & Nasiri, A. (2002) Composition of the essential oil of *Salvia mirzayanii* Rech. f. & Esfand from Iran. Flavour and Fragrance Journal, 17, 465–467

Judžentienė, A. & Būdienė, J. (2008) Volatile constituents from aerial parts and roots of *Cichorium intybus L.*(chicory) grown in Lithuania. Chemija ,19

Jürgens, A. & Dötterl, S. (2004) Chemical composition of anther volatiles in Ranunculaceae: genera-specific profiles in Anemone, Aquilegia, Caltha, Pulsatilla, Ranunculus, and Trollius species. American Journal of Botany, 91, 1969–1980

Kader, A.A. (2008) Flavor quality of fruits and vegetables. Journal of the Science of Food and Agriculture, 88, 1863–1868

Kant, M.R., Ament, K., Sabelis, M.W., Haring, M.A. & Schuurink, R.C. (2004) Differential timing of spider mite-induced direct and indirect defences in tomato Plants. Plant Physiology, 135, 483–495

Khalid, A.K. & El-Gohary, A.E. (2014) Effect of seasonal variations on essential oil production and composition of *Plectranthus amboinicus* (Lour.) grow in Egypt. International Food Research Journal, 21, 1859

Khalid, K. (2006) Influence of water stress on growth, essential oil, and chemical composition of herbs [*Ocimum sp*.]. International Agrophysics, 20

Klimánková, E., Holadová, K., Hajšlová, J., Čajka, T., Poustka, J. & Koudela, M. (2008) Aroma profiles of five basil (*Ocimum basilicum L.*) cultivars grown under conventional and organic conditions. Food Chemistry, 107, 464–472

Kosakowska, O., Węglarz, Z. & Bączek, K. (2019) Yield and quality of 'Greek oregano' (*Origanum vulgare L. subsp. hirtum*) herb from organic production system in temperate climate. Industrial Crops and Products, 141, 111782

Krauze-Baranowska, M., Mardarowicz, M., Wiwart, M., Pobłocka, L. & Dynowska, M. (2002) Antifungal activity of the essential oils from some species of the genus Pinus. Zeitschrift für Naturforschung C, 57, 478–482

Kumar, S., Kumar, S. & Mohapatra, T. (2021) Interaction between macro- and micro-nutrients in plants. Frontiers in Plant Science, 12

Lakušić, D. V., Ristić, M.S., Slavkovska, V.N., Šinžar-Sekulić, J.B. & Lakušić, B.S. (2012) Environment-related variations of the composition of the essential oils of rosemary (*Rosmarinus officinalis L.*) in the Balkan Penninsula. Chemistry & Biodiversity, 9, 1286–1302

Lawless, L.J.R., Hottenstein, A. & Ellingsworth, J. (2012) The McCormick spice wheel: a systematic and visual approach to sensory lexicon development. Journal of Sensory, 27, 37–47

Lazari, D.M., Skaltsa, H.D. & Constantinidis, T. (2000) Volatile constituents of *Centaurea pelia* DC., *C. thessala* Hausskn. subsp. drakiensis (Freyn & Sint.) Georg. and *C. zuccariniana* DC. from Greece. Flavour and Fragrance Journal, 15, 7–11

Le Quere, J.L. & Latrasse, A. (1990) Composition of the essential oils of black currant buds (*Ribes nigrum L.*). Journal of Agricultural and Food Chemistry, 38, 3–10

Lee, S.-J., Umano, K., Shibamoto, T. & Lee, K.-G. (2005) Identification of volatile components in basil (*Ocimum basilicum L.*) and thyme leaves (*Thymus vulgaris L.*) and their antioxidant properties. Food Chemistry, 91, 131–137

Li, J., Zhu, Z. & Gerendás, J. (2008) Effects of nitrogen and sulfur on total phenolics and antioxidant activity in two genotypes of leaf mustard. Journal of Plant Nutrition, 31, 1642–1655

Li, W. & Lan, P. (2017) The understanding of the plant iron deficiency responses in strategy I plants and the role of ethylene in this process by omic approaches. Frontiers in Plant Science, 40

Limberger, R.P., Simões-Pires, C.A., Sobral, M., Menut, C., Bessiere, J.-M. & Henriques, A.T. (2003) Essential oils of six *Gomidesia spp*. from southern Brazil. Flavour and Fragrance Journal, 18, 144–147

Litvin, A.G., Currey, C.J. & Wilson, L.A. (2020) Effects of supplemental light source on basil, dill, and parsley growth, morphology, aroma, and flavor. Journal of the American Society for Horticultural Science, 145, 18–29

Loayza, I., Abujder, D., Aranda, R., Jakupovic, J., Collin, G., Deslauriers, H. & Jean, F.-I. (1995) Essential oils of *Baccharis salicifolia*, *B. latifolia* and *B. dracunculifolia*. Phytochemistry, 38, 381–389

Loreto, F. & Velikova, V. (2001) Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. Plant Physiology, 127, 1781–1787

Loughrin, J.H. & Kasperbauer, M.J. (2001) Light reflected from colored mulches affects aroma and -henol content of sweet basil (*Ocimum basilicum L.*) leaves. Journal of Agricultural and Food Chemistry, 49, 1331–1335

Loughrin, J.H. & Kasperbauer, M.J. (2003) Aroma content of fresh basil (*Ocimum basilicum L.*) leaves is affected by light reflected from colored mulches. Journal of Agricultural and Food Chemistry, 51, 2272–2276

Lucchesi, M.E., Chemat, F. & Smadja, J. (2004) Solvent-free microwave extraction of essential oil from aromatic herbs: comparison with conventional hydro-distillation. Journal of Chromatography, 1043, 323–327

Lucero, M.E., Fredrickson, E.L., Estell, R.E., Morrison, A.A. & Richman, D.B. (2006) Volatile composition of *Gutierrezia sarothrae* (Broom Snakeweed) as determined by steam distillation and solid phase microextraction. Journal of Essential Oil Research, 18, 121–125

Luo, J. & Agnew, M.P. (2001) Gas characteristics before and after biofiltration treating odorous emissions from animal rendering processes. Environmental Technology, 22, 1091–1103

Łyczko, J., Masztalerz, K., Lipan, L., Iwiński, H., Lech, K., Carbonell-Barrachina, Á.A. & Szumny, A. (2021) *Coriandrum sativum L.*-Effect of multiple drying techniques on volatile and sensory profile. Foods, 10

Macku, C. & Shibamoto, T. (1991) Volatile sulfur-containing compounds generated from the thermal interaction of corn oil and cysteine. Journal of Agricultural and Food Chemistry, 39, 1987–1989

Marangoni, C. & Moura, N.F. (2011) Sensory profile of Italian salami with coriander (*Coriandrum sativum L.*) essential oil. Food Science and Technology, 31, 119–123

Maric, S., Maksimovic, M. & Milos, M. (2006) The impact of the locality altitudes and stages of development on the volatile constituents of *Salvia officinalis L.* from Bosnia and Herzegovina. Journal of Essential Oil Research, 18, 178–180

Marongiu, B., Piras, A., Pani, F., Porcedda, S. & Ballero, M. (2003) Extraction, separation and isolation of essential oils from natural matrices by supercritical CO2. Flavour and Fragrance Journal, 18, 505–509

Marongiu, B., Piras, A., Porcedda, S. & Scorciapino, A. (2005) Chemical composition of the essential oil and supercritical CO2 extract of *Commiphora myrrha* (Nees) Engl. and of *Acorus calamus* L. Journal of Agricultural and Food Chemistry, 53, 7939–7943

Marongiu, B., Porcedda, S., Piras, A., Sanna, G., Murreddu, M. & Loddo, R. (2006) Extraction of *Juniperus communis L.* ssp. nana Willd. essential oil by supercritical carbon dioxide. Flavour and Fragrance Journal, 21, 148–154

Marques, F.A., McElfresh, J.S. & Millar, J.G. (2000) Kováts retention indexes of monounsaturated C12, C14, and C16 alcohols, acetates and aldehydes commonly found in lepidopteran pheromone blends. Journal of the Brazilian Chemical Society, 11, 592–599

Mauer, L. & El-Sohemy, A. (2012) Prevalence of cilantro (*Coriandrum sativum*) disliking among different ethnocultural groups. Flavour, 1, 8

McAusland, L., Lim, M.-T., Morris, D.E., Smith-Herman, H.L., Mohammed, U., Hayes-Gill, B.R., Crowe, J.A., Fisk, I.D. & Murchie, E.H. (2020) Growth spectrum complexity dictates aromatic intensity in coriander (*Coriandrum sativum L.*). Frontiers in Plant Science, 11, 462

Meerburg, B.G., Brom, F.W.A. & Kijlstra, A. (2008) The ethics of rodent control experimentation. Pest management science, 64, 1205–1211

Merle, H., Morón, M., Blázquez, M.A. & Boira, H. (2004) Taxonomical contribution of essential oils in mandarins cultivars. Biochemical Systematics and Ecology, 32, 491–497

Mesa-Arango, A.C., Betancur-Galvis, L., Montiel, J., Bueno, J.G., Baena, A., Durán, D.C., Martínez, J.R. & Stashenko, E.E. (2010) Antifungal activity and chemical composition of the essential oils of *Lippia alba* (Miller) N.E Brown grown in different regions of Colombia. Journal of Essential Oil Research, 22, 568–574

Methven, L., Tsoukka, M., Oruna-Concha, M.J., Parker, J.K. & Mottram, D.S. (2007) Influence of sulfur amino acids on the volatile and nonvolatile components of cooked salmon (*Salmo salar*). Journal of Agricultural and Food Chemistry, 55, 1427–1436

Mevy, J.-P., Bessiere, J.-M., Greff, S., Zombre, G. & Viano, J. (2006) Composition of the volatile oil from the leaves of *Ximenia americana* L. Biochemical Systematics and Ecology, 34, 549–553

Miele, M., Dondero, R., Ciarallo, G. & Mazzei, M. (2001) Methyleugenol in *Ocimum basilicum L.* Cv. Genovese Gigante. Journal of Agricultural and Food Chemistry, 49, 517–521

Miller, G., Shulaev, V. & Mittler, R. (2008) Reactive oxygen signaling and abiotic stress. Physiologia plantarum, 133, 481–489

Miyazaki, T., Plotto, A., Goodner, K. & Gmitter Jr, F.G. (2011) Distribution of aroma volatile compounds in tangerine hybrids and proposed inheritance. Journal of the Science of Food and Agriculture, 91, 449–460

Moghaddam, M., Omidbiagi, R. & Sefidkon, F. (2007) Changes in content and chemical composition of *Tagetes minuta* oil at various harvest times. Journal of Essential Oil Research, 19, 18–20

Moosavi, G., Seghatoleslami, M., Ebrahimi, A., Fazeli, M. & Jouyban, Z. (2015) The effect of nitrogen rate and plant density on morphological traits and essential oil yield of coriander. Journal of Ornamental Plants, 3, 95–103

Moretti, M.D.L., Peana, A.T., Passino, G.S., Bazzoni, A. & Solinas, V. (1998) Effects of iron on yield and composition of *Rosmarinus officinalis L.* essential oil. Journal of Essential Oil Research, 10, 43–49

Morteza-Semnani, K., Akbarzadeh, M. & Moshiri, K. (2005) Essential oil composition of *Artemisia fragrans* Willd. from Iran. Flavour and Fragrance Journal, 20, 330–331

Morteza-Semnani, K., Saeedi, M. & Akbarzadeh, M. (2007) Essential oil composition of *Teucrium scordium L.* Acta Pharmaceutica, 57, 499–504

Morteza-Semnani, K., Saeedi, M., Akbarzadeh, M. & Moshiri, K. (2006) The essential oil composition of *Onosma microcarpum* DC. Flavour and Fragrance Journal, 21, 314–316

Mosayebi, M., Amin, G., Arzani, H., Azarnivand, H., Maleki, M. & Shafaghat, A. (2008) Effect of habitat on essential oil of *Achillea filipendulina L.* in Iran. Asian Journal of Plant Sciences, 9, 779-781

Mousavi, S.R., Galavi, M. & Rezaei, M. (2012) The interaction of zinc with other elements in plants: a review. International Journal of Agriculture and Crop Sciences, 4, 1881–1884

Mulas, G., Gardner, Z. & Craker, L.E. (2006) Effect of light quality on growth and essential oil composition in rosemary. Acta Hortic, 723, 427–432

Mulè, A., Moretti, M.D.L., Pirisino, G. & Satta, M. (1996) Studio della resa e della composizione chimica dell'olio essenziale di *Rosmarinus officinalis L.* spontaneo di Calagonone (Sardegna). Rivista Italiana Eppos, 19, 147–157

Munné-Bosch, S. (2007) Aging in perennials. Critical Reviews in Plant Sciences, 26, 123–138

Murárikova, A., Ťažký, A., Neugebauerová, J., Planková, A., Jampílek, J., Mučaji, P. & Mikuš, P. (2017) Characterization of essential oil composition in different basil species and pot cultures by a GC-MS method. Molecules, 22

Nadjafi, F., Damghani, A.M.M. & Ebrahimi, S.N. (2009) Effect of irrigation regimes on yield, yield components, content and composition of the essential oil of four iranian land races of coriander (*Coriandrum sativum*). Journal of Essential Oil Bearing Plants,12, 300–309

Neffati, M. & Marzouk, B. (2008) Changes in essential oil and fatty acid composition in coriander (*Coriandrum sativum L.*) leaves under saline conditions. Industrial Crops and Products, 28, 137–142

Neffati, M. & Marzouk, B. (2008) Changes in essential oil and fatty acid composition in coriander (*Coriandrum sativum L.*) leaves under saline conditions. Industrial Crops and Products, 28, 137–142

Nivinskienė, O., Butkienė, R., Gudalevič, A., Mockutė, D., Meškauskienė, V. & Grigaliūnaitė, B. (2007) Influence of urban environment on chemical composition of *Tilia cordata* essential oil. Chemija, 18

Nogueira, P.C.L., Bittrich, V., Shepherd, G.J., Lopes, A.V. & Marsaioli, A.J. (2001) The ecological and taxonomic importance of flower volatiles of Clusia species (*Guttiferae*). Phytochemistry, 56, 443–452

Nurzyńska-Wierdak, R. (2013) Essential oil composition of the coriander (*Coriandrum sativum L.*) herb depending on the development stage. Acta Agrobotanica, 66, 53–60

Nurzynska-Wierdak, R., Borowski, B., Dzida, K., Zawislak, G. & Kowalski, R. (2013) Essential oil composition of sweet basil cultivars as affected by nitrogen and potassium fertilization. Turkish Journal of Agriculture and Forestry, 37, 427–436

Omidbaigi, R., Hassani, A. & Sefidkon, F. (2003) Essential oil content and composition of sweet basil (*Ocimum basilicum*) at different irrigation regimes. Journal of Essential Oil Bearing Plants, 6, 104–108

Oñate, M., Blanc, J. & Munné-Bosch, S. (2011) Influence of stress history on the response of the dioecious plant *Urtica dioica L*. to abiotic stress. Plant Ecology & Diversity, 4, 45–54

Opara, E.I. & Chohan, M. (2014) Culinary herbs and spices: their bioactive properties, the contribution of polyphenols and the challenges in deducing their true health benefits. International Journal of Molecular Sciences, 15, 19183–19202

Ormeño, E., Céspedes, B., Sánchez, I.A., Velasco-García, A., Moreno, J.M., Fernandez, C. & Baldy, V. (2009) The relationship between terpenes and flammability of leaf litter. Forest Ecology and Management, 257, 471–482

Osakabe, Y., Osakabe, K., Shinozaki, K. & Tran, L.-S. (2014) Response of plants to water stress. Frontiers in Plant Science, 5, 86

Özel, M.Z., Göğüş, F. & Lewis, A.C. (2006) Comparison of direct thermal desorption with water distillation and superheated water extraction for the analysis of volatile components of *Rosa damascena* Mill. using GCxGC-TOF/MS. Analytica Chimica Acta, 566, 172–177

Padalia, R.C. & Verma, R.S. (2011) Comparative volatile oil composition of four *Ocimum* species from northern India. Natural Product Research, 25, 569–575

Parker, J.K., Elmore, J.S. & Methven, L. (2015) Flavour development, Analysis and perception in food and beverages. Food science, technology and nutrition, 273

Patel, M, Lee, R., Merchant, E.V., Juliani, H.R., Simon, J.E. & Tepper, B.J. (2021) Descriptive aroma profiles of fresh sweet basil cultivars (*Ocimum spp*.): relationship to volatile chemical composition. Journal of Food Science, 86, 3228–3239

Patil, J.R., Jayaprakasha, .GK., Chidambara, Murthy, K.N., Tichy, S.E., Chetti, M.B. & Patil, B.S. (2009) Apoptosis-mediated proliferation inhibition of human colon cancer cells by volatile principles of *Citrus aurantifolia*. Food Chemistry, 114, 1351–1358

Pessoa, L.M., Morais, S.M., Bevilaqua, C.M.L. & Luciano, J.H.S. (2002) Anthelmintic activity of essential oil of *Ocimum gratissimum* Linn. and eugenol against *Haemonchus contortus*. Veterinary Parasitology, 109, 59–63

Peter, K.V. & Shylaja, M.R. (2012) Introduction to herbs and spices: definitions, trade and applications. Handbook of Herbs and Spices (Second Edition), Woodhead Publishing, 1–24

Peterson, D.G. & Reineccius, G.A. (2003) Characterization of the volatile compounds that constitute fresh sweet cream butter aroma. Flavour and Fragrance Journal, 18, 215–220

Pintore, G., Usai, M., Bradesi, P., Juliano, C., Boatto, G., Tomi, F., Chessa, M., Cerri, R. & Casanova, J. (2002) Chemical composition and antimicrobial activity of *Rosmarinus officinalis L.* oils from Sardinia and Corsica. Flavour and Fragrance Journal, 17, 15–19

Potter, T.L. & Fagerson, I.S. (1990) Composition of coriander leaf volatiles. Journal of agricultural and food chemistry, 38, 2054–2056

Precone, V., Beccari, T., Stuppia, L., Baglivo, M., Paolacci, S., Manara, E., Miggiano, G.A.D., Falsini, B., Trifirò, A. & Zanlari, A. (2019) Taste, olfactory and texture related genes and food choices: implications on health status. European Review Medical Pharmacological Sciences, 23, 1305–1321

Pushpangadan, P. & George, V. (2012) Basil. Handbook of Herbs and Spices (Second Edition), Woodhead Publishing, 55–72

Putievsky, E. & Galambosi, B. (1999) Production systems of sweet basil. Basil: the genus Ocimum. Harwood Academic Publishers, 10, 39–65

Putra, P.A. & Yuliando, H. (2015) Soilless culture system to support water use efficiency and product quality: a review. Agriculture and Agricultural Science Procedia, 3, 283–288

Raffo, A., Mozzanini, E., Ferrari Nicoli, S., Lupotto, E. & Cervelli, C. (2020) Effect of light intensity and water availability on plant growth, essential oil production and composition in *Rosmarinus officinalis L*. European Food Research and Technology, 246, 167–177

Rahimi-Nasrabadi, M., Gholivand, M.B. & Batooli, H. (2009) Chemical composition of the essential oil from leaves and flowering aerial parts of *Haplophyllum robustum Bge*. (rutaceae). Digest Journal of Nanomaterials & Biostructures, 4

Ramarathnam, N., Rubin, L.J. & Diosady, L.L. (1993) Studies on meat flavor. 3. A novel method for trapping volatile components from uncured and cured pork. Journal of Agricultural and Food Chemistry, 41, 933–938

Ramarathnam, N., Rubin, L.J. & Diosady, L.L. (1993a) Studies on meat flavor. 4. Fractionation, characterization, and quantitation of volatiles from uncured and cured beef and chicken. Journal of Agricultural and Food Chemistry, 41, 939–945

Rao, L.J., Singh, M., Raghavan, B. & Abraham, K.O. (1998) Rosemary (*Rosmarinus officinalis* L.): impact of drying on its flavor quality. Journal of Food Quality, 21, 107–115

Ravi, R., Prakash, M. & Bhat, K.K. (2007) Aroma characterization of coriander (*Coriandrum sativum L.*) oil samples. European Food Research and Technology, 225, 367–374

Reverchon, E., Della Porta. G. & Gorgoglione, D. (1997) Supercritical CO2 extraction of volatile oil from rose concrete. Flavour and Fragrance Journal, 12, 37–41

Rohloff, J. (2006) Essential oil drugs - terpene composition of aromatic herbs. Production Practices and Quality Assessment of Food Crops, 3, 73–128

Rosenthal, G.A. & Berenbaum, M.R. (2012) Herbivores: their interactions with secondary plant metabolites: ecological and evolutionary processes. Elsevier Science

Rostad, C.E. & Pereira, W.E. (1986) Kovats and lee retention indices determined by gas chromatography/mass spectrometry for organic compounds of environmental interest. Journal of High Resolution Chromatography, 9, 328–334

Sabulal, B., Dan, M., John, A., Kurup, R., Chandrika, S.P. & George, V. (2007) Phenylbutanoid-rich rhizome oil of *Zingiber neesanum* from Western Ghats, southern India. Flavour and Fragrance Journal, 22, 521–524

Said-Al Ahl, H.A.H. & Omer, E.A. (2009) Effect of spraying with zinc and/or iron on growth and chemical composition of coriander (*Coriandrum sativum L*.) harvested at three stages of development. Journal of Medicinal Food Plants, 1, 30–46

Saleh, H.A.-R., El-Nashar, Y.I., Serag-El-Din, M.F. & Dewir, Y.H. (2019) Plant growth, yield and bioactive compounds of two culinary herbs as affected by substrate type. Scientia Horticulturae, 243, 464–471

Salido, S., Altarejos, J., Nogueras, M., Saánchez, A. & Luque, P. (2003) Chemical composition and seasonal variations of rosemary oil from southern Spain. Journal of Essential Oil Research, 15, 10–14

Santos, A.P., Lopes, M.C., Limberger, R.P., Apel, M.A., Henriques, A.T. & Moreno, P.R.H. (2004) Analysis of the volatile oil from *Pilocarpus pennatifolius Lemmaire* (Rutaceae) leaves by GC–MS. Flavour and Fragrance Journal, 19, 325–326

Santos, A.S., Andrade, E.H.D.A., Zoghbi, M.D.G.B. & Maia, J.G.S. (1998) Volatile constituents of fruits of *Annona glabra L*. from Brazil. Flavour and Fragrance Journal, 13, 148–150

Sarikurkcu, C., Tepe, B., Daferera, D., Polissiou, M. & Harmandar, M. (2008) Studies on the antioxidant activity of the essential oil and methanol extract of *Marrubium globosum* subsp. globosum (lamiaceae) by three different chemical assays. Bioresource Technology, 99, 4239–4246

Sárosi, S., Sipos, L., Kókai, Z., Pluhár, Z., Szilvássy, B. & Novák, I. (2013) Effect of different drying techniques on the aroma profile of *Thymus vulgaris* analyzed by GC–MS and sensory profile methods. Industrial Crops and Products, 46, 210–216

Sato, F. (2014) Plant Secondary Metabolism. eLS

Scala, A., Allmann, S., Mirabella, R., Haring, M.A. & Schuurink, R.C. (2013) Green leaf volatiles: a plant's multifunctional weapon against herbivores and pathogens. International Journal of Molecular Sciences, 14, 17781–17811

Schöffl, F., Prändl, R. & Reindl, A. (1998) Regulation of the heat-shock response. Plant Physiology, 117, 1135–1141

Schwab, W., Davidovich-Rikanati, R. & Lewinsohn, E. (2008) Biosynthesis of plant-derived flavor compounds. The Plant Journal, 54, 712–732

Schwob, I., Bessière, J.-M. & Viano, J. (2002) Composition of the essential oils of *Hypericum perforatum L*. from southeastern France. Comptes rendus biologies, 325, 781–785

Seigler, D.S. (1998) Plant secondary metabolism. Springer Science & Business Media

Senatore, F., Arnold, N.A. & Bruno, M. (2005) Volatile components of *Centaurea eryngioides* Lam. and *Centaurea iberica* Trev. var. hermonis Boiss. Lam., two Asteraceae growing wild in Lebanon. Natural Product Research, 19, 749–754

Serrano, E., Palma, J., Tinoco, T., Venâncio, F. & Martins, A. (2002) Evaluation of the essential oils of rosemary (*Rosmarinus officinalis L*.) from different zones of "Alentejo" (Portugal). Journal of Essential Oil Research, 14, 87–92

Shahwar, M.K., El-Ghorab, A.H., Anjum, F.M., Butt, M.S., Hussain, S. & Nadeem, M. (2012) Characterization of coriander (*Coriandrum sativum L.*) seeds and leaves: volatile and non volatile extracts. International Journal of Food Properties, 15, 736–747

Sharkey, T.D., Chen, X. & Yeh, S. (2001) Isoprene increases thermotolerance of fosmidomycin-fed leaves. Plant Physiology, 125, 2001–2006

Sharma, N., Acharya, S., Kumar, K., Singh, N. & Chaurasia, O.P. (2018) Hydroponics as an advanced technique for vegetable production: an overview. Journal of Soil and Water Conservation, 17, 364–371

Sifola, M.I. & Barbieri, G. (2006) Growth, yield and essential oil content of three cultivars of basil grown under different levels of nitrogen in the field. Scientia Horticulturae, 108. 408–413

Silva, P.S., Suzuki, É.Y., Moreira, A.P., Raposo, N.R.B., Alves, T.M.A. & Viccini, L.F. (2012) *Stachytarpheta gesnerioides* Cham.: chemical composition of hexane fraction and essential oil, antioxidant and antimicrobial activities. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas, 11, 542–548

Simon, J.E., Morales, M.R., Phippen, W.B., Vieira, R.F. & Hao, Z. (1999) Basil: a source of aroma compounds and a popular culinary and ornamental herb. Perspectives on new crops and new uses, 16, 499–505

Singletary, K. (2016) Coriander: overview of potential health benefits. Nutrition Today, 51

Smelcerovic, A., Spiteller, M., Ligon, A.P., Smelcerovic, Z. & Raabe, N. (2007) Essential oil composition of *Hypericum L*. species from Southeastern Serbia and their chemotaxonomy. Biochemical Systematics and Ecology, 35, 99–113

Socaci, S.A., Tofana, M. & Socaciu, C. (2008) GC-MS analysis of rosemary essential oil. Bulletin UASVM Agriculture, 65, 405–409

Song, C., Lai, W.-C., Reddy, K.M. & Wei, B. (2003) Temperature-programmed retention indices for GC and GC-MS of hydrocarbon fuels and simulated distillation GC of heavy oils. Analytical advances for hydrocarbon research, 147–210

Sulochanamma, G., Ramalakshmi, K. & Borse, B.B. (2009) Stabilization of flavour volatiles of basil (*Ocimum basilicum L*.). Journal of Food Science and Technology, 46, 54-57

Sun, D. & Petracek, P.D. (1999) Grapefruit gland oil composition is affected by wax application, storage temperature, and storage time. Journal of Agricultural and Food Chemistry, 47, 2067–2069

Sylvestre, M., Pichette, A., Longtin, A., Nagau, F. & Legault, J. (2006) Essential oil analysis and anticancer activity of leaf essential oil of *Croton flavens L.* from Guadeloupe. Journal of Ethnopharmacology, 103, 99–102

Szumny, A., Figiel, A., Gutiérrez-Ortíz, A. & Carbonell-Barrachina, Á.A. (2010) Composition of rosemary essential oil (*Rosmarinus officinalis*) as affected by drying method. Journal of Food Engineering, 97, 253–260

Tamura, H., Maeyama, K., Yoshida, E. & Kori, M. (2013) Aroma character of coriander (*Coriandrum Sativum L.*) leaves: limited odor unit method and sensory perception in preference. Nutrition, Functional and Sensory Properties of Foods, 66–73

Tapsell, L.C., Hemphill, I., Cobiac, L., Sullivan, D.R., Fenech, M., Patch, C.S., Roodenrys, S., Keogh, J.B., Clifton, P.M. & Williams, P.G. (2006) Health benefits of herbs and spices: the past, the present, the future.

Telci, I. & Hisil, Y. (2008) Biomass yield and herb essential oil characters at different harvest stages of spring and autumn sown *Coriandrum sativum*. European Journal of Horticultural Science, 73, 267–272

Tellez, M., Estell, R., Fredrickson, E., Powell, J., Wedge, D., Schrader, K. & Kobaisy, M. (2001) Extracts of *Flourensia cernua* (L): volatile constituents and antifungal, antialgal, and antitermite bioactivities. Journal of Chemical Ecology, 27, 2263–2273

Tounekti, T., Vadel, A.M., Ennajeh, M., Khemira, H. & Munné-Bosch, S. (2011) Ionic interactions and salinity affect monoterpene and phenolic diterpene composition in rosemary (*Rosmarinus officinalis*). Journal of Plant Nutrition and Soil Science, 174, 504–514

Turner, L., Lignou S., Gawthrop, F. & Wagstaff, C. (2021) Investigating the relationship of genotype and geographical location on volatile composition and sensory profile of celery (*Apium graveolens*). International journal of molecular sciences, 22, 12016

Turner, L., Lignou, S., Gawthrop, F. & Wagstaff, C. (2021) Consumer acceptability and sensory profile of three new celery (*Apium graveolens*) hybrids and their parental genotypes. International Journal of Molecular Sciences, 22, 13561

Turner, L., Lignou, S., Gawthrop, F. & Wagstaff, C. (2021) Investigating the factors that influence the aroma profile of *Apium graveolens*: a review. Food Chemistry, 345, 128673

Turner, L., Lignou, S., Gawthrop, F. & Wagstaff, C. (2021) Investigating the relationship of genotype and climate conditions on the volatile composition and sensory profile of celery (*Apium graveolens*). Foods,10, 1335

Umar Lule, S. & Xia, W. (2005) Food phenolics, pros and cons: a Review. Food Reviews International, 21, 367–388

Valcourt, A. (2014) The chemistry and biology of purines. American Journal of Psychiatry

Vallverdú-Queralt, A., Regueiro, J., Martínez-Huélamo, M., Rinaldi Alvarenga, J.F., Leal, L.N. & Lamuela-Raventos, R.M. (2014) A comprehensive study on the phenolic profile of widely used culinary herbs and spices: rosemary, thyme, oregano, cinnamon, cumin and bay. Food Chemistry, 154, 299–307

van der Walt, L., Dovey, M., Naudé, Y. & Forbes, P.B.C. (2013) Investigation into the aroma of rosemary using multi-channel silicone rubber traps, off-line olfactometry and comprehensive two-dimensional gas chromatography-mass spectrometry. South African Journal of Chemistry, 66, 21–26

Varlet, V., Serot, T., Cardinal, M., Knockaert, C. & Prost, C. (2007) Olfactometric determination of the most potent odor-active compounds in salmon muscle (*Salmo salar*) smoked by using four smoke generation techniques. Journal of Agricultural and Food Chemistry, 55, 4518–4525

Verma, R.S., Verma, R.K., Chauhan, A. & Yadav, A.K. (2010) Changes in the essential oil composition of *Majorana hortensis Moench*. cultivated in India during plant ontogeny. Journal of the Serbian chemical Society, 75, 441–447

Verpoorte, R. (2000) Secondary metabolism. Metabolic Engineering of Plant Secondary Metabolism, 1–29

Viña, A. & Murillo, E. (2003) Essential oil composition from twelve varieties of basil (*Ocimum spp*) grown in Colombia. Journal of the Brazilian chemical society, 14, 744–749

Wang, Y., Finn, C. & Qian, M.C. (2005) Impact of growing environment on chickasaw blackberry (*Rubus L.*) aroma evaluated by gas chromatography olfactometry dilution analysis. Journal of Agricultural and Food Chemistry, 53, 3563–3571

Wang, Z., Fingas, M. & Li, K. (1994) Fractionation of a light crude oil and identification and quantitation of aliphatic, aromatic, and biomarker compounds by GC-FID and GC-MS, part II. Journal of Chromatographic Science, 32, 367–382

Waterman, P.G. & Mole, S. (1989) Extrinsic factors influencing production of secondary metabolites in plants. Insect-plant interactions, 1, 107–134

Whetstine, M.E.C., Croissant, A.E. & Drake, M.A. (2005) Characterization of dried whey protein concentrate and isolate flavor. Journal of Dairy Science, 88, 3826–3839

Xu, X., Stee, L.L.P., Williams, J., Beens, J., Adahchour, M., Vreuls, R.J.J., Brinkman, U.A. & Lelieveld, J. (2003) Comprehensive two-dimensional gas chromatography (GC × GC) measurements of volatile organic compounds in the atmosphere. Atmospheric Chemistry and Physics, 3, 665–682

Yousif, A.N., Scaman, C.H., Durance, T.D. & Girard, B. (1999) Flavor volatiles and physical properties of vacuum-microwave- and air-dried sweet basil (*Ocimum basilicum L.*). Journal of Agricultural and Food Chemistry, 47, 4777–4781

Yu, E.J., Kim, T.H., Kim, K.H. & Lee, H.J. (2004) Aroma-active compounds of *Pinus densiflora* (red pine) needles. Flavour and Fragrance Journal, 19, 532–537

Zaikin, V.G. & Borisov, R.S. (2002) Chromatographic–mass spectrometric analysis of Fischer–Tropsch synthesis products. Journal of Analytical Chemistry, 57, 544–551

Zawirska-Wojtasiak, R. & Wąsowicz, E. (2009) GC Analysis of rosemary aroma isolated traditionally by distillation and by SPME. Journal of Essential Oil Research, 21, 8–15

Zigene, Z.D., Kassahun, B.M. & Ketaw, T.T. (2012) Effects of harvesting age and spacing on leaf yield and essential oil yield of rosemary (*Rosmarinus officinalis L.*). African Journal of Plant Sciences and Biotechnology, 5, 9–12

Zoghbi, M.G.B., Andrade, E.H.A., Santos, A.S., Silva, M.H.L. & Maia, J.G.S. (1998) Essential oils of *Lippia alba* (Mill.) N. E. Br growing wild in the Brazilian Amazon. Flavour and Fragrance Journal, 13, 47–48

Zunino, M.P., Novillo-Newton, M., Maestri, D.M. & Zygadlo, J.A. (1997) Composition of the essential oil of *Baccharis crispa Spreng*. and *Baccharis salicifolia Pers*. grown in Córdoba (Argentina). Flavour and Fragrance Journal, 12, 405–407

Appendix I – Form submitted by producers with sample submission

FV / PE 455 Herb flavours project

Product life cycle and submission information

1. **Herb species and variety:**

☐Rosemary ☐Coriander ☐Basil

   a. Herb variety:

☐Perigord ☐Miss Jessops ☐Unknown

☐Cruiser ☐Santo ☐Chetchnya

☐Sweet Genovese ☐Other:_____

2. **Grower:**

☐Vitacress ☐Herbs Unlimited ☐Liconlshire ☐Valley Produce ☐J Bond & Son ☐NV Produce ☐Red Deer Herbs

3. **Production Method:**

☐Organic ☐Conventional ☐Hydroponic ☐Soil Protected ☐Pots

4. **Planting date: ___/___/_____**

5. **Harvesting date: ___/___/2021**

6. **Temperature average during growth:**

☐<0 ˚C ☐0-5˚C ☐6-10 ˚C ☐11-15 ˚C ☐16-20 ˚C ☐20-25 ˚C ☐ >25 ˚C ☐No records

7. **Light exposure (protected crops):**

   a. Type: ☐Natural ☐LED ☐HPS ☐MH

      Other:_____

   b. Time of exposure: ____hour(s) of lights on.

8. **Water supply**: ☐Rainfall ☐Irrigation

    a. Quantity (if known) :_____/week

9. **Fertiliser and crop protection product application (please provide records if available)**

☐CAN _____/day ☐CN_____/day ☐SOP_____/day ☐None ☐Records provided

10. **Shipping date: ___/___/2021**

11. **Duration between harvest and cooling**: _____minutes

12. **Average temperature during transport (if known)**

☐<0 ˚C ☐0-5 ˚C ☐6-10 ˚C ☐11-15 ˚C ☐>15 ˚C ☐Unknown

13. **Crop stage/maturity when harvested: (select all that apply)**

    ☐First cut ☐Second cut ☐Fully matured ☐Target____cm

14. **Pot production :product used as soil or growing media:**

☐Peat ☐Coir ☐Mixture ☐Other:_____