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# Light and Temperature Effects on Metabolite Concentration in Selected Herbs and Microgreens

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## Abstract

Increasing evidence from epidemiological studies has shown that vegetable consumption is an important determinant of health. These health benefits are attributed to the presence of phytochemicals in these vegetables or plants that fight and reduce the risk of diseases. Inadequate consumption of vegetables has been linked to increasing incidences of some chronic diseases like cancers and cardiovascular diseases.

Microgreens are edible seedlings of vegetables and herbs with quick production cycles. They are very tender and smaller in size, yet more colourful, flavourful and nutritious than their mature counterparts, thus explaining why they were used in this study.

The objective of this study was to examine the effect of light, in particular photoperiod and temperature on metabolite composition in four microgreens: beet greens, peppercress, lettuce, wheatgrass, and four herbs: basil, coriander, mint, and chives, with the main focus being on metabolites that affect the flavour of the plant and are health related. The analyses were carried out using a derivatisation technique in combination with gas chromatography (GC-MS) based metabolite profiling and a solid phase micro-extraction (SPME) technique for the detection of volatile compounds in the herb species. 208 compounds were identified from the GC-MS metabolite profiling of the species and 38 compounds, i.e. 28 aromatics, 2 terpenes, 3 sugars, 3 acids, and 2 amino acids were selected. Sugars and citric acid were also selected to show trends of changes in central metabolism related to the treatments across the species, while the phenolics play vital roles related to health and food flavour. 17 out of the 28 aromatics were further selected across all the species because they were identifiable by name. Using SPME, 29 volatile compounds were detected in basil, 29 compounds in coriander, 30 compounds in mint, and 35 compounds in chives.

Based on metabolite profiling data, temperature had a stronger effect on metabolite concentration in basil and wheatgrass compared to day length and interaction, while day length had a stronger effect on beet greens, peppercress and mint compared to temperature and interaction. Day length and temperature showed very strong effects on lettuce and under interaction, while chives and coriander showed no effect upon day length and temperature treatment. With SPME in general, day length and temperature had no influence on volatiles in chives and coriander, only (E)-2-hexenal in basil was affected by day length and temperature, while temperature influenced some terpenes in mint volatile composition.

The principal component analyses (PCA) was done on the 17 aromatics and on the whole data set. PCA on the 17 aromatics did not show clear differences across the treatments, but it depicted clear differences on the whole data set across the species clustering based on the treatments.

The overall outcome of this present study showed that metabolite concentration was strongly influenced by day length and temperature, differently across the species. The poor growth conditions of the plants also influenced metabolite concentration across the species.

Cold temperatures and long day lengths generally yielded higher phenolic levels. With the herbs, warm temperatures resulted in higher volatile levels, long day lengths yielded higher levels in coriander and basil, while short day lengths yielded higher levels in mint and chives.



**Keywords:** Temperature, Photoperiod, Microgreens, Herbs, Metabolites, GC-MS, SPME

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# 1. INTRODUCTION

In recent years, consumer interest in vegetable consumption has greatly increased as they contain considerable amounts of nutrients, dietary fibre and phytochemicals, which possess benefits for human health.

There have been some uncertainties over the years as to whether the risk of certain chronic diseases can be reduced by increased consumption of vegetables. However, numerous studies have shown accumulating evidence that vegetable consumption has health promoting properties. Increasing incidences of some chronic diseases have been related to poor or insufficient consumption of vegetables. Epidemiological studies have shown that vegetables in diet are protective against cancer, coronary heart disease, stroke, weight gain (risk factor for type 2 diabetes mellitus), dementia, eye disease and risk of osteoporosis *(Boeing et al., 2012)*.

Amongst these vegetables are microgreens which are a new type of edible vegetables with stems and cotyledons that range in size from 2.5 to 7.5 cm long *(Xiao et al., 2012; Xiao, 2013)*. These plants are very tender and are produced from the seeds of vegetables and herbs. They are very easy to grow and require no experience. They can be grown in growth chambers, urban cultivators, greenhouses and in houses or apartment windows. A wide range of herbs, and seedlings of various crops and garden species are grown as microgreens. They have a quick production cycle, so can be harvested at 7-14 days after germination depending on the species. Although smaller in size, they are generally more flavourful, colourful, have a delicate texture, high nutritional contents, and can also be used to decorate main dishes as seen in master chef cuisines *(Xiao et al., 2012)*. Microgreens are also considered as functional foods, i.e. food products that possess properties beneficial to health, that are additional to their normal nutritional values *(Xiao et al., 2012). Xiao et al. (2012),* reported that the younger leaves of spinach *(Spinacia oleracea L.)* had higher levels of vitamins C, B9, K1, and carotenoids than in their mature leaves. They also found that the concentration of phenolics and antioxidants in young lettuce *(Lactuca sativa* L.*)* seedlings, were higher compared to the older leaves after 7 days germination. In the same study, it was shown that the nutritional content of microgreens can be as high as 4-40 times compared to what the mature vegetable would produce, but taking into consideration that this depends and varies among microgreen species. *Xiao et al. (2012)* reported that according to USDA National Nutrient Database (2011), nutritional concentrations of phytochemicals are higher in microgreens than in their mature counterparts. Microgreens are also rich in minerals like potassium and calcium, and considered as low sodium foods. Since they are mostly consumed raw, they can satisfy the needs of raw foodists.

As mentioned above, herbs grown as microgreens are not only used for culinary purposes, but can further be useful in the production of essential oils for different purposes. Essential oils are odorous volatile compounds naturally found in plants. Their contents are generally very low and hardly exceeds 1%, except for few cases that may reach 10% *(Djilani et al., 2012)*. Most often they are colourless, hydrophobic, soluble in alcohol and slightly soluble in water.

Their extraction can be done through different methods like hydro-distillation and solvent extraction, just to name a few. Some of the volatile constituents in essential oils are hydrocarbons, aldehydes, ketones, alcohols, phenols, lactones, and acids. Their composition and fragrance depend largely on growing conditions of the plant, its geographical area, season and genetics of the plant *(Djilani et al., 2012)*.

The economic value of essential oils cannot be disputed as they have been used since antiquity in many cultures for medicinal and health purposes. They are applied in food industries, perfumeries, cosmetics and pharmaceutics because of their high therapeutic activities (antimicrobial, antioxidative and anti-inflammatory).

## 1.1 Plant Phytochemicals

Phytochemicals are a large group of natural chemical compounds found in all plants known to promote health. Apart from promoting human health, they also protect the plant from drought, stress and pathogenic attacks *(Saxena et al., 2013).* Phytochemicals are not essential nutrients, but may have preventive effects to fight against some diseases, as suggested by some findings that they can reduce the risk of coronary heart diseases, diabetes, cancer and high blood pressure, hence are classified as antioxidant and antibacterial agents (*Saxena et al., 2013).* In another recent study *Ali et al. (2009)* also showed that phytochemicals have antimicrobial, anti-inflammatory and antioxidant properties.

Phytochemicals are also referred to as secondary metabolites. These metabolites are generally produced in smaller quantities compared to primary metabolites in the plant kingdom. Primary metabolites are those compounds essential for plant growth, development, respiration and photosynthesis, and they include: carbohydrates, proteins, lipids, and nucleic acids. Secondary metabolites on the other hand are not essential for plant growth as mentioned above, but are often involved in defense. They include: terpenes, phenolics, alkaloids and glucosinolates. Our focus is therefore on secondary metabolites because of their therapeutic properties. Medicinal plants are very rich in secondary metabolites, and these plants have been used in both the developed and developing countries to date.

Since antiquity, humans have been using plants to treat themselves. Africa is very rich in medicinal herbs, and given the fact that standards of living are low with poor sanitation, bacterial infections are very common. Hence most people resort to treat themselves traditionally in cases where commercial drugs are too expensive and unavailable, e.g. garlic is often used as an antimicrobial agent *(Doughari et al., 2009)*.

Many secondary metabolites have been isolated from plants and developed into drugs, a good example being the antimalarial artemisinin extracted from an aromatic herb called *Artemisia annua (Sholikhah, 2016*). In 1920, the French pharmacists Peletier and Caventou extracted quinine (an important antimalarial drug) from Cinchona (a genus of flowering plants) *(Saxena et al., 2013)*. *Mahdi (2010)* reported that Gerhardt in 1853 produced aspirin from salicin, extracted from willow herb. In the mid-19$^{th}$ century, the German chemist Hoffmann re-discovered the formula and patented it. Due to the numerous side effects in our present-day drugs, many people choose to go back to traditional medicine.

On the other hand, secondary metabolites are also used commercially for their flavours, fragrance and in pharmaceutical industries *(Balandrin et al., 1985)*.

Generally, vegetables can be bought and consumed all year round, but their nutritional qualities are not usually the same in all seasons. Some studies have shown that phytochemical content in vegetables varies greatly within seasons, e.g. in onions and lettuce, carotenoids in spinach, parsley and green *onions (Alarcón-Flores et al., 2015)*. These studies indicate that phytochemical contents are generally higher in plants grown during the summer because of long photoperiods and elevated temperatures. On the other hand, plants grown in the winter have shorter photoperiods and lower temperatures, hence the phytochemicals in the plants tend to reduce but they can be increased in some plants in stress response.

Therefore, since phytochemical concentrations in plants are affected by season, we can eventually say that whether the plants are cultivated in greenhouses, growth chambers or outdoor, these effects remain the same because temperature and light exposure are the main factors affecting phytochemical content in plants. *Alarcón-Flores et al. (2015)* reported that flavonol levels were about 4-5 times higher in vegetables that were grown outdoor compared to those grown in greenhouses. They also showed that summer vegetables yielded more flavonols than winter vegetables.

*Burbott et al. (1967)* showed that peppermint grown under 14 hours of light or less, produced very small amounts of essential oils, and reported that about 15 to 16 hours of light is required to produce peppermint oil, and monoterpenes in peppermint oil are greatly influenced by photoperiod. Just like most plants, the growth of lettuce is also influenced by photoperiod in a controlled environment, as it affects the reproduction of the plant. *Ali et al. (2009)* showed that photoperiod affects secondary metabolites and another study revealed that prolonging photoperiod increases fresh weight for most lettuce cultivars *(Gaudreau et al., 1994).* Also, *Chang et al. (2005)* showed that basil grown at 25°C or 30°C yielded three times more essential oils than when grown at 15°C and eugenol content was also higher at 25°C.

## 1.2 Effect of temperature and light on phytochemical production

It is generally expected that environmental factors like temperature and light would affect plant growth and subsequently, phytochemical production. Since these phytochemicals are found in the growing tissues, the same factors affecting plant growth would also affect the production of these metabolites. High and low temperatures generally affect plant phytochemicals differently, such that when temperatures are not favourable for the plant, they have to adapt to this stress thereby regulating physiological and biochemical processes which can possibly alter the production and concentration of the phytochemicals. In the same way, phytochemical production can increase or decrease depending on photoperiod duration. In a recent study, *Akula et al. (2011)* agrees to this, as they reported that the growing conditions of plants greatly influence the concentration of phytochemicals due to impacts on the metabolic pathways that are responsible to accumulate these compounds.

Therefore, temperature and light stress or even the combination of both can cause distinct changes in metabolic pathways, and they can stimulate the production of a single metabolite

or several compounds involved in the stress response differently. In some cases, certain compounds might increase their levels when the growth conditions are favourable or may decrease when the growth conditions are not favourable.
The methods used to grow microgreens (i.e. soil, compost, hydroponic), can significantly impact the nutritional value of the microgreens. Therefore, in a very recent finding on microgreen cultivation, *Weber (2016)* reported that microgreens grown on vermicompost have a higher concentration of phytochemicals than those grown hydroponically.

For this study, 8 plant species were selected from a list of microgreens and herbs to represent a broader range of plant groups and their uses; Four microgreens: beet greens, peppercress, lettuce, wheatgrass, and four herbs: basil, coriander, mint, and chives.
Thus, this study was carried out on microgreens for the purpose of their higher nutritional contents which could both be used in salads, to decorate dishes and as herbs because they are very flavourful.

## 1.3 Microgreens

### 1.3.1 Beet greens

Beet greens *(Beta vulgaris* L.*),* are leafy vegetables with red stalks that belong to the Amaranthaceae family. The beet plant is either grown for its leaves (beet greens) or for its roots (beet roots). Both contain phytochemicals that are beneficial to human health. It is an herbaceous biennial plant which is well-known and consumed in many parts of the world. The leaves can be consumed in salad. The greens (leaves) are rich in antioxidants, calcium, iron and vitamins A and C, while the roots are good sources of folic acid, fibre and manganese. In a recent study, *Lee et al. (2009)* showed that beets had the highest phenolic content amongst the 23 vegetables they studied, hence are very rich in phenolic acids which can help to prevent age-related diseases. They also mentioned that most studies on the health benefits of beets have been limited to the roots and not the leaves. Wounding and damage caused by bacterial infections can be limited because reactive oxygen species found in beets induces the synthesis of betacyanin *(Lee et al., 2009).*
*Nade et al. (2015)* showed that *Beta vulgaris* can act as an antidepressant, antioxidant, anticonvulsant, agent. It also has cerebroprotective, and hepatoprotective properties. The caloric value in beets is moderate, but they are rich in fibre and sugars *(Antigo et al., 2017)*. Betanin, the main component of the red colourant found in beets contains two groups of pigments; betacyanins (red-violet) and betaxanthin (yellow) *(Antigo et al., 2017)*.

### 1.3.2 Peppercress

Peppercress *(Lepidium sativum* L.*)* also called garden cress is an annual herbaceous herb that belongs to the Brassicaceae family. Peppercress seeds have been used in traditional medicine in India for many years *(Datta et al., 2011).* The seeds have high protein and calorie content while the leaves are rich in vitamin A, C and folic acid. Leaf and seed extracts were found to have anti-inflammatory effect and act again rheumatic pain *(Westphal, 2017)*. Peppercress

has been used in traditional medicine to treat bone fractures, diabetes, inflammations, and bronchial asthma *(Westphal, 2017)*. It is generally an important source of iron, folic acid, calcium, vitamins C, E and A. The seeds are known to contain arachidic and linoleic fatty acids *(Alshammari et al., 2017; Jabeen et al., 2017)* and are used to boost memory loss (*Jabeen et al., 2017).* Its seeds have been shown to reduce the symptoms of asthma and improve lung function in asthmatics (*Paranjape et al*., *2006*)**.** The leaves of the plant can be consumed raw in salads, cooked with other vegetables and used to decorate dishes. According to *Sharma et al. (2011)*, peppercress is a cool seasonal plant which has diuretic and antihypertensive effects, with the main secondary compounds being glucosinolates. They also reported that the essential oils of peppercress have oestrogenic effects because they have been tested on immature rats and showed development in their ovaries, and have antioxidant properties *(Jabeen et al., 2017).* Peppercress seeds have also been reported to regulate irregular menstruation, relieve constipation, and boost haemoglobin. Since they are rich in iron and proteins, they are given to lactating mothers as post-partum to facilitate milk production (*Jabeen et al., 2017)*.

### 1.3.3 Lettuce

Lettuce (*Lactuca sativa* L.) is a leafy vegetable, an annual herb that belongs to the Asteraceae family. It is one of the most widely consumed vegetables worldwide, and is popular for the preparation of salad. All parts of the plant are edible, and it exhibits medicinal properties. It is used in traditional medicine for inflammation, pain, and stomach problems including indigestion and lack of appetite *(Araruna et al., 2010)*. It also has anticonvulsant, sedative-hypnotic and antioxidant properties *(Araruna et al., 2010)*. The nutritional value of lettuce just like most species vary from cultivar to cultivar. They are a rich source in fibre, calcium, potassium and iron *(Baslam et al., 2013).* Lettuce is also rich in vitamins C, E and carotene. It is rich in iron, zinc, calcium, phosphorus, magnesium, manganese, and potassium and other health-promoting bioactive compounds *(Kim et al., 2016)*. Phenolics, carotenoids vitamin C and E are the main compounds in lettuce responsible for the health benefits *(Baslam et al., 2013)*.

### 1.3.4 Wheatgrass

Wheatgrass *(Triticum aestivum* L.*)* belonging to the Poaceae family is known for its high chlorophyll content. It contains phosphorus, magnesium, manganese, iron, copper, zinc, vitamin E, and tocopherols. It is used in traditional medicine and is highly valued for its therapeutic and nutritional properties *(Mohan et., 2013).* About 75% of wheat grass juice is made of chlorophyll. Chlorophyll is said to neutralise infection, heal wounds, overcome inflammation, and get rid of parasitic infection *(Mogra et al., 2013).* Wheatgrass has been proposed to help in blood purification, liver detoxification and colon cleansing, enhance the production of red blood cells, possess anti-oxidant properties, lowers blood pressure, enhance immunity, and restore energy and vitality *(Mogra et al., 2013).* In a recent finding, *Singh et al. (2012)* and *Mohan et al. (2013)* reported that the therapeutic properties of wheatgrass are due to the fact that it is rich in calcium, magnesium, iron, chlorophyll and

vitamin A, C and E. This plant can optimise blood sugar level because it is rich in fibre, and the presence of chlorophyll makes it an anti-diabetic agent with other therapeutic properties as antibacterial, anticarcinogenic and anti-inflammatory *(Rana et al., 2011)*. People suffering from multiple disorders like Parkinson's disease, asthma, hypertension, insomnia, obesity and bronchitis are advised to consume wheatgrass *(Rimple et al., 2016).*

## 1.4 Herbs

### 1.4.1 Basil

Basil *(Ocimum basillicum* L.*),* is a member of the Lamiaceae family. It is both annual and perennial, and is autogamous and herbaceous *(Sarahroodi et al., 2012)*. Basil is an excellent source of essential oils and aromatic compounds *(Simon et al., 1999)*, an herb grown for its strong flavour that is often used for cooking, with an attractive, sweet smelling aroma. Basil has been classified by *Simon et al. (1999)*, into many different cultivars, and further classified the species into 7 types; tall slender types (sweet basil), large leafed (Italian basil), dwarf types (bush basil), compact types (thai basil), purple coloured (sweet flavour), lobed leaves, also purple, (sweet plus clove-like aroma) and citron type (lemon flavoured basil). The taxonomy of basil is confusing and has become difficult to comprehend because of hybridization. This has resulted in many varieties, cultivars and chemotypes that do not really have distinctive morphologies *(Makri et al., 2008; Simon et al., 1999).* These chemotypes produce different aromatic compounds some of which are linalool, eugenol and citral. These chemotypes are commonly known by different names based on geographical origins. Extracts of the plant are used in traditional medicine because they have different properties such as; insecticidal, nematocidal, fungistatic, or antimicrobial *(Simon et al., 1990 Simon et al., 1999)*. In a recent finding, *Sarahroodi et al. (2012)* showed that basil has been traditionally used to treat many disorders such as; headaches and migraines, nerve pains, inflammation, cough, cold, digestive disorders, chest and lung complaints, fever, insect bites, menstrual cramps, sinusitis and as carminative and antispasmodic. It has also been used as cardiotonic, abdominal pain reliever, anti-diarrhoea medicine, hypolipidemic plant hypoglycemic agent, anti-inflammatory and anti-oxidant *(Sarahroodi et al., 2012).* Perfumeries, pharmacies, and food industries use the essential oils of basil, extracted from the leaves and flowers of the plant for their products *(Simon et al., 1999)*.

### 1.4.2 Coriander

Coriander *(Coriandrum sativum* L.*),* is an annual and herbaceous plant that belongs to the Apiaceae family and is sometimes called cilantro. Its leaves and seeds are used in food seasoning. It is the most commonly used spice in India and contains bioactive compounds with potential health benefits. It is an herb that can either be used for cooking or for medicine. This plant has always had high economic value since antiquity, as its flavour is used in cosmetics, drugs, perfumeries and food industries *(Darughe et al., 2012)*. Coriander has also been used traditionally to lower cholesterol, acts as a digestive stimulant, and anti-hypertension *(de Almeida Freires et al., 2014)*. Coriander leaves contain considerable

amounts of vitamin C and A, iron and minerals, low in cholesterol and saturated fats, and rich in zinc and dietary fibre *(Bhat et al., 2014)*. More so, coriander is used to cure diseases like digestive tract disorders, respiratory tract disorders, and urinary tract infections. The essential oils of coriander possesses antioxidant, antidiabetic, anticancerous, antibacterial and antimutagenic properties *(Darughe et al., 2012)*. A recent study suggested that coriander possesses pharmacological activities like anti-diabetic and anti-mutagenic and its powder and essential oils are used to preserve food as it has antibacterial, and antifungal properties *(Bhat et al., 2014)*. In another recent finding, *Sourmaghi et al. (2015)* reported that coriander seeds can be used to treat seasonal fever, convulsion, insomnia, nausea, cough, bronchitis, dysentery, and diarrhea and also have a strong antifungal effect against *Candida* species.
The main components of the volatile oil from the plant are the aliphatic aldehydes which generally have an unpleasant odour. These aldehydes are particularly from the C10 to C16 carbon chain *(Mandal et al., 2015)*.

### 1.4.3 Mint

Mint is the common name for any herbaceous plant in the genus *Mentha* and is a member of the Lamiaceae family. Mints are aromatic, and exclusively perennial plants. Their taxonomy is complex because different classifications have been proposed in the past *(Šarić-Kundalić et al., 2009)*. According to the latest taxonomy, there are about 18 species and an additional 11 hybrids, and its taxonomy is complicated because of the frequent hybridizations as reported by *(Tar et al., 2013; Šarić-Kundalić et al., 2009).* It has been suggested that the five basic species of mint are; *Mentha arvensis* L. (corn mint), *Mentha aquatica* L. (water mint), *Mentha spicata* L. (spearmint), *Mentha longifolia* (L.) Huds. (horse mint) and *Mentha suaveolens* Ehrh. (apple mint) which have given rise to hybrids e.g. *Mentha* x *piperita* (peppermint), a cross of water mint and spearmint, and many others *(Šarić-Kundalić et al., 2009)*. Among the *Mentha* species, peppermint, spearmint, wild mint, curled mint, American mint, bergamot, Korean mint are common *(Park et al., 2016).*
The colour of mint leaves varies from dark green and grey-green to purple, blue, and sometimes pale yellow *(Abbaszadeh et al., 2009)*. Mint is used in the various industries like; pharmaceutical, tobacco, confectionary, food, perfumery and cosmetology, and the plant provides relief from common cold, fever, flu, and indigestion *(Park et al., 2016).* It is commonly used in the treatment of throat irritation, mouth and sore throat, bronchitis and in treatments for minor aches and sprains *(Al-Bayati, 2009)*. It is used to enhance flavour in toothpastes, chewing gums and beverages *(Park et al., 2016).* Menthol is one of the chemical compounds found in the essential oils of mint that gives mint its aroma and cooling sensation. Menthol is also effective against dental bacteria and also has antimicrobial and antifungal effects against ringworm, Escherichia coli, Salmonella and Aspergillus *(Mikaili et al., 2013)*. Recently, *Abbaszadeh et al. (2009)* reported that mint leaves can be used to treat stomach ache, chest pain, and obesity in antiquity, and powdered mint leaves were used to whiten teeth. This is seen even nowadays as some toothpaste made with mint are said to whiten teeth.

### 1.4.4 Chives

Chive *(Allium schoenoprasum* L.*)* is an herbaceous perennial monocot plant and a member of the Amaryllidaceae family. Other members of the genus *Allium* are onions, garlics, leeks and shallots. Chives are aromatic herbs that have both culinary and medicinal uses. They have mild onion flavour, are rich in vitamin A and C, and contain minerals such as iron and calcium. They are used in food as a condiment that provides a milder flavour than other Allium species. The medical properties of chives are like those of garlic, but weaker. They are used to lower blood pressure, relieve sunburn and sore throat pain, and as antimicrobial and antifungal agents *(Parvu et al., 2014)*. The flavour of *Allium* species is attributed to the sulphur-containing compounds in the leaves *(Nicastro et al., 2015)*. In a recent study, it was reported that chives have beneficial effects on the circulatory system, and it has antimicrobial activities against fungi and food-borne pathogens *(Rattanachaikunsopon et al., 2008)*. This same study reported that the health promoting effects and antimicrobial activity of chives are due to the presence of sulphur-containing compounds, specifically diallyl sulfides.

## 1.5 Technology and analyses

The analyses for this study were carried out using a derivatisation technique in combination with gas chromatography-mass spectrometry (GC-MS) based metabolite profiling and the solid phase microextraction technique (SPME) for detection of volatiles in the herb species.

### 1.5.1 Gas chromatography - mass spectrometry based metabolite profiling

GC-MS is a method for qualitative and quantitative detection of metabolites in different sample matrices, including plants. The technique is very fast, specific and highly sensitive. Derivatisation is a step prior to GC-MS, and is the process of chemically modifying compounds to produce new compounds which have properties that are suitable for gas chromatography analysis. The derivatisation process requires a variety of reagents amongst which, the main ones are BSA (N,O-bis (trimethylsilyl)acetamide), MSTFA (N-methyl-N-(trimethylsilyl)tri fluoroacetamide, and BSTFA (N,O-bis (trimethylsilyl) trifluoroacetamide.

Compound derivatisation is either based on silylation, alkylation or acylation reactions *(Rohloff, 2015)*. GC-MS reduces polarity and increases volatility, and thermal stability of metabolites. Gas chromatography separates components in a mixture in the gas phase, while mass spectrometry characterises the separated compounds individually. GC-MS analysis is therefore limited to the metabolites that are or can be made volatile. In a very recent study, *Rohloff (2015)* showed that hundreds of metabolites from different chemical groups can be distinguished in one analytical run after the solvent extraction and the derivatisation step. GC-MS studies are often carried out using the flame ionization detector (FID), but most recent GC-based metabolite profiling studies utilise MS electron impact ionization. *Kopka (2006)* in their study explain that the GC-MS technique can be carried out in six steps; (1) extraction of the metabolites from the plant sample, (2) derivatisation of the metabolites, (3) separation by gas chromatography which is controlled by gas flow, temperature and capillary column, (4) ionisation of the compounds as they elute from the gas chromatograph, (5) mass

separation and detection of fragment ions, and (6) evaluation of the GC-MS file using software.

### 1.5.2 Solid phase microextraction technique

Volatile extraction of the herbs was done by SPME method. In other words, SPME is used in the profiling of volatile compounds (flavour compound analysis). Compared with other conventional methods like steam distillation, which is more time consuming, it is an alternative method to extract essential oil volatiles from plants with the purpose of chromatographic analysis. It is solventless, and the apparatus for extraction is very simple and fast as it combines extraction and concentration of the analytes in one step. It involves the use of a thin fused silica coated fibre in a thin polymer film. It has an extracting phase that extracts the analytes which is then injected into the GC injection port. The choice of the SPME fibre is very important and depends on polarity and volatility of the analytes. SPME technique is highly used in the food industry, quality control, and essential oil analysis, e.g. aroma profiles of basil *(Klimankova et al., 2008)*.

## 1.6 Aim of study

Since antiquity, man has always used herbs as remedy for diseases, and nowadays vegetable consumption has also increased due to their health benefits and their therapeutic properties. The aim of this study was to assess the effects of light and temperature on metabolites in the selected herbs and greens mentioned above, affecting flavour and health-beneficial compounds in the plants and identifying those compounds that are relevant to human health and flavour of the plant. This study will enable us to assess the growth conditions which can affect flavour and health-related compounds.

Temperature and light conditions can independently or in combination affect plant phytochemicals, and this effect varies from species to species. Nevertheless, a combined effect can also influence the composition of phytochemicals in plants in a separate way. These conditions can be easily controlled by growers in an artificial (controlled) environment.

Therefore, since this study is carried out in growth chambers, we can hypothesise that:

- ✓ An increase or a decrease in the length of photoperiod will affect metabolite concentrations
- ✓ High or low temperature, will strongly influence the production of metabolites and
- ✓ A combination of both conditions will affect the metabolites and the composition of essential oils in the plants differently

We intend to verify the effects of photoperiod and temperature levels on the phytochemicals that are flavour and health-related. The results achieved shall serve as guide to growers, to choose the appropriate growth conditions for a particular plant depending on what is desired, since the production of these phytochemicals are very depended on environmental factors.

# 2. MATERIALS AND METHODS

## 2.1. Plant material

Eight different seed types were purchased from Sweden (Impecta Fröhandel, Julita, Sweden), four species of herbs: basil *(Ocimum basillicum),* chives *(Allium schoenoprasum),* coriander *(Coriandrum sativum)*, mint *(Mentha longifolia)*; and four species of greens: lettuce *(Lactura sativa*), beet greens *(Beta vulgaris),* wheatgrass *(Triticum aestivum)* and peppercress *(Lepidium sativum).*

## 2.2. Sowing and germination

The seeds were sown in small pots of 5 x 5 cm in a greenhouse at 20˚C, and about 200 μmol m-2 s-1. The amount of sowing varied from species to species depending on density and seed size. The soil used was a homogenous mixture of vermiculite and ecological soil. Watering was done once a day for the first three days and subsequently when necessary. There was a general delay in germination and the rate of germination was different for all the species. About 14 days later, all the seeds had germinated, and were transferred to growth chambers where growth continued.

## 2.3. Experimental set-up

### 2.3.1. Plants grown under different light and temperature conditions

The plants were grown in two chambers (Percival E36L2, CLF PlantClimatics GmbH). One chamber was set at a temperature of 15°C (cold), while the other was set at a temperature of 25°C (warm). Both chambers were set to have two light treatments, i.e. 18 h of light at the top level and 12 h of light at the bottom level. All the 8 species were grown in both chambers and their positions were randomized. There were 40 replicates for each species, i.e. 20 replicates for each temperature treatment with 10 replicates for each light treatment summing up to 320 pots in total.

### 2.3.2. Preparation of extracted solvents and chemicals used

All the solvents, reagents and consumables needed for extraction, were prepared a day before the sampling. Chemicals used were: ethanol, ribitol, methoxyamine hydrochloride, pyridine, liquid nitrogen and MSTFA. A pyridine mixture (i.e. pyridine + methoxyamine hydrochloride) was prepared and stored at 4°C. A mixture of ethanol and distilled water (solvent mixture) with added ribitol at a concentration of 40μg/ml, was also prepared in the ratio 88ml: 22ml (8800 μl : 2200 μl), respectively. All these mixtures were prepared under a hood, according to the number of samples. Round bottomed 2 ml Eppendorf tubes, glass vials with inserts (for derivatized samples) were labelled accordingly and lids with holes also prepared in advance.

## 2.4. Sampling of plant tissue and extraction

Harvesting was done at the end of each growth period, i.e. when the plant was big enough for sampling. Due to delayed germination in most of the plants, there was a delay in growth and the plants didn't do well as expected. Normally all the plants ought to have been harvested between 3-5 weeks after planting. But during this period, some of the plants leaves turned yellow and there was the presence of insects, mould and fungi. So, to improve the germination and growth conditions, liquid fertilizer was added to the plants once a week until they were big enough for sampling. The application of the fertilizer improved the colour of the leaves to an extent, but not completely. Due to the above shortcomings, we didn't have the same number of plant samples for all species as planned and despite the use of fertilizer, growth was also not properly enhanced for all the species. Therefore, harvesting was done twice at separate times. The first group of plants, i.e. all the herbs and greens in the hot chamber were harvested at 7 weeks after planting. The second group of plants, i.e. all the plants from the cold chamber were harvested at 12 weeks after planting.

Sampling was not done randomly, meaning that it was done in a way that those from the left were distinguished from those on the right in the chamber. The idea was simply to check if the light distribution was even in the chamber, and also to verify if they'll be any difference or not in the results of samples collected on the different sides. We decided to reduce the number of samples, by sampling 2,5 replicates (pots) of each species into 1 centrifuge tube. Therefore, 40 replicates/ 2.5. So, for each species, we collected 16 samples in total. The growth problems explained above, affected the herbs mostly, so the number of samples had to be reduced for herbs species (chives and coriander). No specific measurement of plants fresh weight was carried out because we needed as much as possible of tissue for chemical analyses. The leaves for both herbs and greens were harvested, weighted and transferred into 50 ml centrifuge tubes and stored immediately at -80°C prior to the final sampling into 1.5 ml Eppendorf tubes for extraction which was carried out later. Transferring the plant tissue into the centrifuge tubes was a fast process because once the leaves are cut, enzymes break them down easily. Fresh weight ranged from 0.5 to 9.8 g. All the greens from both chambers were sampled into the centrifuge tubes, but as for the herbs, only half were transferred into those tubes, while the rest were put in small paper envelopes and immediately placed in a drying oven at 30°C for drying. Drying was done for approximately 3 days. The purpose of drying part of the herbs was to extract essential oils (volatiles compounds) from the herbs. Below is a table showing the total number of samples:

**Table 1a and 1b:** Total number of fresh samples in tubes at both temperatures, and number of herbs samples dried in envelopes for the extraction of essential oils respectively.

**Table 1a.**

| Species | Number of tubes | Cold temp 15°C | Warm temp 25°C |
|---|---|---|---|
| Peppercress | 16 | 8 | 8 |
| Lettuce | 16 | 8 | 8 |
| Wheatgrass | 16 | 8 | 8 |
| Beet greens | 16 | 8 | 8 |
| Mint | 8 | 4 | 4 |
| Basil | 8 | 3 | 4 |
| Chives | 4 | 2 | 2 |
| Coriander | 4 | 2 | 2 |
| **Total** | **87** | **43** | **44** |

**Table 1b.**

| Herbs for drying | Number of envelopes | Cold temp 15°C | Warm temp 25°C |
|---|---|---|---|
| Mint | 8 | 4 | 4 |
| Basil | 8 | 3 | 4 |
| Chives | 4 | 2 | 2 |
| Coriander | 4 | 2 | 2 |
| **Total** | **23** | **11** | **12** |

During sampling (for extraction) of the greens and part of the herbs, the samples were kept on liquid nitrogen to keep them frozen. The plant tissues were crushed in the 50 ml centrifuge tubes, and 200 mg of each tissue was sampled in 1.5 ml Eppendorf tubes and stored at -20°C. 1500 µL EtOH:$H_2O$ in the ratio 2.5:1 with internal standard (40 µg ribitol/mL solvent mixture) was added to the sample and vortexed for 10 s. At this point, most enzymatic activity will stop. The tubes were then treated for 60 min at about 50°C in an ultrasonic bath, and then cooled to room temperature before centrifugation. Centrifugation was done at 13,000 rpm for 10 min. 750 µL of the aliquots from the clear supernatant (the polar phase) were transferred into new 1.5 mL round-bottomed Eppendorf tubes. Extra lids with 5 holes each were used to close the tubes. The samples were dried in a SpeedVac overnight, without heating, then were stored at -80°C before further processing prior to derivatization and GC-MS analysis. The samples were dried because derivatisation is not effective with wet substances and ethanol.

### 2.4.1. SPME analysis

Volatile extraction was done by the solid phase micro-extraction method (SPME). 50 mg of dried herbs was transferred into 1.5 ml glass. A syringe was used to make a hole in the septa of the glass vial containing the samples. A 200 ml beaker of water was placed on an agitation plate, and a thermometer was set to measure the temperature in the range when the glass

vial was put half way in the beaker for heating. The SPME fibre was carefully inserted through the hole on the septa half way into the glass vial without touching the sample. It was then incubated in the 200 ml beaker of water at a stable temperature of 50°C, with an agitation of about 750 rpm. The fibre (65 µm PDMS/DVB coated fibre; Supelco) was exposed to the sample for 40 min. The length of extraction is very important because it allows enough time for all the volatiles to be absorbed on the fibre. After this time, the fibre was removed from the sample and the analytes immediately injected into the GC inlet. Injection lasted for 3 min and then the fibre was removed. Within this time, the analytes were eluted by the mobile phase while the complete analysis lasted for 40 min. Semi-quantitative analyses of detected volatiles are based on calculated peak area of the mass spectrometer detector response (arbitrary units). Presented values in figures and tables were adjusted as follows- basil, coriander and mint: detector response $10^{-6}$, chives: detector response $10^{-5}$.

### 2.4.2. Compound derivatization

The dried residues (from the fresh samples) stored at -80°C, were redissolved in 80 µl of 20 mg/ml methoxyamine hydrochloride in pyridine and briefly vortexed. Derivatization was done at 30°C for 30 min in an incubator. The samples were transferred into an ultrasonic bath for 30 min at 30°C, in order to dissolve non-dissolved residues. Samples were then transferred back to the incubator for another 30 minutes. The total time for derivatization was 90 min. Finally, the samples were treated with 80 µl of MSTFA (N-methyl-N-(trimethylsilyl) trifluoroacetamide), vortexed for a few seconds then incubated at 37°C for 30 min. The samples were then transferred to 1.5 ml autosampler vials with glass inserts, and stored at -20°C prior to GC-MS.

## 2.5. Gas chromatography coupled with mass spectroscopy

The separation of samples (SPME and solvent extracts) was performed on an Agilent 6890/5975 GC/MS (Agilent Technologies, Palo Alto, CA) equipped with a HP-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm) (Agilent Technologies, Palo Alto, CA).

SPME: Injection and interface temperature were set to 220°C and 220°C, respectively. The GC temperature program was ramped from 40 to 211°C at 4.5°C/min, and further ramped to 220°C at 50°C/min (run time: 40 min). The MS source was adjusted to 230°C and a mass range of *m/z* 35–350 was recorded (EI mode).

Solvent extracts: Sample volumes of 2 µl were injected with a split ratio of 15:1. The injection and interface temperature were set to 230°C and 250°C, respectively. The GC temperature program was held isothermically at 70°C for 5 min, ramped from 70 to 310°C at 5°C/min, and finally held at 310°C for 7 min (run time: 60 min). The MS source was adjusted to 230°C and a mass range of *m/z* 70–700 was recorded (EI mode).

## 2.6. Statistical analyses

The data was aligned and processed using the MetAlign software (Rikilt, Wageningen, NL), meanwhile the compounds were identified using MS libraries, such as NIST/EPA/NIH MassSpectralLibrary NIST05 (National Institute of Standards and Technology, Gaithersburgh, MD), the Golm Metabolome Database containing MS spectra of derivatized metabolites *(Hummel et al., 2010),* in combination with an in-house retention index library of trimethylsilylated (TMS) metabolites. The GC-MS data was interpreted using the Automated Mass Spectral Deconvolution and Identification System (AMDIS; National Institute of Standards and Technology, Boulder, CO) software. BioStatFlow an online tool (using a two-factor csv script), and the MultiExperiment viewer software (using a two-factor txt script) were used to perform a two-way analysis of variance (ANOVA) where the P-value threshold was 0.05. All figures and standard errors were obtained using Microsoft Excel.

Multivariate analyses for the comparison of these metabolites were performed on the whole data set, and on 17 aromatics pre-selected from the whole data set using principal component analysis (PCA). This was done using Minitab software to visualise the score and loading plots across the metabolites and species.

To eliminate species variation, the logarithm ratio for each value based on the median was calculated to obtain the score and loading plots, i.e. for the 17 aromatics, data points from the GC-MS were divided by the median of the samples for each metabolite and then changing the data to a logarithm scale with base 2 using Microsoft Excel. For the whole data set, the logarithm ratio to median values was also calculated, but on one part, the median was calculated from single metabolite species-wise (species variation eliminated) and on the other part, the median was calculated from single metabolites across all the species (species variation included). Total phenolic level was calculated by calculating the sum of all phenolics in each species under the different treatments using Microsoft Excel.

# 3. RESULTS

For better understanding and clarity, the results for this study were presented separately according to the techniques used. Therefore, I separately presented results from the derivatisation technique, i.e. for the 8 species and those from the SPME technique, i.e. for the 4 herbs. I further presented results by species because the focus here was not to compare the species. The general idea was, if light (photoperiod) or temperature increased the concentration of a compound known to affect the flavour of the plant, or was health related, then the compound will be selected because my focus was on flavour and health related compounds. Compounds that showed a significant difference in the treatments were also included.

The selection was not as easy as expected because compounds with significant values varied from treatment to treatment and under the interaction between the treatments. Also, some compounds that were present in high concentrations and known to have health beneficial effects did not have significant P-values. Narrowing down everything to fit according to my plan was quite challenging. In this case I had no specific criteria of selection, but the compounds presented were those that are flavour and health related.

Therefore, with the SPME technique, given that all the compounds are volatiles, I selected those compounds found in the highest levels and those that were commonly found in previous studies and generally expected. Meanwhile with the derivatisation technique, the selected compounds were generally those related to metabolism, and how they changed or were affected under the different growth conditions. The aromatic amino acids are precursors of secondary metabolites, and were included together with detected phenolic compounds. Nevertheless, the results are presented as simply as possible for the most important compounds that are health and flavour related. The Multiexperiment Viewer software was used to calculate the P-values, and the cut-off value for a significant effect was 0.05. The constraint with the above software was that, it could only analyse even number of samples, so I used the online BioStatFlow software to compensate for this, since it analysed data irrespective of this factor.

For the herbs, the detected volatiles were based on calculated peak area of the MS detector response. The complete tables of all the identified volatiles from mint, chives, basil and coriander together with their P-values are presented in Table A1 to A4 in the appendices. For those species with incomplete samples like chives and coriander, the software could not calculate their interaction effect as seen in the appendices.

For all the figures presented below, **cold** represents temperature treatment at 15°C while **warm** represents temperature treatment at 25°C.

**LD** and **SD** are long and short day length respectively. For this study, long day length was 18 h, while short day length was 12 h.

## 3.1. SPME

### 3.1.1. Mint

SPME detected 30 volatile compounds in mint; 1 alcohol, 1 aldehyde, 1 aliphatic ester, 1 aromatic, 18 monoterpenes and 8 sesquiterpenes.

Out of the 30 volatile compounds from mint, only 5 monoterpenes (sabinene, 1,8-cineole, isopiperitenone, menthyl acetate, piperitenone oxide) and 5 sesquiterpenes (beta-copaene, (E)-muurola-3,5-diene, germacrene D, beta-himachalene, calamenene) were significantly different ($P<0.05$) under temperature, indicating that temperature affected about 1/3 of the volatile compounds in mint species. Compounds like limonene, carvone and beta-myrcene were present at very high levels, but they showed no significant effect as their P-values were above the 0.05 threshold. Piperitenone oxide showed the highest level amongst the monoterpenes while (E)-muurola-3,5-diene had the highest level amongst the sesquiterpenes. On the other hand, day length had little or no significant effect on the volatiles as it affected only one compound, nonanal (an aldehyde), while there was no significant effect under the interactions. Piperitenone oxide showed the highest level among all the volatiles in mint. Linalool was also commonly found in mint but was present at very low levels.

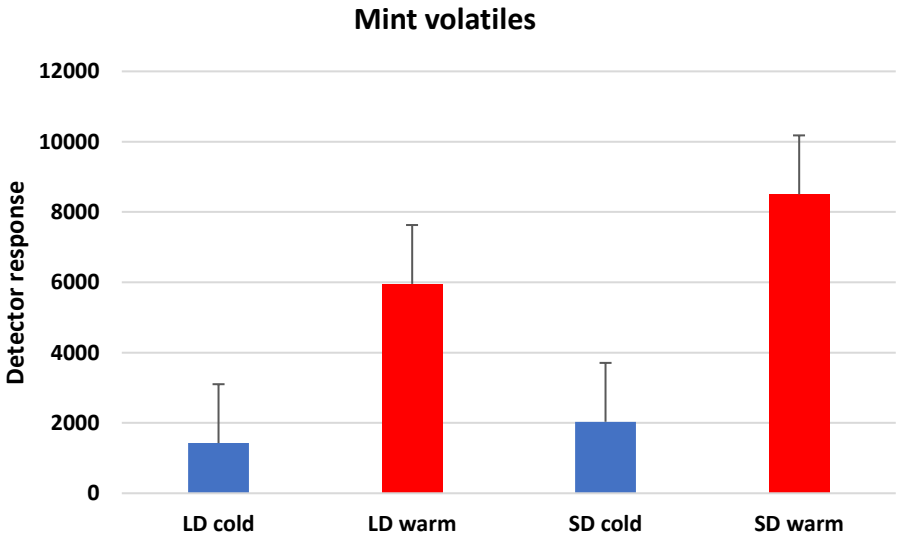


**Fig. 3.1:** Bar chart showing the effect of day length and temperature on the total sum of 30 volatiles detected in mint. Each bar represents mean values of all samples under that treatment. The error bars show the standard error for the whole experiment.

Temperature had a significant effect on mint volatiles. Species grown under warm temperature yielded higher levels of the volatiles than those grown under cold temperature. Short day length under warm temperature yielded the highest levels of the volatiles (Figure 3.1).

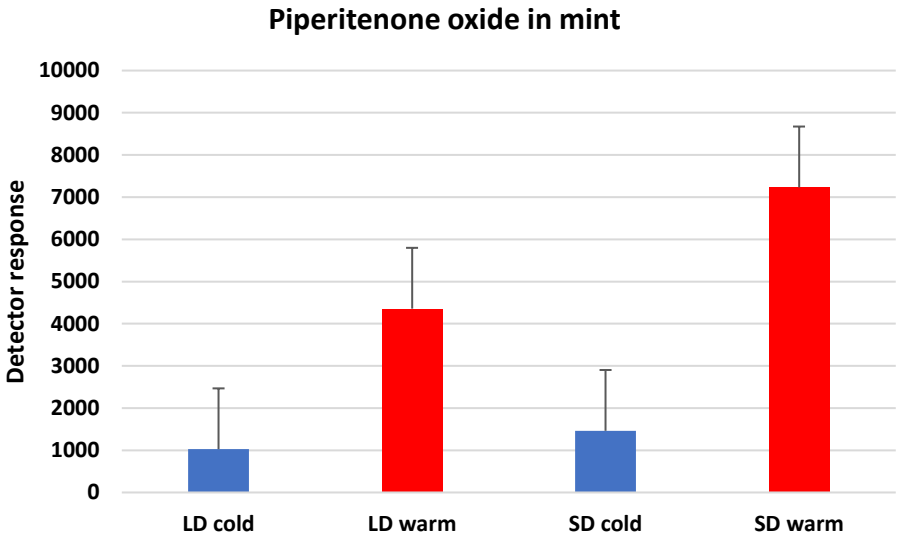


**Fig 3.2:** Effect of day length and temperature on the level of piperitenone oxide in mint. Each bar represents mean values of all samples under that treatment. The error bars show the standard error for the whole experiment.

Temperature and day length had a profound influence on the level of piperitenone oxide. Warm temperature and short day length favoured higher levels of the volatile than cold temperature. (Figure 3.2).

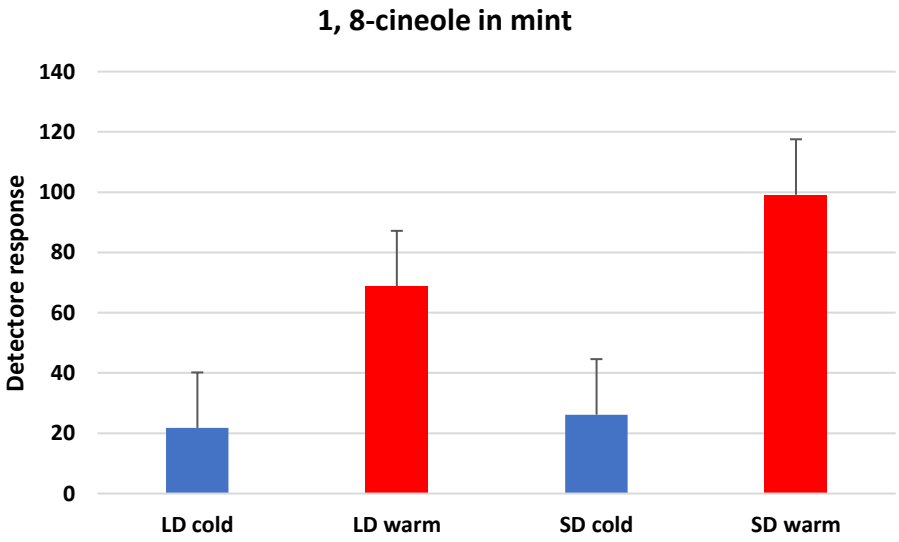


**Fig 3.3:** Day length and temperature effect on the level of 1,8-cineole in mint. Each bar represents mean values of all samples under that treatment. The error bars show the standard error of the whole experiment.

Warm temperature generally yielded about 3 times the levels of 1,8-cineole compared to cold temperatures under both day lengths. Warm temperature and short day length yielded higher levels of this volatile (Figure 3.3).

**Table 3.1:** Mint species under cold temperature at long day (LD) and short day light(SD). CHR1 means cold temperature, high light (LD) and R1 and R2 mean sample harvested at the right side of the chamber under those conditions but divided into 2 portions. CLR1 means cold temperature, low light (SD) and R1 and R2 same as above.

| **Species**<br>Light/Temp<br>**Compound** | Mint<br>LD cold<br>**M-CHR1** | Mint<br>LD cold<br>**M-CHR2** | Mint<br>SD cold<br>**M-CLR1** | Mint<br>SD cold<br>**M-CLR2** |
|---|---|---|---|---|
| Carvone | 60.05 | 23.81 | 57.87 | 175.63 |
| Limonene | 225.08 | 67.80 | 83.22 | 278.94 |
| Beta-myrcene | 139.88 | 60.68 | 51.86 | 249.24 |

The main reason for presenting Table 3.1, was to show that there was a general discrepancy in the values of the sample harvested at the same side of the chamber. For example, a sample harvested under long day from the right side of the chamber and divided into 2 portions showed a great disparity in their volatile levels as can be seen in the table above with carvone, limonene, and beta-myrcene. Many other volatiles showed the same discrepancy, and this disparity was a general trend in this species even with the warm treatment.
It is important to note that this discrepancy was also seen in the other species (chives, coriander and basil) with even larger differences between the values.

### 3.1.2. Chives

The SPME method detected 35 volatile compounds in chives, including 3 acids, 5 alcohols, 9 aldehydes, 1 aliphatic ester, 4 aromatics, 5 ketones and 7 sulfides. Out of the 35 compounds, 6 compounds showed a significant effect under temperature, i.e. pentanal, hexanal, methyl-1-propenyl disulfide, dimethyl trisulfide, benzyl alcohol, and methyl 2-propenyl trisulfide. Two compounds showed a significant effect under the light treatment; pentanal and hexanal. Only two compounds showed a significant effect in both treatments and no compound showed an interaction. In most cases, pinpointing compounds with the highest levels was not easy because the values fluctuated from treatment to treatment and even under the same treatment (as shown in Table 3.1). Nevertheless, the most abundant compounds in chives were hexanal, decanal, pentanal, (E)-2-hexenal, 3-methylbutanal, 2-phenylethyl alcohol and acetic acid. Amongst the sulfides, dimethyl sulfide volatile was detected at highest levels.

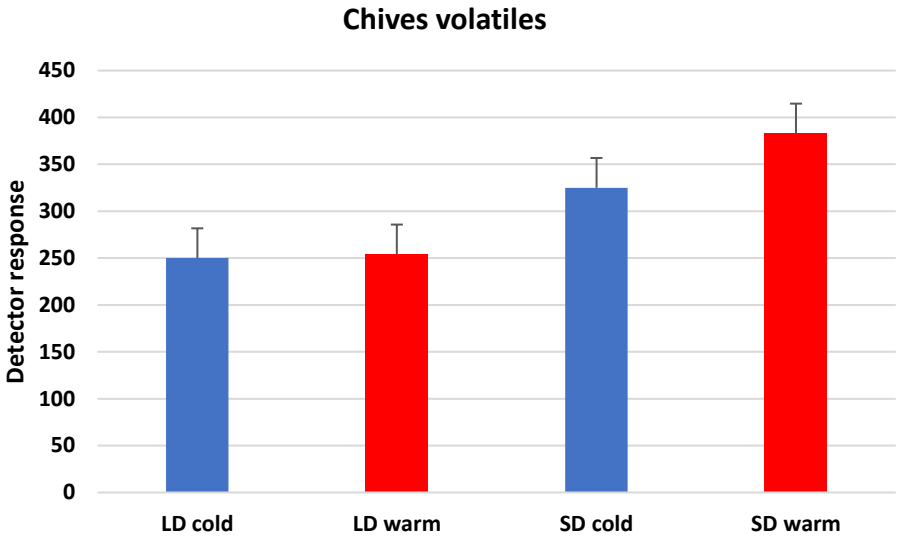


**Fig. 3.4:** Day length and temperature effect on the total sum of 35 volatiles detected in chives. The error bars show the standard error for the whole experiment.

Long day lengths generally had no significant effect on the volatile levels in chives under both temperatures, but volatiles showed highest levels under warm temperature and short day length (Figure 3.4).

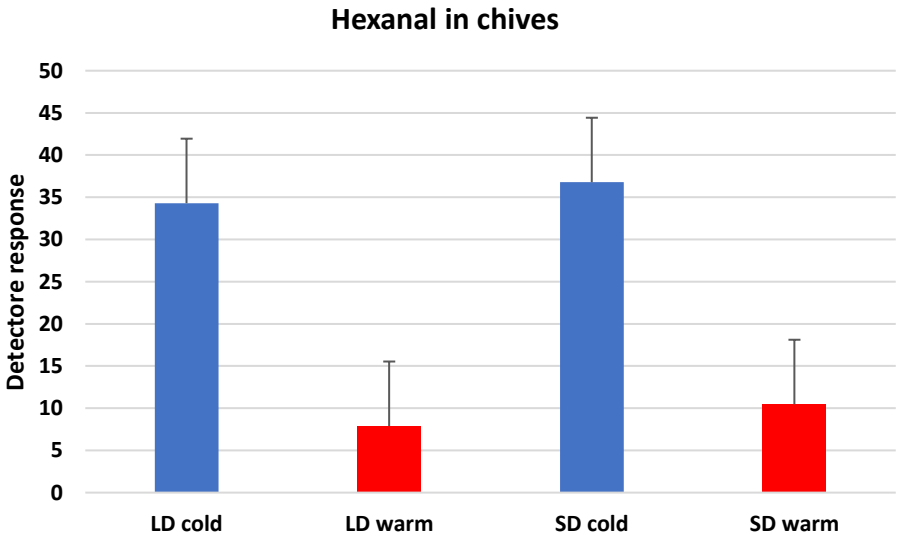


**Fig. 3.5:** Day length and temperature effect on hexanal in chives. The error bars show the standard error for the whole experiment.

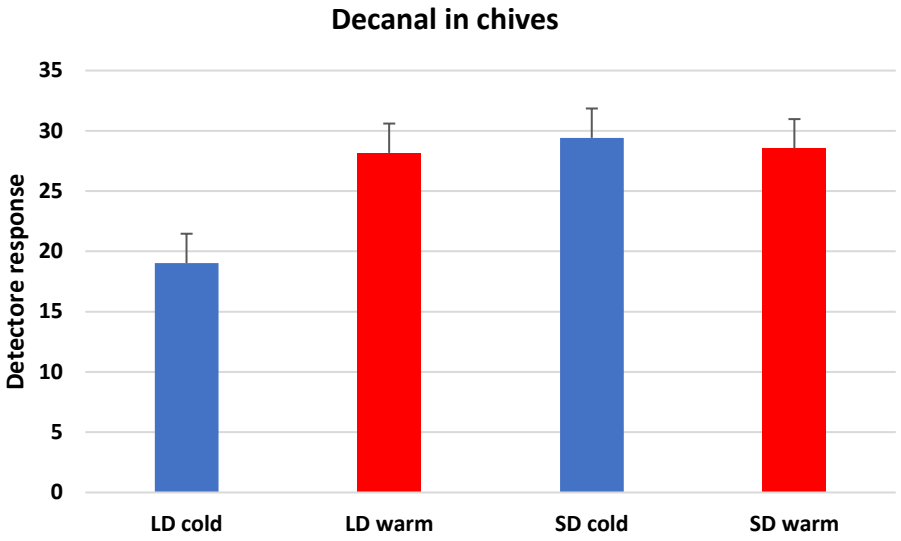


**Fig. 3.6:** Day length and temperature effect on decanal in chives. The error bars show the standard error for the whole experiment.

Figure 3.5 and 3.6 showed how the effect of day length and temperature varied between volatile compounds. Cold temperature greatly favoured considerable amounts of hexanal. Hexanal was higher under short day light at cold temperature and just slightly higher than those under long day light. Unlike hexanal, decanal was slightly influenced by temperature.

### 3.1.3. Coriander

29 volatile compounds were detected in coriander, i.e. 1 aldehyde, 4 aromatics, 14 monoterpenes and 10 sesquiterpenes. Out of these volatiles, none showed a significant effect under the treatments. Decanal, dodecanal, (E)-2-dodecenal and (Z)-9-tetradecenal were coriander volatiles with the highest levels.

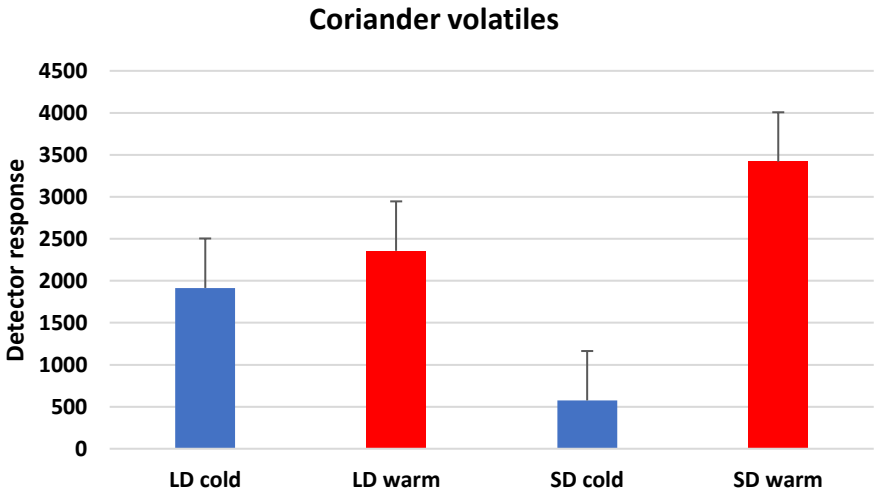


**Fig. 3.7:** Day length and temperature effect on the total sum of 29 volatiles detected in coriander. The error bars show the standard error for the whole experiment.

Short day length and warm temperature generally yielded more volatiles in coriander, but short day length and cold temperature reduced the level of the volatiles. On the other hand, at cold temperatures, volatile levels were higher under long day length (Figure 3.7). Temperature had a significant effect on the production of volatiles in coriander.

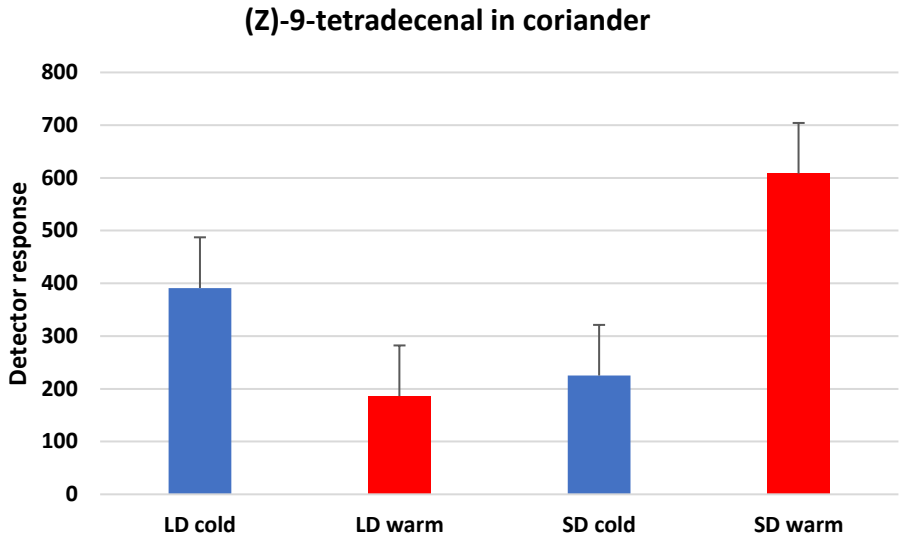


**Fig. 3.8:** Day length and temperature effect on (Z)-9-tetratdecenal in coriander. The error bars show the standard error for the whole experiment.

The higher the temperature, the higher the level of (Z)-9-tetratdecenal under short day length. But under cold temperatures and short day length, the level of the compound dropped and increased when day length was prolonged. Hence showing a significant interaction as we see an opposite effect of temperature depending on day length (Figure 3.8).

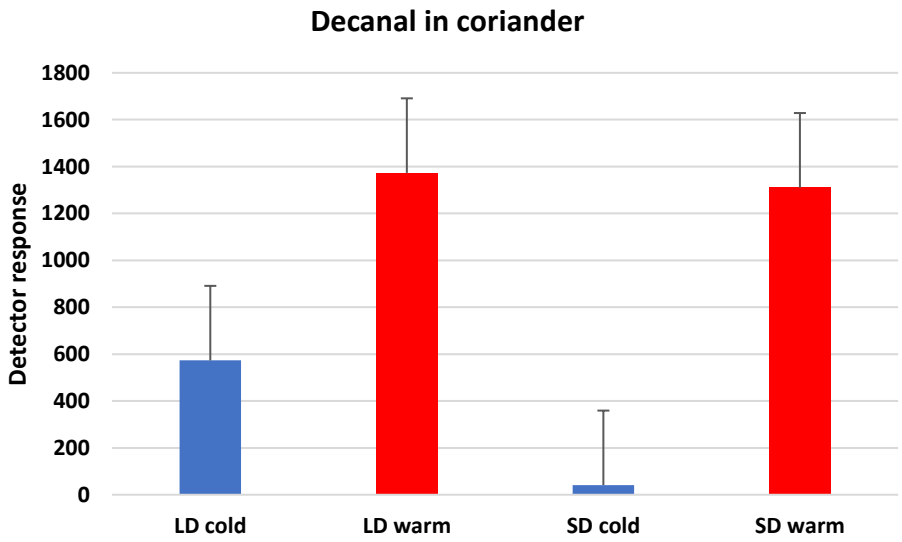


**Fig. 3.9:** Day length and temperature effect on decanal in coriander. The error bars show the standard error for the whole experiment.

Warm temperature yielded higher levels of decanal than cold temperatures. When day length was reduced under warm temperatures, its level dropped slightly. Cold temperatures drastically reduced the level of this compound especially under short day length (Figure 3.9).

### 3.1.4. Basil

29 volatile compounds were detected in basil, i.e. 1 aldehyde, 4 aromatics, 14 monoterpenes and 10 sesquiterpenes. Out of these volatiles, only (E)-2-hexenal was significantly affected by light, temperature and their interaction. Estragole, (E)-β-farnesene, α-bergamotene, eugenol, 1,8-cineole, and germacrene D, were volatiles with the highest levels in basil, while other common volatiles like (E)-beta-ocimene, beta-pinene and linalool were present in lower levels.

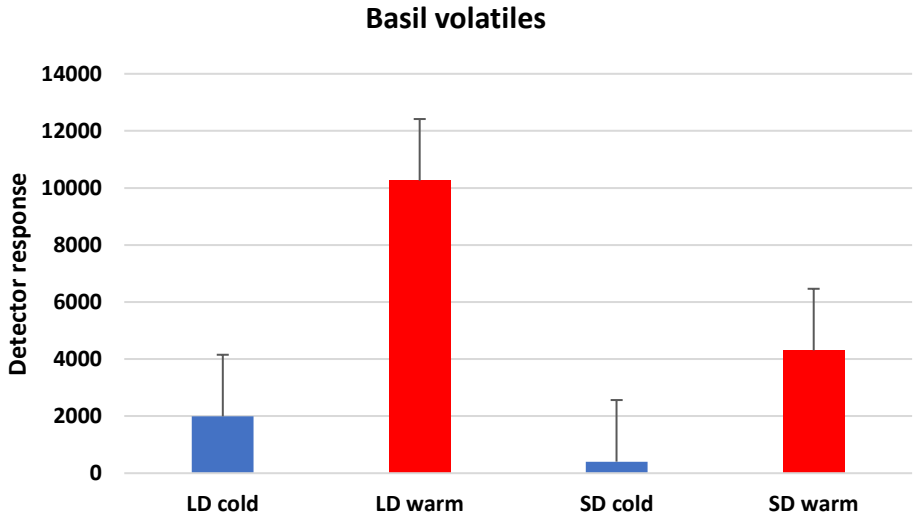


**Fig.3.10:** Day length and temperature effect on the total sum of 29 volatiles detected in basil. Each bar represents mean values of all samples under that treatment. The error bars show the standard error for the whole experiment.

Temperature had a profound influence on the production of basil volatiles. An increase in temperature greatly increased the level of the volatile and long day length increased the levels of the volatiles. Volatile level decreased with decrease in day length under both temperatures (Figure 3.10).

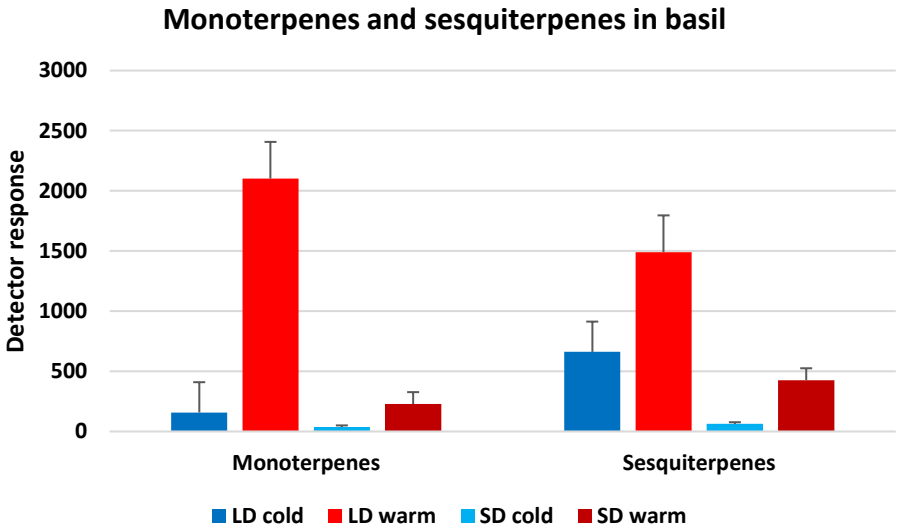


**Fig.3.11:** Effect of day length and temperature on monoterpenes and sesquiterpenes in basil. Each bar represents mean values of all samples under that treatment. The error bars show the standard error for the whole experiment.

Monoterpenes were generally present in lower levels than sesquiterpenes, except under long day length and warm temperature where they were higher compared to the sesquiterpenes. Long day length under warm conditions generally favoured higher levels of terpenes. The lowest levels were registered under short day light at cold temperatures. Day length greatly affected volatile level of the terpenes especially with the monoterpenes. Temperature also affected the volatiles but not as strong as day length. (Figure 3.11).

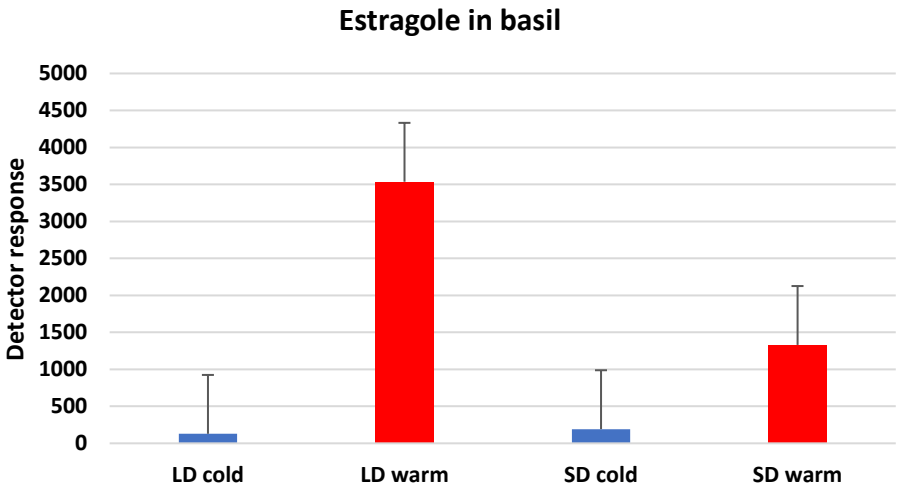


**Fig.3.12:** Effect of day length and temperature on estragole in basil. Each bar represents mean values of all samples under that treatment. The error bars show the standard error for the whole treatment.

Temperature highly influenced estragole in basil, such that warm temperature under long day length increased this volatile to about 2.5 times, while cold temperature yielded very low amounts. Photoperiod had a lower influence on this volatile under warm temperature, and no influence under cold temperature (Figure 3.12).

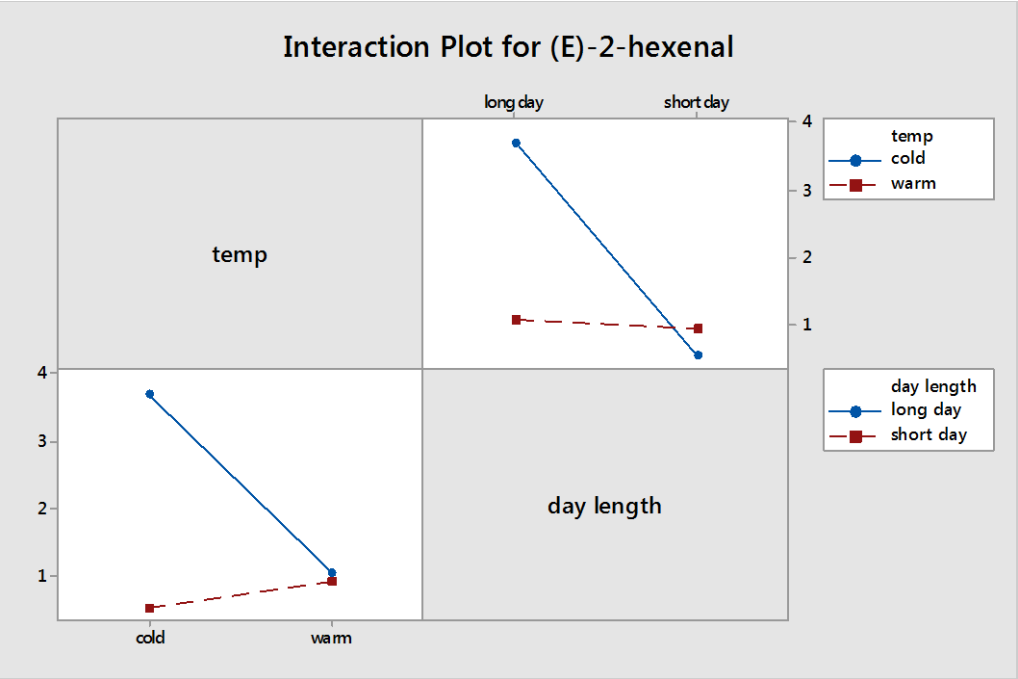


**Fig.3.13:** Interaction plot for (E)-2-hexenal in basil.

The interaction effect for (E)-2-hexenal under day length and temperature showed that cold temperature and long day length had a higher influence on (E)-2-hexenal. The axes represent the detector response, based on peak area (Figure 3.13).

## 3.2. GC-MS metabolite profiling

A total of 208 compounds were identified from the GC-MS metabolite profiling of the species. 131 compounds were identified, 61 compounds were structurally identified, and 17 compounds were not identified. Since this study was mainly focused on the flavour and health related compounds, the list was narrowed down to 38 compounds of interest. It might still seem many, but the idea behind this was also to include important central metabolites such as citric acid and sugars. Since phenolic compounds play vital roles in health and food flavour, they were particularly discussed. Out of the 38 compounds were; 28 aromatics, 2 terpenes, 3 sugars, 3 acids, and 2 aromatic amino acids (precursors of phenolic compounds). Furthermore, 17 compounds were properly identified by name from the 28 aromatics while 11 were unknown. Table 3.2 summarises the above information.

**Table 3.2:** Overview of the 38 compounds selected, for all the species; 17 identified aromatics, 3 sugars, 3 acids, 2 amino acids, 2 terpenes and 11 unidentified aromatics.

| Identified aromatics | Un-identified aromatics | Acids |
|---|---|---|
| Benzoic acid | Phenolic 1 | Shikimic acid |
| Benzyl isothiocyanate | Phenolic 2 | Citric acid |
| p-Coumaric acid | Phenolic 3 | Dehydroascorbic acid dimer |
| Dihydroxyphenylalanine | Phenolic 4 | |
| (E)-Ferulic acid | Phenolic 5 | |

| | | |
|---|---|---|
| (E)-Caffeic acid | Phenolic 6 | **Amino acids** |
| (E)-Sinapic acid | Phenolic 7 | Phenylalanine |
| Rosmarinic acid deriv 1 | Phenolic 8 | Tyrosine |
| Salicylic acid glucopyranoside | Phenolic 9 | |
| Quinic acid deriv 1 | Flavonoid 1 | |
| Luteolin | Flavonoid 2 | **Terpenes** |
| Chlorogenic acid | | Phytol |
| (E)-4-Caffeoylquinic acid | | Ursolic acid |
| Caffeoylquinic acid deriv 1 | **Sugars** | |
| Rosmarinic acid | Sucrose | |
| Rosmarinic acid deriv 2 | Fructose 3 | |
| Caffeoylquinic acid deriv 2 | Glucose 1 | |

Given that the 17 identified aromatics were common to all the 8 species, I simply selected distinct compounds and presented them for each species, and including those with the highest concentrations. Citric acid and sugars (fructose, glucose and sucrose) are generic compounds related to the central metabolism. According to the results, they showed general trends of changes in relation to the photoperiod and temperature treatments across all the species. Hence, they were also presented.

### 3.2.1. Chives

In this species, none of the compounds were significantly affected under both treatments and under interaction. Three distinct compounds were presented for this species; (E)-ferulic acid, (E)-caffeic acid, and dehydroascorbic acid dimer (vitamin C), with citric acid and the sugars. Other important compounds related to health but present in smaller concentrations were; rosmarinic acid, benzoic acid, and chlorogenic acid.

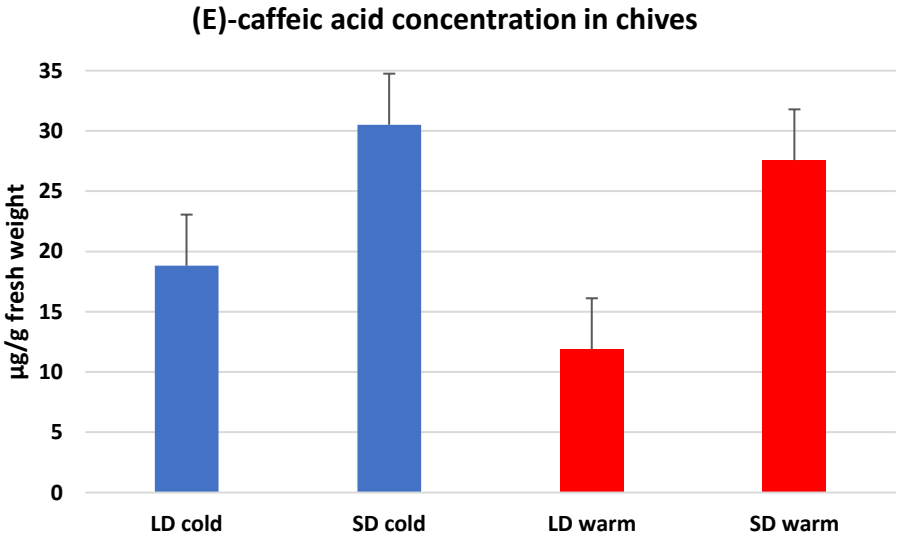


**Fig 3.14.** Effect of day length and temperature on (E)-caffeic acid in chives. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Short day length generally yielded higher concentrations of (E)-caffeic acid under both temperatures but preferably under cold temperature. (Figure 3.14).

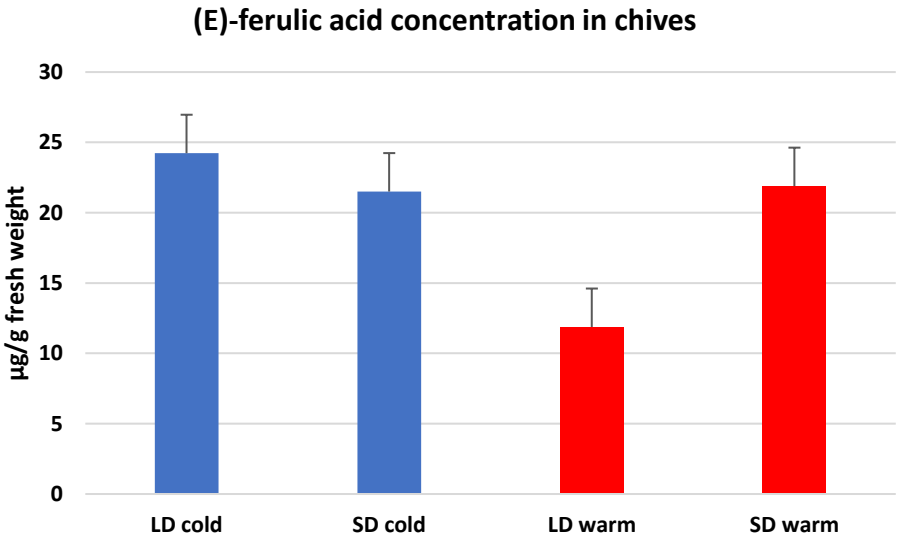


**Fig 3.15.** Effect of day length and temperature on (E)-ferulic acid in chives. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Cold temperature yielded higher concentrations of (E)-ferulic acid than warm temperature. Under warm temperature, short day length was preferable, meanwhile under cold temperature, long day length slightly yielded higher concentrations (Figure 3.15).

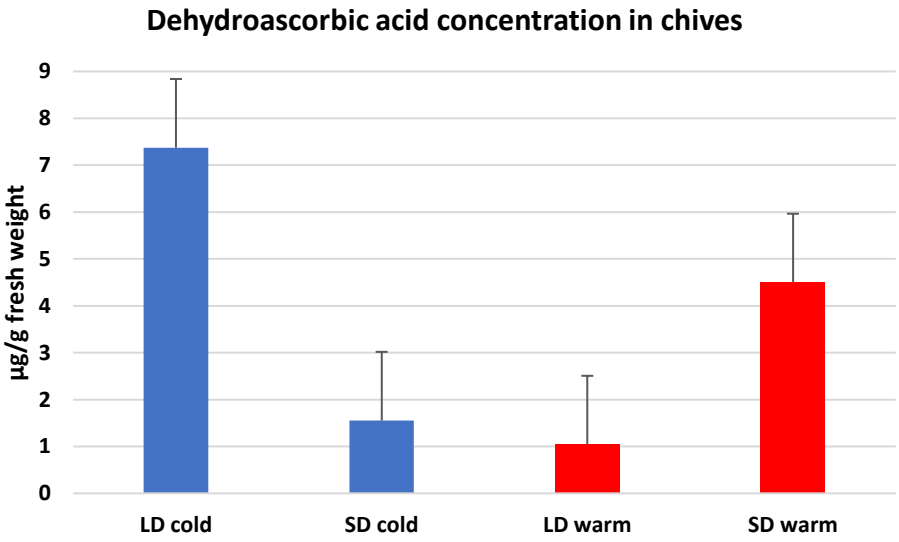


**Fig 3.16.** Effect of day length and temperature on dehydroascorbic acid in chives. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Cold temperature generally favoured higher concentrations of dehydroascorbic acid. Under warm temperature, concentrations under short day length were about 4 times higher. Long

day length under cold temperature was the best condition that yielded higher concentrations of dehydroascorbic acid. This showed a significant interaction as we see an opposite effect of temperature depending on day length (Figure 3.16).

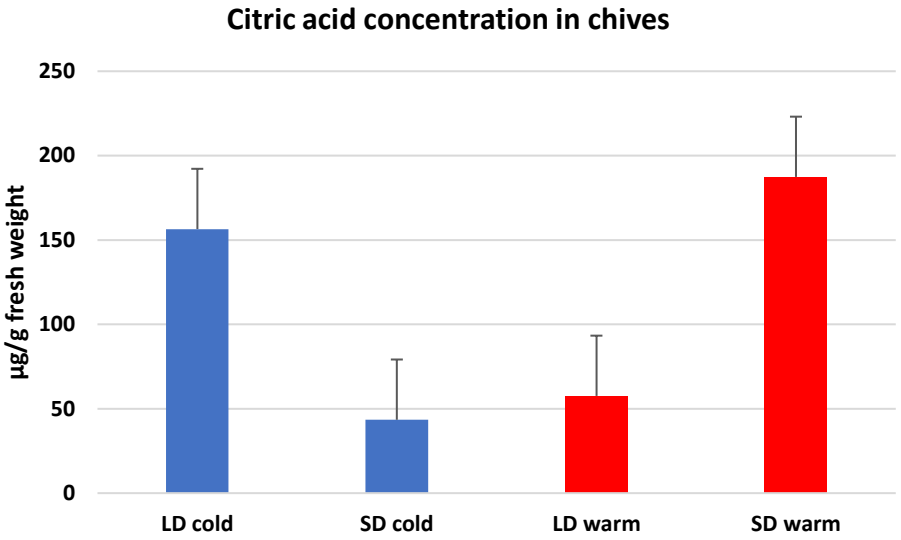


**Fig 3.17.** Effect of day length and temperature on citric acid in chives. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Under cold temperature, long day length favoured the increase of citric acid, and under warm temperature, short day length favoured the production of citric acid. Best condition for citric acid production was short day length under warm temperature (Figure 3.17).

**Table 3.3:** Effect of day length and temperature on fructose, glucose and sucrose in chives. The values are presented in µg/g fresh weight.

| Sugars | LD cold | SD cold | LD warm | SD warm |
|---|---|---|---|---|
| Fructose | 0.78 | 856.86 | 0.57 | 0.75 |
| Glucose | 1122.00 | 497.16 | 749.37 | 1465.29 |
| Sucrose | 3644.87 | 2844.62 | 3160.60 | 3281.38 |

Sucrose was generally produced in far higher amounts than fructose or glucose. Highest levels of sucrose were produced under long day and cold temperature conditions, glucose was highest under short day and warm temperature, while fructose was only produced in reasonable amounts under short day and cold temperature (Table 3.3).

### 3.2.2. Coriander

Generally, no compound showed a significant effect under the treatments except for quinic acid derivative that showed a significant effect under the photoperiod treatment. The distinct compounds for this species were; chlorogenic acid, (E)-caffeic acid, and (E)-ferulic acid, with citric acid and the sugars. Other important compounds but present at lower concentrations were; rosmarinic acid, quinic acid, benzoic acid, and (E)-sinapic acid.

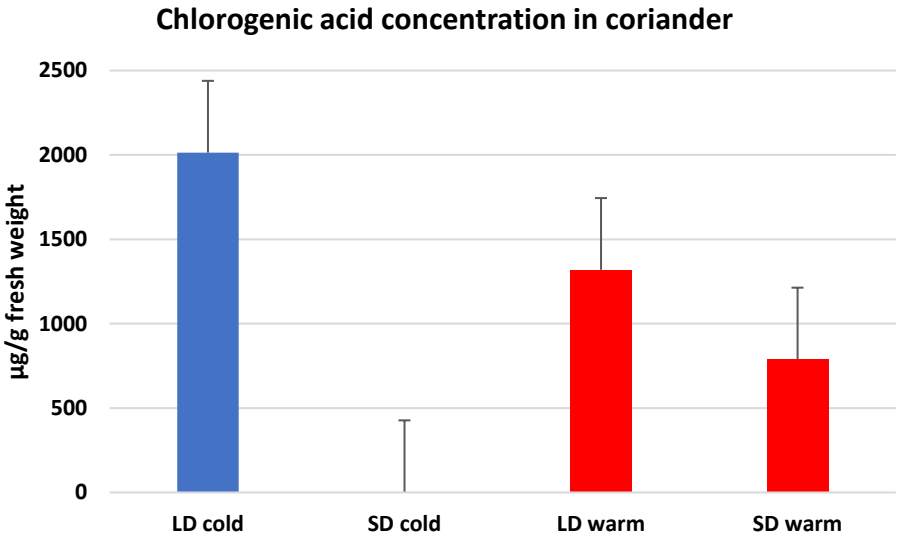


**Fig 3.18.** Effect of day length and temperature on chlorogenic acid in coriander. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Chlorogenic acid was highly produced under long day length and cold temperature. With warm temperature, long day length favoured its production. Therefore, longer day length generally favoured the production of this compound, but preferably under cold temperature (Figure 3.18).

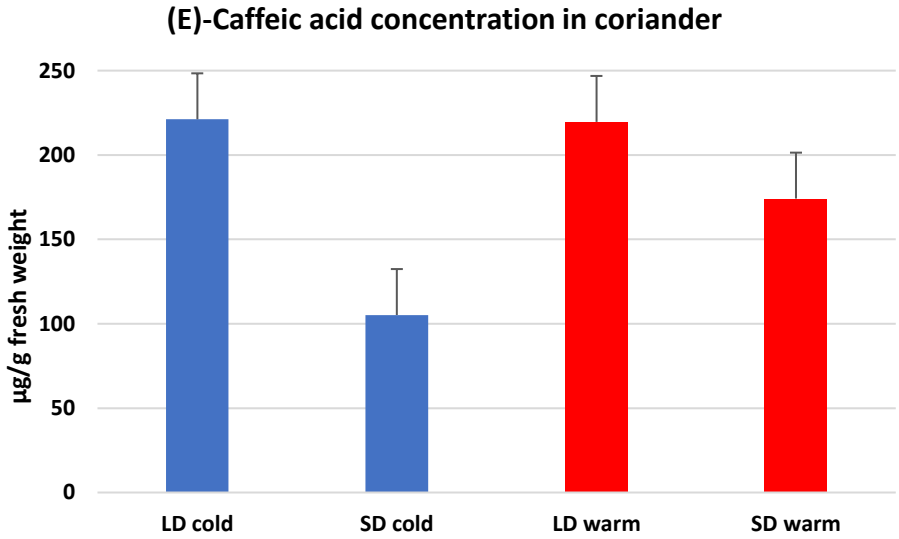


**Fig 3.19.** Effect of day length and temperature on (E)-caffeic acid in coriander. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Generally, (E)-caffeic acid showed highest levels under long day length irrespective of temperature. In contrast, short day length, and warm temperature produced higher levels of this compound. Therefore, temperature had no effect on this compound under long day length (Figure 3.19).

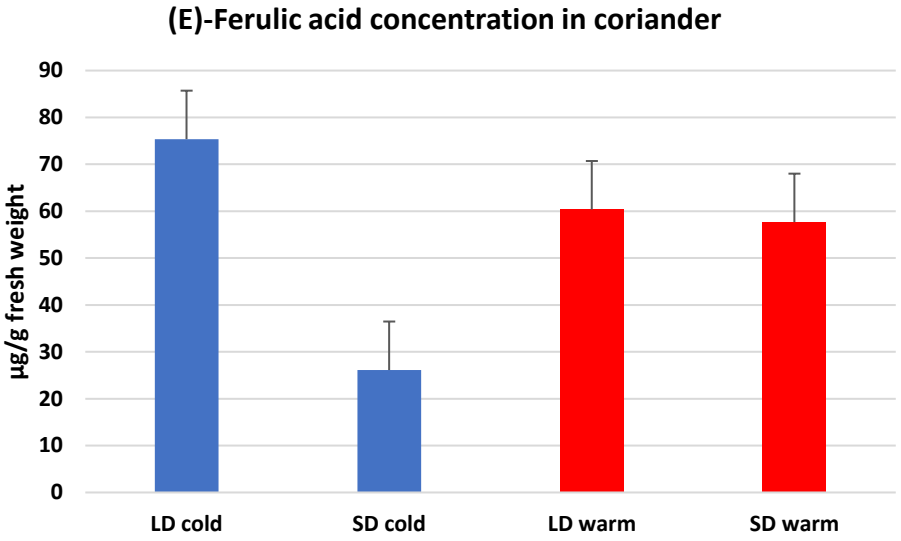


**Fig 3.20.** Effect of day length and temperature on (E)-ferulic acid in coriander. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Day length had little or no effect on (E)-ferulic acid under warm temperature. Under cold temperature and long day length the concentration was almost 3 times higher than at short day length (Figure 3.20).

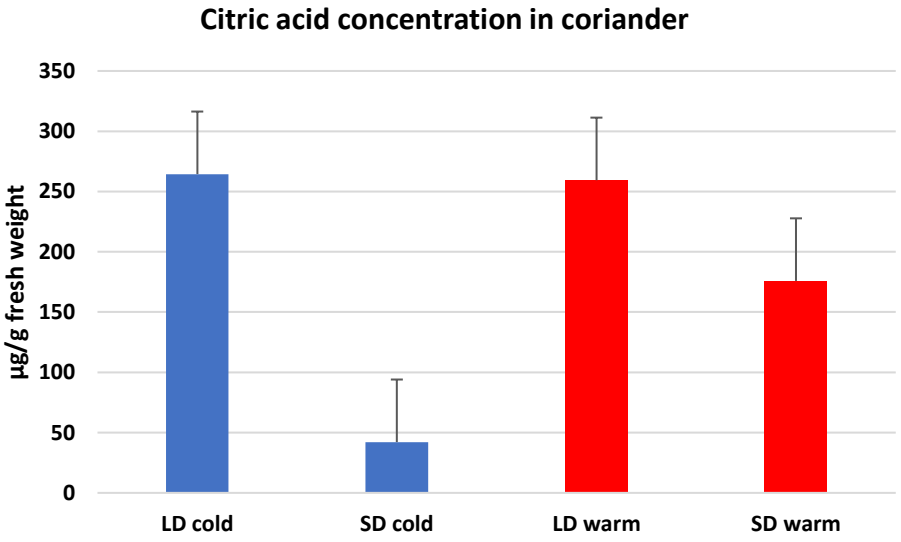


**Fig 3.21.** Effect of day length and temperature on citric acid in coriander. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Long day length had little or no effect on citric acid production under both temperatures. But under short day length, warm temperature yielded more of citric acid than under cold temperature. Warm temperature would on average yield more citric acid than cold temperature (Figure 3.21).

**Table 3.4:** Effect of day length and temperature on fructose, glucose and sucrose in coriander. The values are presented in µg/g fresh weight.

| Sugars | LD cold | SD cold | LD warm | SD warm |
|---|---|---|---|---|
| Fructose | 0.86 | 39.25 | 518.93 | 805.10 |
| Glucose | 884.31 | 36.88 | 892.49 | 964.90 |
| Sucrose | 4708.64 | 3551.83 | 4036.69 | 5693.13 |

Sucrose showed enhanced levels under warm temperature and short day length, and remains relatively higher compared to fructose and glucose. Glucose shows almost the same concentration under warm temperature in general, and under cold temperature long day length, but is not produced under cold temperature short day length. Under cold temperature treatment, only low levels of fructose were produced (Table 3.4).

### 3.2.3. Basil

Most compounds in basil showed a significant effect in both treatments, and under interaction, except for benzoic acid, coumaric acid, (E)-caffeic acid, luteolin, and quinic acid. (E)-sinapic acid only showed an effect under interaction, chlorogenic acid showed no effect under both treatments, while all the sugars showed an effect in both treatments and under interaction. The main compounds presented were; (E)-ferulic acid, rosmarinic acid derivative, and luteolin, with citric acid and the sugars.

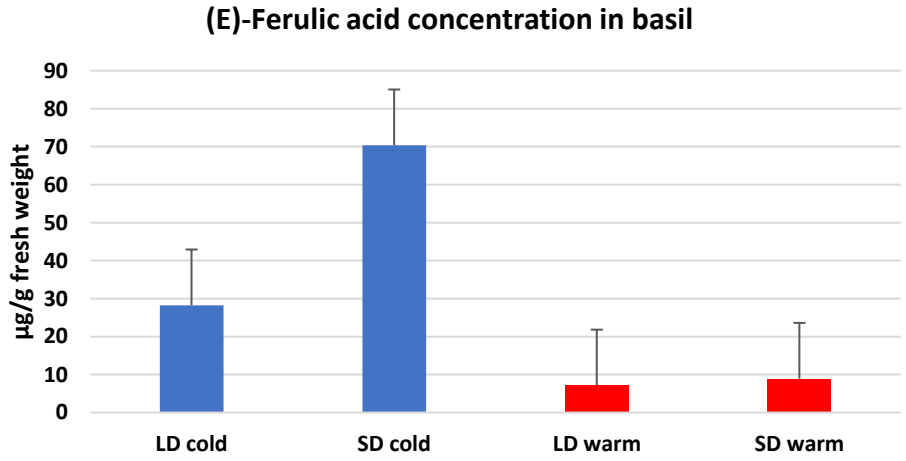


**Fig 3.22.** Effect of day length and temperature on (E)-ferulic acid in basil. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Temperature and photoperiod greatly affected the production of (E)-ferulic acid in basil. Cold temperature generally favoured the production of this compound especially under short day length compared to warm temperature that yielded very low amounts (Figure 3.22).

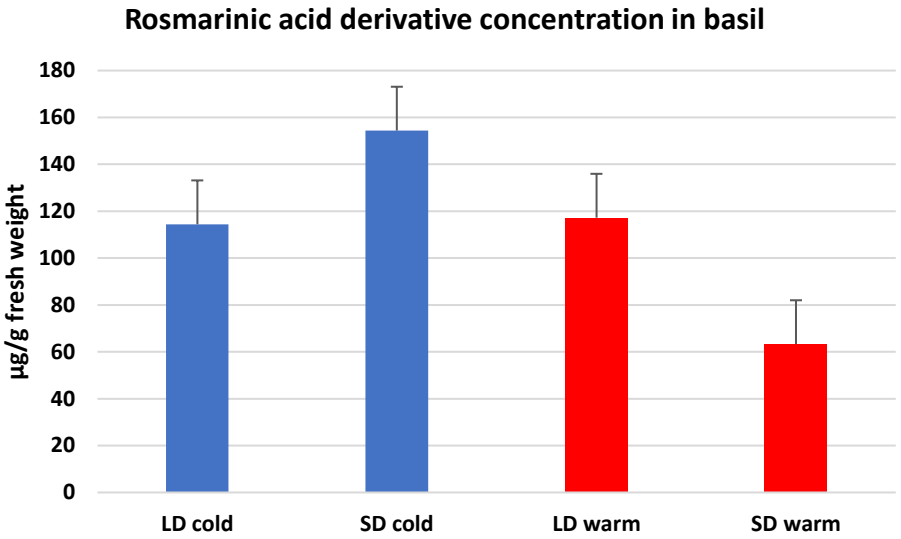


**Fig 3.23.** Effect of day length and temperature on rosmarinic acid derivative in basil. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Long day length under both temperatures yielded almost the same amount of the derivative of rosmarinic acid. But under short day length, cold temperature yielded about 2.5 times more of this compound compared to the warm temperature. Hence, short day length under cold temperature yielded more of rosmarinic acid (Figure 3.23).

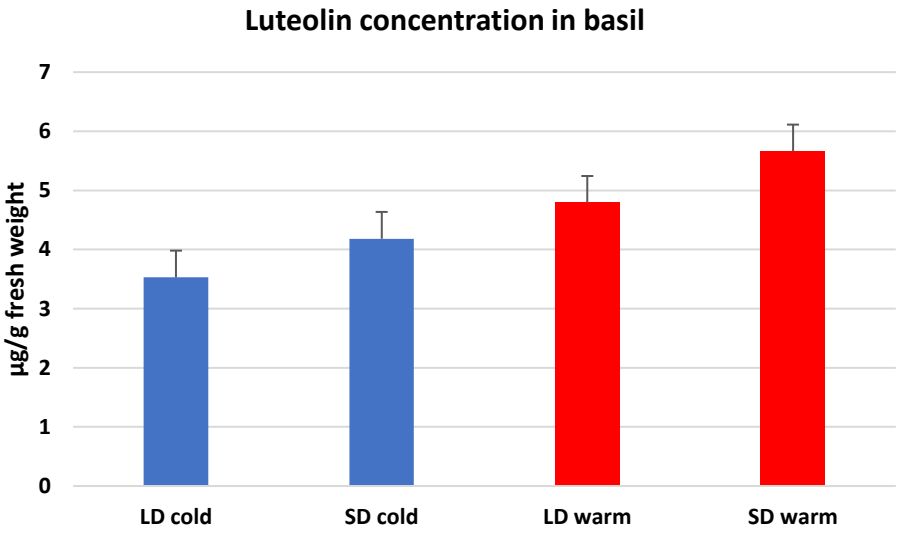


**Fig 3.24.** Effect of day length and temperature on luteolin in basil. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Short day length slightly favoured higher concentration of luteolin under both temperatures. Warm temperature produced more of luteolin than cold temperature. Short day length under warm temperature yielded the highest concentration (Figure 3.24).

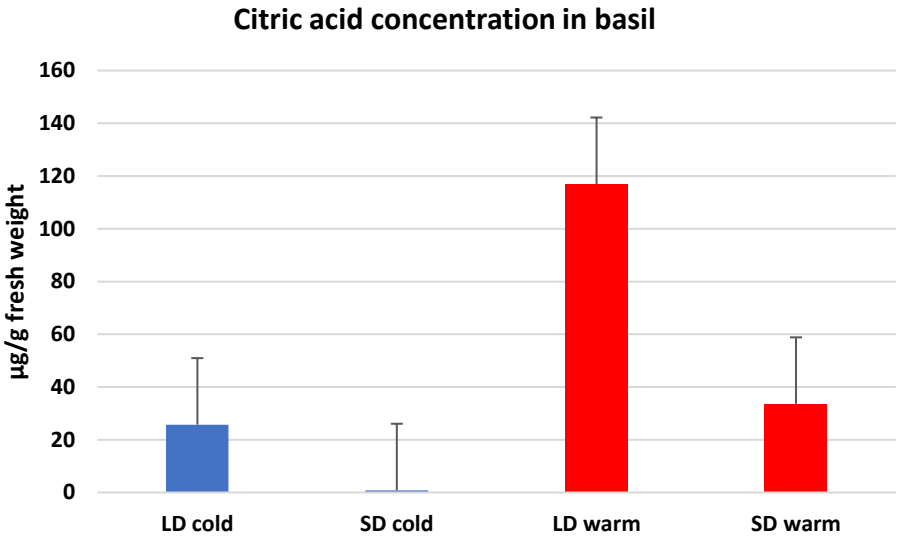


**Fig 3.25.** Effect of day length and temperature on citric acid in basil. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Temperature had a profound effect on citric acid production in basil. Warm temperature under long day length yielded about 3 times the concentration of citric acid compared to warm temperature under short day length. Cold temperature especially under short day length did not favour citric acid production (Figure 3.25).

**Table 3.5.** Effect of day length and temperature on fructose, glucose, and sucrose in basil. The values are presented in µg/g fresh weight.

| Sugars | LD cold | SD cold | LD warm | SD warm |
|---|---|---|---|---|
| Fructose | 559.23 | 2143.160 | 262.89 | 245.73 |
| Glucose | 116.48 | 347.63 | 10.13 | 21.33 |
| Sucrose | 696.34 | 2583.85 | 28.99 | 75.69 |

Highest levels of fructose, glucose and sucrose are produced under cold temperature, particularly under short day length. Warm temperature generally disfavours the production of all the sugars (Table 3.5).

### 3.2.4. Mint

Most compounds in mint showed no significant effect under both treatments, while some showed significant effects in one treatment, but not the other, e.g. (E)- caffeic acid, rosmarinic acid, (E)-4-caffeoylquinic acid and fructose showed an effect only under day length treatment. The main compounds presented were; (E)- caffeic acid, (E)-4-caffeoylquinic acid, and rosmarinic acid derivative, with citric acid and the sugars.

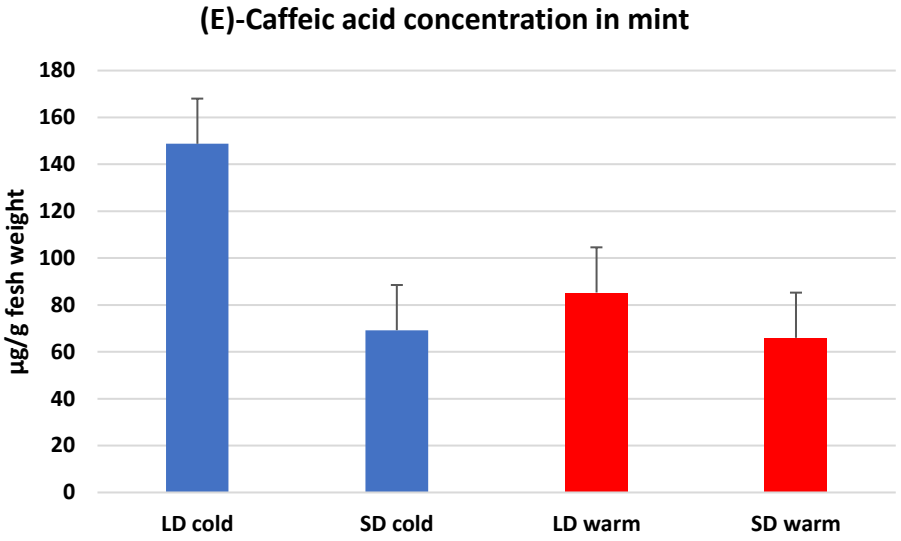


**Fig 3.26.** Effect of day length and temperature on (E)-caffeic acid in mint. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Higher levels of (E)-caffeic acid were produced under cold temperature and long day length. Long day length generally yielded higher concentrations under both temperatures. Under short day lengths, temperature had little or no effect on this compound (Figure 3.26).

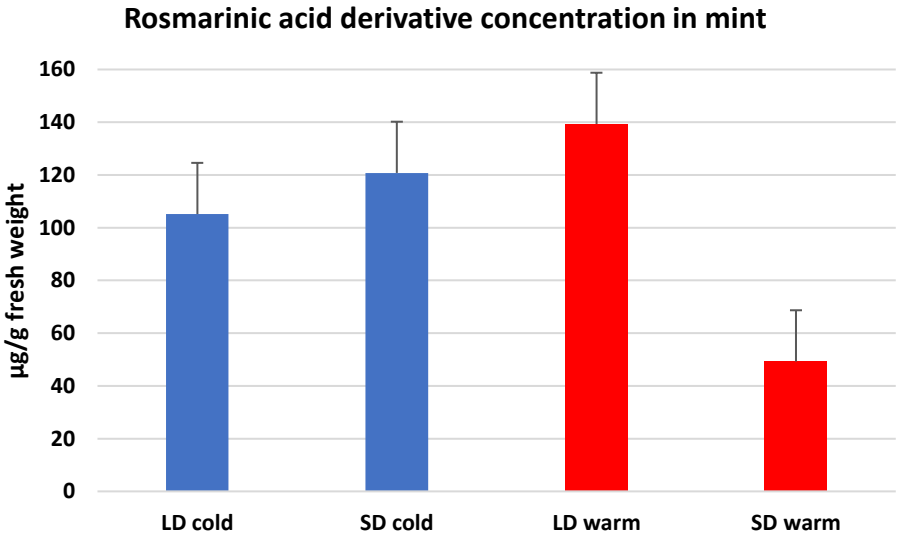


**Fig 3.27.** Effect of day length and temperature on rosmarinic acid derivative in mint. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Long day length under warm temperature yielded higher concentrations of rosmarinic acid. Day length had a slight effect under cold temperature, but had a higher effect under warm temperature (Figure 3.27).

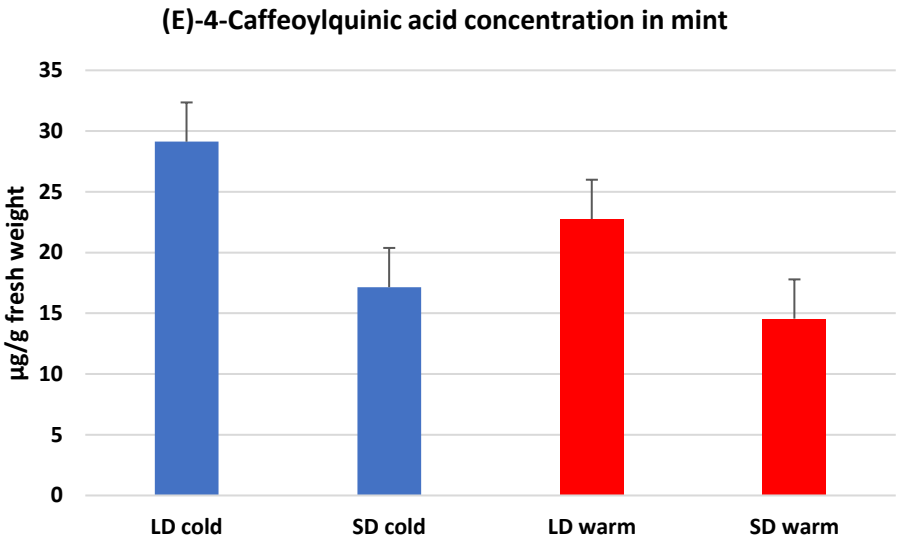


**Fig 3.28.** Effect of day length and temperature on (E)-4-caffeoylquinic acid in mint. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

(E)-4-caffeoylquinic acid production was higher under long day length and cold temperature. Long day length favoured higher concentrations under both temperatures (Figure 3.28).

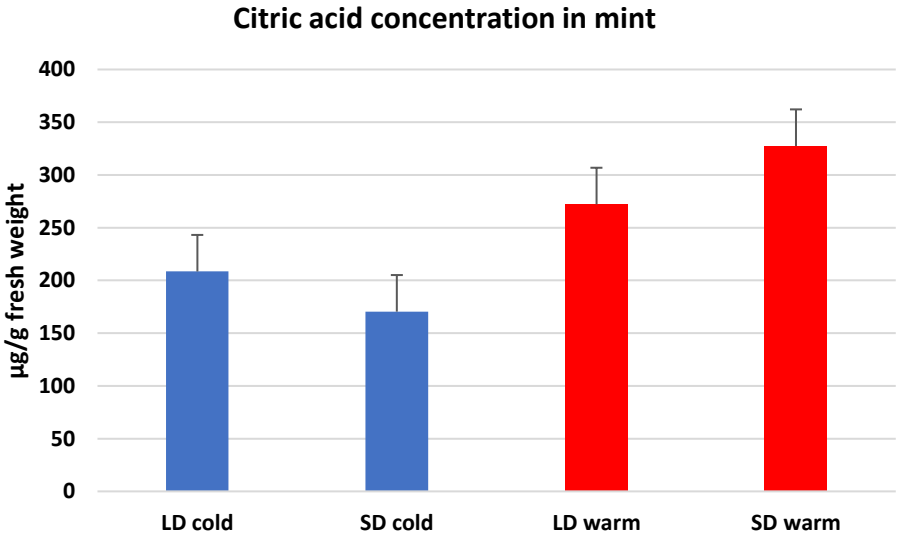


**Fig 3.29.** Effect of day length and temperature on citric acid in mint. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Warm temperature generally favoured higher concentrations of citric acid particularly under short day length than cold temperature. Under cold temperature, long day length yielded more of citric acid (Figure 3.29).

**Table 3.6.** Effect of day length and temperature on fructose, glucose and sucrose in mint. The values are presented in µg/g fresh weight.

| Sugars | LD cold | SD cold | LD warm | SD warm |
|---|---|---|---|---|
| Fructose | 92.89 | 70.74 | 54.56 | 126.18 |
| Glucose | 78.76 | 62.16 | 67.72 | 112.07 |
| Sucrose | 2072.11 | 428.96 | 1882.56 | 1257.94 |

Sucrose showed higher levels under long day lengths at both temperatures, but preferably under cold temperature. The concentrations of fructose and glucose were extremely low compared to sucrose in all the treatments (Table 3.6).

### 3.2.5. Lettuce

Almost all the compounds in lettuce were significantly affected by both treatments and their interaction, except for benzoic acid, chlorogenic acid and (E)-caffeic acid that had no significant effect under temperature and under interaction. Fructose and glucose were not affected under photoperiod treatment. The distinct compounds selected were; (E)-caffeic acid, (E)-ferulic acid, and chlorogenic acid, with citric acid and the sugars.

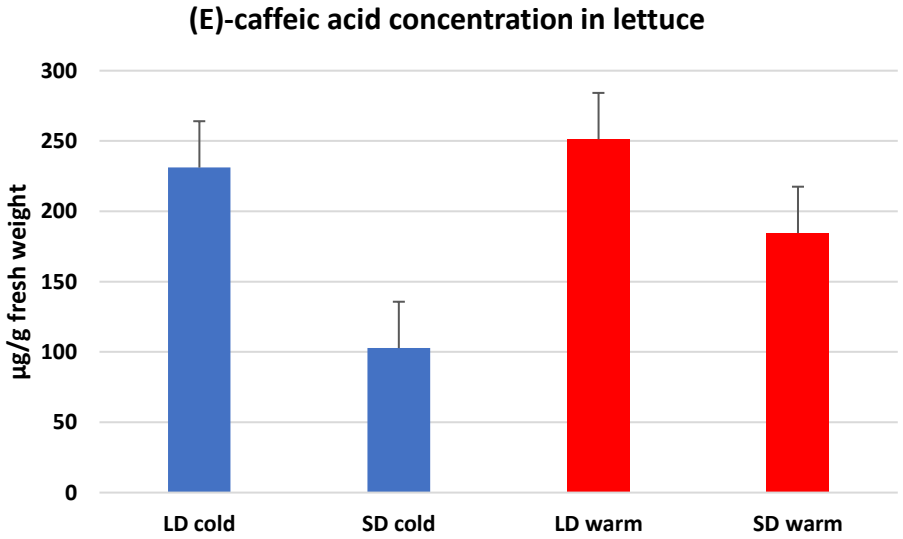


**Fig 3.30.** Effect of day length and temperature on (E)-caffeic acid in lettuce. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Longer day length generally favoured higher concentration of (E)-caffeic acid under both temperatures than shorter day length. This compound showed highest concentrations under warm temperature and long day length (Figure 3.30).

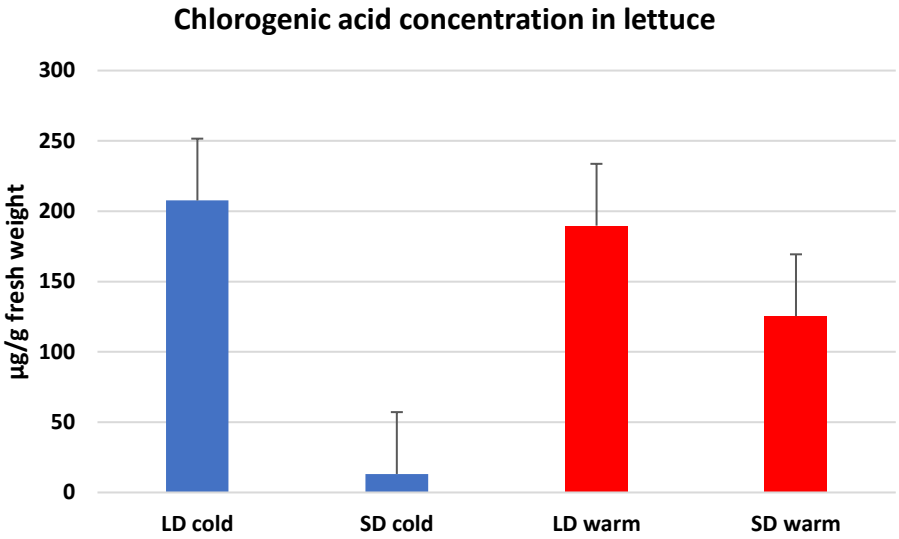


**Fig 3.31.** Effect of day length and temperature on chlorogenic acid in lettuce. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Chlorogenic acid showed highest levels under long day length irrespective of temperature. Under short day length, its concentration was higher when temperature was warm. Best condition for its production according to this study was long day length under cold temperature (Figure 3.31).

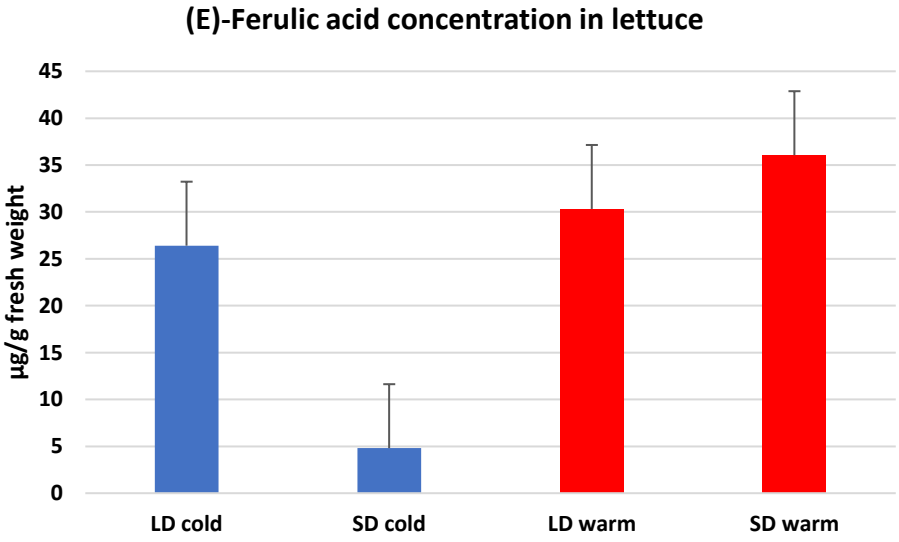


**Fig 3.32.** Effect of day length and temperature on (E)-ferulic acid in lettuce. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Warm temperature under long and short day length, yielded higher concentrations of (E)-ferulic than under cold temperature. The compound showed highest levels under warm temperature under short day length and was produced in very low amounts under short day length and cold temperature (Figure 3.32).

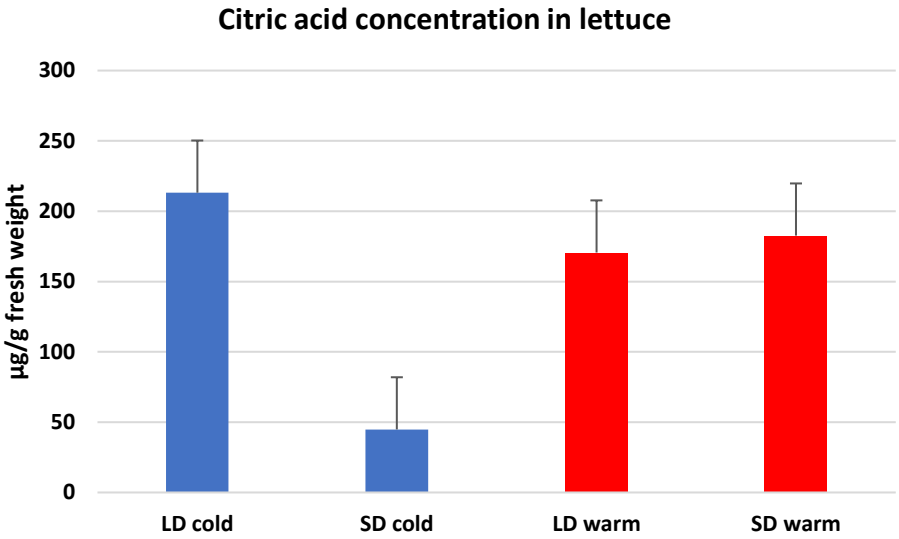


**Fig 3.33.** Effect of day length and temperature on citric acid in lettuce. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Cold temperature under long day length yielded higher concentration of citric acid. Day length had very little effect on citric acid concentration under warm temperature (Figure 3.33).

**Table 3.7:** Effect of day length and temperature on fructose, glucose and sucrose in lettuce. The values are presented in µg/g fresh weight.

| Sugars | LD cold | SD cold | LD warm | SD warm |
|---|---|---|---|---|
| Fructose | 305.12 | 72.52 | 392.95 | 450.00 |
| Glucose | 244.32 | 16.88 | 430.12 | 467.47 |
| Sucrose | 3959.86 | 257.56 | 4477.71 | 3609.54 |

Sucrose concentration was high under long day length for both temperatures, but was higher under warm temperature. Fructose and glucose levels were extremely lower compared to sucrose levels, and they showed almost the same levels in the treatments except under short day length at cold temperature showing lowest concentrations of all treatment combinations (Table 3.7).

### 3.2.6. Peppercress

Most compounds in this species showed no significant effect under both treatments and under interaction. The only compounds that showed a significant effect in all treatments were benzoic acid and (E)-sinapic acid. Citric acid was significantly affected under temperature, (E)-ferulic acid and quinic acid derivative showed significant difference under day length and benzyl isothiocyanate was significantly affected under temperature and interaction. The main compounds presented in peppercress were; (E)-ferulic acid, (E)-sinapic acid, and benzyl isothiocyanate, with citric acid and the sugars. Other important compounds but present in lower concentrations were dehydroascorbic acid and (E)-caffeic acid.

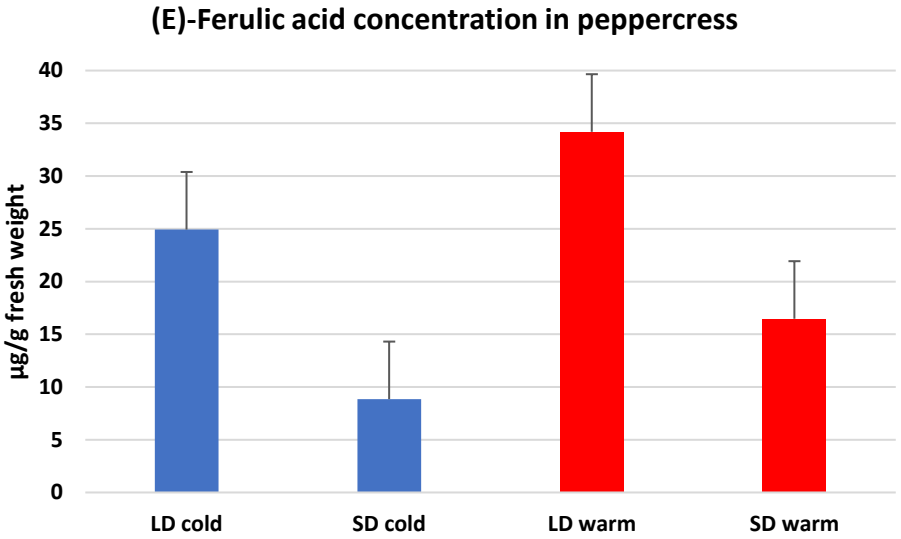


**Fig 3.34.** Effect of day length and temperature on (E)-ferulic acid in peppercress. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Long day length generally yielded higher concentrations of (E)-ferulic acid under both temperatures, but strongly enhanced under long day length and warm temperature (Figure 3.34).

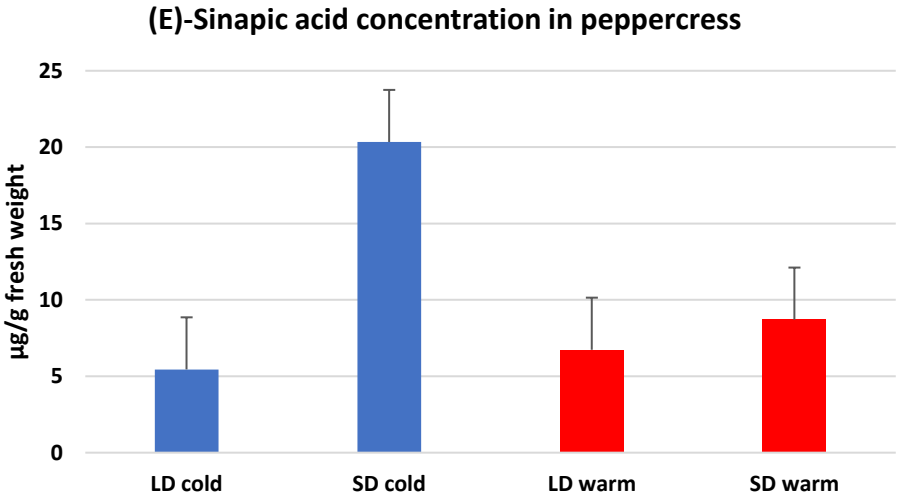


**Fig 3.35.** Effect of day length and temperature on (E)-sinapic acid. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Day length had very little effect on (E)-sinapic acid under warm temperature. Cold temperature and short day length yielded very high concentrations of this compound (Figure 3.35).

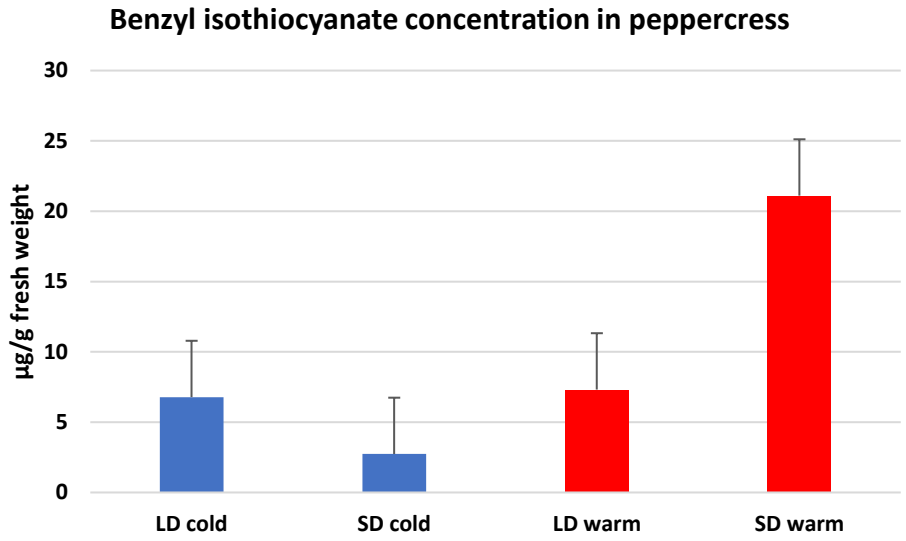


**Fig 3.36.** Effect of day length and temperature on benzyl isothiocyanate in peppercress. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Benzyl isothiocyanate showed highest concentrations under warm temperature and short day length. Generally, temperature had little or no effect under long day lengths (Figure 3.36).

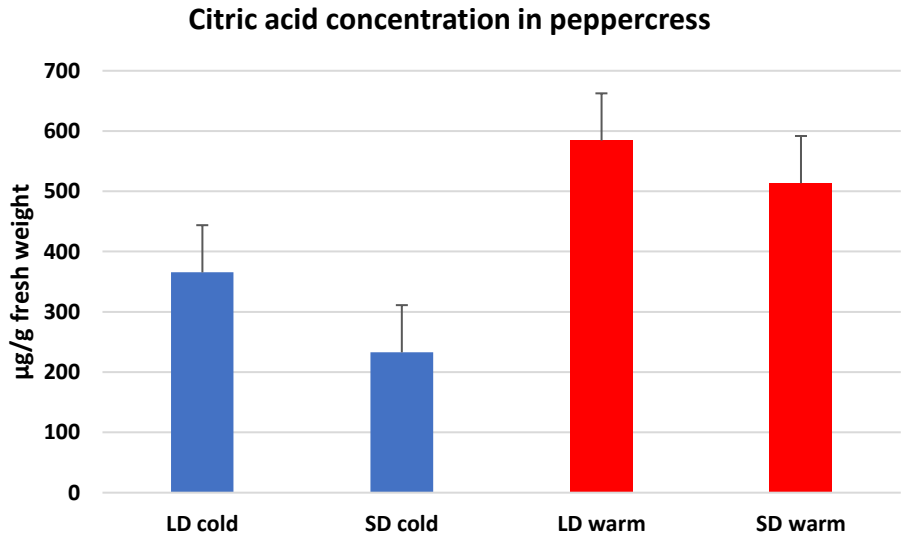


**Fig 3.37.** Effect of day length and temperature on citric acid in peppercress. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Warm temperatures were more favourable for citric acid production in peppercress especially under long day length. Long day length generally enhanced citric acid levels under both temperatures (Figure 3.37).

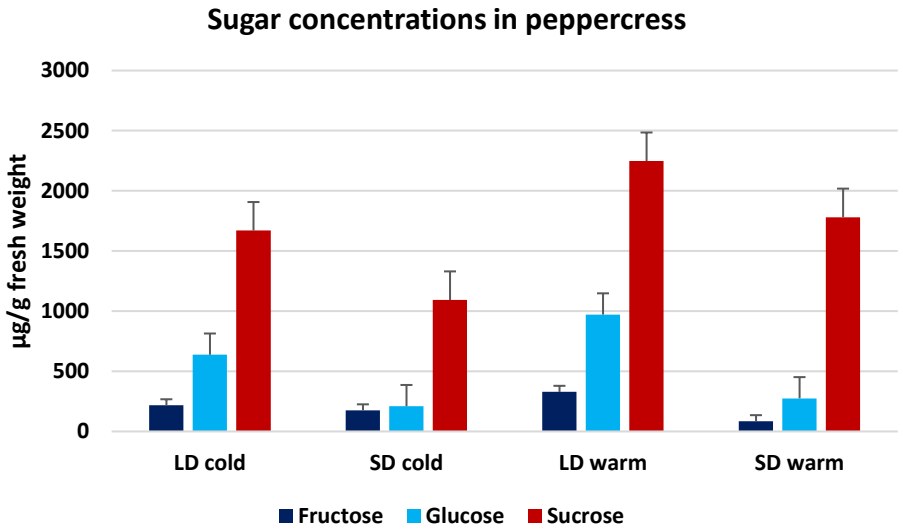


**Fig 3.38.** Effect of day length and temperature on fructose, glucose and sucrose in peppercress. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Warm temperature under long day length generally yielded higher concentrations of sucrose, glucose and fructose. For glucose, temperature had little or no effect under short day length (Figure 3.38).

### 3.2.7. Beet greens

Most compounds in beet greens were not significantly affected by temperature, but rather under light and under interaction. Only citric acid, fructose, and benzyl isothiocyanate had significant effects under temperature. Dehydroascorbic acid had no effect on both treatments and under interaction. The distinct compounds selected were; (E)-ferulic acid and benzoic acid with citric acid and the sugars.

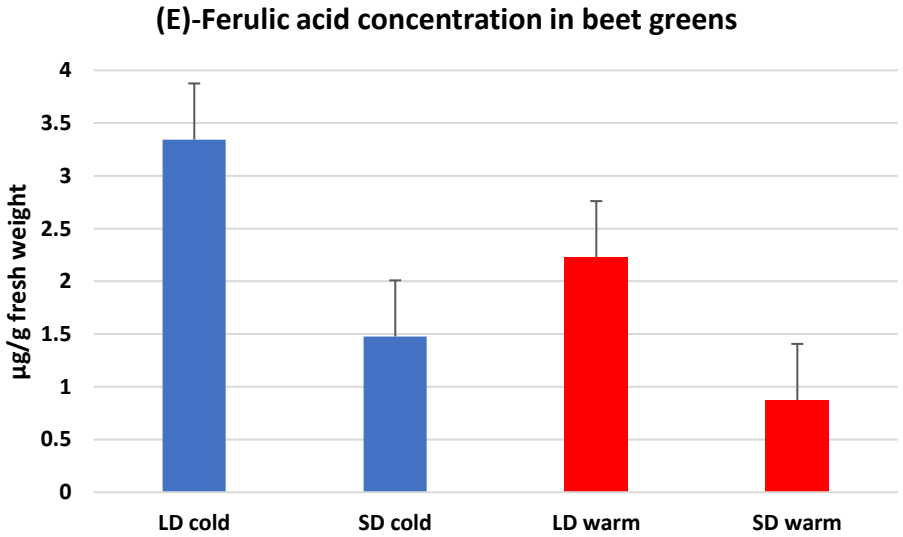


**Fig 3.39.** Effect of day length and temperature on (E)-ferulic acid in beet greens. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Cold temperature favoured the production of (E)-ferulic acid than warm temperature. Long day length generally yielded higher concentrations of the compound under both temperatures. The best condition for (E)-ferulic production was cold temperature under long day length (Figure 3.39).

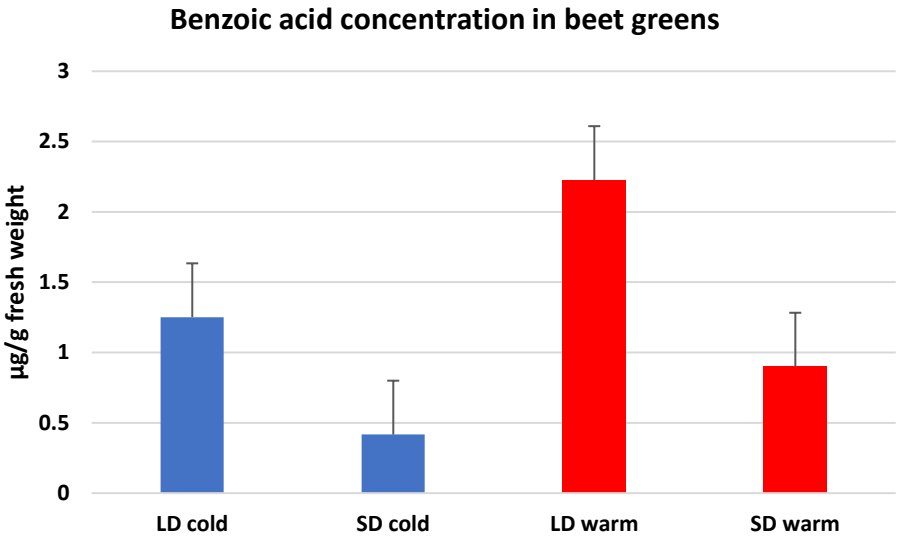


**Fig 3.40.** Effect of day length and temperature on benzoic acid in beet greens. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Long day length generally favoured the production of benzoic acid under both temperatures. Higher levels of this compound were produced under warm temperature and long day length (Figure 3.40).

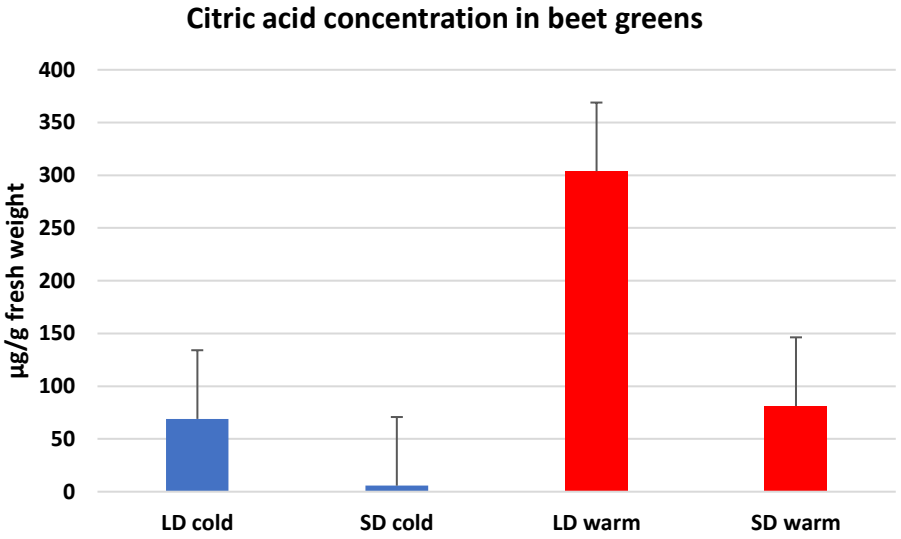


**Fig 3.41.** Effect of day length and temperature on citric acid in beet greens. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Warm temperature generally favoured the production of citric acid, especially under long day length than under cold temperature (Figure 3.41).

**Table 3.8:** Effect of day length and temperature on fructose, glucose and sucrose in beet greens. The values are presented in µg/g fresh weight.

| Sugars | LD cold | SD cold | LD warm | SD warm |
|---|---|---|---|---|
| Fructose | 112.86 | 2.67 | 4.16 | 4.93 |
| Glucose | 324.23 | 24.95 | 146.15 | 66.82 |
| Sucrose | 823.89 | 104.90 | 1172.54 | 288.75 |

Cold temperature under long day length favours higher concentrations of fructose and glucose, while sucrose shows highest levels under warm temperature at long day length. Warm temperatures generally disfavour the production of fructose (Table 3.8).

### 3.2.8. Wheatgrass

Temperature and light affected the compounds in this species almost equally. Some compounds were affected by one treatment and not the other. (E)-caffeic acid and dehydroascorbic acid were the only compounds significantly affected by both treatments and under interaction. The main compounds presented were; (E)-caffeic acid, luteolin and dehydroascorbic acid, with citric acid and the sugars.

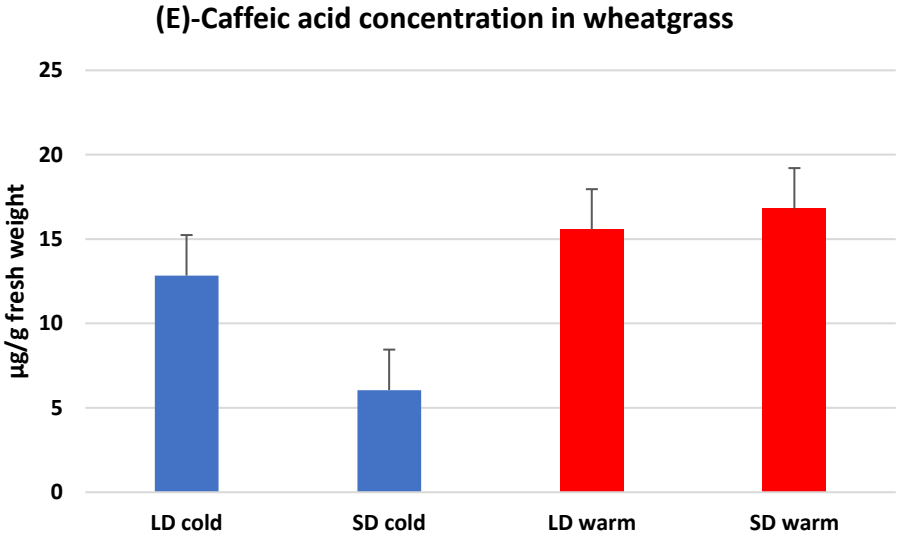


**Fig 3.42.** Effect of day length and temperature on (E)-caffeic acid in wheatgrass. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Warm temperature under long and short day length generally yielded higher concentrations of (E)-caffeic acid than cold temperature (Figure 3.42).

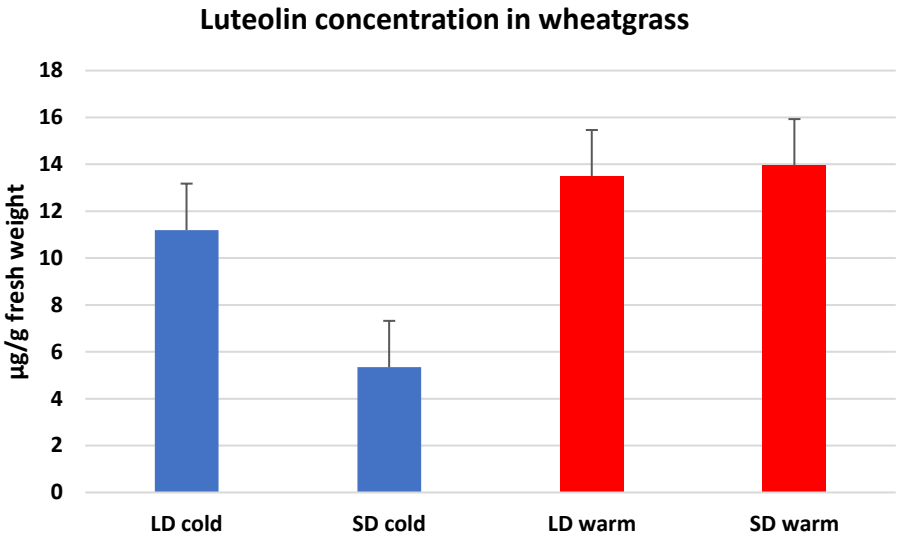


**Fig 3.43.** Effect of day length and temperature on luteolin in wheatgrass. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Warm temperature under long and short day length favoured higher concentrations of luteolin compared to cold temperatures (Figure 3.43).

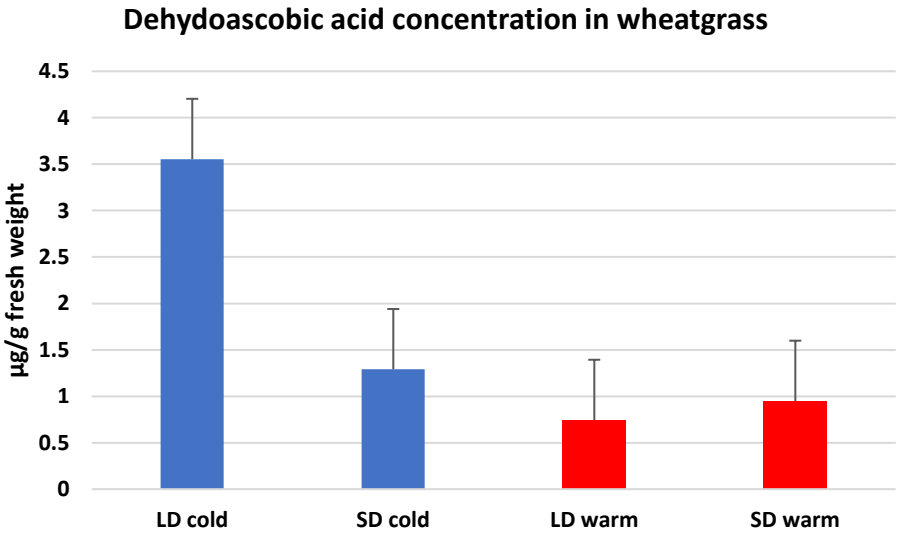


**Fig 3.44.** Effect of day length and temperature on dehydroascorbic acid in wheatgrass. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Dehydroascorbic acid showed highest concentrations under long day length and cold temperature, and the concentration dropped to about 5 times under warm temperature (Figure 3.44).

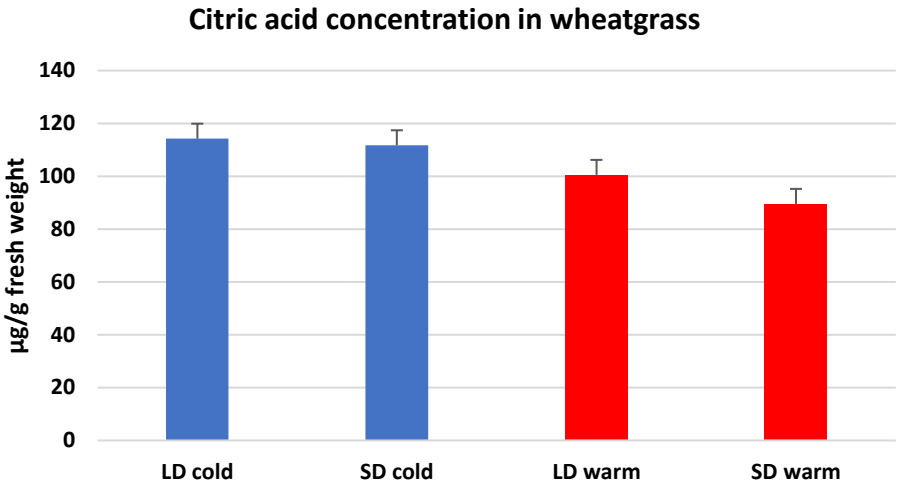


**Fig 3.45.** Effect of day length and temperature on citric acid in wheatgrass. The error bars show the standard error for the whole experiment. Each bar represents mean values of all samples under that treatment.

Generally, day length under both temperatures had little or no effect on the concentration of citric acid. The compound was produced almost in the same amount under both temperatures, but cold temperatures slightly yielded higher concentration of citric acid (Figure 3.45).

**Table 3.9:** Effect of day length and temperature on fructose, glucose and sucrose in wheatgrass. The values are presented in µg/g fresh weight.

| Sugar | LD cold | SD cold | LD warm | SD warm |
|---|---|---|---|---|
| Fructose | 131.62 | 42.87 | 116.72 | 67.94 |
| Glucose | 69.71 | 94.62 | 118.37 | 57.47 |
| Sucrose | 6085.83 | 3010.36 | 8216.92 | 5473.79 |

Long day length yields higher levels of sucrose under warm temperature, meanwhile very low amounts of fructose and glucose are produced under all the treatments in wheatgrass (Table 3.9).

## 3.3. Total aromatics across the species

Since aromatic compounds play vital roles in health and food flavour, the purpose of this section and the table below was to show how the concentration of these aromatics varied from species to species and from treatment to treatment in this study.

**Table 3.10:** Summarised values of total aromatics across the species under the different treatments. The values are presented in μg/g fresh weight.

| Species | LD cold | SD cold | LD warm | SD warm |
|---|---|---|---|---|
| beet greens | 68.06 | 35.05 | 112.64 | 73.94 |
| basil | 591.55 | 671.63 | 636.97 | 460.13 |
| chives | 74.83 | 78.82 | 52.80 | 81.90 |
| peppercress | 88.52 | 67.74 | 149.24 | 88.10 |
| coriander | 2045.96 | 1801.52 | 931.23 | 680.82 |
| lettuce | 550.00 | 130.17 | 549.73 | 428.63 |
| mint | 1281.59 | 621.80 | 831.98 | 538.76 |
| wheatgrass | 87.60 | 47.74 | 100.97 | 103.91 |

The total aromatics here included the identified and un-identified aromatics (Table 3.2). Coriander generally showed far higher concentrations of aromatics under all the treatments compared to the other species. Aromatics were best produced in beet greens and peppercress under long day length and warm temperature, in basil under short day length and cold temperature, in chives and wheatgrass under short day and warm temperature, and in coriander, lettuce and mint under long day and cold temperature (Table 3.10).

## 3.4. Multivariate analyses

Multivariate analyses were performed on the 17 aromatics, and in addition on all the 208 metabolites to give a bigger picture for those species that showed strong effects upon the treatments. The percentages on the axes showed how the principal components varied in the data set. The loading plot of the 17 aromatics in Figure 3.46 showed how the compounds were not evenly distributed along the axes. All the metabolites contributed to the variation on the positive first component.

Benzoic acid, (E)-caffeic acid, (E)-sinapic acid, luteolin and dihydroxyphenylalanine, all contributed to the negative axis on the second component, while rosmarinic acid, (E)-4-caffeoylquinic acid, salicylic acid, and benzyl isothiocyanate contributed to the positive axis of the second component.

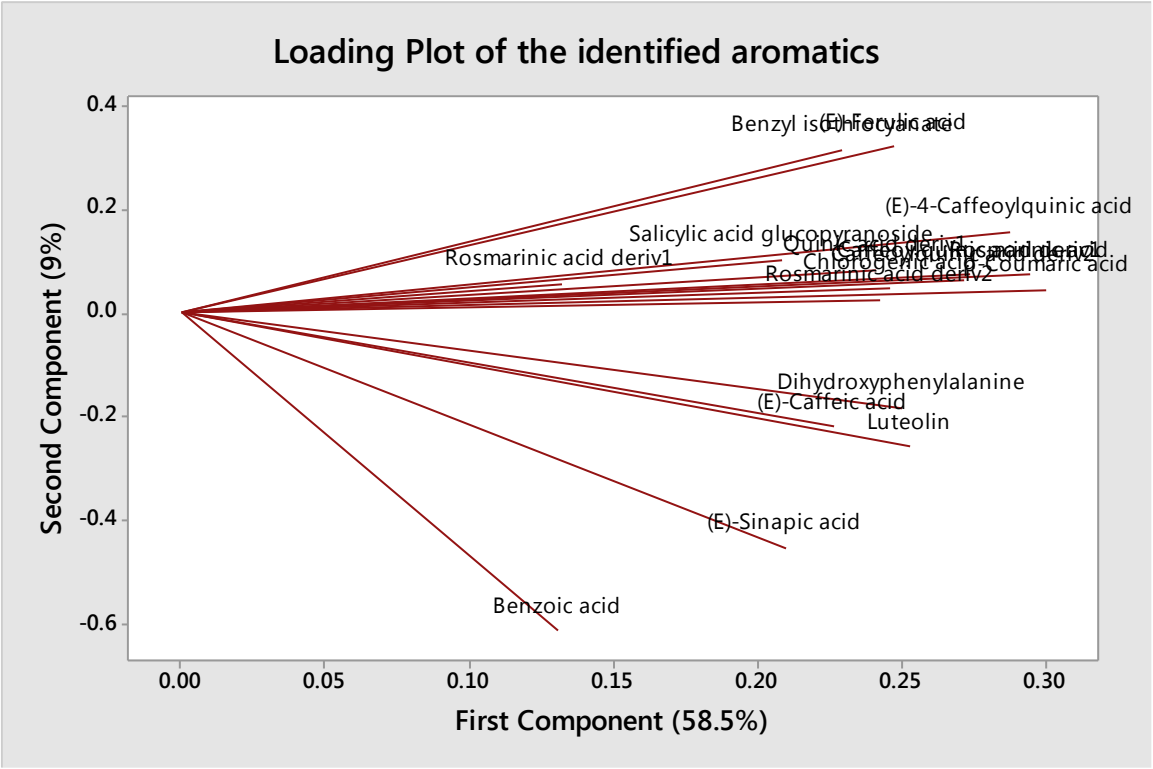


**Fig 3.46.** Principal component analysis (loading plot) of the 17 identified aromatics.

The treatments from Figure 3.46 were summarised into 2 components and distributed into 2 groups under cold and warm temperature (Figure 3.47) and under long and short day length (Figure 3.48). Generally, the samples clustered around the centre of the axes with some outliers. Most of the treatments clustered on the positive first component axis while those on the negative first component axis were a little scattered. In Figure 3.47 and 3.48 the dots represented the samples and their distribution across the coordinate system. With the 208 total metabolites, Figure 3.49 showed a general picture of how photoperiod and temperature influenced sample metabolites composition and clustering, while Figure 3.50 depicted the sample distribution and clustering based on metabolite composition and variation.
Figures 3.47, 3.48 and 3.50 depicted the distribution or segregation of the samples based on the impact of the metabolites (aromatics or whole data set).

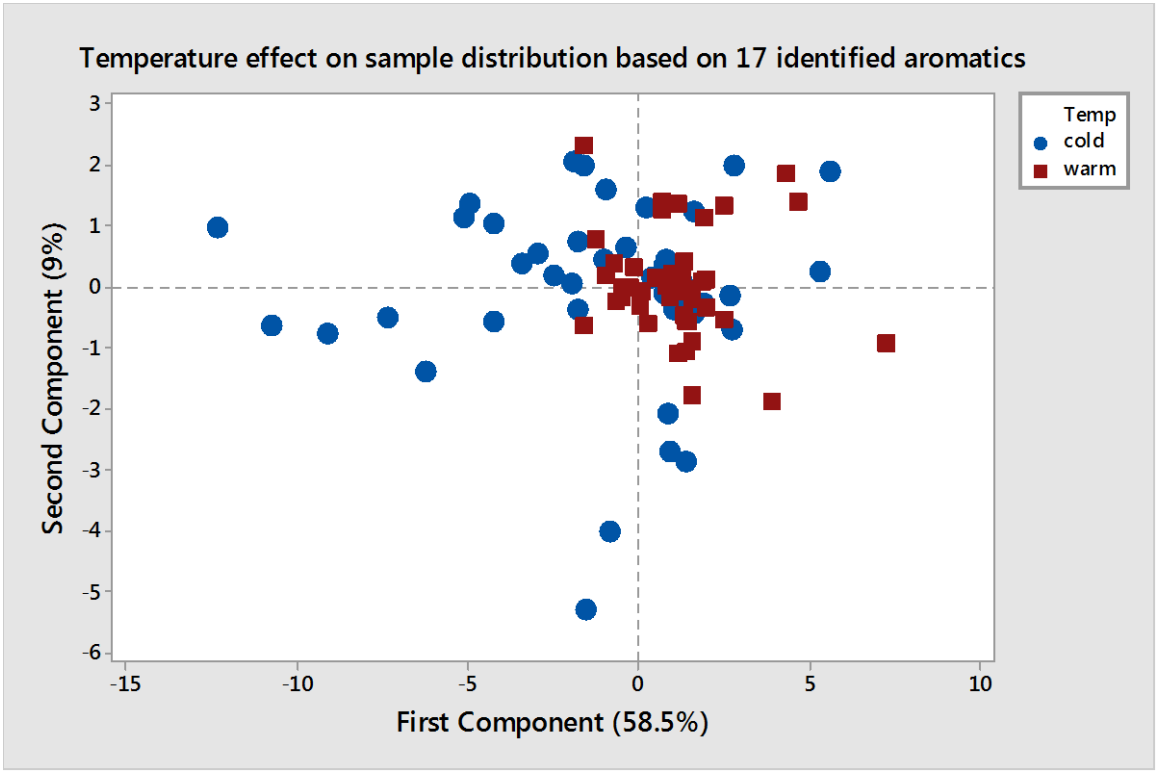


**Fig 3.47.** Principal component analysis (score plot) showing sample distribution as an effect of temperature based on 17 identified aromatics.

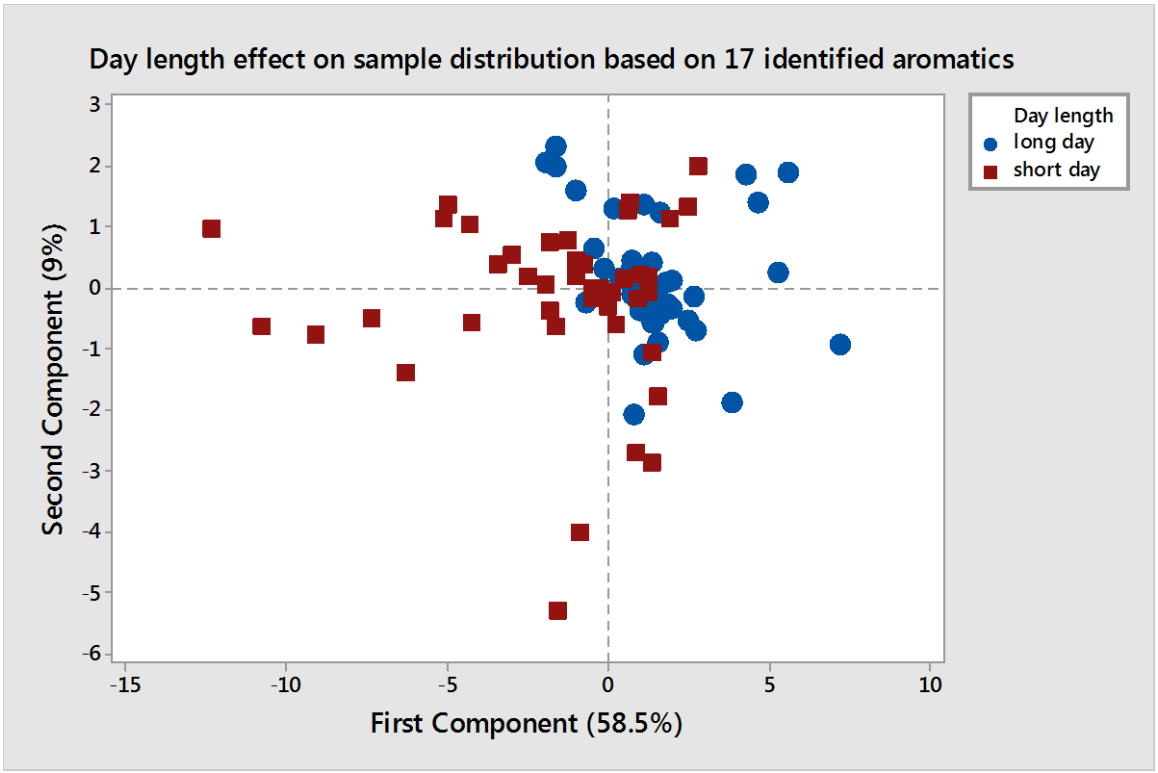


**Fig 3.48.** Principal component analysis (score plot) showing sample distribution as an effect of day length based on 17 identified aromatics.

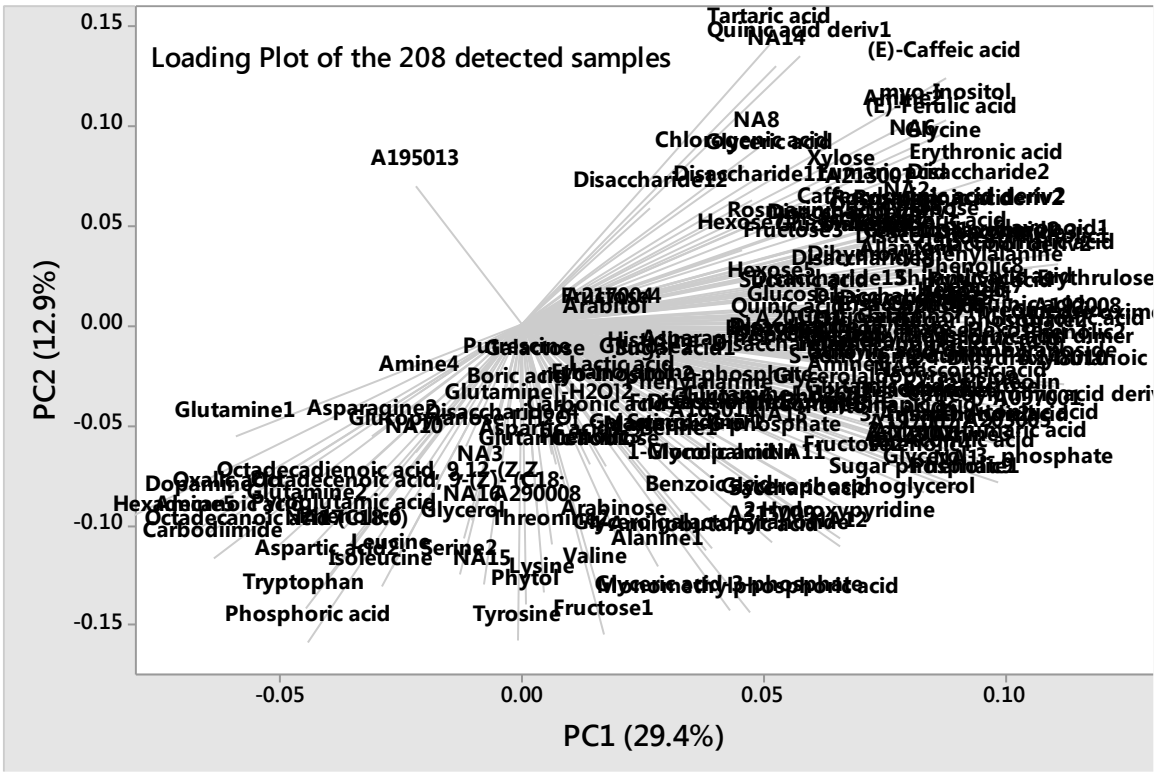


**Fig 3.49.** Principal component analysis (loading plot) showing sample distribution based on the 208 detected metabolites.

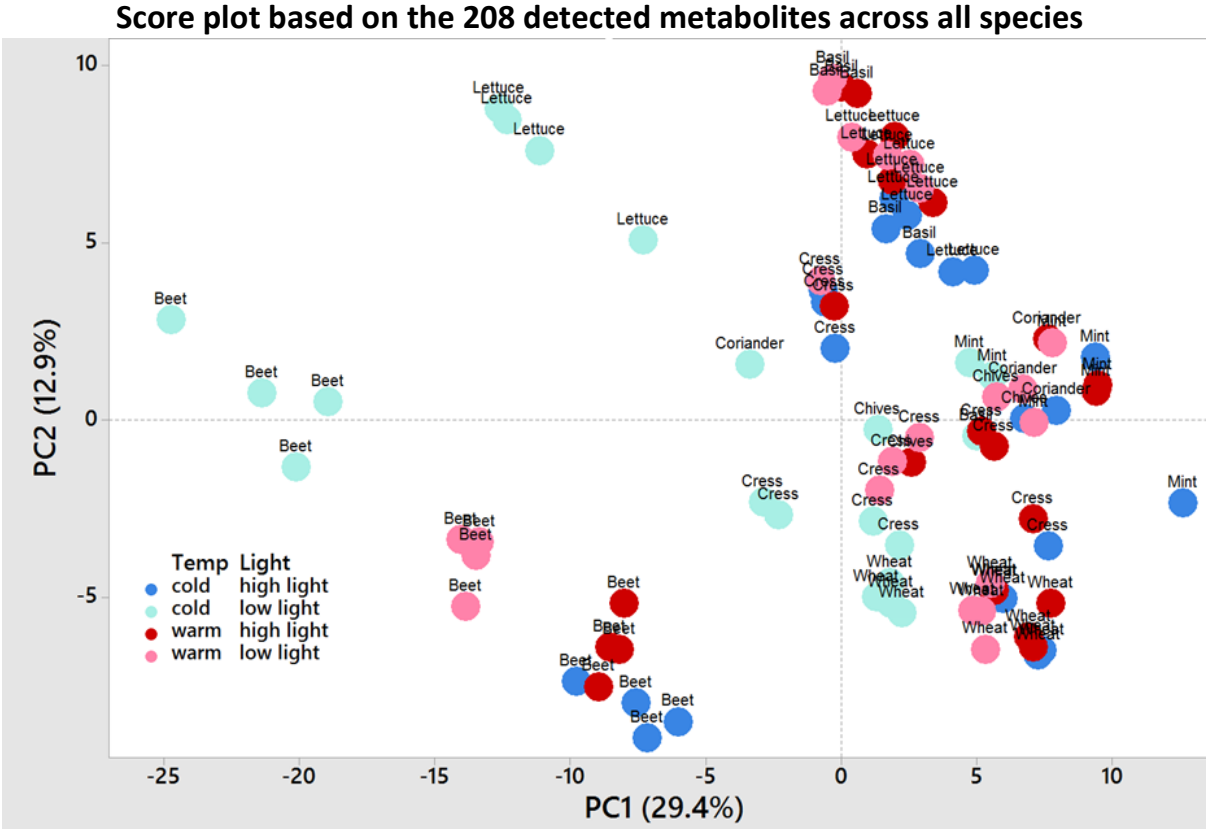


**Fig 3.50.** Principal component analysis (score plot) showing the photoperiod and temperature treatment across the species based on the 208 detected metabolites.

Figure 3.49 showed how the 208 metabolites clustered along the axes. Most of the metabolites contributed to the positive first component and the negative second component. Benzoic acid, caffeic acid, ferulic acid, chlorogenic acid, valine and lysine contributed to the positive first component, while benzoic acid, leucine, glutamine and asparagine contributed to the negative axis of the second component. As shown in Figure 3.46, all the aromatics contributed to the positive axis of the first component.

Figure 3.50 depicted a clear clustering pattern, e.g. in beet greens with the most unfavourable condition being under short day and cold temperature treatment which showed how the samples segregated away from the warm treated ones within the same species. Day length was also clearly demonstrated in beet greens as short day length samples and long day length samples were far away from each other. Distribution across the other species were a little more scattered.

In general, Figures 3.47 and 3.48 showed day length and temperature effects regardless of species differences for the identified aromatics, while Figures 3.49 and 3.50 clearly showed species differences and to some extent within species clustering based on day length and temperature effect.

# 4. DISCUSSION

In this study, I assessed the effects of light and temperature on metabolite composition in 8 plant species. Light and temperature settings were chosen according to potential suitable growing conditions that would not negatively affect the plants.
It is important to note that we might expect some discrepancies with other studies in the levels and concentrations of the compounds because we encountered some problems during germination and growth of the plants. For example, most of the cold chamber plants were harvested at two separate times because of growth delay. As earlier said, the values mentioned are the mean values of all samples of a given compound under the treatment. In a general perspective, studies have shown that day length and temperature greatly influenced the biosynthesis of plant phytochemicals especially those compounds related to taste and colour *(Jaakola et al., 2010)*. This is also reflected in this study.

## 4.1. Effects of light and temperature on the plant metabolites

Plants respond to light, and more particular photoperiod in diverse ways, for example some plants are termed short day plants because they form flowers only when day length is less than 12 hours, while others are termed long day plants because they form flowers only when day length exceeds 12 hours. Some plants favourably accumulate certain compounds under shorter photoperiods and others at longer photoperiods. For example, long day length yielded elevated levels of menthone and menthols in peppermint, while short day length yielded higher levels of menthofuran in peppermint *(Burbott et al., 1967).*
Photoperiod is a very important factor that influences plant growth, development and the accumulation of metabolites *(Bian et al., 2015)*. Light is not only important for plants as a source of energy, but affects on the antioxidant properties of different vegetables.
In our study, the accumulation of the volatile compounds under the different photoperiods varied from species to species, such that the levels of mint and chive volatiles were higher under short day length, while basil volatile levels were higher under long day length. Our study also showed that fluctuation of volatiles in coriander depended on the temperature.
Note should be taken that the mint species used in this study was a special kind of hybrid, so comparisons were made with peppermint in other studies since they all belong to the *Mentha* genus. This may explain why menthone and menthol were not detected in this study. Therefore, with the mint species in general, a 12 h photoperiod yielded more volatile compounds than an 18 h photoperiod in most of the volatiles with a few exceptions. Peperitenone oxide, beta-myrcene and 1,8-cineole in mint showed higher levels under 12 h. *Burbott et al. (1967)* showed that peppermint grown under 14 hours of light or less, produced very small amounts of essential oils, and reported that about 15 to 16 hours of light was required to produce peppermint oil. Monoterpenes are the components responsible for flavour and aroma in mint oils *(Lange et al., 2000),* and in peppermint oil they were greatly influenced by photoperiod *(Burbott et al., 1967)*. Even though we were not dealing with peppermint specifically, we could also conclude that the monoterpenes in the mint species used here were also influenced by photoperiod as short day length favoured their production.

Just like other compounds, monoterpene and sesquiterpene levels vary from species to species due to their genetic makeup, origin and growth conditions. In our study, monoterpene levels in basil were generally lower compared to sesquiterpene levels except under the long day length and warm temperature condition. Short day length favoured higher levels of monoterpenes in mint, but long day length favoured higher levels of monoterpenes in basil. Thus, photoperiod had a strong effect on the production of biosynthesis of monoterpenes depending on the plant.

A geographical region also reflects the plants growing conditions related to cold and warm temperatures. Therefore, the physiology of the plant may relate to temperature and day length yielding strong effects that boost the metabolism of these terpenes.

A recent study conducted by *Kang et al., (2013)* reported that an 18 h photoperiod yielded better growth and higher photosynthetic capacity in lettuce than shorter photoperiod, as was also reflected in our study. In another finding, *Fletcher et al., (2010)* reported that a 12 h photoperiod reduced the accumulation of rosmarinic acid in two of the mint species they used. They further explained that day length had little or no effect on rosmarinic acid in mint, but its accumulation could be maximised under 14 h or above.

Just like light, temperature generally affects metabolite levels in plants with regard to species variation. In this study, warm temperatures resulted in higher levels of metabolites than cold temperatures in all four herbs. Basil is a warm climate plant and the optimum temperature for its growth and volatile oil content is best at 25°C *(Chang et al., 2005)*. In the same study, it was shown that basil grown at 25°C or 30°C yielded three times more essential oils than when grown at 15°C. *Cis*-ocimene and eugenol levels were higher at 25°C while at 15°C camphor and *trans*-farnesene were highest. Similarly, in our study, warm temperature (25°C) also yielded greater amounts of basil volatiles than the cold temperature (15°C). According to our study, day length had a significant effect on rosmarinic acid under warm temperature and slightly under cold temperature. It was also observed that dehydroascorbic acid in chives showed an interaction effect and temperature effect on the concentration on dehydroascorbic acid differed depending on day length.

It was observed that temperature could affect a compound in a species, but would not influence the same compound in another species. This could be because of their genetic makeup and how the plants are adapted to the environment, hence their metabolism and specific pathways would be affected differently. Therefore, the species effect was eliminated, but the light and temperature effect were rather discussed at a general level.

Generally, some plants produce more phenols or aromatics than others, and the composition and levels of phenolic compounds is also different in different species. Therefore, the summary on all the aromatics or total phenolics (Table 3.10) revealed that total phenolic levels across the species were higher under cold temperatures compared to warm temperatures, and under long day length compared to short day length. Since cold acclimation temperature ranges between 0-15°C, our data confirms result of a study by *Akula et al. (2011)* that reported that cold acclimation increased the production of phenolic compounds. Precisely, total phenolic level was highest in coriander under all the treatments. Beet greens showed the lowest concentration under cold temperature and short day length. Total phenolic content in chives remained the same across the treatments, unlike the other

species that showed fluctuations. Temperature showed no effect on total phenolic content in lettuce under long day length.

According to this present study, the citric acid cycle and sugars were represented, and it could be clearly observed how sugar metabolism changed with different temperatures and day lengths. It was also observed that warm temperature generally favoured the production of higher concentrations of citric acid in some species except for coriander, lettuce and wheatgrass. In general, warm temperatures on average under both day lengths increased sucrose concentration in most of the species compared to cold temperatures except for basil where almost no sucrose was produced under warm temperature. But on a more specific consideration, regarding chives and mint, cold temperature under long day length yielded a slightly higher concentration of sucrose even though warm temperature on average showed higher concentrations than the cold temperature. These results were contrary to a study conducted by *Guy et al. (1992)*, as they rather showed that sucrose concentrations were higher under low temperatures. *Guy et al. (1992)* grew their plants - spinach (*Spinacia oleracea*) at 25°C and then transferred them to a constant 5°C under 14 days. Their effects were based on cold acclimation, while the cold temperature in our study would not result in such dramatic changes, hence explaining the disparity in both results.

It was observed in our study that sucrose concentration was generally higher than fructose and glucose concentrations in the species, as also shown by *(Guy et al., 1992)*, except for basil. The difference was such that at cold temperature sucrose level in basil was lower than glucose, but higher than fructose when temperature increased. Sucrose level is generally higher because it is the major transport compound in higher plants produced by photosynthesis, while glucose and fructose are intermediate compounds. A very recent study by *Shwerif (2014)* also reported that low temperature increased the level of sucrose, fructose and glucose. Contrary to the findings of *Guy et al. (1992)* and ours, *Shwerif (2014)* reported that fructose showed the highest concentration compared to glucose and sucrose in lettuce.

Apart from light and temperature, other factors can also influence the levels of compounds. *Jeleń et al. (2015)* therefore explained that the detection of volatile compounds can also be influenced by extraction time, method, fibre type and extraction temperature. This explains why there can be disparities in results sometimes. Therefore, it is imperative to prolong extraction time, so that most of the volatiles can be eluted and detected. *Omer et al. (2008)* also reported that environmental conditions, growing conditions, origin of the plant, and genotype could greatly influence the aromatic character and chemical composition of species e.g. basil. These same conditions apply to other species, as have been reported in other studies that phytochemical accumulation and detection of compounds differed based on the conditions mentioned above. Hence, compound detection in species could also be based on the different chemotypes.

The loading plot from the PCA analysis based on 17 aromatics showed that all the compounds were grouped on the positive first component axis, showing that there is a similar effect in many of the samples. We noticed 3 groups, such that benzyl isothiocyanate and (E)-ferulic acid clustered together, rosmarinic acid, salicylic acid glucopyranoside, (E)-4-caffeoylquinic acid and chlorogenic acid clustered together and luteolin, (E)-caffeic acid and dihydroxyphenylalanine also clustered together. The cluster pattern indicated that those compounds could be closely correlated, i.e. similarly affected by different day length and

temperature treatments. PCA on the aromatics showed a strong variation in the data set. There was a 58.5% variation in the first component, indicating a high spread of the samples along that axis, while there was just a 9% variation in the second component.

In general, the PCA analysis on the aromatics did not reveal clear differences or similarities related to temperature and day length treated samples. Thus, they were not conclusive because of no clear clustering. Therefore, a further step was taken to carry out the PCA on the whole data set, to receive a better picture of how photoperiod and temperature influenced metabolite distribution (loading plot) and sample clustering (score plot) as shown in Figures 3.49 and 3.50.

Figure 3.49 showed metabolite distribution (loading plot), as the species variation was eliminated in the data set to have the treatment effect, and to show the major compounds. Most of the metabolites clustered on the positive first component and negative second component axes. There was low variation in the data set, such that the first component showed a 29.4% variation in metabolite distribution, while the second component showed a 12.9% variation in metabolite distribution. Figure 3.50 showed sample clustering (score plot) with species variation included with the same variations as the loading plot. Species variation was depicted, and to some extent within species, and clustering was based on day length and temperature. Therefore, these figures helped to describe the overall effect of the treatments on metabolism and general physiological effects across all species studied.

The discussion so far, explained in a general manner how photoperiod and temperature affected the metabolites in the varied species. For a better understanding regarding this effect, I couldn't explain one treatment separately without mentioning the other because the effects of light and temperature may not be independent, i.e. the design for this study merged temperature and photoperiod treatments. Meaning that if warm temperature produced considerable amounts of metabolites, I further had to specify under which photoperiod.

Therefore, our further discussion was based on whether the metabolites accumulated highest under 15°C and 12 h, under 15°C and 18 h, under 25°C and 12 h, or under 25°C and 18 h. Given that some metabolites in species are affected by different treatments we can therefore set our growth chambers or other growth mediums by choosing the different growing conditions to favour what we want or need from the herb. A good example of this idea and what I intend to present further, is seen in a recent study by *Engelen et al., (2006)* on watercress, where they reported that long day length and 10°C or 15°C favoured the concentration of the aromatic glucosinolate glucornasturtiin (phenethylglucosinolate), but yielded low fresh weight, while long day length and 20°C or 25°C increased fresh weight but reduced the concentration of glucornasturtiin. Under short days, glucornasturtiin concentration was low, and 10°C or 15°C favoured higher concentrations of this compound. Therefore, growth conditions can be set based on what the grower desires. The following discussion was done in an analogous way to suggest conditions that were best for higher levels of the important metabolites in this study.

## 4.2. Optimal growth conditions for the production of the selected volatiles

### 4.2.1. Cold temperature and short day length treatment (15°C and 12 h)

Amongst the selected volatiles, very few accumulated in elevated levels under the cold temperature, and different volatiles also showed distinct levels depending on photoperiod. The cold temperature and short day treatment was favourable for just a few volatile compounds. These were hexanal, decanal, pentanal and acetic acid all from chives.

Most volatiles in chives show higher levels under cold temperatures, due to the fact that stress might cause the plant to produce more of certain compounds because of the acclimation to the stress. This is confirmed by *Akula et al. (2011)* as they reported that low temperatures can impose a series stresses on plants.

### 4.2.2. Cold temperature and long day length treatment (15°C and 18 h)

Under this treatment, dodecanal from coriander, (E)-2-hexenal and 2-phenylethyl alcohol from chives were abundant. Under the cold treatment, some compounds showed a great reduction in their levels when day length was reduced, e.g. (E)-2-hexenal dropped about 3 times, acetic acid dropped about 3 times, and 2-phenylethyl alcohol dropped about 4 times. Under warm temperature in general, 2-phenylethyl alcohol was not produced at all. So, this compound shows elevated levels specifically under cold temperature (*Akula et al. 2011)*.

### 4.2.3. Warm temperature and short day length treatment (25°C and 12 h)

Most of the selected compounds showed very high levels under the warm temperature. Some increased under shorter photoperiods, while others increased under longer photoperiods. Under this treatment, methyl eugenol, piperitenone oxide, (E)-2-dodecenal, (Z)-9-tetradecenal and 1,8-cineole (from mint), dimethyl sulphide and 3-methylbutanal showed very high levels. Just as the cold treatment, it is important to note that some of the compounds under the same temperature showed a tremendous decrease in level when day length was decreased, e.g. 1,8-cineole dropped about 13 times, eugenol level dropped about 4 times, alpha-bergamotene dropped about 4.4 times, (Z)-9-tetradecenal dropped about 3 times and estragole dropped about 2.5 times.

### 4.2.4. Warm temperature and long day length treatment (25°C and 18 h)

Many volatiles from mint and basil showed higher levels under this treatment, like estragole, eugenol, alpha-bergamotene, limonene, carvone, beta-myrcene, decanal (from coriander), germacrene and 1,8-cineole (from basil). Our finding agreed with that of *Bali et al. (2015)* who reported in their study that long day lengths and high temperatures accumulated oil production in basil. Piperitenone oxide on the other hand decreased about 1.5 times, when day length was increased. Decanal in coriander showed higher levels under warm temperature and long day length, but in chives, levels were enhanced under cold temperature and short day length. Also, 1,8-cineole in mint and basil were best produced under warm temperature but in mint short day length was preferred while long day length was preferred in basil. Decanal and (E)-2-hexenal volatiles were both found in chives and coriander. Decanal was present in very high amounts in coriander compared to very low levels in chives. On the other hand, (E)-2-hexenal was present in lower amounts in coriander compared to chives. Limonene and 1,8-cineole were also both found in basil and mint. Limonene was more

abundant in mint and found in extremely low levels in basil, while 1,8-cineole was extremely low in mint and abundant in basil. Therefore, consuming more of mint benefits the consumer with more of limonene, while consuming more of basil benefits the consumer with more of 1,8-cineole. The interaction effects shown by (E)-hexanal in basil and (Z)-9-tetradecanal in coriander depicted that the effect of temperature on the concentration of both volatiles differed depending on the duration of day length.

## 4.3. Optimal growth conditions for the production of the selected phenolics

Out of the 17 identified aromatics in our study, 10 were selected due to their health beneficial properties. These were; (E)- caffeic acid, (E)-ferulic acid, dehydroascorbic acid, chlorogenic acid, rosmarinic acid, luteolin, (E)-sinapic acid, benzoic acid, benzyl isothiocyanate and (E)-4-caffeoylquinic acid. These compounds were present in all the species and accumulated differently among the species according to the different growth conditions. As mentioned earlier, although the compounds were common to all the species, our focus and objective was not to compare their levels in the varied species, but simply to state how photoperiod and temperature influenced their concentrations in each species. Nevertheless, since they are all important plant phenolics with beneficial health effects, it may be important and informative to point out which amongst the species to choose if we desire to obtain higher amounts of the said compound. Phytochemical accumulation is generally influenced by temperature and literature on some species are limited. Nevertheless, *Rajashekar et al. (2009)* reported that low temperatures have shown to enhance the accumulation of phenolic compounds in different plant species, and *Olenichenko et al. (2005)* also reported that cold hardening could increase phenolic accumulation in plants, like in rape leaves and winter wheat leaves.

In this present study, cold temperatures generally yielded higher phenolic levels, while long day lengths also produced higher phenolic levels across the species. Our study showed that warm temperatures yielded higher phenolic contents in lettuce, but *Lee et al. (2015)* showed in their study that lettuce exposed to low temperature accumulated higher levels of phenolic compounds. *Oh et al. (2009)* also reported in their study that certain phenolic compounds in lettuce like chlorogenic acid were induced by the environmental stresses used in their study. (E)-caffeic acid and (E)-ferulic acid were highly abundant in most of the species, and temperature and light influenced the accumulation of both compounds differently.

(E)-caffeic acid was present in chives, coriander, mint, lettuce, and wheatgrass, and it was observed that cold temperature yielded higher concentrations of this compound in chives and mint compared to warm temperature, while warm temperature yielded higher concentrations of the compound in coriander, lettuce and wheatgrass. Also, long day length was generally more favourable for higher amounts than short day lengths. Therefore, lettuce is a good plant source amongst the presented species providing higher concentrations of (E)-caffeic acid. (E)-ferulic acid was present in chives, coriander, lettuce, peppercress and beet greens. We observed that out of the 5 species, cold temperature favoured higher amounts in 3 species, while longer day lengths yielded higher amounts than short day lengths. Coriander is best recommended among the species presented because of its higher content in (E)-ferulic acid. Dehydroascorbic acid was presented in chives and wheatgrass, and both had higher concentrations under cold temperature and long day length. The concentration of dehydroascorbic acid in chives is twice the amount found in wheatgrass. Chlorogenic acid was

found in coriander and lettuce and both also showed higher concentrations under cold temperature and long day length, with coriander showing much greater concentrations of this compound than lettuce. Rosmarinic acid was found in basil and produced higher amounts under cold temperature and short day length, while mint showed higher concentrations under warm temperature and long day length. In addition, chlorogenic acid level was higher in basil compared to mint. Luteolin was also present in basil and wheatgrass, and both showed higher concentrations under warm temperature and short day length, with wheatgrass concentration almost being three times the concentration detected in basil. (E)-sinapic acid in peppercress showed higher concentrations under cold temperature and short day length, benzoic acid in beet greens showed higher concentrations under warm temperature and long day length, benzyl isothiocyanate showed higher concentrations under warm temperature and short-day length and (E)-4-caffeoylquinic acid showed higher concentrations under cold temperature and long day length.

Unfortunately, there are very limited studies done on the above discussed metabolites, with regard to the effect of day length and temperature, hence comparing our results in this section wasn't feasible. This study showed that light and temperature stress stimulated the production of certain compounds that were involved in the stress response. The concentration of some compounds probably increased because the growth conditions might have favoured them, while the concentration of other compounds decreased probably because the growth conditions weren't favourable for their production. This is because the regulation of plant metabolites is dependent on the stress condition involved, so plants will generally produce high amounts to tolerate these stress conditions. Each plant normally has an optimum temperature for its proper growth and development, the reason why conditions favouring a plant can be stressful for another plant. Thus, the plant feels stress when temperatures are either high (thermotolerance) or low (cold acclimation). These stresses modify the metabolism of the plant and causes imbalance. According to *Yadav (2010),* cold stress (0-15°C) can induce injury after the plant is exposed for about 48 to 72 h. This is seen with poor germination, stunted seedlings and yellowing of leaves. These symptoms were seen in our plants; thus, it is not conclusive if the discrepancies in some of our results were due to seed problems or cold stress. Since studies on cold acclimation are limited for most species, *Cook et al. (2004)* reported that studies on *Arabidopsis* presents the best understood cold response pathway with a central role in cold acclimation. In their study, they examined the changes that occurred in the *Arabidopsis* metabolome in response to low temperature while in a similar way, we also examined in our study the effect of low temperature on metabolite composition.

## 4.4. Health benefits of some important volatiles

### 4.4.1. Limonene

*Sun* (*2007*) reported that the Code of Federal Regulation listed D-limonene as a safe flavouring agent. This compound has been used to add flavour and fragrance in chewing gums, soap, beverages and perfumes. In the same study, it was stated that D-limonene has been used to dissolve cholesterol with gallstone, relieves heartburn, and has been clinically shown to have chemo-preventive properties against cancers.

### 4.4.2. 1,8-cineole

1,8-cineole is also known as eucalyptol with a fresh fragrance. *Miyazawa et al. (2001)* reported that this compound could be used as a disinfectant, an analgesic, food flavouring, and may be used to treat cough, muscular pain, rheumatism and asthma.

### 4.4.3. Estragole

*Silva-Alves et al. (2013)* found out that estragole could be used as an additive in pharmaceutical cosmetics and food industries. They further reported that the compound could show antioxidative and anti-microbial effects and was suspected to show local anaesthetic properties.

## 4.5. Health benefits of some important phenolics

### 4.5.1. Caffeic acid

In a very recent finding, *Dhungyal et al. (2014)* reported that caffeic acid has pharmacological effects such that it could inhibit the proliferation of cancer cells, shows antimicrobial and anti-inflammatory properties and could also be considered a therapeutic agent against diabetes.

### 4.5.2. Rosmarinic acid

Rosmarinic acid is a caffeic acid ester. *Stansbury (2014)* reports this compound as having anti-inflammatory and antioxidant effects. The study further explains that in vivo and in vitro studies have shown this compound to prevent and treat allergies, especially in respiratory allergies, asthma and lung diseases. *Al-Dhabi et al. (2014*) described in their finding that rosmarinic acid could be used to reduce cardiovascular risk when connected with insulin resistance, and could also treat diabetic neuropathy. In a recent study, *Aung et al. (2010)* showed that rosmarinic acid was used as an antidote in Japan for snake poisoning. Hence, it could neutralise snake venoms and inhibit haemorrhage.

### 4.5.3. Salicylic acid

*Arif (2015)* reported that salicylic acid is a lipophilic compound that removes intercellular lipids. It has antihyperplastic effects on the epidermis, hence can be used as a peeling agent that underlies dermal tissues without causing inflammations. *Arif (2015)* further reports that this compound has shown anti-inflammatory and antimicrobial properties. The same author also described salicylic acid as a peeling agent, thus an important agent in cosmetic dermatology that rejuvenates the skin, and treats skin disorders like acne vulgaris, melasma and photodamage. *Bahta (2017)* reported that salicylic acid can be used as a shampoo ingredient to control dandruff while amino salicylic acid can be used to treat tuberculosis.

### 4.5.4. Benzoic acid

*Arif (2015)* described that when benzoic acid is used in combination with salicylic acid in Whitfield's ointment, they show fungicidal properties. *Bahta* (*2017*) also indicates in his study that benzoic acid has been shown to be effective in treating foot skin diseases (foot worm) and skin fungi.

# 5. CONCLUSION

Light, photoperiod and temperature are principal factors affecting phytochemical content in plants, and the nutritional quality in vegetables can vary within seasons due to these factors. The role of vegetables in human health remain indisputable as they have shown to reduce the risk of many chronic diseases. Since antiquity, human health has benefited from secondary metabolites, thus they play vital roles in the healthcare system because of their therapeutic and aromatic properties.

In conclusion, photoperiod and temperature affected the metabolites in the species differently as expected. Based on results from metabolite profiling, it was shown that temperature had a stronger effect on metabolite concentration in basil and wheatgrass than day length and interaction, while day length had a stronger effect on metabolite concentration in beet greens, peppercress and mint compared to temperature and interaction. Light and temperature showed extremely strong effects on lettuce and under interaction, while chives and coriander showed no effect under day length and temperature. Based on data from SPME extraction and analysis, temperature influenced some terpenes in mint volatiles, but day length had no effect on the volatiles except on nonanal. Day length and temperature had no influence on volatiles in chives and coriander, while the only volatile in basil that was affected by day length and temperature was (E)-2-hexenal and under interaction.

The principal component analyses with the score and loading plots on the 17 aromatics did not show any clear difference or similarity across day length and temperature treatments. The score and loading plots on the whole data set clearly showed species differences and clustering based on day length and temperature treatments.

Growing conditions, plant origin, genotype, extraction method and time are other factors that also greatly influence the concentration of plant metabolites and volatile detection, the reason why some predominant compounds and volatiles detected in some species in other studies were not predominant in the same species in our study. E.g. a compound like menthol was not detected in mint in our study but has been detected in other studies.

Therefore, this study has shown that the health and flavour related compounds were affected differently by the growth conditions across the species, such that cold temperatures generally yielded higher phenolic levels than warm temperatures, while long day lengths also yielded higher phenolic levels than short day lengths. Regarding the volatiles, warm temperatures generally resulted in higher volatile levels compared to cold temperatures in the herbs, meanwhile long day lengths led to higher volatile levels in coriander and basil, and short day lengths yielded higher volatile levels in mint and chives. Thus, this knowledge helps growers to choose their growing conditions for the species according to what type of phytochemical is desired.

Some of the detected metabolites have been documented to have potential for therapeutic uses. Thus, choosing specific growth conditions can also influence the potential therapeutic properties of greens and herbs.

# 6. CHALLENGES AND LIMITATIONS

It is very imperative to note that I encountered some challenges during this study in the very beginning. First and foremost, a first experiment was done using Urban Cultivators http://www.urbancultivator.net/ with automated functions, but it was terminated because of the control problems of the chambers. Thus, the design was changed, and I had to run the experiment a second time and also encountered serious problems in seed germination. Most of the seeds took a very long time to germinate, which further affected the growth of the plants. At some point, there was no sign of growth, and the leaves of the very young seedlings turned yellow. Liquid fertilizer was applied to adjust this issue, but it did not boost much. This eventually affected the health of the plants and phytochemical production, consequently limiting the general usefulness and applicability of the results, because there were a lot of differences in the values even under the same treatment as was shown in Table 3.1. Many are the cases where two samples grown under the same light and temperature condition presented extremely different results, one value being extremely lower or higher than the other. This made it very difficult to know which value was more accurate.
It is also important to note that findings on temperature and especially on day length effects on metabolite concentration of the eight-target species in this present study was quite limited. This literature limitation is especially associated to the health related compounds that were chosen in our study.

# 7. RECOMMENDATIONS

Even though there are some positive confirmations with other studies, I strongly suggest that this study be repeated, whereby a first trial run should be done to test the germination efficiency of the seeds. Just as there have been many discrepancies in epidemiological studies, so did I encounter the same in this study. Nevertheless, I believe better results can be obtained if this study is replicated especially taking into consideration the seed variety and its origin. Also, perhaps focusing on few species at a time could be another option, as it permits the researcher to investigate each species in depth. Given that these metabolites play vital roles in health and flavour, it might be interesting to have more detailed researches on them across the species. However, this study serves as a guide to exploring these species in a more detailed approach when repeated in the future.
Also, it might be important to add that more studies be done on day length effects regarding important plant phytochemicals, because most studies have rather been carried out on light quality and intensity. Again, some studies could be carried out by changing temperature and photoperiod during growth to see how far phytochemical production could be enhanced. E.g. growing plants in ideal conditions for a week or two, then transferring them to more stressful conditions like very low temperature, higher photoperiod or both. This might help to increase the yields of some flavour and health related compounds, since most of these compounds are produced in response to stress conditions.

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# Appendix 1

Tables showing all detected volatiles in each herb species with their P-values. RT=retention time, RI= retention index. Significant value threshold is $P \leq 0.05$. With incomplete samples, the interaction effect couldn't be calculated by the software and was shown as NaN.

**Table A1:** Detected volatiles in coriander. None of the compounds were significantly affected.

| Compound | class | RT | RI | P-Temp | P-Light | P-Interaction |
|---|---|---|---|---|---|---|
| (E)-2-hexenal | aldehyde | 6.72 | 827 | 0.867 | 0.389 | NaN |
| (Z)-3-hexen-1-ol | alcohol | 6.78 | 840 | 0.538 | 0.835 | NaN |
| nonane | alcohol | 8.03 | 900 | 0.925 | 0.431 | NaN |
| linalool | monoterpene | 14.47 | 1086 | 0.968 | 0.643 | NaN |
| nonanal | aldehyde | 14.61 | 1091 | 0.325 | 0.814 | NaN |
| decanal | aldehyde | 18.15 | 1185 | 0.142 | 0.425 | NaN |
| (E)-2-decenal | aldehyde | 19.69 | 1252 | 0.448 | 0.520 | NaN |
| decanol | alcohol | 19.98 | 1275 | 0.503 | 0.620 | NaN |
| undecanal | aldehyde | 21.09 | 1307 | 0.488 | 0.213 | NaN |
| dodecanal | aldehyde | 24.21 | 1406 | 0.787 | 0.502 | NaN |
| (E)-2-dodecenal | aldehyde | 25.87 | 1445 | 0.513 | 0.921 | NaN |
| tridecanal | aldehyde | 26.86 | 1505 | 0.902 | 0.441 | NaN |
| (E)-2-tridecenal | aldehyde | 28.47 | 1541 | 0.945 | 0.825 | NaN |
| tetradecanal | aldehyde | 29.56 | 1596 | 0.707 | 0.515 | NaN |
| (E)-2-tetradecenal | aldehyde | 30.62 | 1645 | 0.811 | 0.672 | NaN |
| (Z)-9-tetradecenal | aldehyde | 31.24 | 1671 | 0.813 | 0.739 | NaN |
| (E)-2-hexadecenal | aldehyde | 33.58 | 1857 | 0.845 | 0.741 | NaN |
| (Z)-9-octadecenal | aldehyde | 34.02 | 2010 | 0.956 | 0.936 | NaN |
| (Z)-phytol | diterpene | 34.89 | 2086 | 0.662 | 0.486 | NaN |

UNIT: detector response x $10^{-6}$

**Table A2:** Detected volatiles in mint. The values in red indicate significantly affected compounds.

| Compound | Class | RT | RI | P-Temp | P-Light | P-Interaction |
|---|---|---|---|---|---|---|
| alpha-pinene | monoterpene | 9.12 | 932 | 0.038+ | 0.419 | 0.670 |
| sabinene | monoterpene | 10.35 | 967 | 0.020+ | 0.458 | 0.653 |
| beta-pinene | monoterpene | 10.53 | 972 | 0.061 | 0.607 | 0.476 |
| beta-myrcene | monoterpene | 10.87 | 983 | 0.303 | 0.559 | 0.777 |
| 3-octanol | alcohol | 11.08 | 989 | 0.142 | 0.084 | 0.673 |
| p-cymene | monoterpene | 12.04 | 1014 | 0.245 | 0.892 | 0.649 |
| limonene | monoterpene | 12.31 | 1022 | 0.163 | 0.715 | 0.436 |
| 1,8-cineole | monoterpene | 12.37 | 1027 | 0.017+ | 0.316 | 0.441 |
| (E)-beta-ocimene | monoterpene | 12.72 | 1039 | 0.087 | 0.369 | 0.397 |
| (Z)-sabinene hydrate | monoterpene | 13.54 | 1068 | 0.175 | 0.110 | 0.176 |
| terpinolene | monoterpene | 14.06 | 1079 | 0.631 | 0.444 | 0.727 |
| alpha-p-dimethyl styrene | aromatic | 14.20 | 1081 | 0.385 | 0.727 | 0.252 |

| | | | | | | |
|---|---|---|---|---|---|---|
| linalool | monoterpene | 14.47 | 1086 | 0.060 | 0.065 | 0.316 |
| nonanal | aldehyde | 14.62 | 1091 | 0.053 | 0.018- | 0.336 |
| (Z)-limonene oxide | monoterpene | 15.61 | 1131 | 0.389 | 0.788 | 0.735 |
| (Z)-3-hexenyl isovalerate | aliphatic ester | 18.83 | 1234 | 0.188 | 0.894 | 0.819 |
| carvone | monoterpene | 19.48 | 1246 | 0.290 | 0.670 | 0.558 |
| (Z)-carvone oxide | monoterpene | 19.72 | 1252 | 0.638 | 0.686 | 0.913 |
| (E)-carvone oxide | monoterpene | 19.79 | 1263 | 0.053 | 0.552 | 0.900 |
| isopiperitenone | monoterpene | 20.03 | 1271 | 0.017+ | 0.775 | 0.894 |
| menthyl acetate | monoterpene | 20.48 | 1287 | 0.026+ | 0.520 | 0.944 |
| piperitenone oxide | monoterpene | 23.22 | 1363 | 0.032+ | 0.304 | 0.434 |
| beta-caryophyllene | sesquiterpene | 24.64 | 1419 | 0.083 | 0.369 | 0.375 |
| beta-copaene | sesquiterpene | 25.28 | 1432 | 0.042+ | 0.705 | 0.587 |
| (Z)-muurola-3,5-diene | sesquiterpene | 25.75 | 1440 | 0.075 | 0.562 | 0.400 |
| (E)-muurola-3,5-diene | sesquiterpene | 26.31 | 1454 | 0.022+ | 0.374 | 0.397 |
| germacrene D | sesquiterpene | 27.09 | 1476 | 0.016+ | 0.429 | 0.405 |
| beta-himachalene | sesquiterpene | 27.21 | 1499 | 0.005+ | 0.625 | 0.239 |
| calamenene | sesquiterpene | 27.29 | 1504 | 0.031+ | 0.577 | 0.431 |
| alpha-cadinene | sesquiterpene | 27.71 | 1533 | 0.063 | 0.590 | 0.592 |

UNIT: detector response x $10^{-6}$

**Table A3:** Detected volatiles in chives. The values in red indicate significantly affected compounds.

| Compound | class | RT | RI | P-Temp | P-Light | P-Interaction |
|---|---|---|---|---|---|---|
| dimethylsulfide | sulfide | 2.34 | 532 | 0.064 | 0.636 | NaN |
| acetic acid | acid | 2.62 | 610 | 0.533 | 0.238 | NaN |
| 3-methylbutanal | aldehyde | 3.17 | 651 | 0.726 | 0.729 | NaN |
| 2-methylbutanal | aldehyde | 3.26 | 654 | 0.799 | 0.542 | NaN |
| pentanal | aldehyde | 3.63 | 663 | 0.049- | 0.018- | NaN |
| 3-hydroxy-2-butanone | ketone | 3.77 | 705 | 0.353 | 0.321 | NaN |
| 1,2-propanediol | alcohol | 4.24 | 719 | 0.153 | 0.099 | NaN |
| 1-pentanol | alcohol | 4.76 | 764 | 0.883 | 0.639 | NaN |
| 2,3-butanediol | alcohol | 5.03 | 783 | 0.454 | 0.177 | NaN |
| hexanal | aldehyde | 5.42 | 788 | 0.002- | 0.025- | NaN |
| 2-methylpyrimidine | pyrimidine | 6.03 | 799 | 0.608 | 0.500 | NaN |
| 3-methylbutanoic acid | acid | 6.54 | 839 | 0.163 | 0.556 | NaN |
| (E)-2-hexenal | aldehyde | 6.73 | 827 | 0.334 | 0.515 | NaN |
| 2-methylbutanoic acid | acid | 6.93 | 862 | 0.130 | 0.300 | NaN |
| 2-hydroxyethyl vinyl sulfide | sulfide | 7.59 | 883 | 0.130 | 0.392 | NaN |
| butyrolactone | ketone | 8.30 | 902 | 0.172 | 1.000 | NaN |
| methyl-1-propenyl disulfide | sulfide | 9.24 | 912 | 0.014+ | 0.073 | NaN |
| benzaldehyde | aromatic | 9.98 | 954 | 0.437 | 0.838 | NaN |
| dimethyl trisulfide | sulfide | 10.24 | 961 | 0.024+ | 0.170 | NaN |

| | | | | | | |
|---|---|---|---|---|---|---|
| 6-methyl-5-hepten-2-one | ketone | 10.64 | 986 | 0.144 | 0.102 | NaN |
| octanal | aldehyde | 11.27 | 1006 | 0.317 | 0.595 | NaN |
| 2-ethyl-1-hexanol | alcohol | 12.10 | 1028 | 0.528 | 0.631 | NaN |
| benzyl alcohol | aromatic | 12.33 | 1032 | 0.043+ | 0.150 | NaN |
| benzeneacetaldehyde | aromatic | 12.63 | 1042 | 0.043+ | 0.077 | NaN |
| (E, E)-3,5-octadien-2-one | ketone | 13.45 | 1072 | 0.915 | 0.253 | NaN |
| nonanal | aldehyde | 14.61 | 1091 | 0.587 | 0.422 | NaN |
| 2,6-dimethyl-cyclohexanol | alcohol | 14.85 | 1110 | 0.286 | 0.908 | NaN |
| 2-phenylethyl alcohol | aromatic | 14.91 | 1115 | 0.368 | 0.500 | NaN |
| methyl 2-propenyl trisulfide | sulfide | 16.52 | 1145 | 0.026+ | 0.356 | NaN |
| methyl pentyl disulfide | sulfide | 16.68 | 1154 | 0.160 | 0.542 | NaN |
| decanal | aldehyde | 17.89 | 1185 | 0.561 | 0.477 | NaN |
| hexanoic acid butyl ester | aliphatic ester | 18.28 | 1197 | 0.761 | 0.670 | NaN |
| allyl trisulfide | sulfide | 22.13 | 1307 | 0.273 | 0.272 | NaN |
| dodecanal | aldehyde | 24.01 | 1406 | 0.236 | 0.853 | NaN |
| beta-ionone | ketone | 26.06 | 1492 | 0.139 | 0.669 | NaN |

UNIT: detector response x $10^{-5}$

**Table A4:** Detected volatiles in basil. The values in red indicate significantly affected compounds.

| Compound | Class | RT | RI | P-Temp | P-Light | P-Interaction |
|---|---|---|---|---|---|---|
| (E)-2-hexenal | aldehyde | 6.75 | 827 | 0.013- | 0.024+ | 0.018- |
| alpha-pinene | monoterpene | 9.12 | 932 | 0.411 | 0.314 | 0.470 |
| sabinene | monoterpene | 10.36 | 967 | 0.384 | 0.306 | 0.468 |
| beta-pinene | monoterpene | 10.54 | 972 | 0.488 | 0.291 | 0.515 |
| beta-myrcene | monoterpene | 10.85 | 983 | 0.401 | 0.280 | 0.382 |
| 3-carene | monoterpene | 11.54 | 1013 | 0.159 | 0.761 | 0.422 |
| limonene | monoterpene | 12.22 | 1022 | 0.100 | 0.389 | 0.222 |
| 1,8-cineole | monoterpene | 12.33 | 1027 | 0.404 | 0.335 | 0.471 |
| (E)-beta-ocimene | monoterpene | 12.74 | 1039 | 0.512 | 0.320 | 0.538 |
| (Z)-sabinene hydrate | monoterpene | 13.57 | 1068 | 0.276 | 0.266 | 0.506 |
| terpinolene | monoterpene | 14.09 | 1079 | 0.705 | 0.431 | 0.268 |
| linalool | monoterpene | 14.51 | 1086 | 0.197 | 0.222 | 0.312 |
| camphor | monoterpene | 16.14 | 1148 | 0.304 | 0.201 | 0.368 |
| 4-terpineol | monoterpene | 17.16 | 1177 | 0.337 | 0.278 | 0.423 |
| estragole | aromatic | 17.81 | 1192 | 0.177 | 0.397 | 0.461 |
| bornyl acetate | monoterpene | 20.45 | 1289 | 0.560 | 0.180 | 0.562 |
| eugenol | aromatic | 22.52 | 1362 | 0.088 | 0.143 | 0.243 |
| methyleugenol | aromatic | 24.10 | 1402 | 0.449 | 0.905 | 0.711 |
| alpha-bergamotene | sesquiterpene | 24.84 | 1433 | 0.442 | 0.289 | 0.660 |
| (E)-β-farnesene | sesquiterpene | 25.47 | 1451 | 0.990 | 0.287 | 0.850 |
| alpha-caryophyllene | sesquiterpene | 25.80 | 1460 | 0.083 | 0.149 | 0.257 |

| | | | | | | |
|---|---|---|---|---|---|---|
| germacrene D | sesquiterpene | 26.23 | 1476 | 0.306 | 0.289 | 0.568 |
| patchoulene | sesquiterpene | 26.61 | 1486 | 0.134 | 0.298 | 0.959 |
| β-elemene | sesquiterpene | 26.76 | 1494 | 0.571 | 0.247 | 0.693 |
| alpha-selinene | sesquiterpene | 27.06 | 1499 | 0.156 | 0.184 | 0.390 |
| gamma-murolene | sesquiterpene | 27.26 | 1502 | 0.673 | 0.150 | 0.617 |
| tau-cadinol | sesquiterpene | 30.41 | 1625 | 0.109 | 0.131 | 0.364 |
| beta-eudesmol | sesquiterpene | 30.77 | 1648 | 0.589 | 0.458 | 0.636 |
| (E)-methyl-p-methoxycinnamate | aromatic | 31.12 | 1655 | 0.429 | 0.294 | 0.419 |

UNIT: detector response x $10^{-5}$

# Appendix 2

Figures of loading and score plots of the detected volatiles from each herb species.

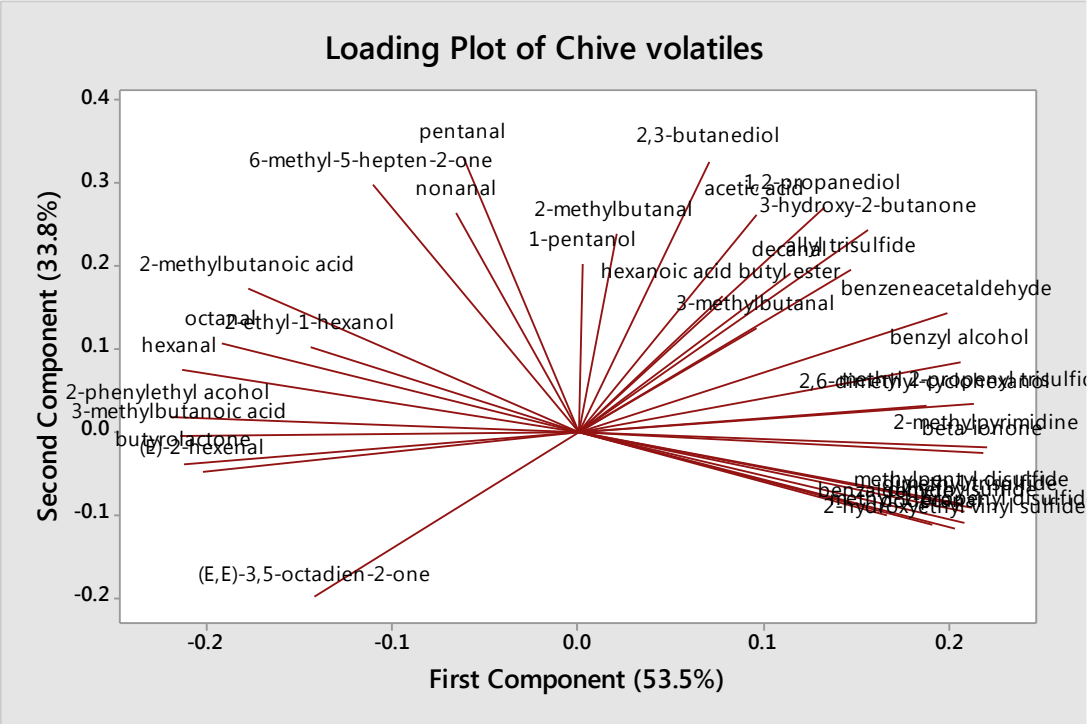


**Figure A1:** Loading plot showing the variation of volatiles in chives.

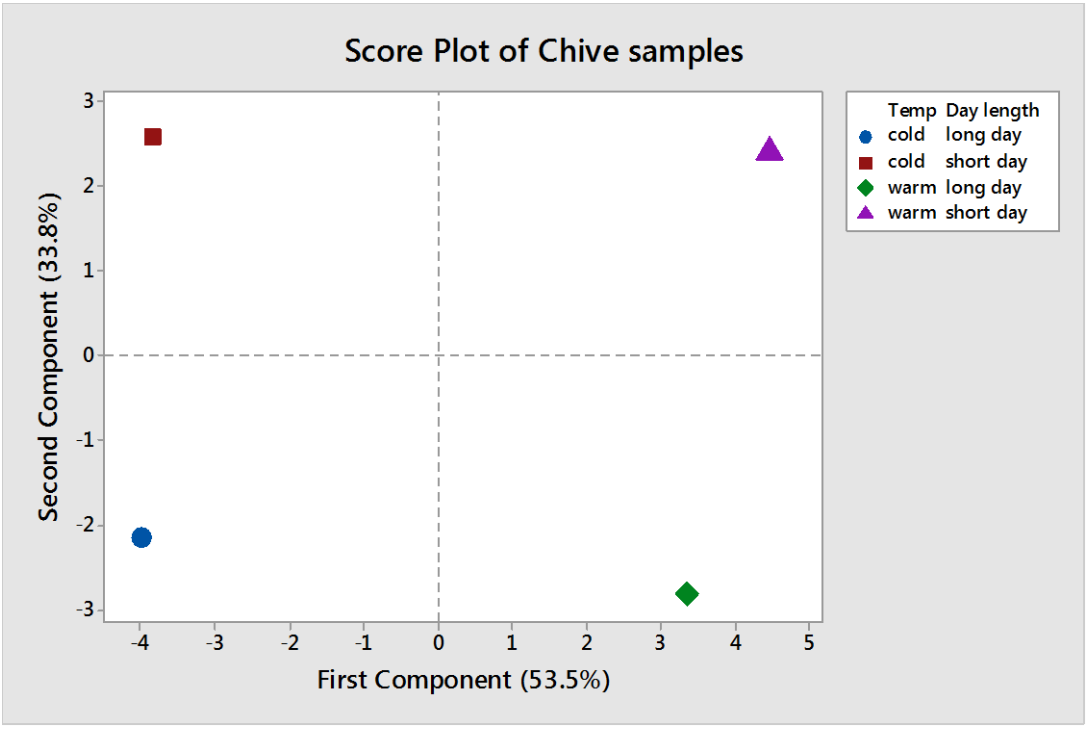


**Figure A2:** Score plot showing light and temperature effect on the distribution of chive samples.

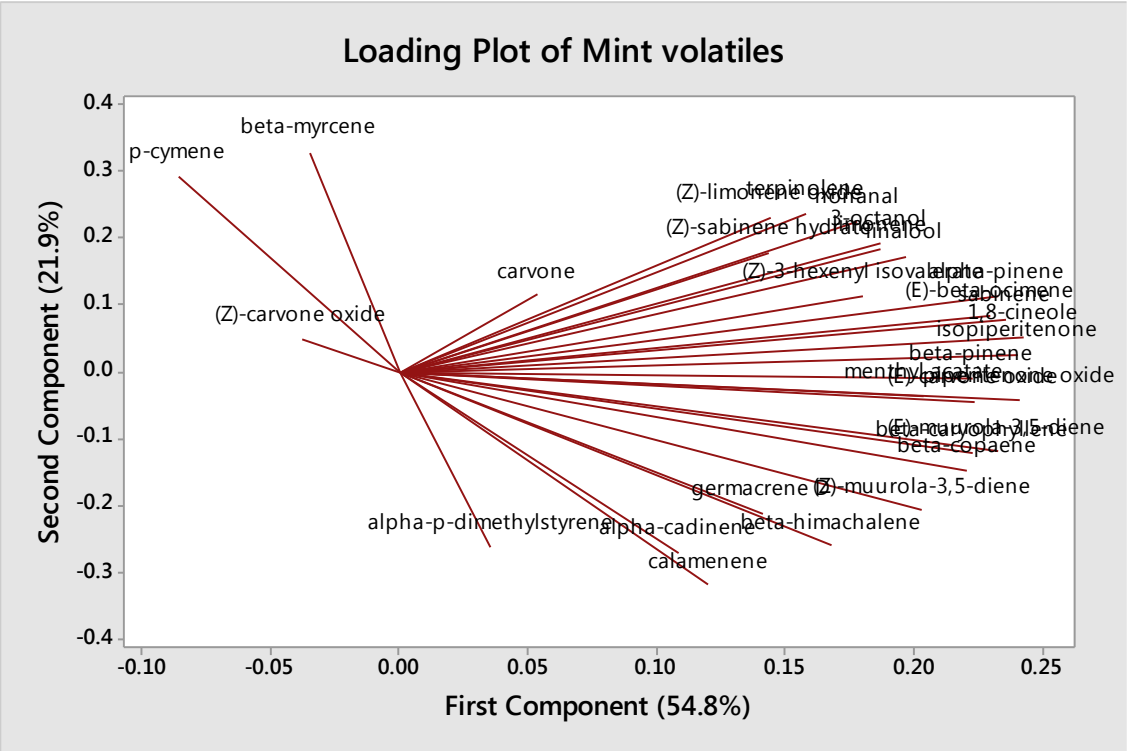


**Figure A3:** Loading plot showing the variation of volatiles in mint.

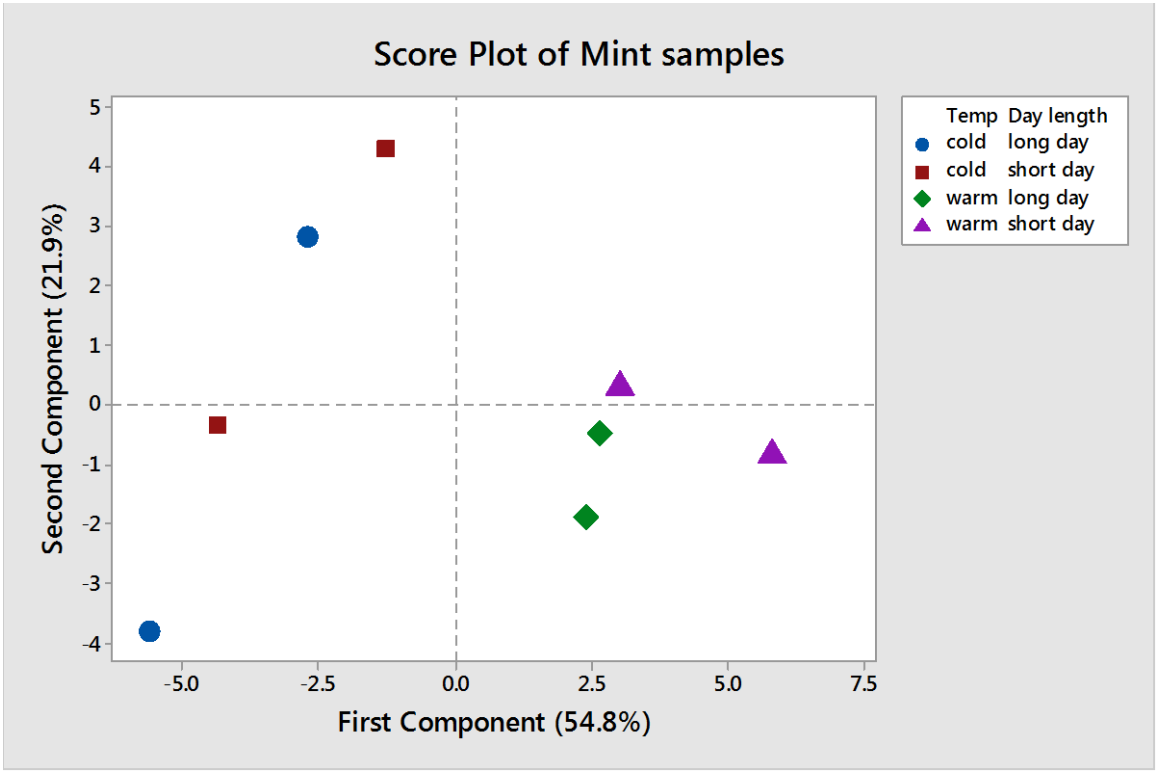


**Figure A4:** Score plot showing how light and temperature affect the distribution of mint samples.

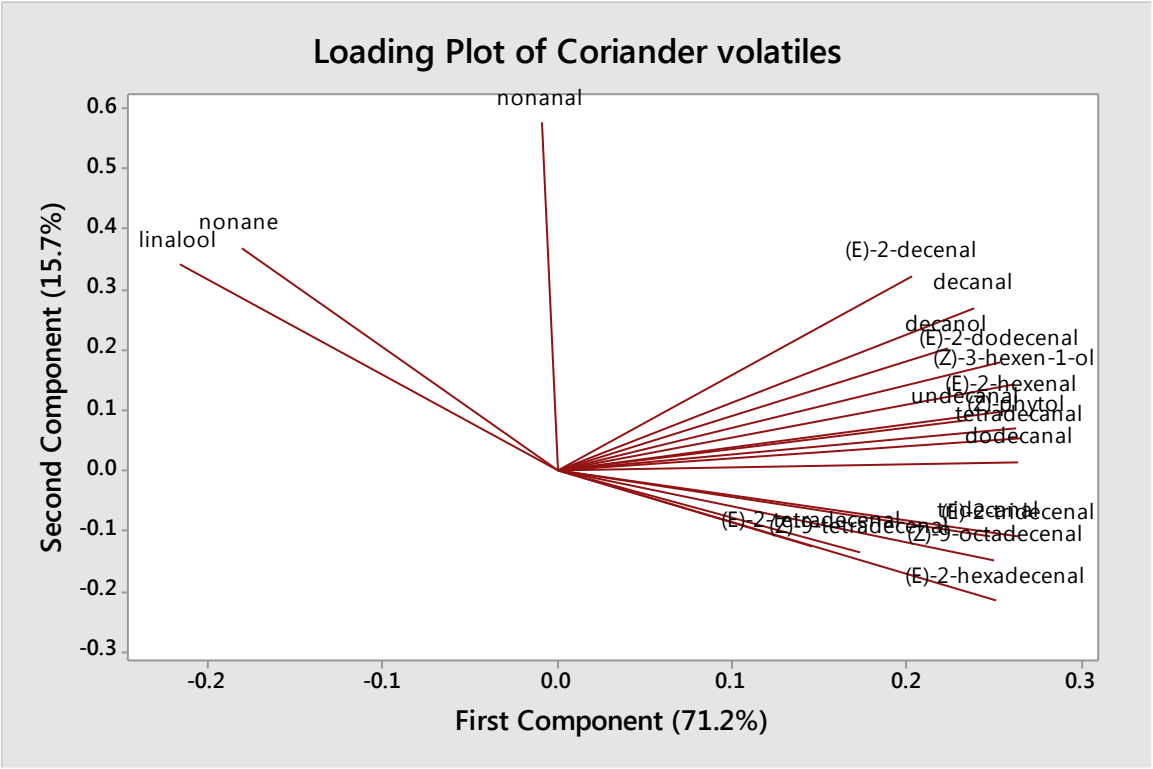


**Figure A5:** Loading plot showing the variation of volatiles in coriander.

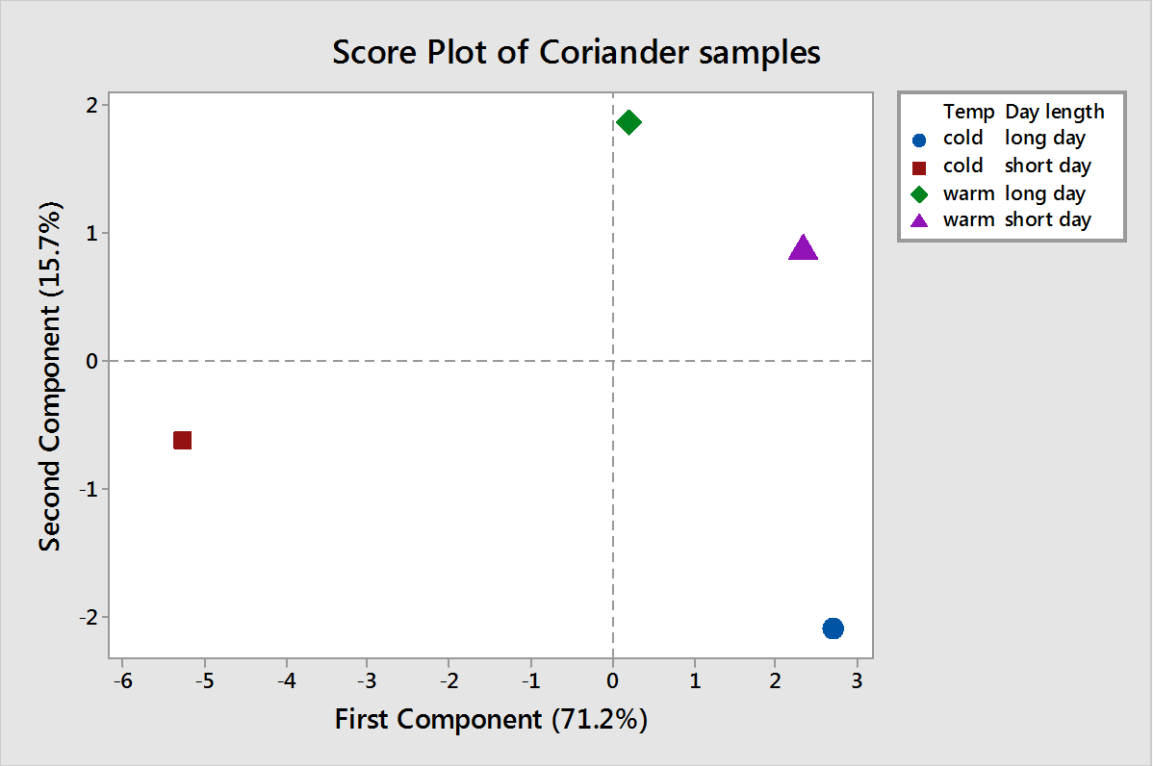


**Figure A6:** Light and temperature effect on the distribution of coriander samples.

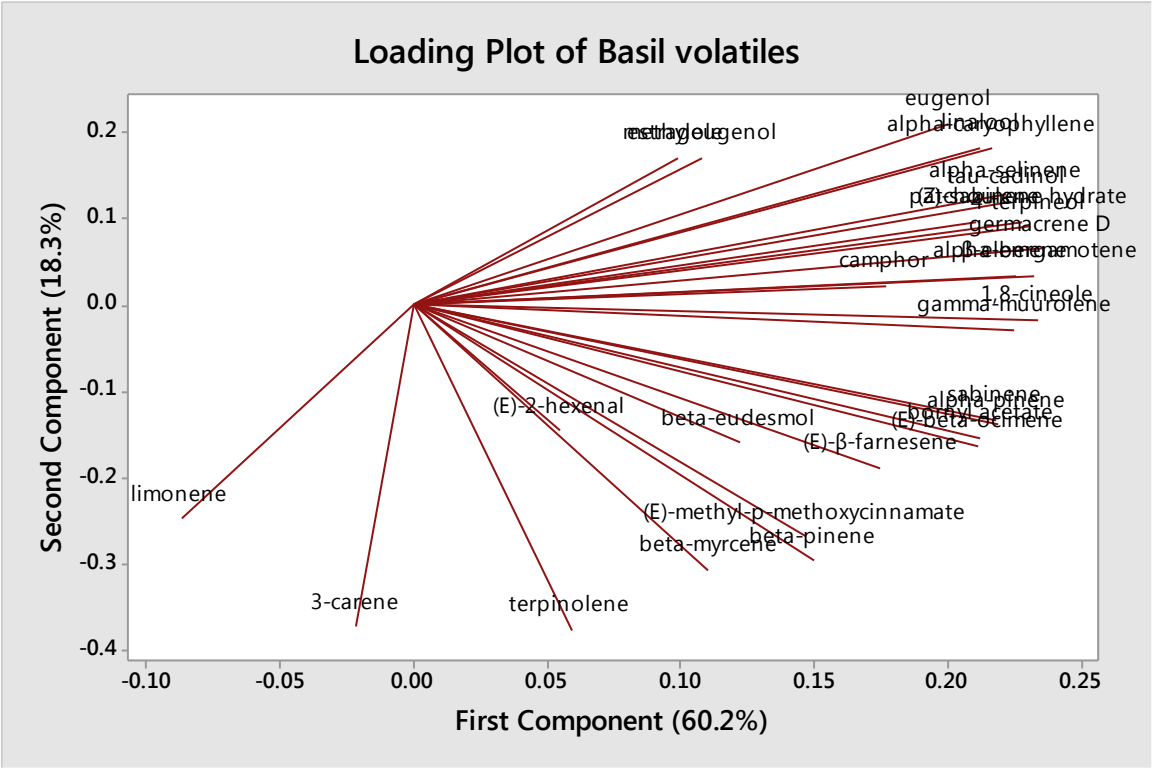


**Figure A7:** Loading plot showing the variation of volatiles in basil.

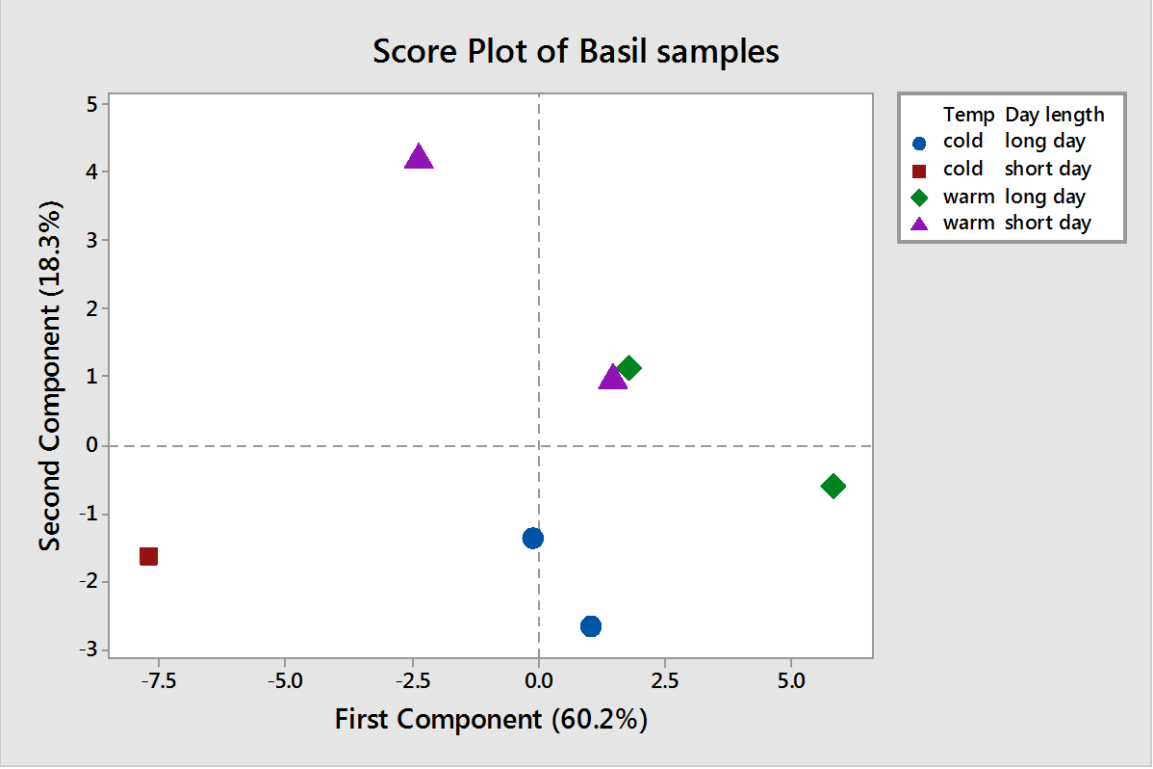


**Figure A8:** Light and temperature effect on the distribution of basil samples.

# Appendix 3

Summary of the 38 selected compounds in all species. Each species is represented to reveal compounds that showed significant effects. Values in red indicate significant effects. The "**+**" sign besides the P-values (temp and light) indicates where high temp or long day yields more of the compound, and the "**-**" indicates a lower yield. The "**+**" or "**-**" under interaction indicates a positive or negative interaction.

**Table A5:** Wheatgrass. Aro=aromatic, aa=amino acid, ac=acid, ter=terpene, hex=hexose, and disac=disaccharide.

| Compound | Class | RT | RI | P-temp | P-light | P-interaction |
|---|---|---|---|---|---|---|
| benzoic acid | aro | 16.55 | 1251.31 | 0.0553 | 0.0172+ | 0.0741 |
| benzyl isothiocyanate | aro | 20.02 | 1361.00 | 0.0002+ | 0.0000+ | 0.8673 |
| phenylalanine | aa | 26.31 | 1629.63 | 0.0396- | 0.0249- | 0.0328+ |
| shikimic acid | ac | 30.35 | 1794.63 | 0.0002+ | 0.7646 | 0.1940 |
| citric acid | ac | 30.49 | 1804.71 | 0.0911 | 0.5036 | 0.6715 |
| dehydroascorbic acid dimer | ac | 31.11 | 1838.43 | 0.0028- | 0.0306+ | 0.0124- |
| fFructose3 | hex | 31.55 | 1856.23 | 0.7124 | 0.0003+ | 0.1636 |
| glucose1 | hex | 32.07 | 1881.63 | 0.7506 | 0.3293 | 0.0321- |
| phenolic1 | aro | 32.14 | | 0.0002+ | 0.0000+ | 0.8673 |
| pPhenolic2 | aro | 32.24 | | 0.0002+ | 0.0000+ | 0.8673 |
| tyrosine | aa | 32.91 | 1932.60 | 0.0505 | 0.0911 | 0.0840 |
| p-Coumaric acid | aro | 32.99 | 1947.53 | 0.0001+ | 0.0005+ | 0.5231 |
| dihydroxyphenylalanine | aro | 35.24 | 2052.83 | 0.0107+ | 0.3452 | 0.1480 |
| (E)-Ferulic acid | aro | 35.80 | 2097.85 | 0.0000+ | 0.0348+ | 0.0041- |
| (E)-Caffeic acid | aro | 36.52 | 2135.55 | 0.0075+ | 0.2116 | 0.0796 |
| phytol | ter | 36.94 | 2131.46 | 0.0002+ | 0.0837 | 0.3188 |
| (E)-Sinapic acid | aro | 37.79 | 2249.43 | 0.2396 | 0.2899 | 0.8822 |
| phenolic3 | aro | 37.85 | | 0.5038 | 0.7594 | 0.9866 |
| rosmarinic acid deriv1 | aro | 40.06 | | 0.0002+ | 0.0000+ | 0.8673 |
| salicylic acid glucopyranoside | aro | 40.62 | 2568.38 | 0.0009+ | 0.8866 | 0.0921 |
| sucrose | disac | 40.98 | 2628.87 | 0.0013+ | 0.0002+ | 0.7675 |
| quinic acid deriv1 | aro | 42.39 | | 0.0002+ | 0.0000+ | 0.8326 |
| phenolic4 | aro | 42.79 | | 0.0002+ | 0.8962 | 0.4837 |
| phenolic5 | aro | 42.88 | | 0.0002+ | 0.0000+ | 0.8673 |
| phenolic6 | aro | 43.12 | | 0.0002+ | 0.0000+ | 0.8673 |
| phenolic7 | aro | 43.21 | | 0.8010 | 0.9053 | 0.0004- |
| phenolic8 | aro | 43.22 | | 0.2023 | 0.7077 | 0.0003- |
| luteolin | aro | 43.88 | 3077.52 | 0.0144+ | 0.1826 | 0.1231 |
| chlorogenic acid | aro | 44.10 | 3099.54 | 0.2788 | 0.0137+ | 0.0353- |
| ursolic acid | ter | 44.25 | 3649.15 | 0.0002+ | 0.0000+ | 0.8673 |
| phenolic9 | aro | 44.41 | | 0.0538 | 0.1616 | 0.1092 |
| (E)-4-Caffeoylquinic acid | aro | 44.56 | 3154.54 | 0.0001+ | 0.0031+ | 0.0740 |
| flavonoid1 | aro | 44.70 | | 0.0431+ | 0.0806 | 0.2160 |
| caffeoylquinic acid deriv1 | aro | 44.75 | | 0.0003+ | 0.0004+ | 0.0028- |
| flavonoid2 | aro | 44.77 | | 0.1554 | 0.1310 | 0.7917 |
| rosmarinic acid | aro | 45.06 | 3400.96 | 0.0002+ | 0.0000+ | 0.8673 |
| rosmarinic acid deriv2 | aro | 45.56 | | 0.0002+ | 0.0000+ | 0.8673 |
| caffeoylquinic acid deriv2 | aro | 45.66 | | 0.0002+ | 0.0000+ | 0.8673 |

**Table A6:** Beet greens. Aro=aromatic, aa=amino acid, ac=acid, ter=terpene, hex=hexose, and disac=disaccharide.

| Compound | Class | RT | RI | P-temp | P-light | P-interaction |
|---|---|---|---|---|---|---|
| benzoic acid | aro | 16.55 | 1251.31 | 0.1631 | 0.0476+ | 0.6223 |
| benzyl isothiocyanate | aro | 20.02 | 1361.00 | 0.0637 | 0.0000+ | 0.0165+ |
| phenylalanine | aa | 26.31 | 1629.63 | 0.0128- | 0.0035+ | 0.0076- |
| shikimic acid | ac | 30.35 | 1794.63 | 0.1229 | 0.0001+ | 0.5903 |
| citric acid | ac | 30.49 | 1804.71 | 0.0000+ | 0.0000+ | 0.0020- |
| dehydroascorbic acid dimer | ac | 31.11 | 1838.43 | 0.2699 | 0.0678 | 0.4727 |
| fructose3 | hex | 31.55 | 1856.23 | 0.0132- | 0.0113+ | 0.0105- |
| glucose1 | hex | 32.07 | 1881.63 | 0.0651 | 0.0001+ | 0.0066- |
| phenolic1 | aro | 32.14 | | 0.0637 | 0.0000+ | 0.0165+ |
| phenolic2 | aro | 32.24 | | 0.0637 | 0.0000+ | 0.0165+ |
| tyrosine | aa | 32.91 | 1932.60 | 0.0002- | 0.0000+ | 0.0000- |
| p-Coumaric acid | aro | 32.99 | 1947.53 | 0.0718 | 0.0001+ | 0.9698 |
| dihydroxyphenylalanine | aro | 35.24 | 2052.83 | 0.0637 | 0.0000+ | 0.0165+ |
| (E)-Ferulic acid | aro | 35.80 | 2097.85 | 0.0915 | 0.0049+ | 0.5954 |
| (E)-Caffeic acid | aro | 36.52 | 2135.55 | 0.0637 | 0.0000+ | 0.0165+ |
| phytol | ter | 36.94 | 2131.46 | 0.0622 | 0.0407+ | 0.5523 |
| (E)-Sinapic acid | aro | 37.79 | 2249.43 | 0.0637 | 0.0000+ | 0.0165+ |
| phenolic3 | aro | 37.85 | | 0.2113 | 0.9457 | 0.7857 |
| rosmarinic acid deriv1 | aro | 40.06 | | 0.0637 | 0.0000+ | 0.0165+ |
| salicylic acid glucopyranoside | aro | 40.62 | 2568.38 | 0.5471 | 0.3599 | 0.6270 |
| sucrose | disac | 40.98 | 2628.87 | 0.0975 | 0.0002+ | 0.5883 |
| quinic acid deriv1 | aro | 42.39 | | 0.0622 | 0.0000+ | 0.0104+ |
| phenolic4 | aro | 42.79 | | 0.0522 | 0.0705 | 0.2958 |
| phenolic5 | aro | 42.88 | | 0.0846 | 0.2134 | 0.8455 |
| phenolic6 | aro | 43.12 | | 0.0171+ | 0.0361+ | 0.8086 |
| phenolic7 | aro | 43.21 | | 0.4752 | 0.0254+ | 0.2640 |
| phenolic8 | aro | 43.22 | | 0.0637 | 0.0000+ | 0.0165+ |
| luteolin | aro | 43.88 | 3077.52 | 0.0637 | 0.0000+ | 0.0165+ |
| chlorogenic acid | aro | 44.10 | 3099.54 | 0.0637 | 0.0000+ | 0.0165+ |
| ursolic acid | ter | 44.25 | 3649.15 | 0.0637 | 0.0000+ | 0.0165+ |
| phenolic9 | aro | 44.41 | | 0.0637 | 0.0000+ | 0.0165+ |
| (E)-4-Caffeoylquinic acid | aro | 44.56 | 3154.54 | 0.0637 | 0.0000+ | 0.0165+ |
| flavonoid1 | aro | 44.70 | | 0.0637 | 0.0000+ | 0.0165+ |
| caffeoylquinic acid deriv1 | aro | 44.75 | | 0.0637 | 0.0000+ | 0.0165+ |
| flavonoid2 | aro | 44.77 | | 0.0637 | 0.0000+ | 0.0165+ |
| rosmarinic acid | aro | 45.06 | 3400.96 | 0.0637 | 0.0000+ | 0.0165+ |
| rosmarinic acid deriv2 | aro | 45.56 | | 0.0637 | 0.0000+ | 0.0165+ |
| caffeoylquinic acid deriv2 | aro | 45.66 | | 0.0637 | 0.0000+ | 0.0165+ |

**Table A7:** Peppercress. Aro=aromatic, aa=amino acid, ac=acid, ter=terpene, hex=hexose, and disac=disaccharide.

| Compound | Class | RT | RI | P-temp | P-light | P-interaction |
|---|---|---|---|---|---|---|
| benzoic acid | aro | 16.55 | 1251.31 | 0.0063- | 0.0015- | 0.0026+ |
| benzyl isothiocyanate | aro | 20.02 | 1361.00 | 0.0030+ | 0.0808 | 0.0045- |
| phenylalanine | aa | 26.31 | 1629.63 | 0.1436 | 0.0442- | 0.3413 |
| shikimic acid | ac | 30.35 | 1794.63 | 0.8768 | 0.4506 | 0.2117 |
| citric acid | ac | 30.49 | 1804.71 | 0.0035+ | 0.1661 | 0.6618 |
| dehydroascorbic acid dimer | ac | 31.11 | 1838.43 | 0.3966 | 0.7556 | 0.4406 |
| fructose3 | hex | 31.55 | 1856.23 | 0.8812 | 0.0654 | 0.1780 |
| glucose1 | hex | 32.07 | 1881.63 | 0.3217 | 0.0130+ | 0.4996 |
| phenolic1 | aro | 32.14 | | 0.0212+ | 0.0084+ | 0.0465- |
| phenolic2 | aro | 32.24 | | 0.2070 | 0.0708 | 0.6229 |
| tyrosine | aa | 32.91 | 1932.60 | 0.3106 | 0.0312- | 0.0378+ |
| p-Coumaric acid | aro | 32.99 | 1947.53 | 0.2574 | 0.1261 | 0.5632 |
| dihydroxyphenylalanine | aro | 35.24 | 2052.83 | 0.1002 | 0.3393 | 0.1714 |
| (E)-Ferulic acid | aro | 35.80 | 2097.85 | 0.1617 | 0.0114+ | 0.8869 |
| (E)-Caffeic acid | aro | 36.52 | 2135.55 | 0.1070 | 0.3922 | 0.1918 |
| phytol | ter | 36.94 | 2131.46 | 0.6737 | 0.4265 | 0.0543 |
| (E)-Sinapic acid | aro | 37.79 | 2249.43 | 0.0013- | 0.0000- | 0.0002+ |
| phenolic3 | aro | 37.85 | | 0.0644 | 0.2315 | 0.1205 |
| rosmarinic acid deriv1 | aro | 40.06 | | 0.3344 | 0.3329 | 0.3368 |
| salicylic acid glucopyranoside | aro | 40.62 | 2568.38 | 0.5625 | 0.3001 | 0.3320 |
| sucrose | disac | 40.98 | 2628.87 | 0.0662 | 0.1217 | 0.8635 |
| quinic acid deriv1 | aro | 42.39 | | 0.0646 | 0.0090+ | 0.8013 |
| phenolic4 | aro | 42.79 | | 0.0878 | 0.3144 | 0.0041+ |
| phenolic5 | aro | 42.88 | | 0.2070 | 0.0708 | 0.6229 |
| phenolic6 | aro | 43.12 | | 0.3126 | 0.1154 | 0.4572 |
| phenolic7 | aro | 43.21 | | 0.6746 | 0.0005+ | 0.1906 |
| phenolic8 | aro | 43.22 | | 0.9409 | 0.0018+ | 0.2672 |
| luteolin | aro | 43.88 | 3077.52 | 0.7921 | 0.5096 | 0.7957 |
| chlorogenic acid | aro | 44.10 | 3099.54 | 0.2070 | 0.0708 | 0.6229 |
| ursolic acid | ter | 44.25 | 3649.15 | 0.1874 | 0.1043 | 0.3664 |
| phenolic9 | aro | 44.41 | | 0.0177- | 0.0115- | 0.0279+ |
| (E)-4-Caffeoylquinic acid | aro | 44.56 | 3154.54 | 0.2070 | 0.0708 | 0.6229 |
| flavonoid1 | aro | 44.70 | | 0.1100 | 0.4708 | 0.7483 |
| caffeoylquinic acid deriv1 | aro | 44.75 | | 0.2213 | 0.0757 | 0.5836 |
| flavonoid2 | aro | 44.77 | | 0.1320 | 0.7570 | 0.8590 |
| rosmarinic acid | aro | 45.06 | 3400.96 | 0.2070 | 0.0708 | 0.6229 |
| rosmarinic acid deriv2 | aro | 45.56 | | 0.2070 | 0.0708 | 0.6229 |
| caffeoylquinic acid deriv2 | aro | 45.66 | | 0.2070 | 0.0708 | 0.6229 |

**Table A8:** Lettuce. Aro=aromatic, aa=amino acid, ac=acid, ter=terpene, hex=hexose, and disac=disaccharide.

| Compound | Class | RT | RI | P-temp | P-light | P-interaction |
|---|---|---|---|---|---|---|
| benzoic acid | aro | 16.55 | 1251.31 | 0.2352 | 0.0430+ | 0.5899 |
| benzyl isothiocyanate | aro | 20.02 | 1361.00 | 0.0036+ | 0.0001+ | 0.0008+ |
| phenylalanine | aa | 26.31 | 1629.63 | 0.0000- | 0.0000- | 0.0000+ |
| shikimic acid | ac | 30.35 | 1794.63 | 0.0000+ | 0.0001+ | 0.2914 |
| citric acid | ac | 30.49 | 1804.71 | 0.0001+ | 0.0000+ | 0.0000- |
| dehydroascorbic acid dimer | ac | 31.11 | 1838.43 | 0.0003+ | 0.0235+ | 0.0058- |
| fructose3 | hex | 31.55 | 1856.23 | 0.0002+ | 0.0660 | 0.0059- |
| glucose1 | hex | 32.07 | 1881.63 | 0.0000+ | 0.0590 | 0.0132- |
| phenolic1 | aro | 32.14 | | 0.0061+ | 0.0005+ | 0.0067+ |
| phenolic2 | aro | 32.24 | | 0.0036+ | 0.0001+ | 0.0008+ |
| tyrosine | aa | 32.91 | 1932.60 | 0.0712 | 0.0014+ | 0.0072+ |
| p-Coumaric acid | aro | 32.99 | 1947.53 | 0.0015+ | 0.0001+ | 0.0012+ |
| dihydroxyphenylalanine | aro | 35.24 | 2052.83 | 0.0036+ | 0.0001+ | 0.0008+ |
| (E)-Ferulic acid | aro | 35.80 | 2097.85 | 0.0000+ | 0.0000+ | 0.0000- |
| (E)-Caffeic acid | aro | 36.52 | 2135.55 | 0.0626 | 0.0020+ | 0.2379 |
| phytol | ter | 36.94 | 2131.46 | 0.0180- | 0.0015- | 0.0123- |
| (E)-Sinapic acid | aro | 37.79 | 2249.43 | 0.0036+ | 0.0001+ | 0.0008+ |
| phenolic3 | aro | 37.85 | | 0.8696 | 0.6511 | 0.9001 |
| rosmarinic acid deriv1 | aro | 40.06 | | 0.0036+ | 0.0001+ | 0.0008+ |
| salicylic acid glucopyranoside | aro | 40.62 | 2568.38 | 0.0036+ | 0.0001+ | 0.0008+ |
| sucrose | disac | 40.98 | 2628.87 | 0.0000+ | 0.0000+ | 0.0003- |
| quinic acid deriv1 | aro | 42.39 | | 0.0010+ | 0.0162+ | 0.0018- |
| phenolic4 | aro | 42.79 | | 0.0036+ | 0.0001+ | 0.0008+ |
| phenolic5 | aro | 42.88 | | 0.0036+ | 0.0001+ | 0.0008+ |
| phenolic6 | aro | 43.12 | | 0.0036+ | 0.0001+ | 0.0008+ |
| phenolic7 | aro | 43.21 | | 0.0012- | 0.0001+ | 0.0000+ |
| phenolic8 | aro | 43.22 | | 0.0037+ | 0.0000+ | 0.0000+ |
| luteolin | aro | 43.88 | 3077.52 | 0.0036+ | 0.0001+ | 0.0008+ |
| chlorogenic acid | aro | 44.10 | 3099.54 | 0.1373 | 0.0009+ | 0.0484- |
| ursolic acid | ter | 44.25 | 3649.15 | 0.0036+ | 0.0001+ | 0.0008+ |
| phenolic9 | aro | 44.41 | | 0.0036+ | 0.0001+ | 0.0008+ |
| (E)-4-Caffeoylquinic acid | aro | 44.56 | 3154.54 | 0.0048+ | 0.0000+ | 0.0001+ |
| flavonoid1 | aro | 44.70 | | 0.0574 | 0.0000+ | 0.0008- |
| caffeoylquinic acid deriv1 | aro | 44.75 | | 0.0008+ | 0.0000+ | 0.0001+ |
| flavonoid2 | aro | 44.77 | | 0.2469 | 0.0000+ | 0.0066- |
| rosmarinic acid | aro | 45.06 | 3400.96 | 0.0036+ | 0.0001+ | 0.0008+ |
| rosmarinic acid deriv2 | aro | 45.56 | | 0.0036+ | 0.0001+ | 0.0008+ |
| caffeoylquinic acid deriv2 | aro | 45.66 | | 0.0036+ | 0.0001+ | 0.0008+ |

**Table A9:** Mint. Aro=aromatic, aa=amino acid, ac=acid, ter=terpene, hex=hexose, and disac=disaccharide.

| Compound | Class | RT | RI | P-temp | P-light | P-interaction |
|---|---|---|---|---|---|---|
| benzoic acid | aro | 16.55 | 1251.31 | 0.0286+ | 0.1617 | 0.3594 |
| benzyl isothiocyanate | aro | 20.02 | 1361.00 | 0.4191 | 0.2514 | 0.3490 |
| phenylalanine | aa | 26.31 | 1629.63 | 0.4314 | 0.3692 | 0.6669 |
| shikimic acid | ac | 30.35 | 1794.63 | 0.0030+ | 0.0029+ | 0.1369 |
| citric acid | ac | 30.49 | 1804.71 | 0.0003+ | 0.4015 | 0.0071- |
| dehydroascorbic acid dimer | ac | 31.11 | 1838.43 | 0.0060- | 0.1440 | 0.1539 |
| fructose3 | hex | 31.55 | 1856.23 | 0.1021 | 0.0036- | 0.0003- |
| glucose1 | hex | 32.07 | 1881.63 | 0.1689 | 0.2975 | 0.0583 |
| phenolic1 | aro | 32.14 | | 0.1232 | 0.3194 | 0.9194 |
| phenolic2 | aro | 32.24 | | 0.5592 | 0.8382 | 0.1485 |
| tyrosine | aa | 32.91 | 1932.60 | 0.0024+ | 0.0373- | 0.8547 |
| p-Coumaric acid | aro | 32.99 | 1947.53 | 0.5489 | 0.0674 | 0.2341 |
| dihydroxyphenylalanine | aro | 35.24 | 2052.83 | 0.0119- | 0.0002+ | 0.0020- |
| (E)-Ferulic acid | aro | 35.80 | 2097.85 | 0.3806 | 0.9340 | 0.7615 |
| (E)-Caffeic acid | aro | 36.52 | 2135.55 | 0.1155 | 0.0411+ | 0.1448 |
| phytol | ter | 36.94 | 2131.46 | 0.2379 | 0.4009 | 0.4829 |
| (E)-Sinapic acid | aro | 37.79 | 2249.43 | 0.2546 | 0.1092 | 0.3625 |
| phenolic3 | aro | 37.85 | | 0.8164 | 0.0241+ | 0.0045- |
| rosmarinic acid deriv1 | aro | 40.06 | | 0.4989 | 0.2124 | 0.1033 |
| salicylic acid glucopyranoside | aro | 40.62 | 2568.38 | 0.3725 | 0.3662 | 0.3742 |
| sucrose | disac | 40.98 | 2628.87 | 0.0403+ | 0.0004+ | 0.0089- |
| quinic acid deriv1 | aro | 42.39 | | 0.0030+ | 0.0793 | 0.6470 |
| phenolic4 | aro | 42.79 | | 0.0199- | 0.0448+ | 0.2127 |
| phenolic5 | aro | 42.88 | | 0.4339 | 0.2593 | 0.3287 |
| phenolic6 | aro | 43.12 | | 0.4191 | 0.2514 | 0.3490 |
| phenolic7 | aro | 43.21 | | 0.0032- | 0.0088+ | 0.0770 |
| phenolic8 | aro | 43.22 | | 0.0049- | 0.0130+ | 0.0509 |
| luteolin | aro | 43.88 | 3077.52 | 0.1416 | 0.1925 | 0.8836 |
| chlorogenic acid | aro | 44.10 | 3099.54 | 0.4191 | 0.2514 | 0.3490 |
| ursolic acid | ter | 44.25 | 3649.15 | 0.1922 | 0.3209 | 0.6471 |
| phenolic9 | aro | 44.41 | | 0.3107 | 0.8774 | 0.4573 |
| (E)-4-Caffeoylquinic acid | aro | 44.56 | 3154.54 | 0.1890 | 0.0235+ | 0.5415 |
| flavonoid1 | aro | 44.70 | | 0.2844 | 0.0376+ | 0.6246 |
| caffeoylquinic acid deriv1 | aro | 44.75 | | 0.3368 | 0.0080+ | 0.2357 |
| flavonoid2 | aro | 44.77 | | 0.0529 | 0.0235+ | 0.0389+ |
| rosmarinic acid | aro | 45.06 | 3400.96 | 0.1390 | 0.0105+ | 0.0643 |
| rosmarinic acid deriv2 | aro | 45.56 | | 0.0013+ | 0.0000+ | 0.0003- |
| caffeoylquinic acid deriv2 | aro | 45.66 | | 0.0063+ | 0.0425+ | 0.7551 |

**Table A10:** Basil. Aro=aromatic, aa=amino acid, ac=acid, ter=terpene, hex=hexose, and disac=disaccharide.

| Compound | Class | RT | RI | P-temp | P-light | P-interaction |
|---|---|---|---|---|---|---|
| benzoic acid | aro | 16.55 | 1251.31 | 0.923 | 0.984 | 0.162 |
| benzyl isothiocyanate | aro | 20.02 | 1361.00 | 0.004- | 0.016- | 0.009- |
| phenylalanine | aa | 26.31 | 1629.63 | 0.010+ | 0.092 | 0.651 |
| shikimic acid | ac | 30.35 | 1794.63 | 0.041+ | 0.705 | 0.938 |
| citric acid | ac | 30.49 | 1804.71 | 0.258 | 0.668 | 0.202 |
| dehydroascorbic acid dimer | ac | 31.11 | 1838.43 | 0.372 | 0.935 | 0.577 |
| fructose3 | hex | 31.55 | 1856.23 | 0.004- | 0.016- | 0.012+ |
| glucose1 | hex | 32.07 | 1881.63 | 0.000- | 0.003- | 0.001+ |
| phenolic1 | aro | 32.14 | | 0.002- | 0.021+ | 0.011+ |
| phenolic2 | aro | 32.24 | | 0.004- | 0.016- | 0.009+ |
| tyrosine | aa | 32.91 | 1932.60 | 0.021- | 0.016- | 0.009+ |
| p-Coumaric acid | aro | 32.99 | 1947.53 | 0.315 | 0.325 | 0.705 |
| dihydroxyphenylalanine | aro | 35.24 | 2052.83 | 0.038+ | 0.140 | 0.703 |
| (E)-Ferulic acid | aro | 35.80 | 2097.85 | 0.001- | 0.005- | 0.003+ |
| (E)-Caffeic acid | aro | 36.52 | 2135.55 | 0.051 | 0.085 | 0.580 |
| phytol | ter | 36.94 | 2131.46 | 0.011- | 0.675 | 0.373 |
| (E)-Sinapic acid | aro | 37.79 | 2249.43 | 0.088 | 0.232 | 0.019+ |
| phenolic3 | aro | 37.85 | | 0.004- | 0.016- | 0.009+ |
| rosmarinic acid deriv1 | aro | 40.06 | | 0.498 | 0.781 | 0.435 |
| salicylic acid glucopyranoside | aro | 40.62 | 2568.38 | 0.004- | 0.016- | 0.009- |
| sucrose | disac | 40.98 | 2628.87 | 0.001- | 0.003- | 0.001+ |
| quinic acid deriv1 | aro | 42.39 | | 0.095 | 0.371 | 0.901 |
| phenolic4 | aro | 42.79 | | 0.011- | 0.016- | 0.009+ |
| phenolic5 | aro | 42.88 | | 0.011- | 0.136 | 0.061 |
| phenolic6 | aro | 43.12 | | 0.004- | 0.016+ | 0.009+ |
| phenolic7 | aro | 43.21 | | 0.000- | 0.007+ | 0.009- |
| phenolic8 | aro | 43.22 | | 0.002- | 0.036+ | 0.031- |
| luteolin | aro | 43.88 | 3077.52 | 0.551 | 0.781 | 0.961 |
| chlorogenic acid | aro | 44.10 | 3099.54 | 0.288 | 0.210 | 0.717 |
| ursolic acid | ter | 44.25 | 3649.15 | 0.907 | 0.984 | 0.039+ |
| phenolic9 | aro | 44.41 | | 0.004+ | 0.016+ | 0.009+ |
| (E)-4-Caffeoylquinic acid | aro | 44.56 | 3154.54 | 0.004- | 0.016- | 0.009- |
| flavonoid1 | aro | 44.70 | | 0.527 | 0.724 | 0.900 |
| caffeoylquinic acid deriv1 | aro | 44.75 | | 0.004- | 0.016- | 0.009- |
| flavonoid2 | aro | 44.77 | | 0.237 | 0.935 | 0.920 |
| rosmarinic acid | aro | 45.06 | 3400.96 | 0.551 | 0.628 | 0.868 |
| rosmarinic acid deriv2 | aro | 45.56 | | 0.500 | 0.209 | 0.272 |
| caffeoylquinic acid deriv2 | aro | 45.66 | | 0.492 | 0.209 | 0.035+ |

**Table A11:** Coriander. Aro=aromatic, aa=amino acid, ac=acid, ter=terpene, hex=hexose, and disac=disaccharide.

| Compound | Class | RT | RI | P-temp | P-light | P-interaction |
|---|---|---|---|---|---|---|
| benzoic acid | aro | 16.55 | 1251.31 | 0.9576 | 0.5000 | NaN |
| benzyl isothiocyanate | aro | 20.02 | 1361.00 | 0.7247 | 0.5000 | NaN |
| phenylalanine | aa | 26.31 | 1629.63 | 0.4586 | 0.5121 | NaN |
| shikimic acid | ac | 30.35 | 1794.63 | 0.8834 | 0.2336 | NaN |
| citric acid | ac | 30.49 | 1804.71 | 0.5240 | 0.2711 | NaN |
| dehydroascorbic acid dimer | ac | 31.11 | 1838.43 | 0.3018 | 0.2070 | NaN |
| fructose3 | hex | 31.55 | 1856.23 | 0.1214 | 0.4151 | NaN |
| glucose1 | hex | 32.07 | 1881.63 | 0.4944 | 0.5543 | NaN |
| phenolic1 | aro | 32.14 | | 0.5619 | 0.6881 | NaN |
| phenolic2 | aro | 32.24 | | 0.7247 | 0.5000 | NaN |
| tyrosine | aa | 32.91 | 1932.60 | 0.5792 | 0.1180 | NaN |
| p-Coumaric acid | aro | 32.99 | 1947.53 | 0.6772 | 0.5245 | NaN |
| dihydroxyphenylalanine | aro | 35.24 | 2052.83 | 0.7247 | 0.5000 | NaN |
| (E)-Ferulic acid | aro | 35.80 | 2097.85 | 0.7828 | 0.4651 | NaN |
| (E)-Caffeic acid | aro | 36.52 | 2135.55 | 0.5141 | 0.2622 | NaN |
| phytol | ter | 36.94 | 2131.46 | 0.5723 | 0.4015 | NaN |
| (E)-Sinapic acid | aro | 37.79 | 2249.43 | 0.7247 | 0.5000 | NaN |
| phenolic3 | aro | 37.85 | | 0.1308 | 0.1018 | NaN |
| rosmarinic acid deriv1 | aro | 40.06 | | 0.7247 | 0.5000 | NaN |
| salicylic acid glucopyranoside | aro | 40.62 | 2568.38 | 0.9915 | 0.5000 | NaN |
| sucrose | disac | 40.98 | 2628.87 | 0.6936 | 0.8881 | NaN |
| quinic acid deriv1 | aro | 42.39 | | 0.1112 | 0.0374+ | NaN |
| phenolic4 | aro | 42.79 | | 0.4760 | 0.2000 | NaN |
| phenolic5 | aro | 42.88 | | 0.7247 | 0.5000 | NaN |
| phenolic6 | aro | 43.12 | | 0.7247 | 0.5000 | NaN |
| phenolic7 | aro | 43.21 | | 0.5059 | 0.7048 | NaN |
| phenolic8 | aro | 43.22 | | 0.7247 | 0.5000 | NaN |
| luteolin | aro | 43.88 | 3077.52 | 0.8500 | 0.5000 | NaN |
| chlorogenic acid | aro | 44.10 | 3099.54 | 0.9606 | 0.3357 | NaN |
| ursolic acid | ter | 44.25 | 3649.15 | 0.8736 | 0.4611 | NaN |
| phenolic9 | aro | 44.41 | | 0.7247 | 0.5000 | NaN |
| (E)-4-Caffeoylquinic acid | aro | 44.56 | 3154.54 | 0.8605 | 0.4298 | NaN |
| flavonoid1 | aro | 44.70 | | 0.6591 | 0.5162 | NaN |
| caffeoylquinic acid deriv1 | aro | 44.75 | | 0.7247 | 0.5000 | NaN |
| flavonoid2 | aro | 44.77 | | 0.6014 | 0.4978 | NaN |
| rosmarinic acid | aro | 45.06 | 3400.96 | 0.7247 | 0.5000 | NaN |
| rosmarinic acid deriv2 | aro | 45.56 | | 0.7247 | 0.5000 | NaN |
| caffeoylquinic acid deriv2 | aro | 45.66 | | 0.7247 | 0.5000 | NaN |

**Table A12:** Chives. Aro=aromatic, aa=amino acid, ac=acid, ter=terpene, hex=hexose, and disac=disaccharide.

| Compound | Class | RT | RI | P-temp | P-light | P-interaction |
|---|---|---|---|---|---|---|
| benzoic acid | aro | 16.55 | 1251.31 | 0.7829 | 0.8982 | NaN |
| benzyl isothiocyanate | aro | 20.02 | 1361.00 | 0.8790 | 0.8100 | NaN |
| phenylalanine | aa | 26.31 | 1629.63 | 0.7705 | 0.6870 | NaN |
| shikimic acid | ac | 30.35 | 1794.63 | 0.5385 | 0.4498 | NaN |
| citric acid | ac | 30.49 | 1804.71 | 0.8832 | 0.9561 | NaN |
| dehydroascorbic acid dimer | ac | 31.11 | 1838.43 | 0.7776 | 0.8415 | NaN |
| fructose3 | hex | 31.55 | 1856.23 | 0.4998 | 0.4999 | NaN |
| glucose1 | hex | 32.07 | 1881.63 | 0.7339 | 0.9568 | NaN |
| phenolic1 | aro | 32.14 | | 0.7746 | 0.5443 | NaN |
| phenolic2 | aro | 32.24 | | 0.8790 | 0.8100 | NaN |
| tyrosine | aa | 32.91 | 1932.60 | 0.6284 | 0.5359 | NaN |
| p-Coumaric acid | aro | 32.99 | 1947.53 | 0.4365 | 0.7952 | NaN |
| dihydroxyphenylalanine | aro | 35.24 | 2052.83 | 0.8790 | 0.8100 | NaN |
| (E)-Ferulic acid | aro | 35.80 | 2097.85 | 0.5196 | 0.6689 | NaN |
| (E)-Caffeic acid | aro | 36.52 | 2135.55 | 0.2433 | 0.0920 | NaN |
| phytol | ter | 36.94 | 2131.46 | 0.8777 | 0.4437 | NaN |
| (E)-Sinapic acid | aro | 37.79 | 2249.43 | 0.8790 | 0.8100 | NaN |
| phenolic3 | aro | 37.85 | | 0.4745 | 0.0936 | NaN |
| rosmarinic acid deriv1 | aro | 40.06 | | 0.8790 | 0.8100 | NaN |
| salicylic acid glucopyranoside | aro | 40.62 | 2568.38 | 0.8790 | 0.8100 | NaN |
| sucrose | disac | 40.98 | 2628.87 | 0.9672 | 0.5954 | NaN |
| quinic acid deriv1 | aro | 42.39 | | 0.8790 | 0.8100 | NaN |
| phenolic4 | aro | 42.79 | | 0.8790 | 0.8100 | NaN |
| phenolic5 | aro | 42.88 | | 0.8790 | 0.8100 | NaN |
| phenolic6 | aro | 43.12 | | 0.8790 | 0.8100 | NaN |
| phenolic7 | aro | 43.21 | | 0.8832 | 0.9733 | NaN |
| phenolic8 | aro | 43.22 | | 0.8790 | 0.8100 | NaN |
| luteolin | aro | 43.88 | 3077.52 | 0.8244 | 0.5404 | NaN |
| chlorogenic acid | aro | 44.10 | 3099.54 | 0.5000 | 0.3286 | NaN |
| ursolic acid | ter | 44.25 | 3649.15 | 0.8790 | 0.8100 | NaN |
| phenolic9 | aro | 44.41 | | 0.8790 | 0.8100 | NaN |
| (E)-4-Caffeoylquinic acid | aro | 44.56 | 3154.54 | 0.8790 | 0.8100 | NaN |
| flavonoid1 | aro | 44.70 | | 0.7025 | 0.1173 | NaN |
| caffeoylquinic acid deriv1 | aro | 44.75 | | 0.8790 | 0.8100 | NaN |
| flavonoid2 | aro | 44.77 | | 0.8982 | 0.6071 | NaN |
| rosmarinic acid | aro | 45.06 | 3400.96 | 0.8790 | 0.8100 | NaN |
| rosmarinic acid deriv2 | aro | 45.56 | | 0.8790 | 0.8100 | NaN |
| caffeoylquinic acid deriv2 | aro | 45.66 | | 0.8790 | 0.8100 | NaN |