

Project title: Determining the basis of variation in herb flavour

Project number: FV/PE 455

Project leader: Prof Carol Wagstaff, University of Reading

Report: Annual report, March 2019

Previous report: N/A

Key staff: Ana Contente
Dr Maria-Jose Oruna-Concha

Location of project: University of Reading

Industry Representative: Philip Dodd, Herbs Unlimited, York

Date project commenced: 12th March 2018

DISCLAIMER

While the Agriculture and Horticulture Development Board seeks to ensure that the information contained within this document is accurate at the time of printing, no warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

© Agriculture and Horticulture Development Board 2019. No part of this publication may be reproduced in any material form (including by photocopy or storage in any medium by 3electronic mean) or any copy or adaptation stored, published or distributed (by physical, electronic or other means) without prior permission in writing of the Agriculture and Horticulture Development Board, other than by reproduction in an unmodified form for the sole purpose of use as an information resource when the Agriculture and Horticulture Development Board or AHDB Horticulture is clearly acknowledged as the source, or in accordance with the provisions of the Copyright, Designs and Patents Act 1988. All rights reserved.

All other trademarks, logos and brand names contained in this publication are the trademarks of their respective holders. No rights are granted without the prior written permission of the relevant owners.

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Professor Carol Wagstaff

Principal Investigator, Head of School Chemistry, Food and Pharmacy

University of Reading

Signature *C. Wagstaff* Date30th April 2019.....

Report authorised by:

Professor Carol Wagstaff

Principal Investigator, Head of School Chemistry, Food and Pharmacy

University of Reading

Signature *C. Wagstaff* Date30th April 2019.....

CONTENTS

GROWER SUMMARY	5
Headline	5
Background	5
Summary	5
Financial Benefits	7
Action Points	7
SCIENCE SECTION	9
Introduction	9
Polyphenols.....	9
Herb Production Systems.....	10
Herb metabolites	11
Aroma Volatile Compounds	11
Materials and methods	15
Sample preparation.....	16
Extraction of aroma volatile compounds by Solid Phase Micro-Extraction (SPME).....	17
Gas Chromatography-Mass Spectrometry (GC-MS) analysis of SPME extracts.....	17
Compound identification	17
Statistical analysis	17
Results	17
Discussion	30
Volatiles analysis	30
Principal Component Analysis.....	32
Conclusions.....	34
Knowledge and Technology Transfer	35
Glossary.....	35
References.....	36
Appendix 1	40

GROWER SUMMARY

Headline

Baseline flavour profiles have been determined for three UK herb crops, basil, coriander and rosemary. Work is ongoing to determine how flavour profiles vary with season and production method.

Background

Herb flavour can vary in its composition as well as intensity. This variation can happen as a result of different cultivars, agronomic practices, season and climate. Consumption of culinary herbs has increased due to pressure to reduce salt content in foods whilst retaining a flavourful eating experience, so the flavour is the most important attribute of the herbs. Therefore, understanding how flavour varies in composition and abundance within a herb species as a result of different production systems and climate conditions, and how these differences are perceived by consumers, will help growers to adjust their practice, to enable the industry to deliver a more consistent and acceptable product.

The overall project aim is to elucidate the chemical profile of commercially important culinary herb crops and understand how varietal choice, season, agronomic practice, cultivation system and environment interact with this. Project objectives are to:

- Profile chemistry of three core herbs: coriander, basil, and rosemary
- Examine the impact of different seasons on flavour profile over three sequential years
- Examine the impact of production system on flavour profile
- Associate flavour profiles with consumer liking

Summary

Three different herbs were selected for study as being of the greatest commercial relevance and covering both annual, perennial, soft and woody herbs: basil, coriander and rosemary. The steering group provided several sites (across West Sussex, Lincolnshire, Berkshire, Worcestershire, Norfolk and Yorkshire) covering a range of production methods. These sites provided samples of herbs produced in protected conditions in pots under glass (Pots), in soil under protection (Soil) or in hydroponics under glass (Hydroponics). Samples from field production (Field) were also provided for analysis. The herbs included at each sampling time depended on what was available for each site / production system. The table below shows which production types were analysed for each of the herbs in summer and autumn 2018, and the corresponding geographical location. Further samples were collected from protected

cropping only, in January 2019. Coriander samples were all of var. Cruiser, and basil was of type Sweet Genovese. Rosemary varieties varied with site.

Table 1. Production systems sampled for three herb species and corresponding geographical location.

	Rosemary	Coriander	Basil
West Sussex	Pots	Pots, Field	Pots, Hydroponics
Lincolnshire		Pots	Pots
Berkshire	Field		
Worcs	Field	Field	Soil
Norfolk	Field		
Yorks	Soil, Field	Field	

To determine flavour profiles, fresh samples were used for head-space volatiles analysis and this was done using solid-phase microextraction (SPME) coupled with gas chromatography-mass spectrometry (GC-MS). The relative abundance of different compounds was calculated using an internal standard. Statistical analyses are ongoing to determine the significance of variation in volatile compounds attributed to production method, location of production and season. This annual report focuses on results from samples collected in summer 2018.

Initial sampling of the three herbs provided baseline data of the key flavour volatiles found in each crop type. For all the three herbs the main compounds mentioned in the literature as being important components of the flavour, were detected in all the samples. In the case of rosemary, these compounds were alpha-pinene, camphene, beta-pinene, 1,8-cineole, linalool, camphor, alpha-terpineol and verbenone, described as giving a fresh, wood, pine, camphor, menthol aroma. For coriander, the compounds provided a soapy, waxy, citrus, fruity aroma, and this was due to the presence of E-2-undecenal, dodecanal, E-2-dodecenal and E-2-decenal. Basil's main compounds were 1,8-cineole, linalool, methyl chavicol and eugenol, providing an aroma described as sweet, herbal, fresh, floral and spicy. Rosemary was the species with the most aroma volatiles detected, followed by basil then coriander. Of the three herbs, basil had the most common compounds detected across samples from different production systems / geographic locations.

From the results so far, it has been possible to observe some broad differences between samples from different herbs, production systems and geographic locations:

- Variation in the flavour profiles of rosemary samples were largely attributed to differences in variety, rather than production systems.
- Pot-produced herbs show significant differences in flavour profile when compared with other types of production such as field cultivation, soil-grown under protection or hydroponics (basil only). This was particularly apparent for coriander samples (Figure A) where principal components analysis was used to correlate flavour components to different production systems.
- Of the three herbs studied, basil is the one with a flavour profile that is most sensitive to environmental conditions, in other words to the geographical location.

More analysis needs to be done in order to reach more definitive conclusions, using samples collected from the same sites during different seasons. Understanding how these differences are perceived by the consumer (project year 2 onwards) will help determine how definite these differences are for those who buy these products.

Financial Benefits

This project will provide UK herb growers with information to help them understand better the variations in their product, and in doing it, help to deliver a more consistent product along the year. This means that the need of importing product from other countries will decrease, leading to an increase in the consumption of UK grown herbs resulting in more profit and less waste.

Action Points

None to date

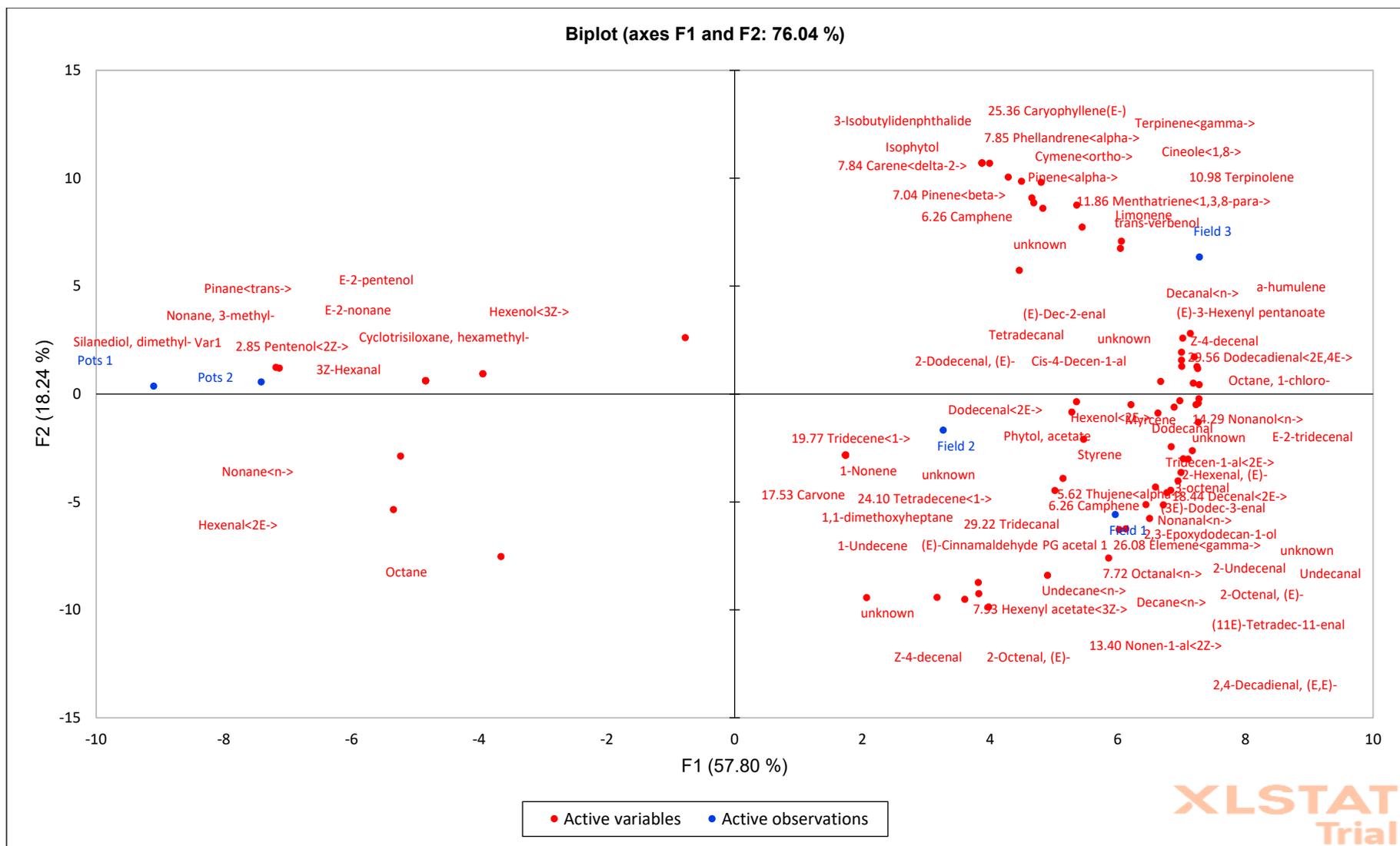


Figure A. Graph correlating the volatile compounds found in coriander (red text) and their relation to the different production methods (blue text).

SCIENCE SECTION

Introduction

The overall project aim is to elucidate the chemical profile of commercially important culinary herb crops and understand how varietal choice, season, agronomic practice, cultivation system and environment interact with this. Project objectives are to:

- Profile chemistry of three core herbs: coriander, basil, and rosemary
- Examine the impact of different seasons on flavour profile over three sequential years
- Examine the impact of production system on flavour profile
- Associate flavour profiles with consumer liking

Work in project year 1 has focussed on the first three objectives, with herb samples (relevant to the season) collected for analyses in summer 2018, autumn 2018 and early 2019. Results provided in this annual report focus on the summer 2018 sampling and associated analyses.

The consumption of herbs has been recently associated with several health benefits like anti-diabetic, anti-inflammatory, anti-carcinogenic properties and also lowers the risk of cardiovascular diseases, they are also known for their antioxidant properties (Chohan, Forster-Wilkins and Opara, 2008; Opara and Chohan, 2014; Bower, Marquez and de Mejia, 2016; Kuban-Jankowska *et al.*, 2018). The replacement of salt by culinary herbs has caused an increase in the frequency of consumption of herbs (Bower, Marquez and de Mejia, 2016). The use of culinary herbs provides flavour to the dishes, and also will contribute to the health of those who consume them due to their high content in polyphenols, despite of their low intake it still provides some beneficial effect (Opara and Chohan, 2014).

Polyphenols

Polyphenols are secondary metabolites of plants and are generally involved in defence against ultraviolet radiation or biotic stressors (Di Ferdinando *et al.*, 2014). They consist of aromatic rings with hydroxyl groups attached (phenol ring). These can be classified into different groups according to the number of phenol rings and other chemical groups that might be connected to this structure. The main groups of polyphenols present in herbs are phenolic acids, flavonoids, stilbenes, lignans, coumarins and tannins (Opara and Chohan, 2014). Dried herbs have higher concentration of polyphenols (Rosemary:2518mg/100g and Coriander:2260mg/100g) when compared with fresh herbs (Rosemary:1082mg/100g and Coriander:159mg/100g) and also when compared with other foods known to have high levels of polyphenols, like dark chocolate (1860mg/100g) (Opara and Chohan, 2014). However,

herbs are used as seasoning, so the amount of intake is very low when compared to other leafy vegetables or even other food that possess any health benefit, which makes the polyphenol intake lower than what would be expected for most leafy vegetables, but this does not mean that there is not going to be any beneficial effect (Opara and Chohan, 2014).

Polyphenols are known for their health benefits the main one being their capacity to reduce the free reactive oxygen present in the body, this antioxidant activity is thought to be related with other defence mechanisms like anti-diabetic, anti-inflammatory, anticancer and also slowing down other cardiovascular diseases. This happens because polyphenols affect the production and activity of immune system cells, affecting the defence mechanisms (Kuban-Jankowska *et al.*, 2018).

Herb Production Systems

There has been an increase in consumer interest to purchase fresh herbs in supermarkets, due to their desirable aromas and flavours. Herb crops grown outdoors come mainly from warm climates or are produced under warmer seasons in the UK. When this climate is not available the crops are exported from warmer countries or produced under protected environments in the UK. Protected production systems include potted herbs, soil grown and hydroponic production under glass. Crops grown in glasshouses can use a lot of energy resource due to the use of supplementary lighting systems. The most common ones are high-pressure sodium (HPS) and metal halide (MH) due to their low costs, HPS also contributes to the temperature since they provide heat. Light-emitting diode (LED) lights are another option since they are energy efficient and wave lengths and light colour can be personalized, however these lights require higher investment (Seely, 2017).

The hydroponic system is a method that does not require soil to grow plants, where the nutrients are provided via salts dissolved in water through an irrigation system. Here the roots can be partially or completely submersed in water. In this system the growers can control the concentration of the fertilizers supplied to the roots of the plant and have a more even distribution of the nutrients. This type of production gives growers a higher control of the phenotype of their crops (Putra and Yuliando, 2015).

Fresh herbs, as for any other type of fresh produce, suffers from degradation and spoilage after it has been harvested. That is why for many years, and still in many cases currently, most herbs are consumed dried. Fresh herbs are comprised mainly of water (75-80%) thus it needs to be reduced in order to preserve them for longer. In order to decrease this perishability, the herbs go through a drying process; this affects their properties, like their appearance, losses in volatiles and increase in polyphenols concentration (Hossain *et al.*, 2010; Opara and

Chohan, 2014). For this reason, fresh herbs are described as having better flavour (Hossain *et al.*, 2010).

Flavour comes from the essential oils present in the oil glands (trichomes) on the leaf and stem. Variation in the composition and abundance of the compounds present in the essential oil has a significant impact in the flavour characteristics. The analysis of the aroma is more efficient when done on fresh herbs compared to dried herbs, since when herbs are dried there is a loss in the volatile content (10-30%) (Díaz-Maroto *et al.*, 2004; Ravi, Prakash and Bhat, 2007).

Herb metabolites

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. These metabolites are universal to the plants and do not confer uniqueness to individual varieties (Rosenthal and Berenbaum, 2012). Secondary metabolites are smaller compounds, with simpler structures 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 as well (Rosenthal and Berenbaum, 2012). Within the secondary metabolites of relevance to herbs it is possible to divide them into terpenes, alkanes, phenolics and aldehydes (Rohloff, 2006).

Aroma Volatile Compounds

Some secondary metabolites are volatile compounds, and get dispersed through the air, allowing the plant to communicate with the environment and other living organisms around the plant.

Volatiles are complex structures, with a broad variety, 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, and they also present a low detection threshold for their target. Volatiles like isoprenoids are a result of an enzymatic process; for instance to achieve geraniol, the activity of geranyl diphosphate synthetase is required in the synthesis pathway. Geranyl diphosphate is a precursor for different monoterpenes including geraniol (Valcourt, 2014). In Figure 1 it is possible to see a simple pathway of the synthesis of some terpenes.

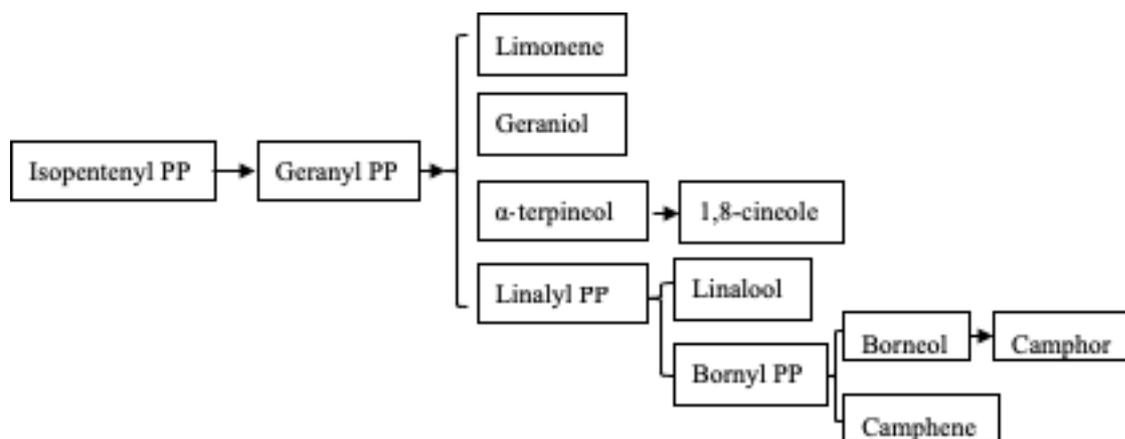


Figure 1. Simplified synthesis pathway of some terpenes. (PP) = diphosphate

Plants like herbs, which produce essential oils in their trichomes or glandular cells, produce some volatile compounds including monoterpenes and sesquiterpenes. These are produced and stored in specific structures of leaves, flowers and seeds and only in certain types of plant families. Because of this, their contribution to the air volatiles is very low, especially when compared to isoprenes which are volatiles that are produced by every plant in every green cell (Valcourt, 2014).

Volatiles can also be classified as the plant pheromones, since they are signalling molecules that are involved in the defence mechanism of the plant. There are two types of defence mechanisms, direct and indirect, in the first one certain compounds are produced in order to repel or intoxicate the pests. The indirect mechanism is where volatiles are involved, and they work as a calling signal to predators of the organism that is threatening the plant. Aldehydes, like other volatiles, take part in this type of defence mechanism. The production of these volatiles is induced when the plant is wounded or attacked by insects (Chehab *et al.*, 2008; Meerburg, Brom and Kijlstra, 2008). Aldehydes are produced through two enzymatic reactions, involving lipoxygenase and hydroperoxide lyase. They act in different stages, the substrate being polyunsaturated fatty acids, the first enzyme catalyses their oxygenation originating unstable compounds that are then split into aldehydes and oxo acids. Aldehydes can be transformed into alcohols. Besides being a defence mechanism, aldehydes also contribute to the flavour of the plant (Meerburg, Brom and Kijlstra, 2008), usually conferring a green and waxy type of flavour.

Rosemary

Rosemary (*Rosmarinus officinalis*) comes from the family Lamiaceae (the same as mint); 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 the rosemary is constituted mainly by

monoterpenes, like camphor, 1,8-cineole and alpha-pinene (Pintore *et al.*, 2002). However, the essential oil can also be described as primarily borneol and 1,8-cineole, followed by camphor and limonene. When it comes to the oil composition, Pintore *et al.* (2002) distinguish two groups, oils with over 40% 1,8-cineole and equal ratios of 1,8-cineole, α -pinene and camphor. Rosemary extract has been described as having health benefits like being antidiabetic and anticarcinogenic (Opara and Chohan, 2014).

Coriander

Coriander (*Coriandrum sativum*) is a plant from the Umbelliferae family. India is the world's largest coriander producer and exports to other countries. Coriander flavour is mainly given by primary compounds, unlike most of the herbs which are defined by their secondary metabolites, which means coriander flavour is less affected by environmental changes (Chadwick, 2018b). Aroma and flavour come from the essential oil present in the oil gland on the leaf. International standard of oil/coriander is 70% linalool content. Coriander with strong sweet, floral odour has been attributed to the presence of geranyl acetate in higher amounts (Ravi, Prakash and Bhat, 2007). Coriander leaves and seeds are consumed as seasoning, and both have shown to have beneficial effects on health when consumed (Opara and Chohan, 2014), however there is not a lot of human trials investigating this effect and the knowledge is based on diets that have been practiced like the Mediterranean diet.

Basil

Basil (*Ocimum basilicum*) is from the Lamiaceae (mint) family and it is highly cultivated in Mediterranean areas, and it is used both fresh and dried. Basil can be classified as different subspecies according to the content of certain volatiles. Chemical composition of the essential oil of basil is very variable with the many constituents being linalool, estragole, eugenol and/or methyl cinnamate. Basil synthesises and stores its essential oil on the leaf and stem surface in peltate trichomes. As described above, drying basil will affect its appearance as well as flavour, since the process leads to changes in the volatile profile. Diaz-Maroto (2004) observed that volatiles losses were 28.6% in oven dried, 27.4% in freeze-dried and 13.6% in air dried. However, an increase of sesquiterpenes during drying process has been described in basil and some other herbs. Samples dried at ambient temperature have similar composition to fresh samples (Díaz-Maroto *et al.*, 2004).

Figure 2 shows some of compounds mentioned above, with their corresponding chemical structures.

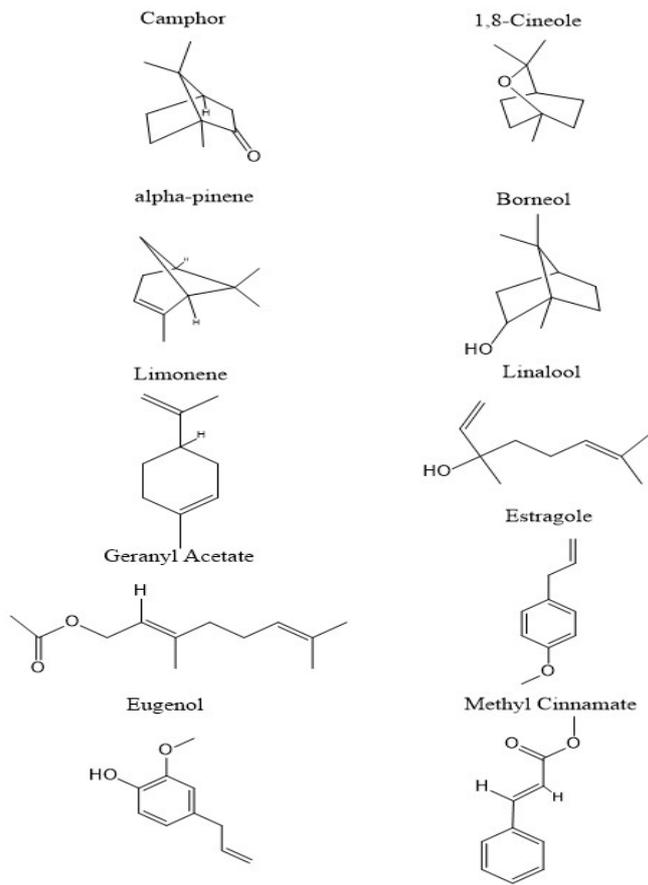


Figure 2. Structures of some of the compounds described in the literature as part of the three herbs volatile profile.

Materials and methods

Herbs including, rosemary (*Rosmarinus officinalis*), coriander (*Coriandrum sativum* var. Cruiser) and basil (*Ocimum basilicum* var. Sweet Genovese), were provided by different growers across the United Kingdom (UK), and for each herb more than one type of agronomic production was considered. The type of agronomic practice for the samples consisted of herbs grown in pots under protected conditions (Pots), produced in soil, protected under glass (Soil), grown in open field subject to weather conditions (Field) and using the hydroponics system (Hydroponics). For rosemary, samples were sent from available crops such that there may have been varietal differences; growers were not always aware of the crop variety that they were growing. Coriander samples were of the variety Cruiser and basil samples were of the Sweet Genovese type. Table 1 shows the different samples analysed for each of the herbs with the respective locations, these can also be seen in Figure 3. Each sample, from the different types of production and location, was analysed in quintuplicate (n=5). Information about the cultivation variables was collected using a form (Appendix 1) and completed by the growers. All the samples were harvested at commercial maturity and sent by a courier in boxes with cooling packs and stored at 5°C (cut samples) or at room temperature (pot samples) to the University of Reading. Analyses were carried out within 4 days of receipt.

Table 2. Sample information for rosemary, coriander and basil, including type of production with their corresponding geographical location and time of harvesting for summer 2018

	Rosemary	Coriander	Basil
West Sussex	Pots	Pots 1, Field 2	Pots 1, Hydroponics
Lincolnshire		Pots 2	Pots 2
Reading	Field 1		
Worcs	Field 2	Field 3	Soil
Norfolk	Field 3		
York	Soil, Field 4	Field 1	
Harvesting	June 2018	July 2018	August 2018

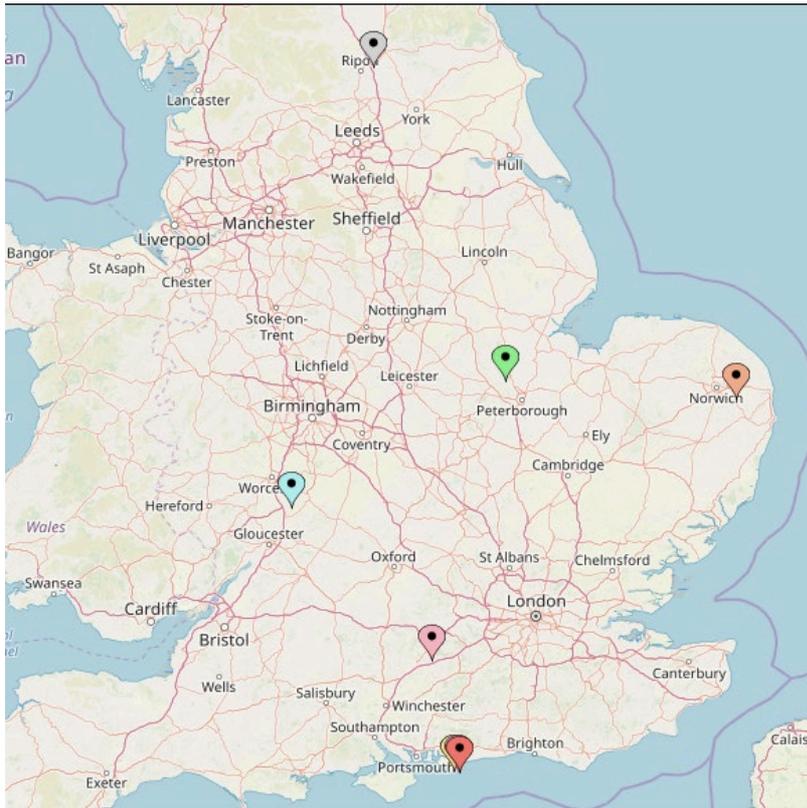


Figure 3. Map showing the distribution of all the production sites for rosemary, coriander and basil.

Grey- Yorks

Green- Lincolnshire

Blue-Worcs

Orange- Norfolk

Pink- Reading

Red- West Sussex

Sample preparation

2 g of fresh herb (including leaves and stems) were hand cut making sure that different maturities of leaves were included in the sample for analysis and were ground with 2.8 mL of saturated calcium chloride solution (as it helps to stabilize plant cells and preserve the samples for longer) for 1 minute using a pestle and the mortar.

From this mixture, 5 g of ground herb and solution were transferred to a SPME vial and kept at 4 °C until extraction. Just before loading the samples into the SPME, 50 µL of propyl propanoate (internal standard) at 100 ppm was added into each vial.

Extraction of aroma volatile compounds by Solid Phase Micro-Extraction (SPME)

The samples were loaded to a cooling tray so that they were kept at 4-5 °C until extraction time. The volatiles extraction was done using a stationary phase composed of 75 µm divinylbenzene/Carboxen™ on polydimethylsiloxane fibre. Using an automated system, each sample was incubated at 35 °C with an agitation of 500 rpm for 10 minutes. After incubation a needle was inserted into the vial and a stationary phase fibre was exposed to the headspace of the vial so that the volatiles could be extracted, this extraction was done at 35 °C with a 500 rpm agitation for 30 minutes.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of SPME extracts

The fibre was then inserted into the inlet port of the GC-MS, using an Agilent 110 PAL injection systems and an Agilent 7890 gas chromatograph with 5975C mass spectrometer. Using this equipment, the volatiles were removed in a splitless mode for 36 minutes and passed through a DB-5 column.

Compound identification

The relative concentration for each of the compounds found was calculated relative to the known amount of internal standard (propyl propionate).

The volatile compounds were identified using spectral databases (ADAMS, NIST and INRAMSS), linear retention indices (LRIs) were calculated using a set of known alkanes and compared with data in the literature.

Statistical analysis

For the purpose of statistical analysis, the compound data found across all the samples for each herb, were analysed using a one-way analysis of variance (ANOVA) and for those showing significant difference, post hoc Tukey test was used to determine which of the samples were significantly different from each other (p-value<0.05). Principal component analysis was also applied to the whole set of data.

Results

GC-MS chromatograms for each of the herbs showed a clear difference from each other (Figure 4). It can be seen that rosemary presents a more clustered graph, representing more detected compounds. On the other hand, coriander out of the three herbs is the one with a

chromatogram with fewer peaks and the least number of high (abundant) peaks. As for basil, the graph complexity falls between rosemary and coriander, not showing clustered peaks but with clearly more peaks than coriander.

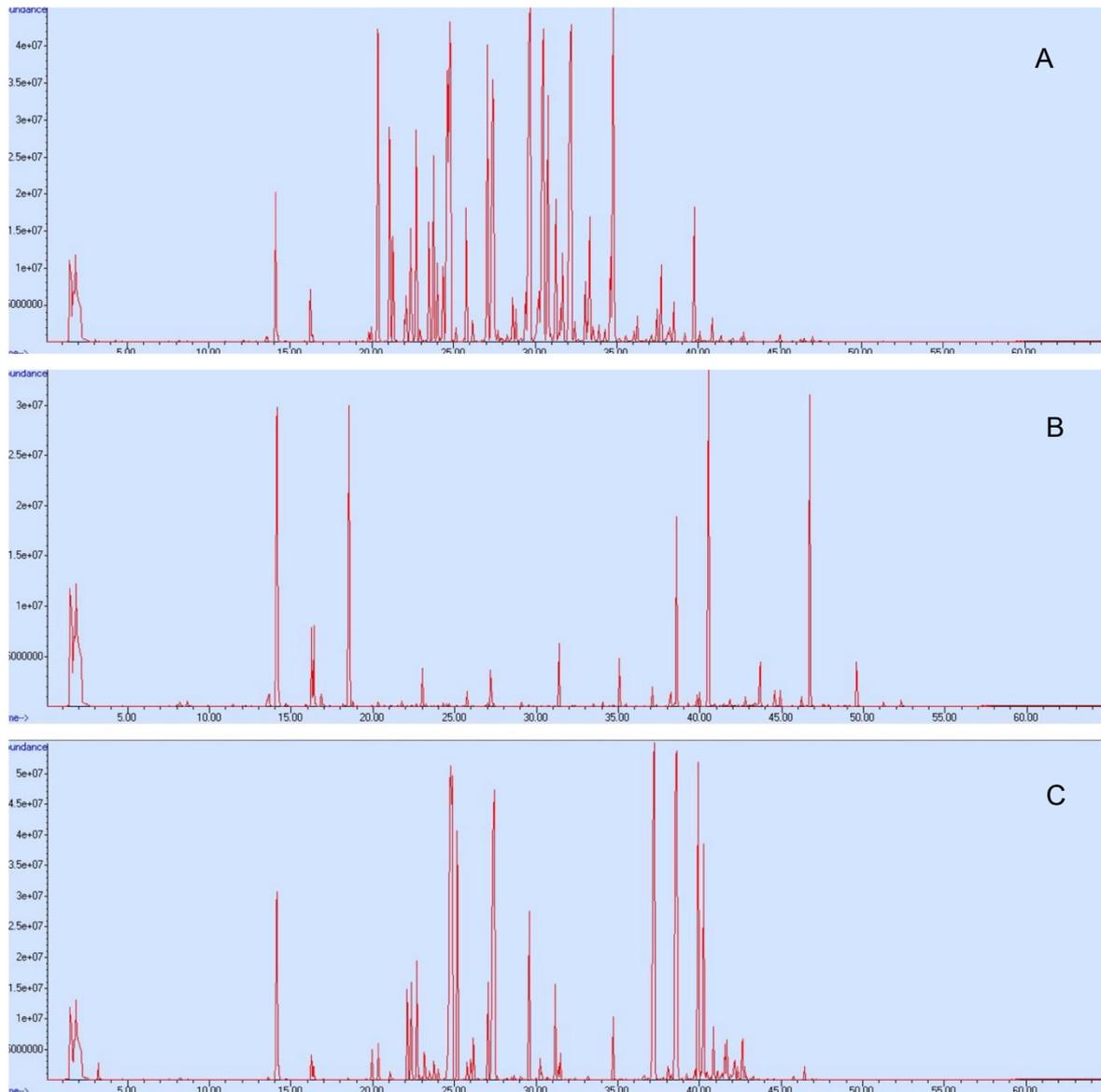


Figure 4. GC-MS chromatograms for the three herbs analysed: A-rosemary; B-coriander; C-basil

As previously stated, different number of samples per herb were provided (Table 1; Figure 3) which had been grown using different production systems and each sample was analysed in quintuplicate ($n=5$). An average of the replicates was calculated as well as standard deviation. In the case of rosemary, 111 compounds were detected across the six different samples

analysed, however only 36 of these compounds were common to all the samples. For coriander, 81 compounds were detected across the five different samples analysed with 29 of those being present in all the samples. As for basil, 60 compounds were common to all the four samples analysed out of a total of 88 compounds detected.

The volatile compounds were identified using a spectral database and LRIs were calculated and compared to literature data and publications. Below, in Table 3, Table 4 and Table 5, are the calculated linear retention indices, with the corresponding searched indices, references for this information are found in the corresponding tables. The volatile compounds were listed accordingly to the class of compound that they belong to.

The compounds found in all the samples for each herb were analysed and their relative concentration was calculated using the internal standard. Using one-way ANOVA, the significant differences ($p\text{-value} < 0.05$) between samples were evaluated. In order to identify which samples were significantly different, a post-hoc test was applied, and the differences were registered. The results for each of the herbs are summarized in the tables below (Table 3, Table 4 and Table 5).

In order to analyse if the compounds found within each of the herbs, had any correlation to each other, principal component analysis (PCA) was done and the results for this analysis are showed in the graphs presented below (Figure 5, Figure 6 and Figure 7) for rosemary, coriander and basil respectively. From this analysis, it was possible to see that some compounds have a positive correlation with each other, and some have a negative correlation. With this analysis it was also possible to relate the samples with the compounds and access which samples are the most similar and which are more different.

Table 3. List of volatiles compounds identified in rosemary samples, expressed in the relative amount per 2 g of plant. Significant difference (p-value<0.05) between production methods is also shown (a,b,c,d).

Compound	Type of compound	RT (min)	Exp. LRI	Lit. LRI	Average Relative Amount ($\mu\text{g}/2\text{ g}$)											
					Pots		Soil		Field 1		Field 2		Field 3		Field 4	
4-methyl-3-Penten-2-one	enone	13.56	799		3.93	a	25.25	a	76.34	b	14.15	a	19.36	a	24.19	a
E-2-Hexenal	aldehyde	16.24	854	855 ^A	31.1	a	85.45	ab	130.82	b	94.51	ab	135.8	b	101.13	ab
1-Methyl-2-pyrrolealdehyde	aldehyde	23.67	1015		115.5	c	23.91	ab	17.49	a	7.9	a	15.67	a	33.25	b
Tricyclene	monoterpenoid	19.8	929	928 ^B	5.58	a	108.13	d	71.44	bc	38.66	ab	47.4	ab	91.94	cd
Thuja-2,4(10)-diene	monoterpenoid	21.3	962	960 ^B	63.28	c	35.69	a	75.47	bc	27.57	a	38.85	ab	42.42	abc
alpha-Phellandrene	monoterpenoid	23.51	1011		71.35	a	135.69	a	822.69	b	787.4	b	827.32	b	119.11	a
trans-Sabinene hydrate	monoterpenoid	26.19	1075	1098 ^A	10.02	a	189.06	b	220.71	b	185.91	b	221.71	b	380.48	c
Chrysanthenone	monoterpenoid	28.63	1135	1124 ^A	28.72	a	78.83	b	53.21	ab	49.21	ab	76.49	b	157.88	c
<i>m</i> -cymen-8-ol	monoterpenoid	30.45	1182	1182 ^B	401.1	a	1143.7	bc	1564	bc	825.89	ab	1011.9	abc	1529.4	c
1-Terpinen-4-ol	monoterpenoid	30.76	1190		196.8	a	616.69	b	379.76	a	279.81	a	283.82	a	652.62	b
Myrtenol	monoterpenoid	31.61	1213	N/A	51.72	b	14.3	a	17.3	a	10.12	a	11.89	a	18.28	a
Geranial	monoterpenoid	33.93	1276	1264 ^A	9.93	a	74.54	b	17.26	a	11.87	a	19.33	a	70.55	b
Bornyl acetate	monoterpenoid	34.8	1300	1296 ^A	327	a	2057.9	b	2509.3	b	1249.9	ab	1340.1	ab	1846.1	b
Eucarvone	monoterpenoid	38.26	1401	1146 ^A	14.12	a	19.97	a	13.83	a	11.84	a	25.25	ab	53.16	b
alpha-Thujene	monoterpene	19.95	932	932 ^B	8.46	a	669.63	b	204.02	a	116.24	a	138.52	a	868.47	c
alpha-Pinene	monoterpene	20.38	942	942 ^C	266.6	a	2889	c	1471.3	ab	1173.2	b	1318.5	b	2779.4	c
Camphene	monoterpene	21.13	958		132.2	a	1931	d	1495.7	bc	869.97	b	1007.6	b	1766.7	cd
beta-Pinene	monoterpene	22.39	986	982 ^C	78.56	a	1871.9	c	1760.5	bc	700.03	ab	844.57	ab	1608.6	bc

Compound	Type of compound	RT (min)	Exp. LRI	Lit. LRI	Average Relative Amount ($\mu\text{g}/2\text{ g}$)											
					Pots		Soil		Field 1		Field 2		Field 3		Field 4	
r-Cymene	monoterpene	24.03	1024	1024 ^B	44.08	a	550.81	c	299.64	b	247.18	b	263.02	b	367.29	b
1,8-Cineole	monoterpene	24.75	1041	1043 ^D	334.2	a	4230.4	b	4605.6	b	2678	b	3091.8	b	4430	b
E-beta-Ocimene	monoterpene	25.16	1051		10.11	a	23.24	bc	36.1	c	41.15	d	11.29	a	16.74	ab
Camphor	monoterpene	29.68	1162	1141 ^A	528.8	a	2704	b	3395.4	b	1815.9	ab	2031.8	b	2693.5	b
Myrcene	monoterpene	31.51	1210	N/A	20.66	a	32.6	ab	37.87	bc	20.92	a	28.35	ab	45.99	c
Verbenone	monoterpene	32.11	1226	1205 ^A	415.2	a	997.05	b	458.59	a	461.2	a	603.89	a	1021.3	b
Beta-Phellandrene	monoterpene	32.41	1234	1025 ^A	10.73	a	156.8	c	75.01	b	42.03	ab	52.54	ab	65.76	b
3-Octanol	fatty alcohol	22.95	998		8.32	a	47.2	b	99.47	c	9.27	a	12.5	a	95.19	c
gamma-Terpinene	terpene	25.81	1066	1054 ^A	68.54	a	939.17	c	699.23	b	455.59	b	551.21	b	622.78	bc
(E)-2-Hexenal diethyl acetal	acetal	27.08	1096		203.7	a	981.3	c	478.94	a	388.92	a	394.28	a	682.57	b
Linalool	monoterpene alcohol	27.41	1104		327.7	a	578.97	ab	1047.3	b	287.53	a	224.77	a	607.19	ab
alpha-Terpineol	monoterpene alcohol	31.25	1203	1203 ^E	86.95	a	820.56	bc	846.39	bc	539.55	b	568.23	b	966.71	c
Piperitone	monoterpene ketone	33.62	1267	1249 ^A	11.15		15.28		14.23		9.33		8.61		16.86	
Isophorone	ketone	34.27	1285	1118 ^A	6.32	a	13.45	ab	8.73	ab	6.97	a	12.41	ab	26.68	b
E-Caryophyllene	sesquiterpene	39.76	1447	1417 ^A	81.82	a	642.09	b	456.51	ab	433.9	b	539.9	b	393.55	b
alpha-Humulene	sesquiterpenoid	40.86	1481	1452 ^A	14.05	a	137.79	a	896.19	b	70.01	a	88.68	a	66.99	a
Bulnesol	sesquiterpenoid	46.49	1668	1668 ^B	2.4		9.08		19.9		9.01		14.66		8.03	
8-cedren-13-ol	sesquiterpene alcohol	47.01	1686	1686 ^F	3.39	a	16.45	a	37.56	b	16.46	a	24.4	ab	13.76	a

A-University of Reading flavour centre database; B- Lucero et al., 2006; C- Lucero, Estell and Sedillo, 2005; D- Lucero et al., 2004; E- Bouzouita et al., 2003; F- Tellez et al., 1997

Table 4. List of volatiles compounds identified in coriander samples, expressed in the relative amount per 2 g of plant. Significant difference (p-value<0.05) between production methods is also shown (a,b,c).

Compound	Type of compound	RT(min)	Exp.LRI	Lit.LRI	Average Relative Amount ($\mu\text{g}/2\text{ g}$)									
					Pots 1		Pots 2		Field 1		Field 2		Field 3	
(Z)-3-Hexen-1-ol	fatty alcohol	16.4	857	855 ^A	18.98	a	48.33	b	28.52	ab	32.45	ab	32.16	ab
(E)-2-Hexen-1-ol	fatty alcohol	16.84	866	867 ^A	2.83	a	14.3	b	15.55	b	20.64	b	17.02	b
Nonane	alkane	18.49	900	900 ^B	82.53	c	68.76	bc	64.44	bc	25.78	a	38.88	ab
Decane	alkane	23	999		5.16	a	4.39	a	21.01	b	5.74	a	6.56	a
Undecane	alkane	27.19	1099		7.52	a	6.12	a	22.76	b	11.27	a	10.52	a
Tetradecane	alkane	38.22	1400		2.53	a	3.46	a	49.62	bc	36.19	b	59.74	c
beta-Myrcene	monoterpene	22.66	992	991 ^A	1	a	1.22	a	31.33	b	5.7	a	27.38	b
Limonene	monoterpene	24.51	1035	1034 ^A	0.95	a	1.14	a	30.98	ab	54.75	b	107.06	c
gamma-Terpinene	terpene	25.74	1064	1063 ^A	2.21	a	1.67	a	7.26	ab	3.07	a	61.46	b
Nonanal	aldehyde	27.4	1104	1107 ^A	0.48	a	2.24	a	86.48	c	57.67	bc	51.01	b
Decanal	aldehyde	31.47	1209		8.42	a	39.22	a	863.39	b	758.46	b	1281.09	c
Undecanal	aldehyde	35.11	1309		5.51	a	12.58	a	255.76	c	112.9	b	158.92	b
E-2-Undecenal	aldehyde	37.17	1369	1368 ^A	1.89	a	6.96	a	633.71	c	387.88	b	403.05	b
Dodecanal	aldehyde	38.58	1411	1414 ^A	29.67	a	29.91	a	194.01	b	78.43	a	172.74	b
E-2-Dodecenal	aldehyde	40.61	1473	1464 ^A	52.37	a	85.42	a	859.92	bc	595.29	b	885.48	c
Tridecanal	aldehyde	41.84	1512	1518 ^E	1.08	a	1.96	a	15.84	c	7.6	b	9.29	b
Tetradecanal	aldehyde	44.93	1614	1618 ^E	2.68	a	3.24	a	11.51	b	8.8	b	12.43	b

Compound	Type of compound	RT(min)	Exp.LRI	Lit.LRI	Average Relative Amount ($\mu\text{g}/2\text{ g}$)									
					Pots 1		Pots 2		Field 1		Field 2		Field 3	
<i>trans</i> -verbenol	monoterpene alcohol	29.08	1147	1147 ^C	1.26	a	1.38	a	3.98	ab	7.17	b	11.11	c
(E)-3-Hexenyl pentanoate	fatty esters	34.11	1281		0.88	a	1.14	a	6.58	bc	4.26	b	8.57	c
gamma-Elemene	sesquiterpene	39.85	1450	1434 ^A	1.59		1.64		14.98		6.65		7.5	
α -humulene	sesquiterpenoid	40	1455	1455 ^D	1.77	a	2.55	a	47.64	bc	34.09	b	60.08	c
E-2-Tridecen-1-al	fatty aldehyde	43.69	1573	1567 ^A	5.58	a	11.72	a	110.61	b	85.19	b	91.76	b
unknown		42.81	1544		1.61	a	1.75	a	6.52	bc	4.16	ab	7.7	c
unknown		44.59	1602		2.84	a	3.64	a	14.07	b	17.72	bc	18.62	c
unknow		46.24	1659		1.37	a	1.89	a	9.28	b	11.91	bc	12.87	c
unknown		46.75	1677		54.24	a	67.97	a	317.51	b	321.73	b	340.46	b
unknown		49.58	1779		5.66	a	12.44	a	49.68	b	56.91	b	45.49	b
unknown		51.21	1840		0.71	a	1.22	ab	2.62	bc	5.5	d	3.28	c
unknown		52.3	1882		1.01	a	1.43	a	7.47	b	9.59	b	8.42	b

A-University of Reading flavour centre database;

B- Tellez et al., 1998

C- Lucero et al., 2006

D- Lucero, Estell and Fredrickson, 2003

E- Ramarathnam, Rubin and Diosady, 1993

Table 5. List of volatiles compounds identified in basil samples, expressed in the amount per 2 g of plant. Significant difference (p-value<0.05) between production methods is also shown (a,b,c).

Compound	Type of compound	RT(min)	Exp. LRI	Lit. LRI	Average Relative Amount ($\mu\text{g}/2\text{ g}$)							
					Pots 1		Pots 2		Soil		Hydroponics	
E-2-Hexenal	aldehyde	16.2	853	855 ^A	6.39	ab	9.54	b	4.24	a	13.03	c
n-Octanal	aldehyde	23.12	1002	1001 ^C	24.32	b	8.94	a	8.32	a	2.65	a
Perilla aldehyde PG acetal (isomer 2)	aldehyde	43.15	1555		3.92	ab	2.02	a	5.95	b	7.95	b
(Z)-3-Hexen-1-ol	fatty alcohol	16.34	856	855 ^A	3.81	a	5.75	ab	14.85	c	12.5	bc
n-Octanol	fatty alcohol	26.07	1072	1072 ^B	11.99	a	9.52	a	14.23	a	27.86	b
Methyl hexanoate	fatty acid methyl ester	19.89	931		14.27		10.17		11.65		15.12	
alpha-Pinene	monoterpene	20.28	940	939 ^A	33.15	b	17.98	a	19.15	a	21.41	a
Camphene	monoterpene	21.02	956	958 ^A	9.05	b	4.64	a	5.38	a	6.92	ab
beta-Pinene	monoterpene	22.3	984		80.35	b	49.1	a	49.22	a	53.76	a
myrcene	monoterpene	22.66	992	992 ^B	82.66	ab	58.68	a	97.45	b	89.86	b
delta-3-Carene	monoterpene	23.71	1016	1017 ^A	10.49	b	4.66	a	2.11	a	3.61	a
delta-2-Carene	monoterpene	23.96	1022	1001 ^A	8.15	b	4.96	a	9.41	b	12.95	c
1,8-Cineole	monoterpene	24.71	1040	1039 ^A	580.57	ab	448.66	a	427.18	a	674.38	b
E-beta-Ocimene	monoterpene	25.15	1050	1050 ^D	203.51	b	139.13	a	128.43	a	165.26	ab
gamma-Terpinene	monoterpene	25.74	1064	1062 ^C	14.26	b	6.65	a	10.88	ab	14.37	b
Linalool	monoterpene	27.44	1105	1103 ^A	683.54	a	522.6	a	736.12	a	1133.84	b
neoallo-ocimene	monoterpene	29.02	1145	1145 ^F	5.11		2.83		4.97		5.2	
Camphor	monoterpene	29.57	1160	1159 ^A	167.62	b	91	a	31.98	a	56.19	a
Nerol	monoterpene	32.33	1232	1231 ^A	2.06	ab	1.32	a	3.62	bc	4.53	c

Compound	Type of compound	RT(min)	Exp. LRI	Lit. LRI	Average Relative Amount ($\mu\text{g}/2\text{ g}$)							
					Pots 1		Pots 2		Soil		Hydroponics	
dehydro-1,8-Cineole	pyran	22.81	995	988 ^A	2.32	a	2.51	ab	3.97	b	3.37	ab
Rose oxide 2	pyran	28.49	1132		1.55	ab	1.3	a	1.92	bc	2.28	c
Sabinene	monoterpenoid	22.07	979	980 ^A	68.61	b	49.62	a	39.24	a	46.97	a
alpha-Phellandrene	monoterpenoid	23.43	1010	1011 ^A	7.64	bc	4.5	a	6.18	b	7.84	c
1,3,8-para-Menthatriene	monoterpenoid	28.63	1136	1108 ^A	3.3		2.35		2.82		3.11	
1-Terpinen-4-ol	monoterpenoid	30.69	1188	1189 ^A	20.82		1.14		1.57		3.25	
Bornyl acetate	monoterpenoid	34.7	1297		68.21	a	36.95	a	138.81	b	81.28	a
Terpinolene	terpenoid ketone	27.01	1095	1086 ^A	73.71	b	35.65	a	39.62	a	41.28	a
Borneol	monoterpene alcohol	30.34	1179	1177 ^G	14.07	a	18.07	a	15.6	a	64.88	b
alpha-Terpineol	monoterpene alcohol	31.16	1200	1195 ^H	91.41	c	55.7	ab	39.65	a	68.14	bc
Estragole	phenylpropanoid	31.35	1205	1203 ^A	4.41		4.82		1.16		5.72	
Eugenol	phenylpropanoid	37.22	1370	1373 ^J	703.38	b	434.97	ab	260.21	a	596.88	ab
Octanol acetate	ester	31.46	1208	1211 ^A	7.99	ab	6.67	ab	10.1	b	2.43	a
alpha-Cubebene	sesquiterpenoids	36.61	1352	1352 ^I	10.24	b	4.42	a	11.64	b	21.1	c
alpha-Copaene	sesquiterpenoids	38.1	1396	1394 ^K	19.86		9.7		23.13		28.41	
Sesquithujene	sesquiterpenoids	38.27	1401	1405 ^A	7.99		4.07		6.27		9	
Z- β -farnesene	sesquiterpenoids	39.72	1446	1446 ^I	15.2	ab	5.2	a	9.76	ab	22.42	b
E-beta-Farnesene	sesquiterpenoids	40.26	1462	1460 ^M	255.57	b	111.06	a	98.42	a	178.12	ab
Aromadendrene	sesquiterpenoids	40.38	1466	1463 ^N	10.53	ab	4.5	a	22.37	bc	36.18	c
trans-cadina-1,(6),4-diene	sesquiterpenoids	40.67	1475	1475 ^I	4.94	b	2.3	a	3.86	ab	6.17	b
trans-Muurolo-4(14),5-diene	sesquiterpenoids	41.09	1488	1491 ^O	19.96	ab	8.07	a	16.99	ab	27.23	b
gamma-Muuroloene	sesquiterpenoids	41.38	1497	1494 ^H	18.23	ab	7.47	a	29.91	bc	41.98	c

Compound	Type of compound	RT(min)	Exp. LRI	Lit. LRI	Average Relative Amount ($\mu\text{g}/2\text{ g}$)							
					Pots 1		Pots 2		Soil		Hydroponics	
d-selinene	sesquiterpenoids	41.69	1507	1506 ^F	106.15	b	37.3	a	59.36	ab	93.26	ab
alpha-Bulnesene	sesquiterpenoids	42.35	1529	1526 ^P	36.44	ab	16.4	a	67.48	ab	80.59	b
alpha-cadinene	sesquiterpenoids	42.63	1538	1538 ^Q	97.57	ab	43.55	a	95.81	ab	140	b
trans-Calamenene	sesquiterpenoids	42.89	1547	1542 ^R	5.51	ab	2.61	a	7.61	b	11.47	c
alpha-Cadinene	sesquiterpenoids	43.3	1560	NA	6.37	ab	2.62	a	9.71	bc	14.2	c
cubenol	sesquiterpenoids	45.77	1643	1643 ^F	5.88	ab	3.33	a	8.57	b	16.03	c
isocomene	sesquiterpene	37.77	1386	1386 ^D	6.18		2.66		1.47		2.34	
cis-a-bergamotene	sesquiterpene	38.71	1415	1415 ^D	5.33		2.45		4.3		6.81	
β -caryophyllene	sesquiterpene	38.89	1420	1420 ^I	1.89		1.33		2.08		2.83	
Germacrene D	sesquiterpene	39.61	1443	1451 ^L	5.15	ab	2.07	a	5.23	b	6.64	b
alpah-cis-Bergamotene	sesquiterpene	39.89	1451	1411 ^A	284.69	b	233.45	ab	103.98	a	235.42	ab
y-curcumene	sesquiterpene	40.86	1481	1475 ^D	73.29	bc	26.25	a	42.01	ab	108.92	c
beta-Cedrene	sesquiterpene	46.43	1666	NA	24	a	12.72	a	33.97	a	71.64	b
Methyl eugenol	phenylpropanoid	38.55	1410	1409 ^A	470.23		282.18		125.62		551.81	
Ethylvanillin	hydroxybenzaldehyde	40.47	1469		16.52	ab	6.79	a	14.01	ab	24.91	b
Methylisoeugenol isomer #2	dimethoxybenzenes	41.56	1503		45.21	b	24.35	ab	16.06	a	28.39	ab
Isopropyl cinnamate	acid ester	42.15	1523		73.31	ab	28.45	a	75.07	ab	112.76	b
unknown		41.9	1514		10.81	ab	4.75	a	22.16	bc	34.6	c
unknown		42.78	1543		25.21	ab	10.21	a	44.88	bc	65.01	c

A- University of Reading flavour centre database; B-Lucero et al.; C- Merle et al., 2004; D- Tellez et al., 1998; E- Adams et al., 2006; F- Tellez et al., 1997; G- Avato et al., 2004; H- Rao et al., 2000; I- Lucero et al., 2006; J- Hazzit et al., 2006; K- Jalali-Heravi, Zekavat and Sereshhti, 2006; L- Marongiu et al., 2006; M- Merle et al., 2004; N- Zoghbi et al., 1998; O- Adams and Nguyen, 2005; P- Bouzouita et al., 2003; Q- Lucero, Estell and Fredrickson, 2003; R- Loayza et al., 1995

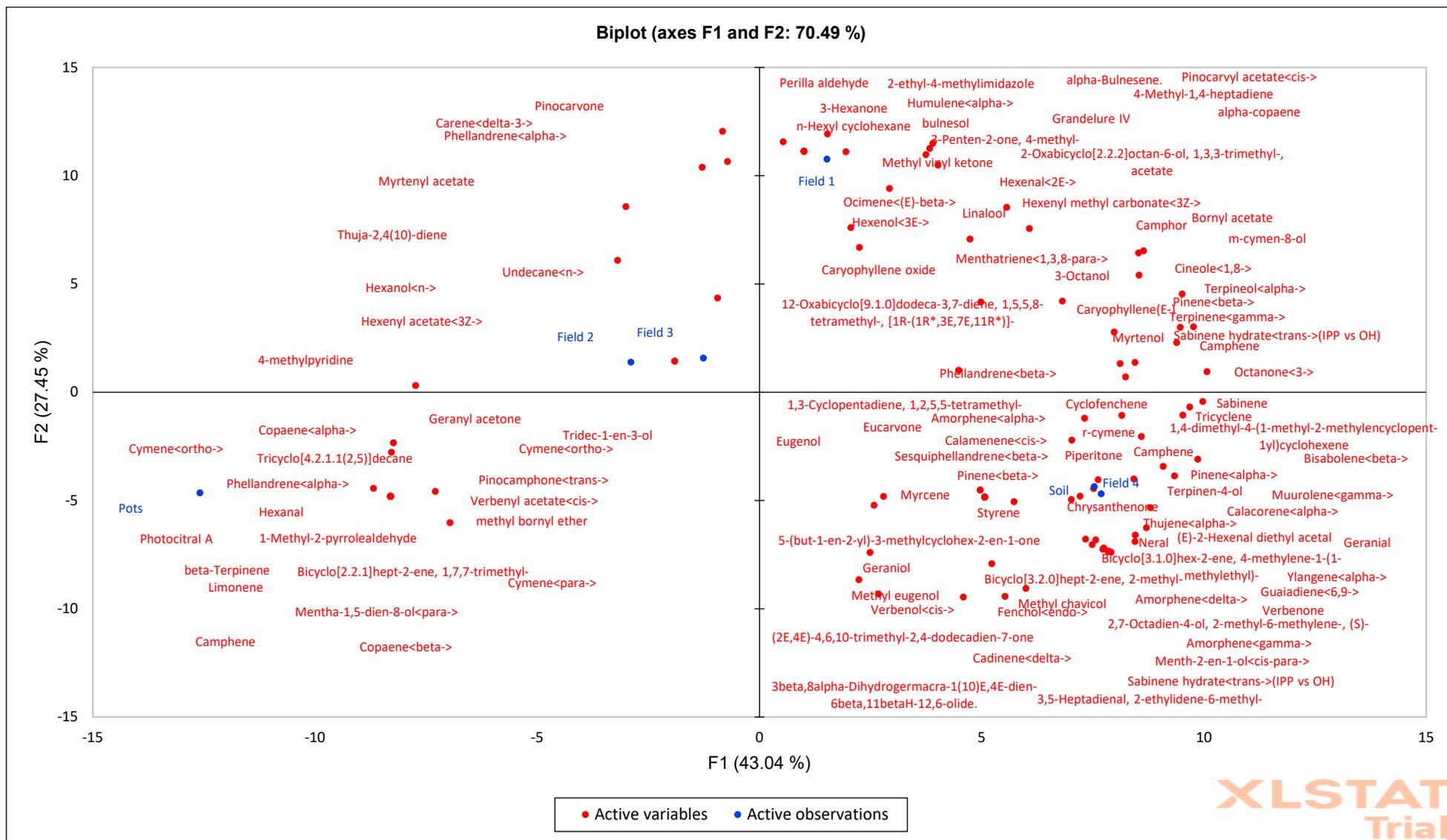


Figure 5. Biplot graph correlating the volatile compounds found in rosemary (red text) and their relation to the different production methods (blue text).

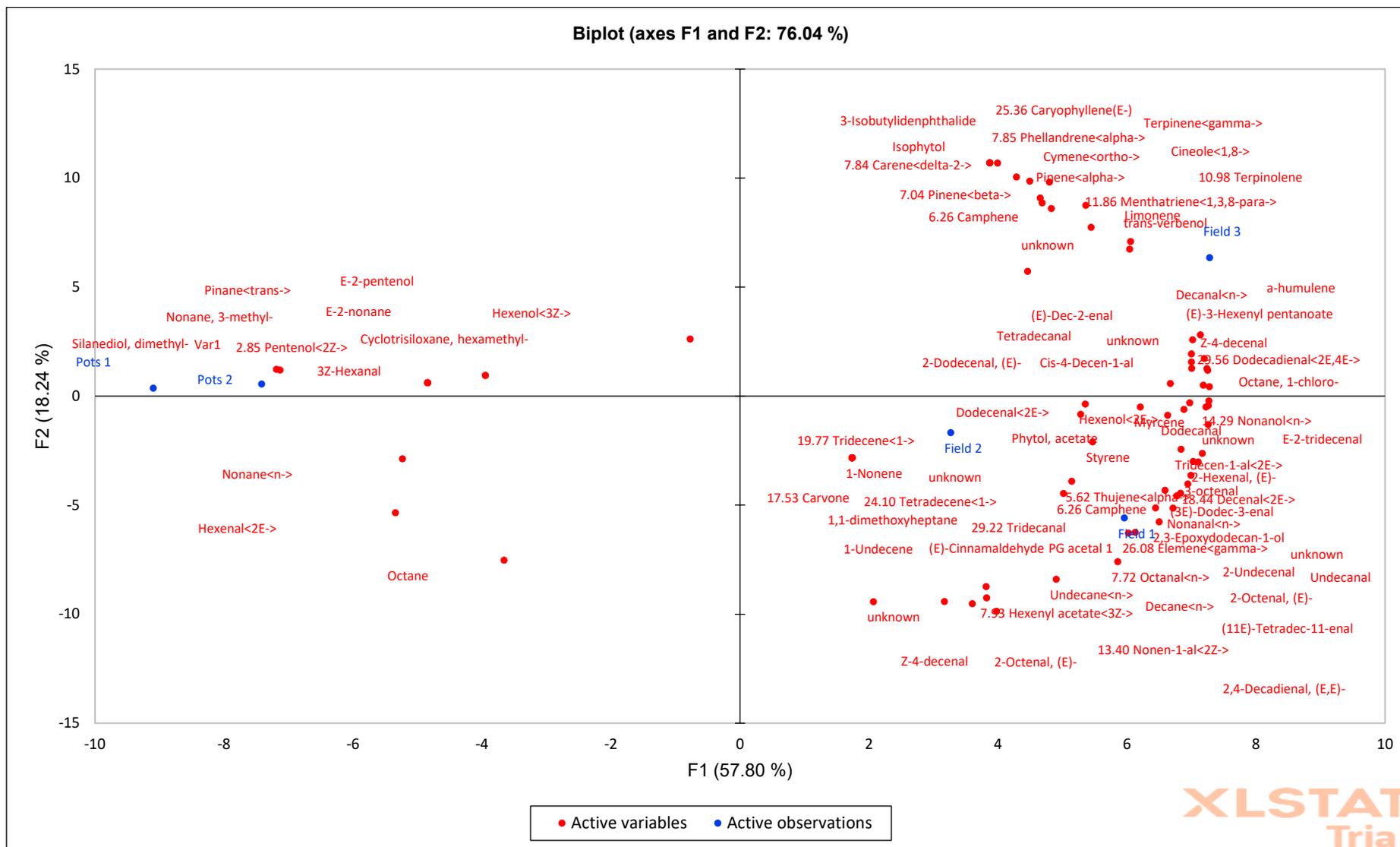


Figure 6. Biplot graph correlating the volatile compounds found in coriander (red text) and their relation to the different production methods (blue text).

Discussion

From the results it is possible to see that rosemary out of the three herbs is the one with more volatiles detected, followed by basil and finally coriander. Also, coriander has fewer common volatile compounds across all the samples tested and basil has more mutual volatile compounds that are common to all samples. This means that most of the compounds found on a basil sample are common to any sample of basil, whilst for rosemary and coriander the compounds mutual to all samples are a smaller percentage of the total of compounds detected. In the case of rosemary, this might be because the samples received are probably from different varieties (growers sent rosemary samples from available crops, not always knowing varietal information). This would explain why so many different compounds were observed and only a small percentage were common. However, this does not explain coriander, since the samples were from the same variety, so this is suggestive of the coriander volatile profile being highly influenced by production method.

Looking at the chromatograms presented in Figure 4, it is possible to see that rosemary has more highly abundant peaks, more peaks and they are concentrated in similar retention times. Coriander is the opposite, having less peaks and lower abundance peaks out of the three herbs.

Volatiles analysis

Rosemary

Table 4 shows the volatile compounds that were detected in all the samples, the corresponding retention time average of relative abundance, compared to the internal standard, and the significant difference relation between the samples. For rosemary, only two compounds (piperitone and bulnesol) have no significant differences. Furthermore, it is possible to see that most of the pot grown samples were significantly different from the rest (soil protected and outside field), leading to the interpretation that the type of production has had an impact on the volatile abundance and diversity. Verbenone, when present in rosemary, has been described as having the best flavour (Chadwick, 2018c). Looking at Table 4, samples Pot and Field 4 are the ones with higher contents of this compound and do not show any significant differences between each other, leading to the suggestion that these samples might have 'better' flavour. If the sample has verbenone in its composition, alpha-pinene should also be present since this is a substrate for the production of verbenone (Chadwick, 2018c), however, all the substrate might have been used to produce the second compound, explaining why the substrate might not be present. In the case of the compound 1,8-cineole, when present there should also be alpha-terpineol as it can be observed in Table 4 (both are present). The samples Soil and Field 4 were produced by the same grower and

at the same location, resulting in the majority of volatiles not being significantly different. This is most likely due to the samples being produced in the same location with the same soil type, however it could also be due to the consistency in agronomic methods such as the application of water, fertilizer and crop protection chemicals from a single grower. Looking at the samples that were produced in the field outside (Field 1, Field 2, Field 3), it is possible to see that most of the compounds are not significantly different from each other, however, Field 4 shows significant differences when compared to the other three. The first three field productions are different growers but located around the south of England, whilst the fourth sample is located more north, this indicates that the location influences the abundance of the compounds, this might also be due to variables like temperature, irrigation, light intensity and soil type used for cultivation. Site differences in rosemary flavour profile are also likely due to varietal differences.

Coriander

Coriander results are presented in Table 5 where only one of the 29 compounds identified shows no significant difference between all the samples; this compound is gamma-Elementene, described with a green, woody and oily flavour. It can also be observed that for almost all the compounds identified, there are no significant differences between the samples that were pot grown. These samples have the same type of production but are from different locations/growers, however the difference in location is not as marked as the one described for rosemary. This leads to the conclusion that only big differences in location/environment influence the abundance of aroma volatile compounds. Examining the significance results for the samples produced in the field, there does not appear to be any correlation, in other words, there is no common behaviour between the samples that can be associated with any clear variable. Even from the same type of production they do not show any similarities and the same can be observed when comparing by geographical location. In the case of coriander, the only conclusion that is possible to establish is that pot grown herbs do not show significant differences between each other, but exhibit differences when compared with the field production method. There is not a lot of information about coriander flavour, especially for plants produced for culinary purposes and within the UK. It is known that most of the mechanisms to produce the aroma volatile compounds are from oxidation of fatty acids (Chadwick, 2018b).

Basil

The results presented in Table 6 show the compounds identified in basil. Eleven of these compounds do not show any statistical difference between the production methods for pot grown, soil protected and hydroponics. This means that besides being present in all the

samples, they have similar abundance, indicating that these eleven compounds might be the core contributors to the aroma volatile profile for the *Sweet Genovese* variety of basil. The compounds that characterize basil flavour are produced as UV protectants as the samples analysed were produced during the summer, thus following what would be expected (Chadwick, 2018a). Samples from pot production have fewer similarities, between each other than what would be expected, as some of the compounds are significantly different. Despite being from the same type of production, they are produced in different locations by different producers. If what is affecting the profile is the location and not the producer this might indicate that, in the case of basil, a small difference in location affects the aroma volatile profile composition. This supports what is found in literature, since basil is described as very sensitive to climate conditions (Chadwick, 2018a). For some of the compounds, there are similarities between Pots 2 and Soil samples which are closer in terms of location, supporting what has been stated before. This leads to the conclusion that in the case of basil, location has higher influence on the aroma volatile compounds than the type of production. In the case of hydroponic production, there is no apparent relation to the other types of production, significant differences were only observed for some compounds. However, curcumin (floral flavour) and isopropyl cinnamate (balsamic, fruity, tropical flavour) were present at very high levels, showing significant differences with Pots 2.

Further analysis needs to be done, to allow a more definitive conclusion and also to establish what variables are influencing the flavour. Future experiments including sensory analysis with trained panel and consumer perception, will also link these aroma volatile profiles with perception by the consumer, in order to correlate both results. The seasonal variation will shed some light on how climate influences the herbs aroma. The basil grown in the UK are characterised by having high content of linalool and eugenol (Chadwick, 2018a), which can be seen in Table 6 as the highest compounds present followed by 1,8-cineole. Methyl eugenol, a derivative of eugenol, can also be found in the samples. Both compounds are present which agrees with their pathway. With that in mind, the same should be expected for estragole and chavicol, however the last was not detected in all the samples, this might be due to the fact that all the substrate might have been used up (Chadwick, 2018a).

Principal Component Analysis

The characteristic aroma volatile profile identified in each of the herbs, allows us to understand and identify the differences in terms of compound composition between samples of the same herb. This also allows us to establish possible similarities between samples, and to relate this with some of the characteristics of the growing conditions, like type of production and geographical location.

Principal component analysis allows a graphical vision of the relation between compounds and the samples. This will enable the discovery of which samples are more similar to each other and which compounds are more associated with them.

Rosemary

PCA results for rosemary are shown in the graph presented in Figure 5 and account for 70% of the variation. It is very clear that pot grown samples are different from the rest, being in a different quadrant. Rosemary potted samples are expected to have different volatiles profile when compared with rosemary produced in fields (protected and unprotected), since the latter is cut more than once per crop which has an impact on the flavour (Chadwick, 2018c). This explains what might cause the differences between pot grown and unprotected field grown samples. Field 2 and Field 3 samples are placed close to each other and apart from Field 1, this might be due to the geographical location, since the last one is located in the south of England, whilst the others are more in the centre of the country. As it was noticed before, Soil sample and Field 4 sample, are similar when it comes to compounds composition and have a positive correlation. These two samples come from the same producer and geographical location, this leads to the interpretation that geographical location has more impact on the volatile composition than the growing system. However, the varieties of the rosemary samples are not known, but what is known is that both samples from the same grower are from the same variety. This means that it is not possible to determine what is causing the differences between the samples i.e. whether it is the agronomic practice and geographical location or if it is the fact that the samples might be from different varieties. The variety of rosemary is described as having high influence on the flavour composition (Chadwick, 2018c). Therefore, the similarity between samples Soil and Field 4 might be because both samples are from the same variety. Before it was mentioned that verbenone is a product of alpha-pinene (Chadwick, 2018c). In the PCA plot it is possible to see that they have a positive correlation and that they are associated with the samples Soil and Field 4. The same applies to 1,8-cineole and alpha-terpineol, the first is a product of the second. Statistical analysis was carried out and there is positive correlation (0.985) between the two.

Coriander

There is not a lot of information in relation to what influences coriander flavour, but it is known that variety is a major factor. All the samples analysed in this study are from the same variety so differences between samples must be attributed to another factor (Chadwick, 2018b). Coriander's PCA results (Figure 6) account for 76% of the variation and show a clear separation of pot grown samples and field grown samples, each type being in opposite ends of the graphs. This supports what been discussed before, that potted coriander is significantly

different from the ones grown outdoors in the field. Another thing to notice is that field grown crops can go through a few cuts which leads to the development of odd flavour compounds like nonane, which has been detected in all the samples but does not seem to have a positive correlation with other compounds. Nonane shows a slight association with pot grown samples, which contradicts what would be expected, since cut herbs were exposed to more stress (Chadwick, 2018b). It is also clear that most of the compounds detected are associated with the field samples. Looking at the field samples, it is possible to see that Field 1 and Field 2 have a correlation, leading to the conclusion that they might be similar, however when analysing the data with a plot from a different perspective (explaining 74% of the variation), this relationship disappears, and Field 1 shows a correlation with Field 3. Field 2 sample is the one produced more in the south of the UK and if this is in fact significantly different from the others, it might be due to the geographical location. More analysis needs to be done in order to understand if the field samples are in fact different and what is causing this difference, since it is not currently possible to draw any other conclusion besides that pots are clearly different.

Basil

Figure 7 shows the PCA plot for basil accounting for 85% of the variation. Each of the four basil samples appear in different quadrants of the plot, however pot samples can be found on the left side of the graph, meaning that they have some similarities (even when looking at a different plot perspective, these samples keep the same correlation – data not shown). Looking at the graph it is possible to see that hydroponic grown basil is associated with the highest proportion of the compounds, and the Soil sample is the one with less correlation compared to the others. Hydroponic grown herbs also seem to be associated with the compounds most relevant to the variety flavour (Chadwick, 2018a). However, when looking at other perspectives of the plot, the protected Soil sample is positioned closer to Pots 1; what is causing this similarity is not known and more analyses relating the growing conditions needs to be done.

Conclusions

The results gathered so far have helped to better define the volatile profiles of the three herbs. From what has been previously stated, this first stage of the experiments has given some light on how the volatile profile might change for the three herbs, giving a better idea on what significantly influences the compound abundance. As mentioned before, although we requested information from the growers in order to collect information about the growing

conditions, unfortunately, some information was not provided uniformly across all growers, making it difficult to link that information with the results.

It is clear that basil is the herb that varies the least in the profile of compounds across its samples, the differences being in the abundance of each rather than presence or absence. Type of production has an effect when it comes to rosemary and coriander, while geographical location (and by consequence the environment) also shows influences being more significant in basil. For rosemary, it was apparent that variety has a big influence on the flavour profile.

The results have given some light on how volatiles present in the herbs vary and is a step forward for the growers to understand their product better. Other analyses need to be done in order to complement what has been found and also to draw more definitive conclusions, as well as other comparisons. Understanding how these differences are perceived by the consumer (project year 2 onwards) will help determine how definite these differences are for those who buy these products.

Knowledge and Technology Transfer

Industry engagement:

- Project start-up meeting with steering group, 11 April 2018?
- Project progress meeting with steering group, 1 October 2018
- Project progress meeting and presentation to BHTA R&D Committee, 5 March 2019

Presentations:

- Nursten Symposium, 2018
- AHDB PhD conference, 2018
- Food and Nutritional Sciences Department Seminar, 2019
- Food and Nutritional Sciences Research Symposium, 2019

Courses:

- AFTP Fresh Produce: Postharvest Quality Management
- AFTP Flavour: Farm to Fork & Beyond
- Using Liquid Nitrogen Safely

Glossary

ANOVA: Analysis of variance

SPME: Solid-Phase Microextraction

GC-MS: Gas Chromatography- Mass Spectrometry

UK: United Kingdom

HPS: High-Pressure Sodium

MH: Metal Halide

LED: Light-Emitting Diode

LRIs: Linear Retention Indices

PCA: Principal Component Analysis

References

Adams, R. P. *et al.* (2006) 'DNA fingerprinting and terpenoid analysis of *Juniperus blancoi* var. *huehuentensis* (Cupressaceae), a new subalpine variety from Durango, Mexico', *Biochemical Systematics and Ecology*, 34(3), pp. 205–211. doi: <https://doi.org/10.1016/j.bse.2005.11.004>.

Adams, R. P. and Nguyen, S. (2005) 'Infra-specific variation in *Juniperus deppeana* and *J. sperryi* in the Davis mountains of Texas: Variation in leaf essential oils and random amplified polymorphic DNAs (RAPDs)', *Phytologia*. Bronx Park, New York, H.A. Gleason and H.N. Moldenke, 87(2), pp. 97–109. Available at: <https://www.biodiversitylibrary.org/part/220277>.

Avato, P. *et al.* (2004) 'Essential oils of *Varthemia iphionoides* from Jordan', *Flavour and Fragrance Journal*. John Wiley & Sons, Ltd, 19(6), pp. 559–561. doi: 10.1002/ffj.1351.

Bouzouita, N. *et al.* (2003) 'Antimicrobial activity of essential oils from Tunisian aromatic plants', *Flavour and Fragrance Journal*. John Wiley & Sons, Ltd, 18(5), pp. 380–383. doi: 10.1002/ffj.1200.

Bower, A., Marquez, S. and 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(16), pp. 2728–2746. doi: 10.1080/10408398.2013.805713.

Chadwick, M. (2018a) *Optimising flavour in basil*. Available at: <https://horticulture.ahdb.org.uk/publication/optimising-flavour-basil>.

Chadwick, M. (2018b) *Optimising flavour in coriander*. Available at: <https://horticulture.ahdb.org.uk/publication/optimising-flavour-coriander>.

Chadwick, M. (2018c) *Optimising flavour in rosemary*. Available at: <https://horticulture.ahdb.org.uk/publication/optimising-flavour-rosemary>.

- Chehab, E. W. *et al.* (2008) 'Distinct roles of jasmonates and aldehydes in plant-defense responses', *PLoS ONE*, 3(4). doi: 10.1371/journal.pone.0001904.
- Chohan, M., Forster-Wilkins, G. and 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(2), pp. 47–52. doi: 10.1007/s11130-007-0068-2.
- Cruickshank, B. (2012) *Improving the quality of cut herbs through optimisation of the growing environment*. University of Reading.
- Díaz-Maroto, M. C. *et al.* (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(15), pp. 2070–2076. doi: 10.1002/jsfa.1921.
- Di Ferdinando, M. *et al.* (2014) 'Multiple functions of polyphenols in plants inhabiting unfavorable Mediterranean areas', *Environmental and Experimental Botany*, 103, pp. 107–116. doi: <https://doi.org/10.1016/j.envexpbot.2013.09.012>.
- Hazzit, M. *et al.* (2006) 'Composition of the Essential Oils of Thymus and Origanum Species from Algeria and Their Antioxidant and Antimicrobial Activities', *Journal of Agricultural and Food Chemistry*. American Chemical Society, 54(17), pp. 6314–6321. doi: 10.1021/jf0606104.
- Hossain, M. B. *et al.* (2010) 'Effect of drying method on the antioxidant capacity of six Lamiaceae herbs', *Food Chemistry*. Elsevier Ltd, 123(1), pp. 85–91. doi: 10.1016/j.foodchem.2010.04.003.
- Jalali-Heravi, M., Zekavat, B. and Sereshti, H. (2006) 'Characterization of essential oil components of Iranian geranium oil using gas chromatography–mass spectrometry combined with chemometric resolution techniques', *Journal of Chromatography A*, 1114(1), pp. 154–163. doi: <https://doi.org/10.1016/j.chroma.2006.02.034>.
- Kuban-Jankowska, A. *et al.* (2018) 'Potential Health Benefits of Olive Oil and Plant Polyphenols', *International Journal of Molecular Sciences*, 19(3), p. 686. doi: 10.3390/ijms19030686.
- Loayza, I. *et al.* (1995) 'Essential oils of *Baccharis salicifolia*, *B. latifolia* and *B. dracunculifolia*', *Phytochemistry*, 38(2), pp. 381–389. doi: [https://doi.org/10.1016/0031-9422\(94\)00628-7](https://doi.org/10.1016/0031-9422(94)00628-7).
- Lucero, M. *et al.* (2004) 'Differences in Volatile Profiles Between Populations of *Ceratoides lanata* var. *subspinosa* (Rydb.) J.T. Howell', *Shrubland Dynamics—Fire and Water*, pp. 142–146. Available at: https://www.fs.fed.us/rm/pubs/rmrs_p047.pdf.

- Lucero, M. E. *et al.* (2006) 'Volatile Composition of *Gutierrezia sarothrae* (Broom Snakeweed) as Determined by Steam Distillation and Solid Phase Microextraction', *Journal of Essential Oil Research*. Taylor & Francis, 18(2), pp. 121–125. doi: 10.1080/10412905.2006.9699039.
- Lucero, M. E., Estell, R. E. and Fredrickson, E. L. (2003) 'The Essential Oil Composition of *Psoralea scoparius* (A. Gray) Rydb.', *Journal of Essential Oil Research*. Taylor & Francis, 15(2), pp. 108–111. doi: 10.1080/10412905.2003.9712083.
- Lucero, M. E., Estell, R. E. and Sedillo, R. L. (2005) 'The Composition of *Dalea formosa* Oil Determined by Steam Distillation and Solid-Phase Microextraction', *Journal of Essential Oil Research*. Taylor & Francis, 17(6), pp. 645–647. doi: 10.1080/10412905.2005.9699022.
- Marongiu, B. *et al.* (2006) 'Extraction of *Juniperus communis* L. ssp. *nana* Willd. essential oil by supercritical carbon dioxide', *Flavour and Fragrance Journal*. John Wiley & Sons, Ltd, 21(1), pp. 148–154. doi: 10.1002/ffj.1549.
- Meerburg, B. G., Brom, F. W. A. and Kijlstra, A. (2008) 'The ethics of rodent control EXPERIMENTATION', *Pest management science*, 64(June 2007), pp. 1205–1211. doi: 10.1002/ps.
- Merle, H. *et al.* (2004) 'Taxonomical contribution of essential oils in mandarins cultivars', *Biochemical Systematics and Ecology*, 32(5), pp. 491–497. doi: <https://doi.org/10.1016/j.bse.2003.09.010>.
- Opara, E. I. and 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(10), pp. 19183–19202. doi: 10.3390/ijms151019183.
- Pintore, G. *et al.* (2002) 'Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. oils from Sardinia and Corsica', *Flavour and Fragrance Journal*, 17(1), pp. 15–19. doi: 10.1002/ffj.1022.
- Putra, P. A. and Yuliando, H. (2015) 'Soilless Culture System to Support Water Use Efficiency and Product Quality: A Review', *Agriculture and Agricultural Science Procedia*. Elsevier Srl, 3, pp. 283–288. doi: 10.1016/j.aaspro.2015.01.054.
- Ramarathnam, N., Rubin, L. J. and 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*. American Chemical Society, 41(6), pp. 933–938. doi: 10.1021/jf00030a019.
- Rao, Y. R. *et al.* (2000) 'Composition of essential oil of citronella (*Cymbopogon winterianus*

jowitt) grown in coastal Orissa', *Fafai Journal*, 2(4), pp. 29–31.

Ravi, R., Prakash, M. and Bhat, K. K. (2007) 'Aroma characterization of coriander (*Coriandrum sativum* L.) oil samples', *European Food Research and Technology*, 225(3–4), pp. 367–374. doi: 10.1007/s00217-006-0425-7.

Rohloff, J. (2006) 'Essential Oil Drugs — Terpene Composition of Aromatic Herbs', *Production Practices and Quality Assessment of Food Crops*, 3, pp. 73–128. doi: 10.1007/1-4020-2534-3_4.

Rosenthal, G. A. and Berenbaum, M. R. (2012) *Herbivores: Their Interactions with Secondary Plant Metabolites: Ecological and Evolutionary Processes*. Elsevier Science (Herbivores: their interactions with secondary plant metabolites). Available at: <https://books.google.co.uk/books?id=g6BlbAfDveQC>.

Seely, A. (2017) *Determining aroma differences among basil, parsley, and dill grown under varied supplemental light wavelengths using consumer sensory and flash gas chromatograph- electronic nose analyses*. Iowa State University.

Tellez, M. R. *et al.* (1997) 'Essential Oil of *Dyssodia acerosa* DC.', *Journal of Agricultural and Food Chemistry*. American Chemical Society, 45(8), pp. 3276–3278. doi: 10.1021/jf9701502.

Tellez, M. R. *et al.* (1998) 'Essential Oil of *Chrysothamnus pulchellus* (Gray) Greene ssp. *pulchellus*', *Journal of Essential Oil Research*. Taylor & Francis, 10(2), pp. 201–204. doi: 10.1080/10412905.1998.9700880.

Valcourt, A. (2014) *The Chemistry and Biology of Purines*, *American Journal of Psychiatry*. doi: 10.1176/ajp.115.6.570.

Zoghbi, M. das G. B. *et al.* (1998) 'Essential oils of *Lippia alba* (Mill.) N. E. Br growing wild in the Brazilian Amazon', *Flavour and Fragrance Journal*. John Wiley & Sons, Ltd, 13(1), pp. 47–48. doi: 10.1002/(SICI)1099-1026(199801/02)13:1<47::AID-FFJ690>3.0.CO;2-0.

Appendix 1

Form given to growers to provide information about the herb sample.

FV / PE 455 Herb flavours project

Product life cycle and submission information

Herb species and variety	
Grower contact name and business address	
Location of production: GPS	
Type of production method (organic, conventional, hydroponic, soil, protected etc)	
Planting date	
Harvesting date	
Temperature average and range during growth period	
Light exposure (for protected crops)	
Type of light	
Time of exposure	<p>Time lights on:</p> <p>Time lights off:</p> <p>Does the above change during the production cycle? If so, please provide a record sheet if one is available</p>
Water supply	
Quantity/rainfall (if known)	Please provide a record sheet if one is available – amounts per day if possible or total/daily average over production cycle if more detailed data are not available.

Fertiliser and crop protection product application	Please provide a record sheet if one is available
Date of shipping to Reading	
Duration between harvest and cooling	
Time of the day that herbs were harvested	
Temperature range (°C) during transport (stipulated by courier or if you have any records)	
Use of oxygen scavengers	Don't use these
Crop stage / maturity of the plant when harvested	
Pot production: What product do you use (e.g. peat, coir or a mixture) as soil or growing media?	