



Characterization of key aroma compounds in microgreens and mature plants of hydroponic fennel (*Foeniculum vulgare* Mill.)

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ABSTRACT

Fennel is a popular culinary herb known for its unique flavor. In this study, we identified key aroma-active compounds in fresh fennel (*Foeniculum vulgare* Mill.) and its microgreens. Fennel was cultivated hydroponically with soilless substrates. Upon harvesting, samples were ground with liquid nitrogen, and headspace solid phase microextraction (SPME) gas chromatography-mass-spectrometry-olfactometry (GC-MS-O) was used for volatile analysis. Thirty-two and 28 key aroma-active compounds were identified in fennel microgreens and mature leaves, respectively. Phenylpropenes, especially (*E*)-anethole, were the predominant aroma-active compound in all samples (36.3–83.4 %). Quantitation results showed that fennel microgreens contained a significantly higher amount of monoterpenes, showing an 81.4–98.1 % increase when compared to mature fennel. Principal component analysis (PCA) of identified volatiles indicated a distinctive difference in the overall aroma profile between microgreens and mature leaf. The changes in aroma contents over different growth stages revealed the underlying volatile biosynthesis discrepancy. This study provided baseline information for understanding the aroma evolution from microgreen to mature fennel herbs.

1. Introduction

The COVID-19 pandemic has shifted food preferences towards healthier and more sustainable choices (Skalkos & Kalyva, 2023; Timpanaro & Cascone, 2022). Driven by this change, there is a growing interest and consumer demand for high-quality fresh produce with potential health benefits (Celik & Dane, 2020; Filimonau et al., 2022; Maire et al., 2022). Diets rich in plant-derived foods have been shown to promote health by reducing the risk of chronic diseases (Wallace et al., 2020). Against this backdrop, new food sources such as microgreens are emerging as novel culinary ingredients with health-promoting functions (Choe, Yu, & Wang, 2018; Zhang et al., 2021).

Microgreens are young, tender greens produced from the seeds of herbs, vegetables, or grains (Xiao et al., 2012). Microgreens consist of the stem, seed leaves (cotyledons), and young true leaves. With a short production cycle, microgreens can be harvested 7 to 21 days after seed germination, depending on the species (Kyriacou et al., 2016). Microgreens production can be easily adopted into controlled environment agriculture (CEA) due to their short production cycle, and they are

usually grown hydroponically (Zhang et al., 2021). CEA refers to a controlled plant production space where environmental factors such as light, nutrients, and temperature are precisely regulated (Folta, 2019; Kozai & Niu, 2016). CEA enables year-round production of fresh produce and supports the supply of local food. Hydroponic production has the advantages of reduced water usage and recycling of organic waste (Folta, 2019). Recent advances have shown that many culinary herbs, such as chives, dill (González-García et al., 2009), basil (Walters & Currey, 2015), and lemon balm (Son et al., 2021) adapt well to CEA and hydroponic production.

Studies have shown that microgreens have higher levels of bioactive phytochemicals than their mature counterparts, making them reliable sources of functional compounds (Choe et al., 2018). In other words, a small amount of microgreens may provide equal nutritional effects similar to a large quantity of mature plants. For example, compared to mature cilantro, cilantro microgreens contained a 10 times higher concentration of lutein and zeaxanthin, which are two major carotenoids that function as antioxidants and can prevent age-related eye disease (Xiao et al., 2012). Recently, microgreens of plants belonging to

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Brassicaceae, Apiaceae, and Lamiaceae families, have also been identified as excellent sources of dietary antioxidants, such as vitamin C and polyphenols (Ghoora, Haldipur, & Srividya, 2020).

However, compared to numerous studies focusing on the nutritional value of microgreens, their aroma remains largely unexplored, despite the importance of aroma in sensory quality and food consumption. So far, the comparison of aroma compounds of microgreens and their mature counterpart has only been studied in a few species, such as cilantro (Oruña-Concha et al., 2017) and perilla mint (Dimita et al., 2022). Moreover, the differences in aroma compounds between herb microgreens and their mature plants are species-specific, and no information is found for fennel.

Fennel (*Foeniculum vulgare* Mill.) is a widely used culinary herb native to the Mediterranean region. The plant contains many volatile compounds with industrial interest (Guillén & Manzanos, 1996; Valduga et al., 2019), and its aroma characteristics have been previously studied. Bilia et al. (2002) identified anethole and anisaldehyde as the main constituents of fennel tea essential oil using gas chromatography-mass spectrometry (GC-MS). Díaz-Maroto et al. (2005) characterized odor-active compounds in dried fennel seeds with gas chromatography-mass spectrometry-olfactometry (GC-MS-O), finding (*E*)-anethole, estragole, fenchone, and 1-octen-3-ol as the most intense aroma compounds. Zeller and Rychlik (2006) conducted aroma extract dilution analysis (AEDA) on fennel seeds and tea, identifying (*E*)-Anethole, estragole, and fenchone as the compounds with the highest odor activity value (OAV). Saharkhiz and Tarakeme (2011) found that the essential oil content of fennel seeds varies largely with plant growth stage. Xiao et al. (2017) identified seven sensory terms correlated to aroma constituents in fennel essential oil through GC-MS-O. Most recently, Afifi et al. (2021) analyzed the volatile composition of fennel seeds from 12 varieties using solid phase microextraction (SPME), though GC-O or detailed volatile quantitation was not conducted.

Notably, most previous studies have focused primarily on fennel seeds rather than fresh leaves. Barros et al. (2010) analyzed the nutritional composition of different fennel parts (shoots, leaves, stems, and inflorescences), but did not evaluate volatile compounds. Fennel seeds were found to exhibit low to moderate antioxidant capacity due to phenolic compounds (Lu et al., 2011). In contrast, fennel microgreens have been shown to possess high antioxidant capacities (Ghoora et al., 2020) and nutritional potential (Uher et al., 2023), yet their aroma profile remains uncharacterized.

Our research aims to fill this gap by identifying and comparing the key aroma compounds in hydroponically grown fennel microgreens and mature plants using SPME-GC-MS-O, multiple internal standards, and the standard addition method. These techniques minimize matrix effects and ensure accurate quantitation (Penton, 1999). This approach has demonstrated good precision and accuracy for the quantitation of volatile aroma compounds in different food samples (Zhang et al., 2015; Fortini et al., 2017; Su & Yin, 2021). Understanding the differences in these important secondary metabolites during plant growth can enhance the quality of hydroponic fennel, helping herb growers determine the optimal harvest time.

2. Materials and methods

2.1. Plant materials and growth conditions

Fennel (*Foeniculum vulgare* Mill., cv. Grosfruchtiger) seeds were purchased from Johnny's Selected Seeds (Winslow, ME). Seeds were sown into rockwool propagation cubes saturated with water in a germination tray. The trays were placed in a glass-glazed greenhouse in Blacksburg, VA (lat. 37.2°N) and the plant growth experiment lasted from October to December 2022. Seedlings were irrigated daily with water and allowed to grow for 3 weeks before being transplanted to the NFT hydroponic systems (NFT0406, CropKing, Lodi, OH). Fennel microgreens were harvested 3 weeks after seed germination. The rest of

the seedlings from the same batch were transplanted into NFT hydroponic systems, which included nutrient solution supplemented with 2-1-6 and 5-0-1 (nitrogen-phosphate potash) liquid fertilizers (FloralGro and FloraMicro, General Hydroponics, Sebastopol, CA).

To investigate the changes in key aroma compounds of fennel plants during the vegetative growth stage, twelve mature fennel plants were destructively harvested at 8, 9, and 10 weeks after seed germination. During the plant material collection, data on plant height, growth index (GI), fresh biomass, number of branches, and apparent moisture content were also collected. Plant height was measured perpendicularly from the surface of the growing substrate to the tallest point of the canopy. Growth index was calculated using the formula $GI = \frac{W1+W2}{H}$, where W1 and W2 represent two perpendicular canopy widths, and H represents plant height. Fresh biomass was recorded as the fresh weight of aerial parts of the plant upon harvesting. Number of branches was recorded as the total count of branches originating from fully expanded nodes. Apparent moisture content (AMC) was determined by placing a specific amount of fresh tissue in a forced-air oven at 105 °C for 2 h (Ahn et al., 2014), recording the dry weight, and calculating using the formula $AMC = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100\%$. Growth parameters were measured for all twelve plants, and six plants (three from hydroponic system 1 and three from hydroponic system 2) were used for aroma characterization by GC-MS. For microgreens, three replicates from germination tray 1 and three replicates from germination tray 2 were analyzed by GC-MS. Immediately after harvesting, the fennel samples were stored at −80 °C until further analysis.

During the growth experiment, the day and night temperature of greenhouse were set to 21 °C and 24 °C, respectively. The greenhouse temperature was maintained with an environmental controller (Step Control, Wadsworth Control Systems, Arvada, CO). The temperature and humidity were recorded at 5 min intervals by a data logger (Watchdog 1650 Micro Station, Spectrum Technologies, Aurora, IL). Light was measured with a quantum PAR light sensor (3668I, Spectrum Technologies, Aurora, IL) in a radiation shield (3663A, Spectrum Technologies, Aurora, IL). The greenhouse environmental conditions, such as average daylight hours, daily light integral (DLI), maximum and minimum greenhouse temperature, and humidity were shown in Supplemental Table 1.

The pH and electrical conductivity (EC) of each NFT unit were monitored daily, and pH of the nutrient solution was maintained at the range of 5.8–6.2 while EC was maintained at the range of 1.4–1.6 dS·m^{−1} with a portable pH/EC meter (HI98131, HANNA Instruments, Smithfield, RI). The pH was adjusted with phosphoric and citric acid (pH Down, General Hydroponics, Sebastopol, CA) and potassium hydroxide and potassium carbonate (pH Up, General Hydroponics, Sebastopol, CA). EC was adjusted with deionized water and 2-1-6 and 5-0-1 liquid fertilizers.

2.2. Chemicals and standards

The chemicals and authentic standards are purchased from the following companies: α -Pinene (98 %), α -phellandrene (≥ 75 %), 1-octen-3-ol (≥ 98 %), anisketone (≥ 97 %), β -ionone (96 %), 3-octanol acetate (≥ 98 %), (*Z*)-3-hexen-1-ol (≥ 98 %), carvacrol (≥ 99 %), γ -terpinene (≥ 95 %), ($\pm$)-camphor (≥ 95.5 %), estragole (≥ 96 %), fenchyl acetate (≥ 96 %, mix of α - and β - isomers), *p*-cymenene (≥ 98 %), *p*-anisaldehyde (≥ 97.5 %), phenyl acetaldehyde (≥ 95 %), *p*-propyl-anisole (≥ 99 %), methyl eugenol (≥ 98 %), linalool (≥ 97 %), myristicin (≥ 97 %), (*E*)-anethole (≥ 99 %), decane (≥ 99.8 %), 2-octanol (≥ 97 %), 1-phenylethanol (98 %), and *n*-alkanes (C₇–C₃₀) were purchased from Sigma-Aldrich (St. Louis, MO). (*Z*)-3-Hexenal (50 % in triacetin) and (*E*)-2-hexenal (98 %) were provided by Bedoukian Research Inc. (Danbury, CT). Tridecane (> 99 %) and 4-methoxycinnamaldehyde (> 97 %) were purchased from Tokyo Chemical Industry Co., Ltd. America (Portland, OR). (−)-Bornyl acetate (95 %) was purchased from Alfa

Aesar (Haverhill, MA). (*Z*)-Anethole was purchased from Toronto Research Chemicals (Toronto, Canada) and methanol (HPLC grade, 99.9 %) was purchased from Fisher Scientific (Fair Lawn, NJ).

2.3. Aroma compound extraction by solid phase micro extraction (SPME)

Frozen fennel leaves were ground with a mortar and a pestle in liquid nitrogen to fine powders. Fennel powder (50 ± 0.5 mg) was weighed in a 20 mL clear and odorless vial (Supelco, Bellefonte, PA), spiked with 1 μ L internal standard (IS) and then sealed with a PTFE lined screw cap (Supelco, Bellefonte, PA). Specifically, the internal standard solution consisted of 36.4 ng/ μ L decane, 4.8 ng/ μ L 2-octanol, and 99.0 ng/ μ L 1-phenylethanol dissolved in methanol. The IS were selected based on the following principles (McNair, Miller, & Snow, 2019; Xia et al., 2023): (1) the IS are not present in fennel samples; (2) they are stable and do not interfere with any sample components; (3) the peak of IS does not overlay with peak of any compounds of interest. The vial containing sample was then kept in the autosampler agitator for 10 min at 50 °C with a stirring speed of 250 rpm so that the headspace was equilibrated. After equilibration, a 1 cm 50/30 divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (Supelco, Bellefonte, PA) was exposed to the headspace of the vial for 30 min at 50 °C with a stirring speed of 250 rpm to extract the volatile compounds. The SPME-GC-MS measurement was performed in six replicates for each harvest time, with each replicate containing leaf tissue from different fennel plants.

2.4. Gas chromatography-mass spectrometry-olfactometry

The system consisted of an Agilent 6890 N GC (Santa Clara, CA) equipped with a DB-WAX capillary column (30 m $\times$ 0.25 mm i.d., film thickness = 0.25 μ m) (Supelco, Bellefonte, PA), a 5975B mass selective detector (MSD) (Agilent Technologies Inc., Santa Clara, CA) and a PHASER sniff port (GL Sciences, Eindhoven, the Netherlands). The injection port was held at 230 °C in splitless mode and the column temperature was programmed as follows: 5 min at 40 °C, followed by a temperature increase at 8 °C/min to 80 °C, 5 °C/min to 152 °C, 8 °C/min to 225 °C, and hold 15 min at 225 °C. The carrier gas was helium at the flow rate of 2 mL/min (67.9 cm/s). The interface temperature of MSD is 230 °C; the ionization energy was 70 eV; the mass range was 33–500 amu; the EM voltage was Atune + 200 V; and the scan rate was 5.15 scans/s. Column effluent was split 1:1 between sniff port and MSD using the 0.25 mm i.d. deactivated capillary column. The sniff port temperature was 230 °C and the eluting volatile compounds were combined with humidified air at the flow rate of 5 mL/min.

GC-O of fennel samples was performed by three trained panelists (two females, one male, aged between 20 and 27 years old). Each panelist participated in three training sessions where they sniffed pure standards of common aroma compounds found in fennel through the sniff port. The purpose of training was for panelists to recognize and describe different aroma attributes of odorants (Jonas & Schieberle, 2021). For each sample, panelists recorded retention time, aroma descriptors, and aroma intensity of each perceived odorant. Panelists rated the intensity of odorant on a five-point scale: very weak (1), weak (2), medium (3), strong (4), and very strong (5). A volatile compound is considered aroma-active if only (1) two or more panelists smelled the compounds; (2) only one panelist smelled the aroma compound, the odor intensity was weak or above. The results of three panelists were averaged. The fennel samples used for GC-O were not spiked with IS.

2.5. Identification of aroma-active compounds

Aroma-active compounds were first selected based on the results of GC-O. These compounds were then identified by: (1) matching electron-impact (EI) mass spectra with NIST Mass Spectral Library (NIST 17), (2) matching retention indices on the polar GC column with previous references, (3) matching odor characteristics with authentic standards.

Retention indices were calculated based on the equation described by a previous publication (van Den Dool & Kratz, 1963) with the retention time of a C₇-C₃₀ alkane solution.

2.6. Quantitation of aroma-active compounds with standard addition method

The quantitation of identified aroma-active compounds was performed by GC-MS with standard addition method. Fennel powder (50 ± 0.5 mg) was spiked with IS (1 μ L, containing 36.4 ng/ μ L decane, 4.8 ng/ μ L 2-octanol, and 99.0 ng/ μ L 1-phenylethanol) and the aroma compounds were extracted by SPME as described in section 2.3. To build a calibration curve of each aroma-active compound, a known concentration of authentic standards of aroma-active compounds were first mixed and dissolved in methanol, and then the stock solution was stepwise diluted to levels of known concentrations. Levels of standards mixture solution (1 μ L) were added to an “odorless” or “blank” fennel powder, which was achieved by incubating the sample under 50 °C for about 15 h with regular nitrogen purge of the headspace (Su & Yin, 2021). The “blank” state of fennel powder was confirmed when GC-MS peaks were negligible. The “odorless” fennel powder was also spiked with the same amount of IS (1 μ L, containing 36.4 ng/ μ L decane, 4.8 ng/ μ L 2-octanol, and 99.0 ng/ μ L 1-phenylethanol). The regression equation was constructed based on the peak area ratio and mass ratio of a target aroma-active compound and its internal standard (McNair et al., 2019). At each dilution point, GC-MS was performed in duplicate.

The aroma-active compounds were divided into three major groups against particular IS applied. For each group, the IS has similar molecular structure and/or chemical formula to the target analytes. For the compounds using decane as the IS, the stock solution contained 7.16 μ g/ μ L α -pinene, 24.23 μ g/ μ L α -phellandrene, 9.88 μ g/ μ L γ -terpinene, 0.38 μ g/ μ L tridecane, and 1.67 μ g/ μ L *p*-cymene. The stock solution was then diluted with methanol at 1:2, 1:4, 1:20, 1:200, and 1:400 ratios. For the compounds using 2-octanol as the IS, the stock solution contained 3.33 μ g/ μ L (*E*)-2-hexenal, 0.42 μ g/ μ L (*Z*)-3-hexen-1-ol, 0.08 μ g/ μ L 1-octen-3-ol, 0.17 μ g/ μ L linalool, 0.20 μ g/ μ L phenyl acetaldehyde, 2.18 μ g/ μ L *p*-anisaldehyde, and 0.10 μ g/ μ L 4-methoxycinnamaldehyde. The stock solution was, likewise, diluted with methanol at 1:2, 1:4, 1:20, 1:200, and 1:400 ratios. For the compounds using 1-phenylethanol as the IS, the stock solution contained 2.39 μ g/ μ L camphor, 3.76 μ g/ μ L bornyl acetate, 1.46 μ g/ μ L carvacrol, 0.08 μ g/ μ L 3-octanol acetate, 0.36 μ g/ μ L *p*-propyl-anisole, 14.27 μ g/ μ L methyl eugenol, and 1.56 μ g/ μ L anisketone. It was diluted with methanol at 1:2, 1:4, 1:20, 1:200, 1:400, and 1:4000 ratios.

The following standards were run individually with IS because their authentic standards contained isomers: the concentration of stock solution of 3-hexenal, fenchyl acetate, and β -ionone are 0.08 μ g/ μ L, 28.22 μ g/ μ L, and 3.19 μ g/ μ L, respectively. (*E*)-Anethole and estragole were also run individually with IS due to their high concentrations in fennel samples. The stock solution concentration of (*E*)-anethole and estragole was 294.03 μ g/ μ L and 76.05 μ g/ μ L, respectively. (*Z*)-Anethole and myristicin were run individually with IS because their authentic standards came in small quantity (50 mg and 10 mg, respectively) and the total volume of their stock solution was lower than other standards mixtures. For (*Z*)-anethole, the concentration of stock solution is 10.67 μ g/ μ L and for myristicin, the concentration of stock solution is 0.78 μ g/ μ L. The above-mentioned stock solutions were diluted with methanol at 1:2, 1:4, 1:20, 1:200, and 1:400 ratios. For compounds without authentic standards, compounds with similar chemical structures were used to build the calibration curve (Su & Yin, 2021; Wei et al., 2018). Specifically, allo-ocimine, γ -muurolene, germacrene D, and α -cadinene were quantified against *p*-cymene; methyl isoeugenol and apiol were quantified against methyl eugenol.

The quantitation capability of each calibration curve was validated with limit of detection (LOD) and limit of quantitation (LOQ) based on the lowest concentration point of each calibration curve. The equations

for the calculation of LOD and LOQ are as follows (MacDougall et al., 1980; Su & Yin, 2021), where SD is the standard deviation of the response and b is the slope of calibration curve:

$$LOD = \frac{3SD}{b}$$

$$LOQ = \frac{10SD}{b}$$

2.7. Calculation of odor activity value (OAV)

The odor active value was calculated by dividing the concentration of aroma-active compounds in fennel samples and their previous published odor detection threshold in water (Erten & Cadwallader, 2017).

2.8. Statistical analysis

Concentration of aroma-active compounds in fennel microgreens and mature plants were compared by one-way analysis of variance (ANOVA) based on Tukey's honest significant difference (HSD) test for post hoc comparisons using JMP (JMP® Pro 16.0.0). The significance level was set at $p < 0.05$. For multivariate analysis, principal component analysis (PCA) was conducted to reduce the dimensionality of data and to identify patterns in the overall variances in key aroma compounds across different harvest stages. In addition, a heatmap with hierarchical clustering was generated using the pheatmap package (Kolde & Kolde, 2015) in R (version 4.3.1) to visualize the relative concentration of the key aroma compounds in samples harvested at different growth stages. The dendrogram in the heatmap was constructed based on Ward's method and Euclidean distances as the similarity measure.

3. Results and discussion

3.1. Growth and morphological evaluation

The plant growth and morphological data is shown in Table 1. The apparent moisture content was similar between microgreens and earlier harvested mature fennel, but decreased in week 10 samples, in addition to significant increases in plant height, number of branches, and fresh biomass. Plant fresh biomass and the number of branches on each plant gradually increased with growth period. Notably, the average fresh biomass of week 10 sample (43.60 g/plant) is nearly three times as the week 9 sample (15.21 g/plant), suggesting drastic vegetative growth during this period. The fresh biomass of hydroponic fennel is

Table 1
Morphological and growth data of fennel at different growth stages.

Weeks after germination	Microgreen	Mature Herbs		
	3	8	9	10
Apparent moisture content (%)	88.66 ± 0.78 ^a	90.24 ± 1.00 ^a	88.95 ± 0.35 ^a	83.03 ± 2.69 ^b
Fresh biomass (g)	0.06 ± 0.02 ^d	7.76 ± 2.45 ^c	15.21 ± 4.46 ^b	43.60 ± 19.79 ^a
Number of branches	2.96 ± 0.27 ^c	6.58 ± 0.51 ^b	9.00 ± 1.76 ^b	12.83 ± 6.01 ^a
Height (cm)	5.56 ± 1.11 ^c	20.35 ± 3.19 ^b	19.02 ± 3.57 ^b	22.75 ± 6.55 ^a
Growth index	1.35 ± 0.43 ^b	1.24 ± 0.19 ^b	1.73 ± 0.30 ^a	2.14 ± 0.72 ^a

^{abcd} Represent the mean separation between harvest time by Tukey's HSD at $p < 0.05$. The results are expressed as mean ± SD. Growth index (GI) was calculated as $GI = \frac{W1 + W2}{H}$, where W1 and W2 are two widths of plant canopy perpendicular with each other, and H is plant height.

comparable to that of field-grown fennel. According to Moosavi et al. (2015), the fresh biomass of field-grown fennel ranged from 4.27 g/plant to 48.31 g/plant, depending on plant density in the field and sowing date. Plant height was relatively consistent for mature fennel ranging from week 8 to week 9, with a small increase at week 10. However, the growth index of fennel exhibited a clear increase trend during late harvest, which indicated that greater growth changes on branches length and width, instead of plant height, were happening for later harvested samples. At week 10, we also observed a larger standard deviation in all growth parameters, suggesting there were larger variances due to differences in growth conditions. Nevertheless, positive plant growth data suggested the fennel cultivar (Grosfruchtiger) used in this study was well suited to hydroponic cultivation and produced good yield.

3.2. Aroma-active compounds in fennel microgreens and mature plants

The aroma-active compounds found in fennel microgreens and mature plants, the identification methods, their odor descriptors, and the intensity are shown in Table 2. A total of 35 aroma-active compounds were identified in fennel microgreens and mature plants, including 5 monoterpenes, 3 sesquiterpenes, 1 alkane, 3 alcohols, 5 aldehydes, 4 monoterpeneoids, 1 ester, 2 ketones, 8 phenylpropenes, and 3 unknown compounds that cannot be identified by GC-MS due to invisible peaks. The peaks not identified by GC-MS were below the detection limit of mass spectrometer but above the human sensory threshold. The number of identified aroma-active compounds is similar to previous studies focusing on fennel. Twenty-seven and 30 aroma compounds were identified by GC-MS-O from fennel seeds (Díaz-Maroto et al., 2005) and commercial fennel essential oils (Xiao et al., 2017), respectively. However, in both studies, volatile compounds were extracted using either simultaneous distillation extraction (SDE) or supercritical fluid extraction (SFE) instead of SPME. In our study, fourteen compounds, namely *p*-cymene, γ -muurolene, germacrene D, α -cadinene, tridecane, (*E*)-2-hexenal, phenyl acetaldehyde, 4-methoxycinnamaldehyde, bornyl acetate, carvacrol, 3-octanol acetate, *p*-propyl-anisole, methyl eugenol, and apinol, were first identified as aroma-active compounds by GC-MS-O in fennel.

Fennel microgreens contained more aroma-active compounds compared to mature leaves. Thirty-two aroma compounds were identified in fennel microgreens, while 23, 23, and 28 aroma-active compounds were identified in mature fennel harvested at week 8, 9, 10, respectively. (*E*)-Anethole was the most potent and characteristics aroma-active compound among all samples, with a strong and distinct sweet, anise aroma. (*E*)-Anethole was also reported by previous work as the main aroma constituent of fennel tea and fennel seeds (Díaz-Maroto et al., 2005; Zeller & Rychlik, 2006). Due to its sweet, anise-like aroma, (*E*)-anethole is commonly used in the cosmetics and food industries (Wang et al., 2020). In addition, (*E*)-anethole has been reported to have several bioactive functions, such as antioxidant and antibacterial activities (Senatore et al., 2013). In a recent study, (*E*)-anethole was added to the emitting sachets inside the packages of ready-to-eat lettuce, and the results showed that the added (*E*)-anethole reduced discoloration, and improved overall freshness and odor of lettuce (Wiecznińska & Cavoski, 2018). *p*-Cymene and myristicin were only identified in fennel microgreens. *p*-Cymene exhibited a medium woody, terpenic aroma, and myristicin exhibited a weak woody, spicy aroma. Allo-cimene and anisketone were only identified in early harvest of mature fennel. Allo-cimene exhibited a medium to strong herbal, terpenic aroma and anisketone exhibited a weak licorice, sweet aroma. 3-Octanol acetate was only detected in week 10 fennel with a medium woody, herbal aroma. Six aroma compounds had a stronger intensity in mature fennel, namely α -pinene (pine, woody), γ -muurolene (spice, woody), germacrene D (spice, clove), 1-octen-3-ol (earthy, mushroom), (*E*)-2-hexenal (leafy, green), and β -ionone (floral, sweet). On the other hand, carvacrol (herbal, oregano) showed a stronger odor intensity in

Table 2
Aroma-active compounds identified in fennel microgreens and mature plants.

no.	Compound	IS	RI	RI Ref. ^a	Identification Method ^b	Odor Description	Odor Intensity ^c			
							Week 3	Week 8	Week 9	Week10
	Monoterpenes									
1	<i>α</i> -pinene	decane	1011	1011	MS, RI, OD, AS	Pine, woody	W	M*	W	M*
2	<i>α</i> -phellandrene	decane	1153	1157	MS, RI, OD, AS	Herbal, woody	M*	M**	W*	M
3	<i>γ</i> -terpinene	decane	1239	1233	MS, RI, OD, AS	Oily, herbal	M	W	W*	M
4	allo-ocimene	decane	1371	1366	MS, RI, OD	Herbal, terpenic		S*	M*	
5	<i>p</i> -cymenene	decane	1435	1435	MS, RI, OD	Woody, terpenic	M*			
	Sesquiterpenes									
6	<i>γ</i> -muurolene	decane	1697	1690	MS, RI, OD	Spice, woody	W*	W*	M*	S*
7	germacrene D	decane	1719	1717	MS, RI, OD	Spice, clove	W*	M*	M*	S*
8	<i>α</i> -cadinene	decane	1816	1815	MS, RI, OD	Woody, fresh	M	M**	M**	M
	Alkane									
9	tridecane	decane	1303	1300	MS, RI, OD, AS	Mushroom, gasoline-like	M*	W**	W**	M*
	Alcohols									
10	(<i>Z</i>)-3-hexen-1-ol	2-octanol	1384	1377	MS, RI, OD, AS	Leafy, green	M			M*
11	1-octen-3-ol	2-octanol	1451	1451	MS, RI, OD, AS	Earthy, mushroom	M	M	M	S
12	linalool	2-octanol	1552	1552	MS, RI, OD, AS	Floral	M*	M*		
	Aldehydes									
13	(<i>Z</i>)-3-hexenal	2-octanol	1130	1134	MS, RI, OD, AS	Leafy, grassy	M	S	W	M*
14	(<i>E</i>)-2-hexenal	2-octanol	1218	1218	MS, RI, OD, AS	Leafy, green	W*	M**		M*
15	phenyl acetaldehyde	2-octanol	1634	1632	MS, RI, OD, AS	Floral, sweet	M**			M*
16	<i>p</i> -anisaldehyde	2-octanol	2019	2016	MS, RI, OD, AS	Floral, sweet	M	M*	M	M
17	4-methoxycinnamaldehyde	2-octanol	2554	2544	MS, RI, OD	Cinnamon, sweet	M			M*
	Monoterpenoids									
18	fenchyl acetate	1-phenylethanol	1495	1481	MS, RI, OD, AS	Herbal, pine	W**			W**
19	camphor	1-phenylethanol	1518	1519	MS, RI, OD	Camphor	W			W**
20	bornyl acetate	1-phenylethanol	1583	1580	MS, RI, OD, AS	Woody, pine	M	M	W	M
21	carvacrol	1-phenylethanol	2231	2233	MS, RI, OD	Herbal, oregano	M	W	W*	W
	Ester									
22	3-octanol acetate	1-phenylethanol	1335	1344	MS, RI, OD	Woody, herbal				M**
	Ketones									
23	<i>β</i> -ionone	1-phenylethanol	1940	1940	MS, RI, OD, AS	Floral, sweet	M	S**	S*	S*
24	anisketone	1-phenylethanol	2150	2170	MS, RI, OD, AS	Licorice, sweet			W**	
	Phenylpropenes									
25	<i>p</i> -propyl-anisole	1-phenylethanol	1612	1606	MS, RI, OD, AS	Anise, green	M**	W**		
26	estragole	1-phenylethanol	1669	1661	MS, RI, OD, AS	Anise, herbal	M	W	M	S
27	(<i>Z</i>)-anethole	1-phenylethanol	1754	1732	MS, RI, OD	Sweet, anise	M	M	M	W
28	(<i>E</i>)-anethole	1-phenylethanol	1846	1838	MS, RI, OD, AS	Sweet, anise	S	VS	S	VS
29	methyl eugenol	1-phenylethanol	2000	2003	MS, RI, OD, AS	Clove, sweet	M	M	M*	M
30	methyl isoeugenol	1-phenylethanol	2174	2176	MS, RI, OD	Clove, woody	M*		W*	M*
31	myristicin	1-phenylethanol	2262	2257	MS, RI, OD	Woody, spicy	W*			
32	apiol	1-phenylethanol	2361	2431	MS, RI, OD	Parsley, green	W*	W**		M**
	Unknown									
33	Unknown 1	NA	1538	NA	OD	Woody, floral	M*		W*	W**
34	Unknown 2	NA	2058	NA	OD	Fruity, metal	M	M	M*	M*
35	Unknown 3	NA	2300	NA	OD	Floral, fruity	W*		W*	
Total number of aroma-active compounds identified							32	23	23	28

^a Reference retention indices are from a NIST publication (Babushok, Linstrom, & Zenkevich, 2011).
^b Identification method: mass spectra (MS), retention index (RI), odor descriptors (OD) against authentic aroma standards (AS).
^c Odor intensity: very weak (VW), weak (W), medium (M), strong (S), and very strong (VS).
* Two out of three panelists smelled the compound.
** One out of three panelists smelled the compound.

fennel microgreens compared with mature fennel. While the differences in aroma profiles observed across different stages of fresh fennel are evident, it remains uncertain whether these differences are perceptible to consumers. A future sensory study may be necessary to validate the

differences, although this is beyond the scope of the current investigation.

3.3. Quantification of aroma-active compounds and odor activity values

The 32 known aroma-active compounds were quantitated with standard addition methods (SAM). The representative SPME-GC-MS total ion chromatograms of aroma compounds from microgreen and mature fennel were shown in Supplemental Fig. 1. The regression equations of aroma-active compounds built from SAM and their details were shown in Table 3. The quantification results and odor-active values (OAV) of aroma-active compounds in fennel microgreens and mature fennel leaves were shown in Table 4. Six replicates were measured at each growth stage, with each replicate analyzed twice by GC-MS. The large variation in the concentration of some compounds was due to individual differences between plants (as shown in Supplemental Tables 2–5), not the analytical method. Quantitatively, (*E*)-anethole was present in all fresh fennel samples with the highest concentration above 492 µg/g. Two aroma compounds with concentrations above 100 µg/g were α -phellandrene and apiol. Other aroma compounds ranging from 1 to 100 µg/g were α -pinene, γ -terpinene, (*Z*)-3-hexenal, (*E*)-2-hexenal, *p*-anisaldehyde, 4-methoxycinnamaldehyde, β -ionone, anisketone, (*Z*)-anethole, methyl eugenol, and methyl isoeugenol. It is worth noting that microgreens contained a significant higher level of α -phellandrene (herbal, woody), γ -terpinene (oily, herbal), *p*-cymenene (woody, terpenic), germacrene D (spice, clove), tridecane (mushroom, oily), linalool (floral), and apiol (parsley, green), while mature fennel contained a higher level of (*Z*)-3-hexen-1-ol (leafy, green). The concentration of (*Z*)-3-hexenal and (*E*)-2-hexenal also seemed to gradually decrease when plants were allowed to grow older. Both (*Z*)-3-hexenal and (*E*)-2-hexenal reached the highest concentration in the samples taken at week 8, an early mature stage. The differences suggested the discrepancy of secondary metabolites biosynthesis at different growth stages.

The contribution of aroma compounds depends on the concentration as well as their odor detection threshold. OAV of aroma compounds was calculated based on previous published odor detection thresholds in water (moisture content of fresh fennel > 83 %) (Jonas et al., 2021). (*E*)-Anethole also possessed a high OAV, with week 8 sample being the highest value. It is greatly contributing to the “anise”, “sweet” odor of both fennel microgreens and mature plants. (*Z*)-3-Hexenal was the compound with the highest OAV because of its low odor detection threshold, despite its concentration being relatively low. It was contributing a “leafy”, “grassy” odor of fennel. Other aroma compounds with a high OAV and contribution to fennel aroma profiles were α -pinene (OAV 4044–7134), estragole (OAV 3027–5550), β -ionone (OAV 2097–3415), (*E*)-2-hexenal (OAV 591–1604), α -phellandrene (OAV 441–985), *p*-anisaldehyde (OAV 139–248), which was also in agreement with GC-O results. These compounds contributed to the “woody”, “herbal”, “leafy”, and “floral” notes of fennel aroma. What’s more, the results were in accordance with previous reports published by other researchers. For example, Xiao et al. (2017) also found that (*E*)-anethole, estragole, *p*-anisaldehyde, α -phellandrene, and α -pinene were compounds with high OAV in fennel essential oils produced in different regions of China. Similarly, Zeller and Rychlik (2006) reported (*E*)-anethole and estragole as the main aroma contributing compounds of tea made from bitter fennel from Hungary. However, there were some aroma compounds that have been reported but were not found in our study, such as fenchone and limonene (Miraldi, 1999; Zeller & Rychlik, 2006), which suggested that the aroma constitutes of fennel may be related to variety and geographical origins.

The comparison of different aroma constituents of fennel over harvest time was shown in Fig. 1. In general, the total concentration of all aroma-active compounds in fennel was not significantly different between samples (Fig. 1A), but numerically fennel microgreens contained

Table 3
Regression equations for selected aroma-active compounds in fennel by standard addition method.

No.	Compound	IS	Regression Equation ^a	R ²	Linear range (µg/g)	LOD	LOQ
1	α -pinene	decane	y = 0.3418x + 0.0527	0.9975	0.36 ~ 143.28	0.39	1.29
2	α -phellandrene	decane	y = 0.7269x + 3.6977	0.9947	1.21 ~ 484.50	0.11	0.38
3	γ -terpinene	decane	y = 1.1434x + 1.8957	0.9948	0.49 ~ 197.60	0.33	1.10
4	allo-ocimene*	decane	y = 3.3571x - 1.0863	0.9941	0.08 ~ 33.32	0.01	0.04
5	<i>p</i> -cymenene	decane	y = 3.3571x - 1.0863	0.9941	0.08 ~ 33.32	0.01	0.04
6	γ -muurolene*	decane	y = 3.3571x - 1.0863	0.9941	0.08 ~ 33.32	0.01	0.04
7	germacrene D*	decane	y = 3.3571x - 1.0863	0.9941	0.08 ~ 33.32	0.01	0.04
8	α -cadinene*	decane	y = 3.3571x - 1.0863	0.9941	0.08 ~ 33.32	0.01	0.04
9	tridecane	decane	y = 2.3394x - 0.4302	0.9941	0.02 ~ 7.52	0.00	0.01
10	(<i>Z</i>)-3-hexen-1-ol	2-octanol	y = 0.2213x + 0.6373	0.9970	0.02 ~ 8.33	0.04	0.14
11	1-octen-3-ol	2-octanol	y = 0.4363x + 1.3773	0.9955	0.01 ~ 1.65	0.16	0.54
12	linalool	2-octanol	y = 0.5871x - 0.1086	0.9965	0.01 ~ 3.34	0.01	0.05
13	(<i>Z</i>)-3-hexenal	2-octanol	y = 0.0035x + 0.0266	0.9942	0.01 ~ 16.00	0.07	0.25
14	(<i>E</i>)-2-hexenal	2-octanol	y = 0.1129x + 3.1690	0.9886	0.17 ~ 66.64	1.17	3.90
15	phenyl acetaldehyde	2-octanol	y = 0.0836x + 1.4775	0.9957	0.01 ~ 3.91	0.20	0.67
16	<i>p</i> -anisaldehyde	2-octanol	y = 0.0581x - 0.7592	0.9822	0.11 ~ 43.68	0.65	2.15
17	4-methoxycinnamaldehyde	2-octanol	y = 0.0105x - 0.0005	0.9766	0.01 ~ 1.94	0.03	0.10
18	fenchyl acetate	1-phenylethanol	y = 1.2779x + 18.9400	0.9729	1.41 ~ 564.48	0.69	2.29
19	camphor	1-phenylethanol	y = 1.3442x + 1.7364	0.9861	0.12 ~ 47.75	0.08	0.26
20	bornyl acetate	1-phenylethanol	y = 2.0570x + 0.8921	0.9966	0.19 ~ 75.24	0.07	0.22
21	carvacrol	1-phenylethanol	y = 0.8875x - 0.0478	0.9989	0.07 ~ 29.11	0.02	0.05
22	3-octanol acetate	1-phenylethanol	y = 3.6567x + 0.1126	0.9847	0.01 ~ 1.69	0.00	0.02
23	β -ionone	1-phenylethanol	y = 0.5634x + 0.3565	0.9971	0.16 ~ 63.84	0.02	0.07
24	anisketone	1-phenylethanol	y = 0.4849x - 0.0254	0.9973	0.08 ~ 31.14	0.03	0.11
25	<i>p</i> -propyl-anisole	1-phenylethanol	y = 5.3344x - 0.1859	0.9982	0.02 ~ 7.13	0.00	0.00
26	estragole	1-phenylethanol	y = 3.7210x - 3.4301	0.9999	3.80 ~ 1520.96	0.38	1.01
27	(<i>Z</i>)-anethole	1-phenylethanol	y = 3.4311x - 14.1430	0.9799	0.53 ~ 213.41	0.04	0.11
28	(<i>E</i>)-anethole	1-phenylethanol	y = 2.9579x + 62.2620	0.9935	14.70 ~ 5880.60	1.96	6.52
29	methyl eugenol	1-phenylethanol	y = 0.7534x - 0.4722	0.9996	0.07 ~ 285.38	0.18	0.61
30	methyl isoeugenol*	1-phenylethanol	y = 0.7534x - 0.4722	0.9996	0.07 ~ 285.38	0.18	0.61
31	myristicin	1-phenylethanol	y = 0.2460x + 0.0209	0.9970	0.04 ~ 15.52	0.01	0.03
32	apiol*	1-phenylethanol	y = 0.7534x - 0.4722	0.9996	0.07 ~ 285.38	0.18	0.61

^a Regression equation: $y = \frac{\text{Peak area of analyte}}{\text{Peak area of IS}} \times \frac{\text{Mass of analyte}}{\text{Mass of IS}}$.
* Pure standards not available, calibration curve was built based against compounds with similar chemical structure. Specifically, allo-ocimene, γ -muurolene, germacrene D, and α -cadinene were quantified against *p*-cymenene; methyl isoeugenol and apiol were quantified against methyl eugenol.

Table 4Concentration ($\mu\text{g/g}$ based on fresh weight) of aroma-active compounds found in leafy fennel.

No.	Compound	Odor threshold in water ($\mu\text{g/g}$)	Concentration ($\mu\text{g/g}$ fennel, fresh weight)				OAV			
			Microgreen fennel		Mature fennel		Microgreen fennel		Mature fennel	
			Week 3	Week 8	Week 9	Week 10	Week 3	Week 8	Week 9	Week 10
1	α -pinene	0.006 ^A	24.26 $\pm$ 11.85 ^a	42.80 $\pm$ 15.30 ^a	29.04 $\pm$ 18.96 ^a	33.97 $\pm$ 26.38 ^a	4044	7134	4840	5661
2	α -phellandrene	0.2 ^B	196.92 $\pm$ 56.56 ^a	90.71 $\pm$ 26.16 ^b	99.48 $\pm$ 35.21 ^b	88.17 $\pm$ 26.82 ^b	985	454	497	441
3	γ -terpinene	1 ^D	28.49 $\pm$ 5.83 ^a	3.90 $\pm$ 2.41 ^b	6.02 $\pm$ 4.13 ^b	3.65 $\pm$ 3.20 ^b	28	4	6	4
4	allo-ocimene	0.727 ^F	0.27 $\pm$ 0.01 ^a	0.25 $\pm$ 0.01 ^a	0.26 $\pm$ 0.05 ^a	0.25 $\pm$ 0.01 ^a	0.4	0.3	0.4	0.3
5	<i>p</i> -cymenene	11.4 ^G	0.30 $\pm$ 0.03 ^a	0.26 $\pm$ 0.01 ^b	0.26 $\pm$ 0.01 ^b	0.26 $\pm$ 0.01 ^b	0.03	0.02	0.02	0.02
6	γ -muurolene	ND	0.53 $\pm$ 0.08 ^a	0.54 $\pm$ 0.19 ^a	0.38 $\pm$ 0.06 ^a	0.43 $\pm$ 0.14 ^a	ND	ND	ND	ND
7	germacrene D	ND	0.85 $\pm$ 0.16 ^a	0.36 $\pm$ 0.03 ^b	0.36 $\pm$ 0.08 ^b	0.61 $\pm$ 0.25 ^{ab}	ND	ND	ND	ND
8	α -cadinene	ND	0.25 $\pm$ 0.01 ^a	0.28 $\pm$ 0.02 ^a	0.26 $\pm$ 0.02 ^a	0.26 $\pm$ 0.02 ^a	ND	ND	ND	ND
9	tridecane	ND	0.51 $\pm$ 0.18 ^a	0.15 $\pm$ 0.01 ^b	0.14 $\pm$ 0.00 ^b	0.14 $\pm$ 0.01 ^b	ND	ND	ND	ND
10	(<i>Z</i>)-3-hexen-1-ol	0.07 ^A	0.12 $\pm$ 0.07 ^c	1.29 $\pm$ 0.69 ^{ab}	2.40 $\pm$ 1.22 ^a	0.36 $\pm$ 0.31 ^{bc}	2	19	34	5
11	1-octen-3-ol	0.001 ^A	ND	ND	ND	0.04	ND	ND	ND	35
12	linalool	0.006 ^A	0.09 $\pm$ 0.05 ^a	0.03 $\pm$ 0.01 ^b	0.03 $\pm$ 0.01 ^b	0.04 $\pm$ 0.03 ^b	15	5	4	7
13	(<i>Z</i>)-3-hexenal	0.00025 ^A	4.56 $\pm$ 2.96 ^{ab}	6.75 $\pm$ 1.41 ^a	4.12 $\pm$ 2.22 ^{ab}	1.97 $\pm$ 1.41 ^b	18,233	27,011	16,485	7865
14	(<i>E</i>)-2-hexenal	0.017 ^A	22.92 $\pm$ 6.16 ^{ab}	27.27 $\pm$ 9.37 ^a	14.97 $\pm$ 5.26 ^{bc}	10.05 $\pm$ 7.37 ^c	1348	1604	881	591
15	phenyl acetaldehyde	0.004 ^A	ND	ND	ND	ND	ND	ND	ND	ND
16	<i>p</i> -anisaldehyde	0.047 ^E	9.15 $\pm$ 2.74 ^a	11.66 $\pm$ 9.17 ^a	6.54 $\pm$ 4.33 ^a	6.91 $\pm$ 3.01 ^a	195	248	139	147
17	4-methoxycinnamaldehyde	ND	1.70 $\pm$ 0.55 ^a	1.59 $\pm$ 0.79 ^a	1.26 $\pm$ 1.02 ^a	1.22 $\pm$ 0.52 ^a	ND	ND	ND	ND
18	fenchyl acetate	ND	3.17 $\pm$ 4.30	ND	ND	ND	ND	ND	ND	ND
19	camphor	1.47 ^E	0.77	0.78	ND	0.52	0.5	0.5	ND	0.4
20	bornyl acetate	0.075 ^A	0.74 $\pm$ 0.35 ^a	0.04 ^a	ND	0.43 ^a	10	0.5	ND	6
21	carvacrol	1.7 ^F	0.22 $\pm$ 0.07 ^a	0.20 $\pm$ 0.05 ^a	0.16 $\pm$ 0.03 ^a	0.19 $\pm$ 0.06 ^a	0.1	0.1	0.1	0.1
22	3-octanol acetate	ND	ND	ND	ND	0.02 $\pm$ 0.02	ND	ND	ND	ND
23	β -ionone	0.0005 ^B	1.11 $\pm$ 0.39 ^a	1.71 $\pm$ 0.89 ^a	1.05 $\pm$ 0.80 ^a	1.56 $\pm$ 0.93 ^a	2212	3415	2097	3127
24	anisketone	1.8 ^E	2.30 $\pm$ 0.49 ^a	2.92 $\pm$ 1.71 ^a	1.71 $\pm$ 1.26 ^a	2.02 $\pm$ 1.19 ^a	1	2	1	1
25	<i>p</i> -propyl-anisole	ND	0.08 $\pm$ 0.00 ^a	0.12 $\pm$ 0.08 ^a	0.08 $\pm$ 0.01 ^a	0.09 $\pm$ 0.01 ^a	ND	ND	ND	ND
26	estragole	0.0075 ^B	22.70 $\pm$ 4.58 ^a	31.42 $\pm$ 17.13 ^a	22.81 $\pm$ 20.09 ^a	41.62 $\pm$ 43.91 ^a	3027	4190	3041	5550
27	(<i>Z</i>)-anethole	ND	9.54 $\pm$ 0.19 ^b	12.35 $\pm$ 1.75 ^a	10.65 $\pm$ 2.40 ^{ab}	10.06 $\pm$ 1.36 ^{ab}	ND	ND	ND	ND
28	(<i>E</i>)-anethole	0.073 ^E	543.11 $\pm$ 140.97 ^a	765.66 $\pm$ 506.40 ^a	492.08 $\pm$ 548.75 ^a	514.75 $\pm$ 450.89 ^a	7440	10,488	6741	7051
29	methyl eugenol	0.82 ^A	1.52 $\pm$ 0.12 ^{ab}	1.47 $\pm$ 0.06 ^{ab}	1.40 $\pm$ 0.08 ^b	1.69 $\pm$ 0.23 ^a	2	2	2	2
30	methyl isoeugenol	1.6 ^E	2.67 $\pm$ 0.37 ^a	3.00 $\pm$ 1.18 ^a	1.93 $\pm$ 0.78 ^a	2.03 $\pm$ 1.00 ^a	2	2	1	1
31	myristicin	0.025 ^C	3.85 $\pm$ 2.76 ^a	0.08 $\pm$ 0.07 ^a	0.21 ^a	3.05 $\pm$ 6.04 ^a	154	3	9	122
32	apiol	ND	151.60 $\pm$ 32.91 ^a	4.59 $\pm$ 4.05 ^b	2.31 $\pm$ 0.48 ^b	2.98 $\pm$ 1.44 ^b	ND	ND	ND	ND

^{abc} Represent the mean separation between harvest time by Tukey's HSD at $p < 0.05$. Results are expressed as mean $\pm$ SD (unless the compound is detected in only one of the six replicates). ND = not determined.

^A Odor threshold obtained from Leffingwell and Associates (1999) (<http://www.leffingwell.com/odorthre.htm>).

^B Odor threshold obtained from literature (Rychlik et al., 1998).

^C Odor threshold obtained from literature (Buttery et al., 1968).

^D Odor threshold obtained from literature (van Gemert, 2003).

^E Odor threshold obtained from literature (Zeller & Rychlik, 2006).

^F Odor threshold obtained from literature (Zhang et al., 2023).

^G Odor threshold obtained from literature (Liang et al., 2022).

the highest average value of total concentration of aroma-compounds. Phenylpropenes, including (*E*)-anethole, (*Z*)-anethole, estragole, methyl eugenol, methyl isoeugenol, *p*-propyl-anisole, myristicin, and apiol, were the most abundant group of aroma-active compounds in fennel. They largely determined the total concentration of aroma-active compounds and showed similar trend over different harvest times (Fig. 1B). Although not significantly different, fennel microgreens also contained the highest average value of phenylpropenes (735.09 $\mu\text{g/g}$), higher than the phenylpropene content of all mature fennel. Among the aroma compounds, myristicin exhibited the highest OAV in microgreens at week 3, while (*E*)-anethole had the highest OAV in early mature samples at week 8, and estragole showed the highest OAV in late mature samples at week 10. In contrast, the OAVs of methyl eugenol and methyl isoeugenol did not show clear trends across harvest times.

Monoterpenes were the second largest group of aroma-active compounds in fennel, and the average value ranging from 126.30 to 250.23

$\mu\text{g/g}$ among all samples. One-way ANOVA results showed that microgreens contained a significantly higher level of monoterpenes than mature fennel (Fig. 1C). α -Phellandrene was the monoterpene with highest concentration, but α -pinene had the highest OAV due to its low odor detection threshold, making it an important contributor to the overall fennel aroma profile. The other monoterpenes, such as γ -terpinene, allo-ocimene, and *p*-cymenene, had relatively lower concentrations and OAV values, therefore, were considered not as important as α -phellandrene and α -pinene. α -Pinene exhibited the highest OAV in early mature samples at week 8, while α -phellandrene and γ -terpinene exhibited the highest OAVs in microgreens at week 3.

Aldehydes were also found to have relatively high concentrations in fennel. Fennel sample from week 8 harvest showed the highest content in aldehydes, significantly higher than other mature samples, and numerically higher than the aldehyde content of fennel microgreens (Fig. 1D). (*Z*)-3-hexenal and (*E*)-2-hexenal were aldehydes with highest

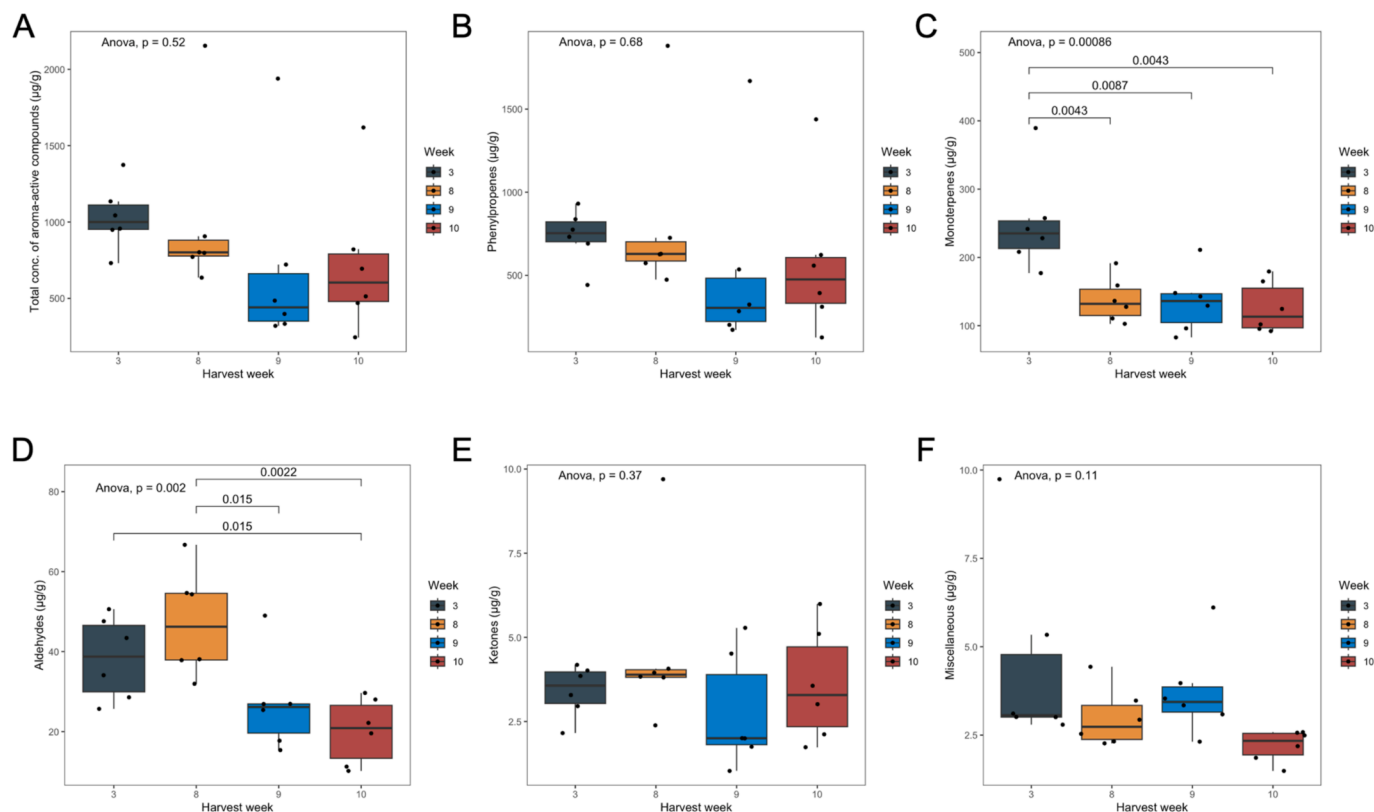


Fig. 1. Concentration of (A) total aroma-active compounds, (B) phenylpropenes, (C) monoterpenes, (D) aldehydes, (E) ketones, and (F) miscellaneous compounds found in fennel sample harvested at different weeks.

OAV and they contributed a green, leafy aroma. Both (*Z*)-3-hexenal and (*E*)-2-hexenal showed the highest OAV in early mature samples at week 8, and the OAV continued to decrease as growth time increased. Phenyl acetaldehyde and *p*-anisaldehyde were aldehydes contributing to the floral and sweet note of fennel aroma profile. Similar to other aldehydes, *p*-anisaldehyde also exhibited the highest OAV in the samples collected at week 8. β -Ionone and anisketone were the two ketones identified as aroma-active compounds in this study, and no significant differences were found in the level of ketones over microgreens and mature fennel samples (Fig. 1E). However, both ketones showed the highest OAVs at early mature fennel plants harvest at week 8. Other miscellaneous compounds, including sesquiterpenes, monoterpenoids, alcohols, ester, and alkane, only took up less than 1 % of total aroma-active compounds, and showed no significant differences between fennel samples collected at different times (Fig. 1F).

Phenylpropanoids are a part of the chemical defense mechanism and contribute to plant resistance against pests and herbivores (Chang & Ahn, 2002; Sousa et al., 2013). Additionally, phenylpropanoids and terpenoids found in plant essential oils exhibit activity against a wide range of fungi and pathogens (Basaid et al., 2021). These defense compounds, derived from specialized metabolism, are typically found in higher concentrations in young plants to protect them from abiotic and biotic stresses (Gershenson & Ullah, 2022). This may explain why fennel microgreens contain higher levels of phenylpropanoids and monoterpenes.

3.4. Multivariate statistical analysis of aroma-active compounds in fennel microgreens and mature plants

To further evaluate the overall variance in aroma profiles of fennel microgreens and mature plants, principal component analysis (PCA) was applied to the concentrations of different aroma-active compounds and the results were shown in Fig. 2A and Fig. 2B. Five compounds, namely

1-octen-3-ol, phenyl acetaldehyde, fenchyl acetate, camphor, and 3-octanol acetate, were excluded due to the presence of many zero values, which could bias the model due to zero-inflation. The samples were well differentiated by the first two principal components (PC1 and PC2), which accounted for 30.1 % and 24.0 % of the total variance, respectively. As shown in Supplemental Tables 2-5, individual differences among plant samples could contribute to the overall variance. Considering the total variances explained by the first two principal components were less than 60 %, we also visualized the variances explained by other principal components (Supplemental Fig. 2). Subsequent principal components, such as PC3 and PC4, also captured significant portions of the variance (9.2 % and 6.7 %, respectively), reflecting the complex interaction among variables. In total, the first ten principal components covered more than 90 % of the total variances. While some overlap was observed among mature fennel samples from different growth stages, no overlap occurred between microgreens and mature fennels (Fig. 2). This indicates a clear distinction in the aroma-active compounds between microgreens and mature plants. All samples representing fennel microgreens were in the positive side of PC1, which were also positively correlated to a higher concentration of certain aroma-active compounds, such as α -phellandrene, γ -terpinene, germacrene D, tridecane, linalool, and apiol. Supplemental Fig. 3A and Supplemental Fig. 3B showed the sample distribution on the first and third principal components. All points representing microgreens were located on the positive side of PC1, while most points for mature fennel were located on the negative side of PC1. Additionally, all points representing Week 10 samples were located on the positive side of PC3, while samples from week 3, week 8, and week 9 were predominantly on the negative side. These results indicated that the aroma-active compounds associated with PC1 more effectively distinguish between microgreens and mature fennel, while the aroma-active compounds associated PC3 better differentiate week 10 samples from earlier time points (week 3, 8, and 9). Supplemental Fig. 3C and Supplemental

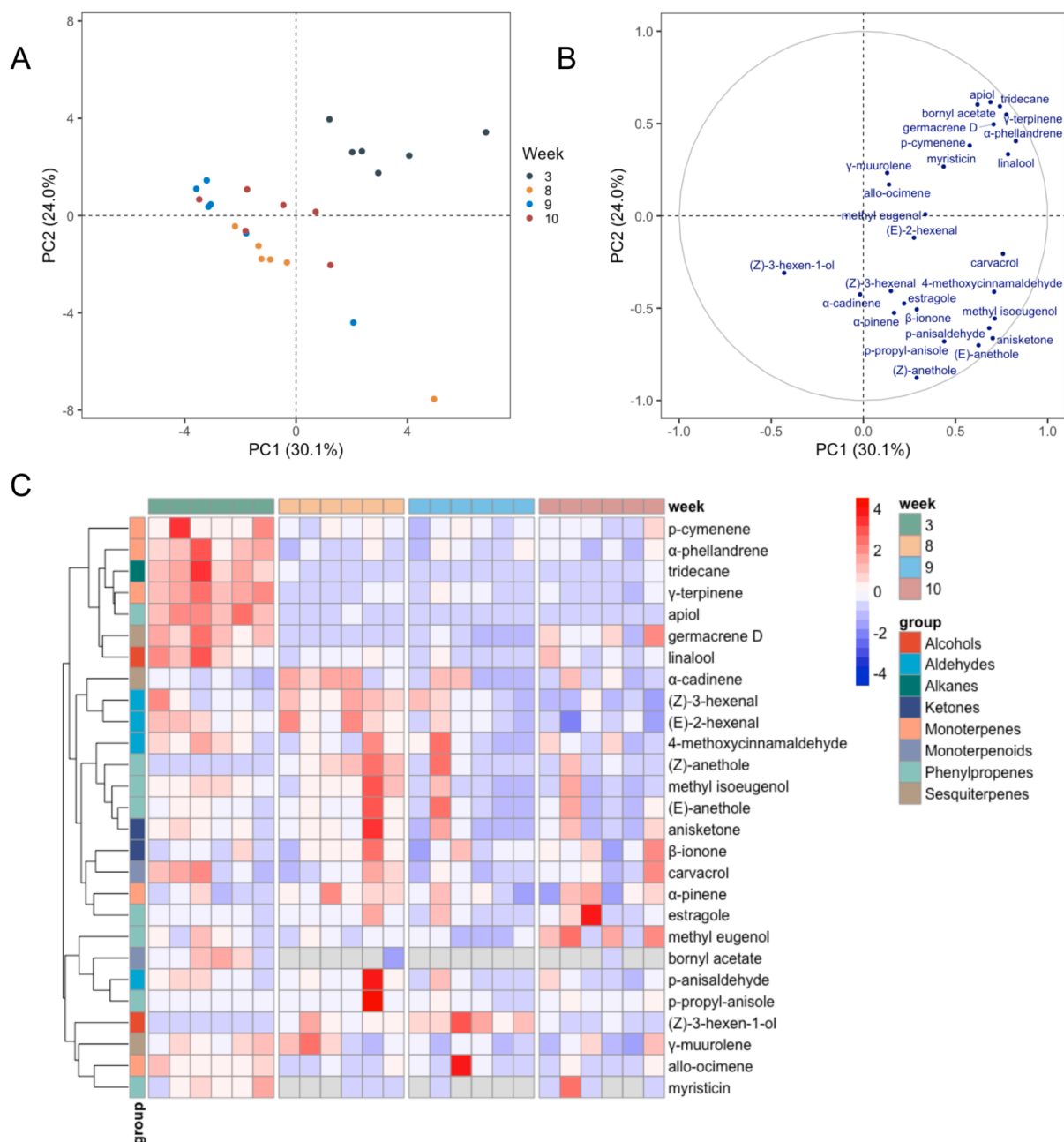


Fig. 2. Multivariate statistical analysis of aroma-active compounds found via SPME-GC-MS in fennel samples. (A) Principal component analysis (PCA), (B) the loading plot of PCA, and (C) heatmap of aroma-active compounds in fennel from different harvest week.

Fig. 3D showed the distribution of samples on the second and third principal components. All the data points representing microgreens were located on the positive side of PC2, while most points representing mature fennels were positioned on the negative side of PC2. The results suggest that fennel microgreens are positively associated with higher concentrations of aroma-active compounds such as bornyl acetate and α -phellandrene, whereas mature fennel contains lower amount of these compounds.

To visually illustrate the pattern of change in aroma-active compounds present in fennel samples from different harvest time, a heatmap with hierarchical clustering analysis was applied. The results are shown in Fig. 2C, where aroma-active compounds were labeled by chemical groups, indicated by different colors on the left side of the heatmap. It can be observed that seven compounds, specifically *p*-cymenene, α -phellandrene, tridecane, γ -terpinene, apiol, germacrene D, and

linalool, in the same cluster showed a clear decreasing pattern from week 3 to week 10. It is also worth mentioning that more than half of these compounds are terpenes. This pattern is similar to findings in dill, another Apiaceae family herb, where terpenes such as α -phellandrene and apiol also decreased with harvest time (Huopalahti & Linko, 1983).

In addition, a decreasing trend was observed for 4-methoxycinnamaldehyde, methyl isoeugenol, (E)-anethole, and anisaketone. Young plants tend to accumulate high levels of certain volatile compounds, such as phenylpropanoids, as part of their defense mechanism; however, the specific levels and types of the secondary metabolites can vary largely depending on factors including plant cultivar, phenotype, physiological traits, the type of secondary metabolites, and the specific stress conditions (Brunetti et al., 2020; Francini, Giro, & Ferrante, 2019; Petruřová, Duřaiová, & Repčák, 2014; Wang et al., 2023). Methyl isoeugenol and (E)-anethole are phenylpropanoids synthesized from the

aromatic amino acid phenylalanine through enzymatic reactions. In contrast to the observation for fennel, methyl isoeugenol was found only in mature leaves of orange carrot, another Apiaceae plant, and not in young leaves (Yahya et al., 2019). This suggests that volatile composition is influenced not only by growth stage but also by the specific plant species.

A similar decreasing trend was observed for (*Z*)-3-hexenal and (*E*)-2-hexenal in another cluster. Both compounds are C-6 aldehydes that have a leafy, grassy odor produced by linoleic acid peroxidation via lipoxygenase (Thomas, 2017). Interestingly, (*Z*)-3-hexen-1-ol, a C6 alcohol and another green leaf volatile, showed an increasing trend from week 3 to week 9, followed by a decrease thereafter. These findings indicate a metabolic shift during plant growth, with volatile compounds serving different biological functions at various stages.

Additionally, in Fig. 2C, some mature samples, such as sample 5 from week 8 (the fifth column of week 8) and sample 2 from week 9 (the second column of week 9), showed higher concentrations of compounds than other replicates, suggesting variability in growth conditions among individual plants.

4. Conclusion

In this study, the aroma profile and volatile constituents of hydroponic fennel at different growth stages were investigated. In general, more aroma-active compounds were detected in microgreens, and the overall content of aroma-active compounds was also higher in fennel microgreens. In terms of the volatile profiles, phenylpropenes were the major aroma constituents of hydroponic fennel, regardless of growth stage. Compared with mature fennel, microgreens showed a significantly higher level of monoterpenes, and early harvested mature fennel contained a higher level of aldehydes. The results from multivariate statistical analysis revealed clear differences in the overall aroma profile of fennel microgreens and mature plants, and many aroma-active compounds, especially terpenes, exhibited a decrease trend with growth period. Although distinct differences in aroma profiles across growth stages of fresh fennel leaves were identified, it is unclear whether these differences are perceivable to consumers. A future sensory study could be necessary to confirm consumer perception of these differences. Our study determined for the first time the differences in aroma quality between fennel microgreens and mature fennel and provided information on aroma compounds to herb growers for the cultivation of hydroponic fennel. Other than aroma quality, farmers and herb growers are encouraged to take production cycle and cost into consideration when deciding the best harvest time. Future studies could investigate the impact of different cultivation techniques and environmental factors on the aroma and quality of hydroponic herbs, and the identification and regulation of genes and enzymes responsible for the biosynthesis of key aroma volatiles.

CRediT authorship contribution statement

Jingsi Liu: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Song Li:** Writing – review & editing, Resources. **Sean O’Keefe:** Writing – review & editing. **Ken Hurley:** Writing – review & editing. **Laban Rutto:** Writing – review & editing. **Renee Eriksen:** Writing – review & editing. **Yun Yin:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodres.2024.115229>.

Data availability

Data will be made available on request.

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