Final Report

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Title: Culinary herbs: determining the basis of variation in herb flavour

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1. Industry Summary

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 (group size/population (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.
- Higher abundances of monoterpenes and phenylpropanoids were found desirable in rosemary and basil by the consumer, and aldehydes for coriander.
- The influence of soil, water source and lighting was herb specific.
- Herbs with higher abundances of aroma compounds were described as having higher flavour and aroma intensity by the sensory panel.

The results from the present study show how different growing conditions affect the flavour of the herbs and consumers' preference. This allows growers to better understand their products and be aware of how changes in production will influence the final product and how this will be received by the final consumer.

2. Introduction

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, most 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 herbs material used in this project 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 project 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.

3. Materials and methods

3.1. 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 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 project analysed the aroma profile of herbs and the contribution of seasonal variation on herb profile. Seasonal variation was evaluated for two seasons (summer and autumn) and in multi-year analysis (2018, 2019, 2020,2021). 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 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)

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.

3.2. 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).

3.3. 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).

3.4. 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.

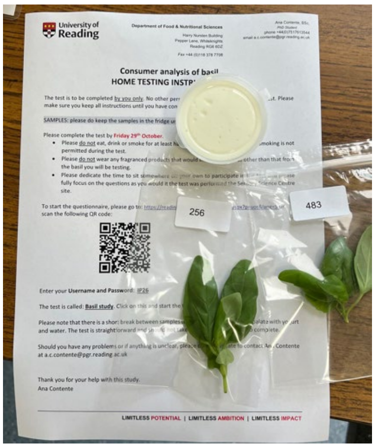
The relative concentration for each volatile compound was analysed and relative concentration was calculated in relation to the IS (propyl propanoate). The volatile compounds were identified by comparing each mass spectra with the mass spectra of compounds analysed in our laboratory (The Flavour Centre, 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.

3.5. Sensory profiling

Quantitative descriptive analysis (QDA) was carried out during the summer season of 2019, 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.

3.6. Consumer sensory

Consumer study was conducted with coriander and basil samples harvested in autumn season of 2021. This herbs were selected due to consumer’s consumption of these herbs as fresh leaves. 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 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. Finally, participants were asked to rank the samples according to their preference (ranking from most preferred to least preferred), if they liked the herb, whether they regularly consumed or purchased the herbs and how usually they consume them. 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.



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Consumer analysis of basil
HOME TESTING INSTR

The test is to be completed by you only. No other per st. Please make sure you keep all instructions until you have con

SAMPLES: please do keep the samples in the fridge u

Please complete the test by Friday 29th October.

- Please do not eat, drink or smoke for at least h moking is not permitted during the test.
- Please do not wear any fragranced products that would other than that from the basil you will be testing.
- Please dedicate the time to sit somewhere on your own to participate in fully focus on the questions as you would if the test was performed the Sensory Science Centre site.

To start the questionnaire, please go to: https://readi scan the following QR code:

256

483

Enter your Username and Password: IP26

The test is called: Basil study. Click on this and start the

Please note that there is a short break between samples palate with yogurt and water. The test is straightforward and should not take o complete.

Should you have any problems or if anything is unclear, please ate to contact Ana Contente at a.c.contente@pgr.reading.ac.uk

Thank you for your help with this study.
Ana Contente

LIMITLESS POTENTIAL | LIMITLESS AMBITION | LIMITLESS IMPACT

Figure 1: At-home test kit collected by participants.

3.7. 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 multiple factor analysis (MFA) 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 ($p < 0.05$).

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.

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. Results

4.1. Basil

4.1.1. Seasonal influence in the aroma

In total, 109 compounds were detected in the headspace of basil samples across two growing seasons during four years of harvest. The compounds detected included 23 monoterpenes (M), 13 other compounds (O), 15 sesquiterpenes (S), four phenylpropanoids (P), nine aldehydes (AL) and 38 unidentified compounds (U). 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.

Multiple factor analysis was used to visualise the differences in environmental factors and the chemical composition observed for the season of autumn (A) and summer (S), for the years of 2018, 2019, 2020 and 2021 (Figure 2). 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 2., 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. 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 2), 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.

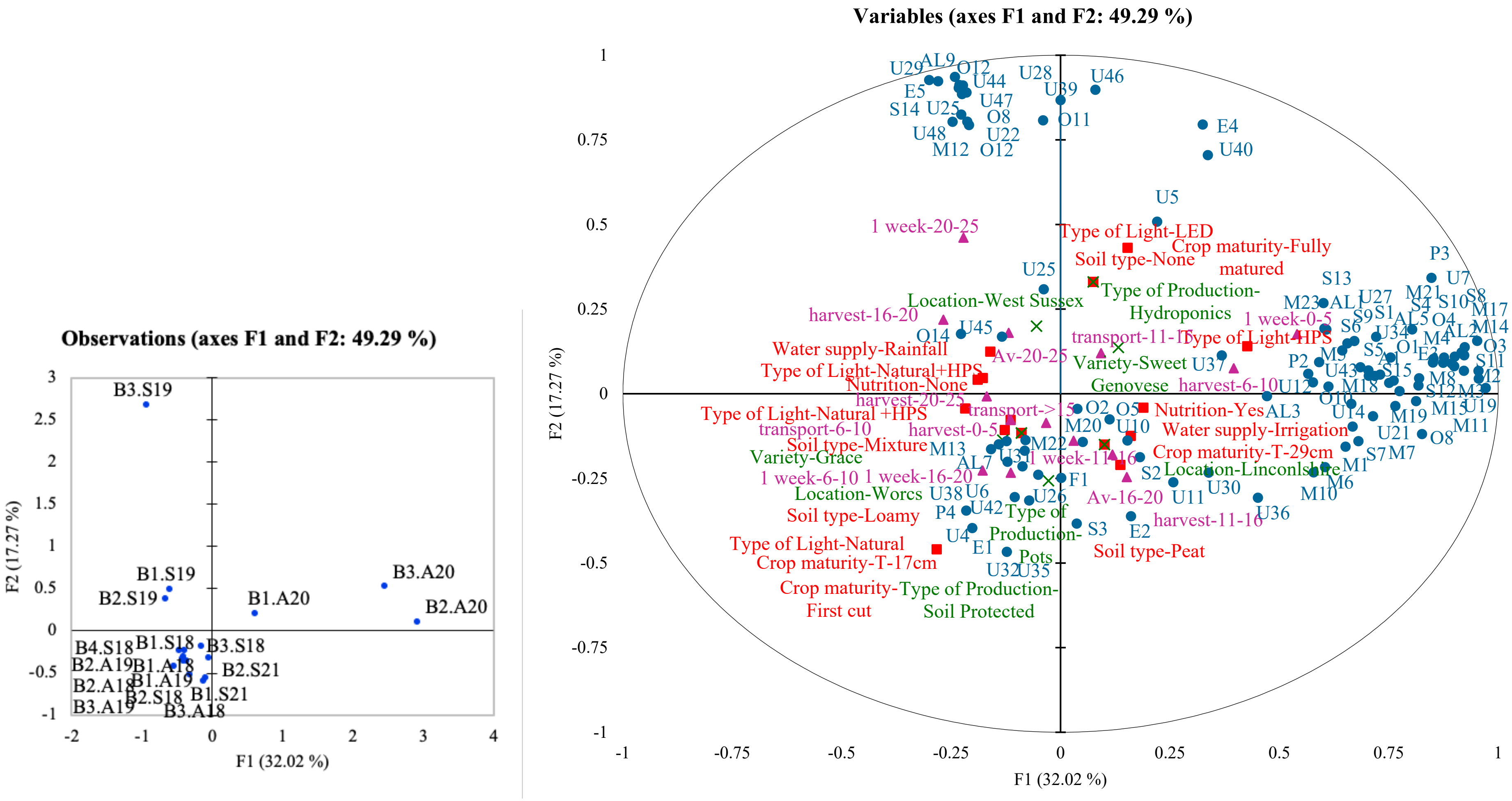
Different types of soil will result in different soil properties like water holding capacity and mineral composition thus affecting the production of primary and secondary compounds. As it can be seen in Figure 2, basil produced in loamy soil, mixture or peat was highly associated with compounds including 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 2). 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 2). 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 2), 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 2) 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 2: 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.

4.1.2. Sensory profiling

The sensory profile of three basil samples was analysed during the summer season of 2019. The profile 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 2) and out of the 27 attributes that were profiled, 10 of these were found to be significantly different between the three basil samples.

Table 2: 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). 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: 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 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.

4.1.3. Consumer evaluation

One hundred and seventeen consumers evaluated the basil samples, and demographic data is in Table 3. 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 3: 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 4) 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, suggesting consumers prefer a basil with higher monoterpene content.

Table 4: 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.

4.2. Coriander

4.2.1. Seasonal influence in the aroma

In total, 92 compounds were detected in the headspace of coriander samples for two growing seasons and three years of harvest. The compounds detected included 16 aldehydes (AL), seven other (O), seven alcohol (A), six alkanes (AK) and 38 unidentified compounds (U). 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.

Multiple factor analysis was used to visualise the differences in environmental factors and the chemical composition observed for the autumn (A) and summer (S) seasons, and for the years of 2018, 2019, 2020 and 2021 (Figure 4). 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). 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.

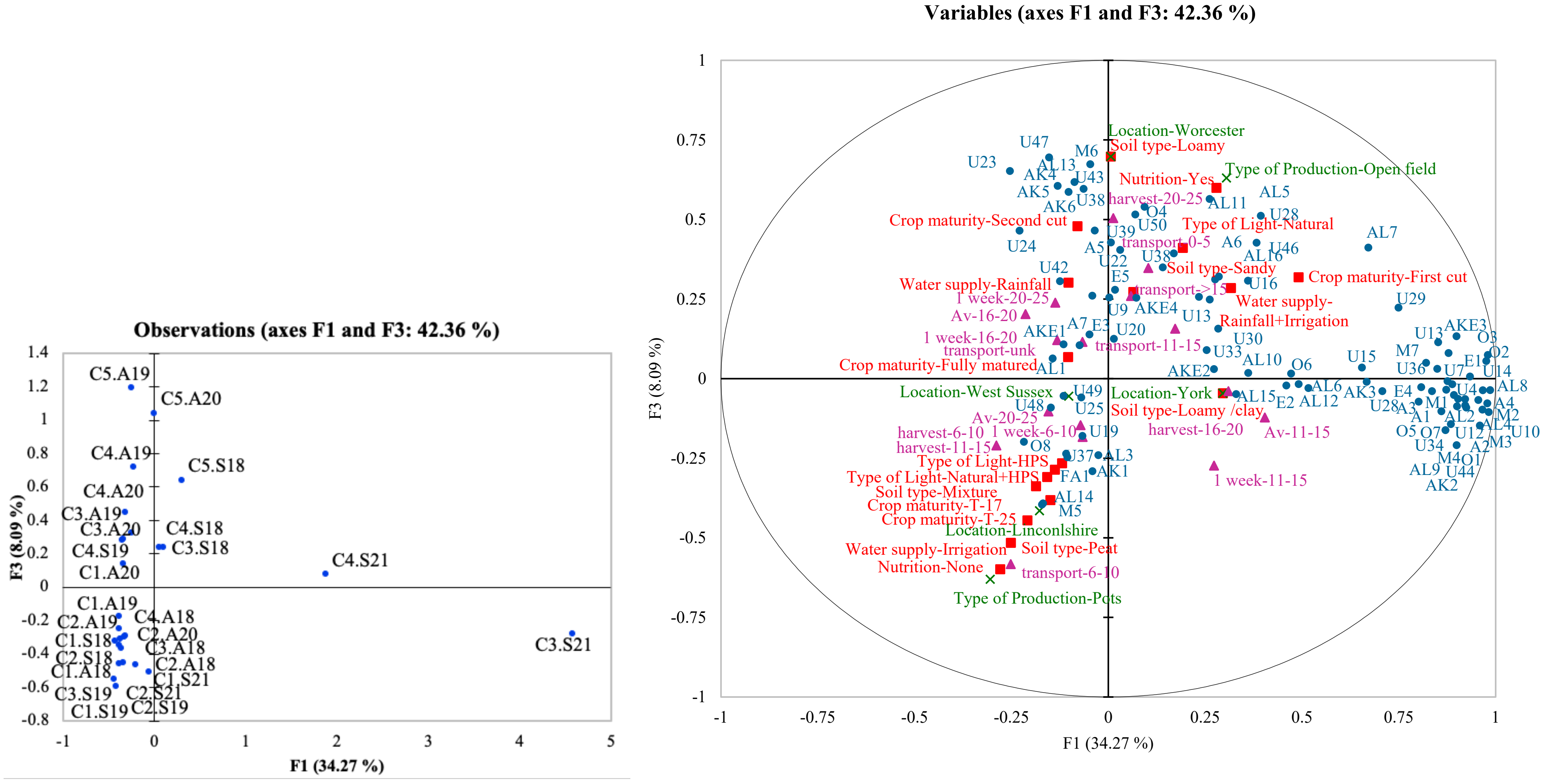
Contrary to the basil results, the application of fertilizers to coriander was positively correlated with most compounds and highly associated with (*E*)-2-decenal (AL11), tetradecanal (AL16) and nonanal (AL5), whereas no application resulted in high association with some minor compounds (Figure 4). 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, 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), 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). 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). 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).

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: 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.

4.2.2. Sensory profiling

The sensory profile of four coriander samples harvested during the summer season of 2019 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 5) and out of all attributes that were profiled, 17 of these were significantly different between the four coriander samples.

Table 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 5). 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 (Figure 4).

Figure 5: 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.

4.2.3. Consumer evaluation

One hundred and six consumers evaluated the coriander samples harvested during the autumn season of 2021, and demographic data is in Table 6. 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: 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 7) 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 7: 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).

4.3. Rosemary

4.3.1. Seasonal influence in the aroma

In total, 125 compounds were detected in the headspace of rosemary samples for two growing seasons over three years. Detected compounds included 45 monoterpenes (M), eight sesquiterpenes (S), three alcohol (A), three aldehydes (AL) and 46 unidentified compounds (U). 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.

Multiple factor analysis was used to visualise the differences in environmental factors and the chemical composition observed for the season of autumn (A) and summer (S), for the years of 2018, 2019, 2020 and 2021 (Figure 6). 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 6).

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 6) 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).

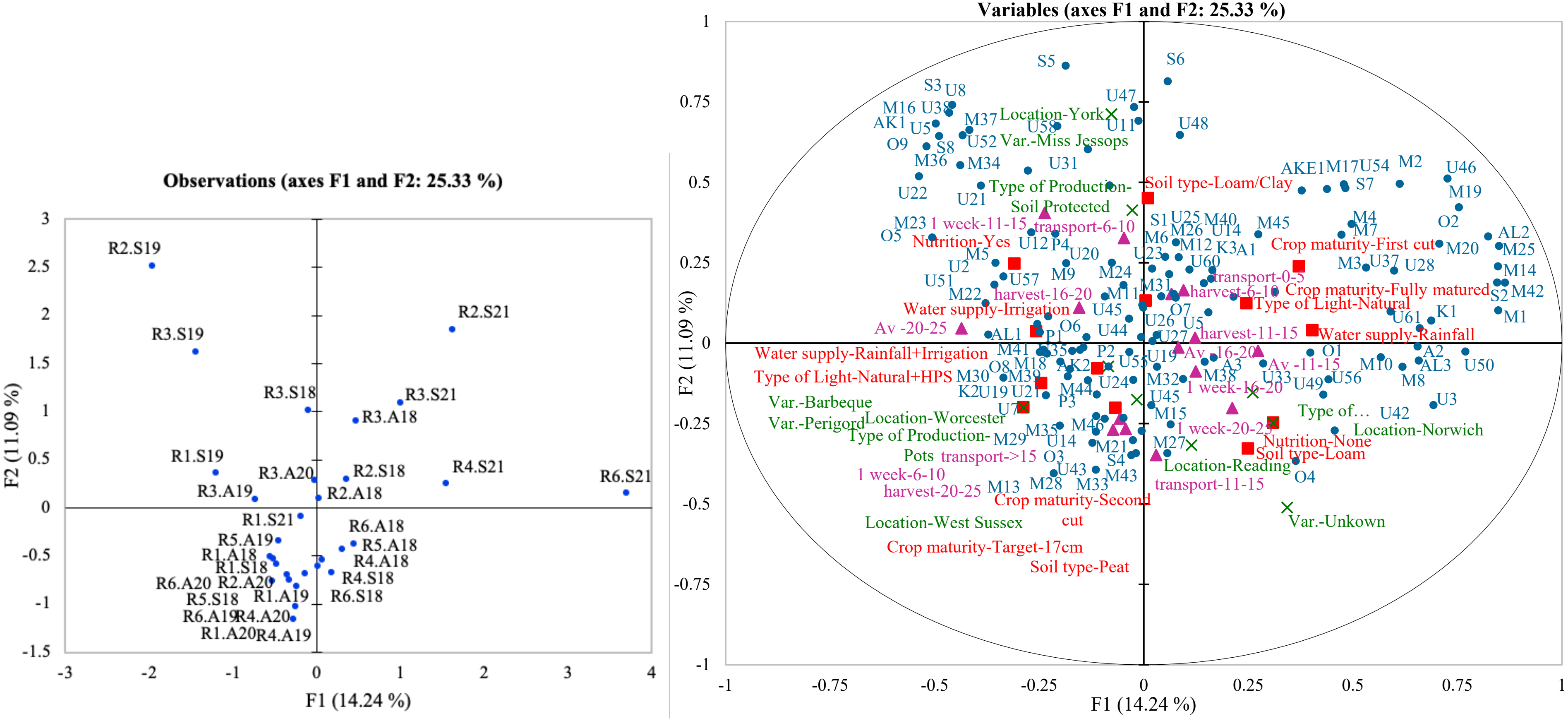
The influence of application or non-application of fertilizers on the aroma profile was examined (Figure 6), where the application of fertilisers was negatively correlated with main compounds and highly associated with minor compounds including gamma-terpineol (M34) and neral (M37), conversely not using 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 6) 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 6), 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 6: 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.

4.3.2. Sensory profiling

Three samples of rosemary harvested during the summer season of 2019 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 8) and all the attributes were profiled, out of these 24 were found to be significantly different between the three rosemary samples.

Table 8: 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 7). 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.

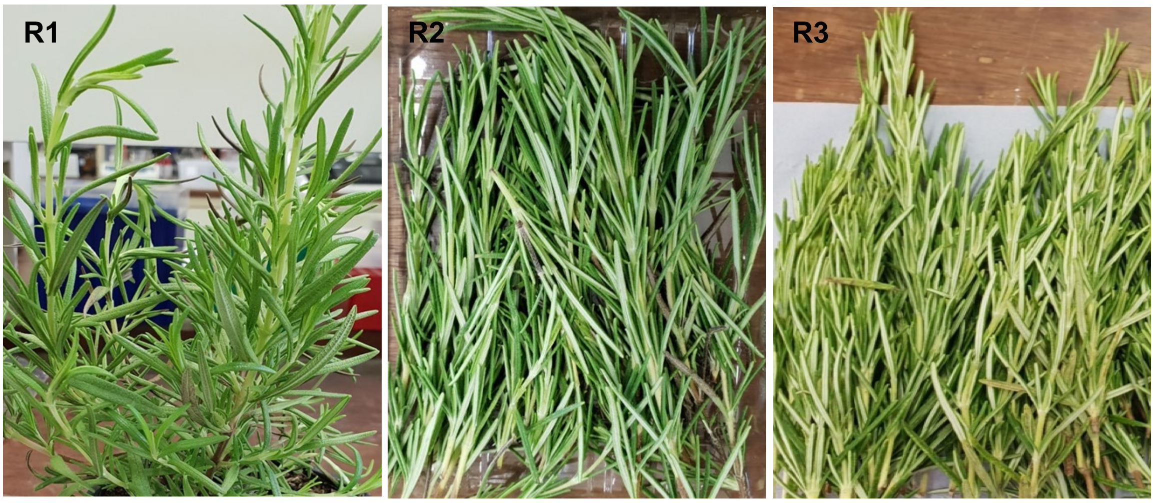


Figure 7: Images of the leaves of the three rosemary samples used in this study.

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.

5. Discussion

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 project 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). A single known variety was examined for basil and coriander. 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. 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. 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 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.

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 an increase in 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.

5.1. 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.

5.2. Final Remarks

This project 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.

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