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## Research Article

### Variation Trend of Flavor Components at Different Growth Stages of Chinese Chives *Allium tuberosum*

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#### ABSTRACT

Chinese chives are valued for their culinary and medicinal properties. Chinese chive possesses anti-inflammatory, antibacterial, antioxidant, illness prevention, and other health benefits, according to global studies. Studies analysed the complete plant for volatiles with varied food processing and therapeutic properties, but little is known about volatile levels at different plant growth stages. This study examined flavour component variation in Chinese chives at different development phases. The study found that total volatile chemicals of Fu Jiu Bao F1 increased initially and subsequently dropped, approaching peak at 30 DAT; whereas Jiu Xing 22 climbed gradually and approaching peak at fruiting. Fujibao F1 has 9, 18, and 15 volatiles in leaf sheath, leaf, and root. Thus, Fujibao F1 was suitable for harvesting vegetative organs, while Jiuxing 22 was suitable for harvesting chives stem, and its seeds and inflorescence had greater condiment potential. Overall volatile components of Chinese chive changed dynamically at different phases, although overall contents in different organs were consistent. In variety Jiu Xing 22, total volatile contents varied abnormally, while variety Fu Jiu Bao F1 had considerable constancy in volatile contents at all organ stages. Fu Jiu Bao F1 leaves had greater volatile content than roots and leaf sheath during vegetative development (seedling, 30 days after transplanting, and harvesting). However, Jiu Xing 22 root volatiles were greater than leaf sheath and leaves. During flowering, Fu Jiu Bao F1 mature flowers had the greatest volatile chemicals (530.6µg/g) while Jiu Xing 22 had the highest in the leaf sheath. At fruiting, Fu Jiu Bao F1 had more volatile concentration (252.16µg/g) in seeds than other organs, while Jiuxing 22 had higher volatiles in leaf sheath. The E-Nose examination discovered aromatic, broad range, sulphur organic, hydrogen, arom-aliph, broad methane, broad alcohol, and methane aliph, although plant growth stages may affect flavour, fragrance, and other features.

**Keywords:** Chinese Chive, Volatile Organic Compounds, Growth Stages, Organs, HS-SPME/GC-MS, E-Nose Analyses, PCA.

#### INTRODUCTION

Vegetables contain a large amount of nutrients required by the human body, because for maintaining the human health, vitamins, minerals and antioxidants are fundamental. Similarly, the plants has essential role to maintain balanced diet and health of humans.



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Chinese chive seeds contained high amounts of oil (15.8%), dietary fibre (18.2%) and crude protein (12.3%); while oil of seed was composed of 10.1% saturated and 90.0% unsaturated fatty acids, 69.1% linoleic and 7.0% palmitic (Alan et al., 2018; Imahori et al. 2014). The crude proteins widely differ in seed and leaves of Chinese chives; the variation in crude protein may also be linked with the genetic makeup of cultivars used by different researchers under different ecologies. Because of its attractive colored flower, the plant is also recognized as an ornamental plant (Devi et al., 2014). Apart from the culinary and medicinal use of *A. tuberosum*, its significance as spice and source of libido stimulation (sexual urge) is also well recognized in this region. Moreover, they use *A. tuberosum* to treat erectile dysfunction in the traditional Chinese medicine. Hence, *A. tuberosum* it is considered as aphrodisiac (Tang et al., 2017). The properties of *A. tuberosum* plant are effectively lower cholesterol and locally in India it is used as a hair tonic (Kim et al., 2008). Traditionally the plant is widely used for its aphrodisiac property (Benkeblia and Lanzotti, 2007; Da Silva et al., 2012; Hu et al., 2006). Some experimental data also demonstrated its efficacy against impotency (Da Silva et al., 2012).

Alkaloids, phenols, carotenoids and organosulfur compounds are the phytochemical groups of great importance (Liu, 2004). In Chinese chive (*Allium tuberosum*), the organosulfur compounds are the core flavor forerunners, and this makes the Chinese chives and other associated allium species important in nutritional and medicinal values (Yabuki et al., 2010). Thiosulfates enhance the expression of apoptosis inducing factor (Jannat et al., 2019), lessen expression of antiapoptotic protein Bcl-2, inhibit human prostate cells (Hasanuzzaman et al., 2017). Seeds of the plant contain a phytochemical which was studied on human promyelocytic leukemia cell line, showing inhibitory effect on growth of the cell line (Sang et al., 2002; Son et al., 2012). Many varieties of *A. tuberosum* have been evolved either for leaf, flower stem and yield improvement (Arreola, 2015). In Asia, more emphases have been given to improve its culinary characteristics, while in western world, more emphasis is given to improve it as an ornamental plant (Wali, 2022; Yan, 2009; Mostafa, 2020). Flavonoids contain antifungal properties and are involved in pathogen defense (Napal et al., 2009); while flavonoids and phenolics are antioxidants (Nayak et al., 2015; Soppelsa et al., 2018). The extract of *A. tuberosum* could be considered as the most effective agent to improve hair growth in mice. This extract was used to dorsal skin area of mice that augmented hair follicle count due to stimulated insulin expression like growth factor-1 (Park et al., 2007). *A. tuberosum* extract is commonly used as hair tonic in India (Kim et al., 2008) and in Bangladesh (Jannat et al., 2019). The hormones control human body for metabolism, growth, development, digestion, reproduction, mood and sleep. Hormonal balance is essential to a healthy body and mind which is generally disrupted by excessive stress, poor diet, ailment, excessive exercise, remedial medicines and toxic overload (D'Amico and Roehrborn, 2007). The extract caused an increase in serum testosterone and nitric oxide levels. The nitric oxide is main factor for penile psychogenic erection. Because of increase in testosterone level and nitric oxide, the study concluded that *A. tuberosum* plant extract can develop a potential cure of certain hormonal imbalances (Benkeblia and Lanzotti, 2007).

In Chinese chive consistent pattern of flavour distribution in different varieties has been studied and no flavor components were determined in outer dried scales, while flavor concentration increases from minimum value in outer leaf to innermost tissue and the stem (Yabuki et al., 2010). Often the latter one contains multiple times greater concentration than outer leaf. The concentration pattern in roots and edible portions is different suggesting that the flavor components at different growth stages may differ in different plant parts. The flavor intensity is increased from outer leaf to inner tissues and generally, the values of flavor components are similar in root and stem (Ahmed and Shivhare, 2001). The pattern of flavour distribution in chives was different to that of other *Allium* species. Some aspects of metabolism of sulphur compounds in roots and other tissues of Chinese chives and transport of the corresponding metabolites is studied commonly. Baek et al. (2021) found volatile compound contents in several Chinese chive cultivars and isolated as fresh leaves had higher volatile compound contents than leaf sheath. Reyes-Díaz et al. (2016) analysed sulfur compounds by GC-MS of leaf extract detecting disulfides as major sulfur compound and suggested potential use of fresh *A. tuberosum* leaves as condiment and preservative in food industry. In *A. tuberosum* there are still numerous unknown substances to be explored using different accurate methods of detection. Baek et al. (2021) found dimethyl disulphide and dimethyl trisulphide in Chinese chive varieties. The factors affect flavor intensity and quality include of the compounds included the procedure of detection, processing and analysis (Kang et al., 2012). Furthermore, E-nose is a sensing system comprised of sampling, an array of chemical gas sensors and classification of the pattern. E-nose analysis reveals that distinguishing volatiles were found scattered as aromatic, broad range, sulfur organic, hydrogen, arom-aliph, broad methane, broad alcohol and methane aliph. Wei et al. (2021) categorically designated volatiles as aromatic, pungent, fruity, fatty and floral by the

radar fingerprint chart. The pungent odor could be considered as the strongest scent and ethers have main role to form this odor (Medhia Hanif et al., 2022). The odor descriptions of dimethyl disulfide and dimethyl trisulfide are generally described as *Allium* and sulfurous aroma and these were determined in entire samples having vital role in development of fragrance in *Allium* species.

The studies are commonly conducted on the detection of volatile organic sulfur compounds in Chinese chives; but the experiments on volatile compound contents at different growth stages of Chinese chives is scarce. Thus, this experiment was conducted to characterize composition of volatiles in typical two varieties of Chinese chive for their growth stage specific contents of compounds.

## MATERIALS AND METHODS

This experiment was conducted on the analysis of flavor in two specific Chinese chives varieties at different growth stage. Chinese chive varieties i.e. Fu Jiu Bao F1 (high texture) and Jiu Xing 22 (Low texture) were selected to determine and compare for their growth stage specific sulfur containing compounds (Stage I: seedling, Stage II: 30 d after transplanting, Stage III: vegetative harvesting, Stage IV: flowering and Stage V: fruiting stage) in different plant organs such as leaf sheath, leaves, root, floral axis, mature flower and seed. Chinese chive varieties Fu Jiu Bao F1 and Jiu Xing 22 were used, one is rich flavor; one is light flavor (based on previous data of six varieties). The flavor of Chinese chives was measured by GC-MS and electronic nose. The samples of leaf, root, leaf sheath, floral axis, inflorescence stalk with young flowers, mature flowers, seeds were taken at different growth stages including vegetative period and flowering and fruiting stage. The seed of these two genotypes were obtained from different markets, such as seed of Fu Jiu Bao F1 variety was obtained from Alibaba Platform, while the seed of variety Jiu Xing 22 was obtained from Henan Jiuxing Biotechnology Research Institute Co. Ltd. The seeds were sown initially for nursery raising; and after one month age of this nursery, the seedlings were transplanted in pots in three replicated CRD. In order to prepare samples for analysis, mature leaves were picked and crushed for each variety for detection of volatile Sulfur-containing compounds. The leaves were picked in such a way to avoid interference of external impurities as suggested by Yabuki *et al.* (2010).

### Characterization of volatile sulfur-containing compounds

Three randomly harvested plants each of two Chinese chive genotypes were soaked in water; and after smearing off the leaves, they were separately shredded gently, followed by crushing in a mortar with 10 ml of deionized water and a small quantity of sea sand to achieve 10 g sample for each plant.

### GC-MS analysis of volatiles leaf extract

Characterization of volatile sulfur-containing compounds was done by GC-MS analysis of volatiles leaf extract (Yabuki et al., 2010). An Agilent 7890B Gas Chromatograph coupled with an Agilent 7000D Quadrupole Mass Spectrometric Detector (Agilent, USA); and standard mass Spectrometry Library workstation were used for the separation and identification of the Volatile organic compounds (VOCs). ADB-WAX elastic quartz capillary column (30 m × 0.25 mm, 0.25 μm) was used as stationary phase (Agilent, USA). The VOCs of Chinese chive were analyzed using an Agilent 7890B/7000D GC-MS under the following conditions: capillary column, DB-WAX (30 m × 0.25 mm, 0.25 μm) with He (≥99.999% purity) as the carrier gas at a flow rate of 1 mL/min and splitless mode; initial temperature 40°C held for 1 min, raised to the 80°C at 8°C/min, then raised to 130°C at 2°C/min, and finally raised to 220°C at 6°C/min held for 3 min; total analysis time, 49 min; MS ionization, EI, 70eV; MS source, 230 °C; scan area, 30-660 amu. After the program started, the VOCs were separated and identified by the GC-MS with automatic Deconvolution System (AMDIS) and Mass Spectrometry Library (NIST, 2014). Compared with the Mass Spectrometry Library, only those with a matching score of more than 70 were identified. The concentration of VOCs was analyzed by the internal standard method, using the formula:

$$\text{VOCs } (\mu\text{g/kg}) = \frac{A_1}{A_2} \times \frac{M_1}{M_2} \times 1000$$

Where A1 and A2 are the peak areas of determinant and the internal standard, respectively; M1 and M2 are the amount of the internal standard and sample, respectively.

### Electric-Nose Analysis

The determination and analysis was performed with a commercial PEN3.5 E-nose (Airsense Analytics, GmbH Schwerin). The system contained ten metal oxide sensors. Prior to detection, each sample (1.5 g of Chinese chives) was placed in 15mL headspace glass vial and closely capped with a PTFE-silicon stopper. The samples then were kept at 70°C for approximately 65 minutes (equilibrium time). The detection time of sample was 120 seconds, the cleaning time of sensor was 60 seconds, and the adjustment time of automatic zero was 10 seconds. All samples were run with three repetitions.

Table 1. Material type and performance description represented by sensors.

Sensor number	Sensor name	Sensor sensitivity and general description	Detection limits (mg kg <sup>-1</sup> )
1	W1C	Aromatic organic compounds	Toluene, 10
2	W5S	Very sensitive, broad range sensitivity, reacts to nitrogen oxides	NO <sub>2</sub> , 1
3	W3C	Ammonia, also used as sensor for aromatic compounds	Benzene, 10
4	W6S	Detection on mainly hydrogen gas	H <sub>2</sub> , 0.1
5	W5C	Alkanes, aromatic compounds	Propane, 1
6	W1S	Sensitive to methane	CH <sub>4</sub> , 100
7	W1W	Detection on inorganic sulfur compounds	H <sub>2</sub> S, 1
8	W2S	Detection on alcohol	CO, 100
9	W2W	Aromatic compounds, inorganic sulfur and organic compounds	H <sub>2</sub> S, 1
10	W3S	Sensitive to methane and aliphatic organic compounds	CH <sub>3</sub> , 10

### Statistical analysis

The data are mentioned as mean ± standard error (SE) for different experiments, and the genotypes were assessed for their variation in morphological and quality traits by using ANOVA and Tukey's comparison test ( $P < 0.05$ ). The PCA analysis were performed as suggested by Jolliffe, 2002).

## RESULTS

### Growth stage specific total volatiles distribution pattern in Chinese chive

The total volatile contents in different organs of Chinese chive varieties Fu Jiu Bao F1 (A) and Jiu Xing 22 (B) were quantified and the results (Figure 1) showed that in leaf sheath of variety Fu Jiu Bao F1, the total volatile contents at seedling stage were 40.15µg/g, which reached at peak at 30 DAT stage (595.95µg/g), sustaining upto vegetative harvesting stage (449.7µg/g) and later declined at flowering stage (21.496µg/g) and fruiting stage (13.52µg/g). In leaf, the total volatiles were highest at seedling stage (210.88µg/g), decreased at 30 DAT stage (89.7µg/g) and declined at vegetative harvesting stage (15.62µg/g), flowering stage (16.26µg/g) and fruiting stage (10.26µg/g). Similarly, in root, the total volatiles were higher at seedling stage (73.26µg/g) as compared to 30 DAT stage (61.77µg/g), vegetative harvesting stage (9.954µg/g), flowering stage (11.19µg/g) and fruiting stage (19.75µg/g). In case of variety Jiu Xing 22, leaf sheath contained highest level of total volatile contents (764.19µg/g) at fruiting stage, followed by flowering stage (77.54µg/g), vegetative harvesting stage (75.18µg/g), seedling stage (34.41µg/g) and least was at 30 DAT stage (26.07µg/g). In leaf, the total volatiles were highest at 30 DAT stage (48.06µg/g), relatively lower at seedling stage (41.58µg/g) and declined at vegetative harvesting stage (12.65µg/g), flowering stage (16.19µg/g) and fruiting stage (13.73µg/g). Likewise, in root, the total volatiles were higher at flowering stage (288.01µg/g), followed by flowering stage (168.49µg/g), 30 DAT stage (128.3µg/g), fruiting stage (61.83µg/g) and least at vegetative harvesting stage (12.93µg/g).

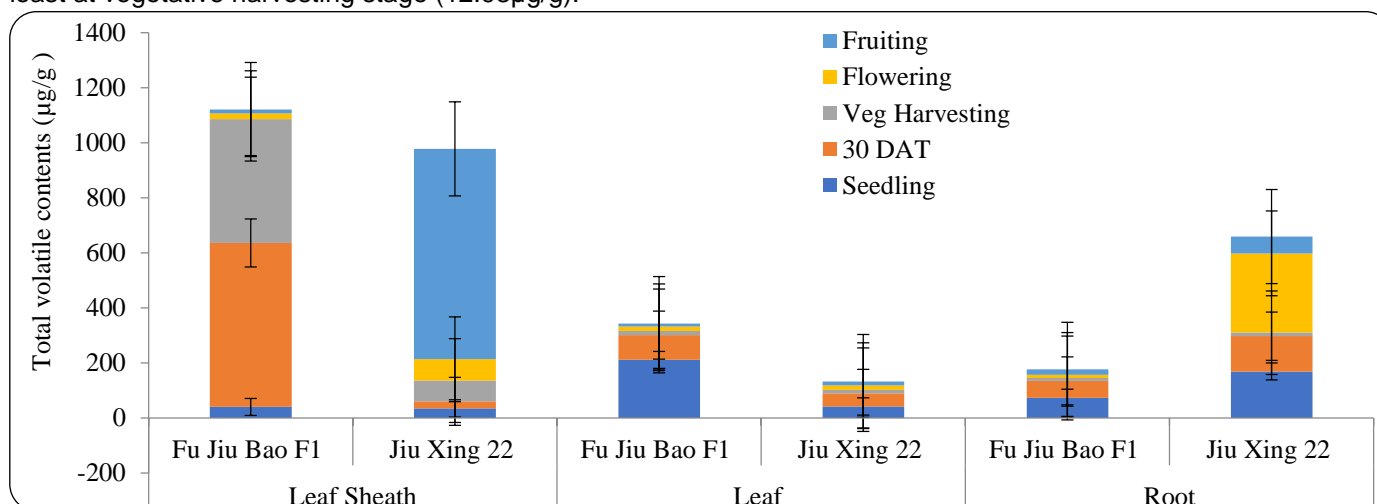


Figure 1. Growth stage specific total volatile contents in different plant organs of Chinese chive varieties Jiu Xing 22 and Fu Jiu Bao F1.

**Distribution pattern of volatiles in different classes at various growth stages**

**Seedling stage**

The figures 2 and 3, showing the total volatile compounds contents at seedling stage (Stage-I) in different plant organs in Chinese chive varieties Fu Jiu Bao F1 Jiu Xing 22 respectively, illustrated that the volatile contents in leaves of variety Fu Jiu Bao F1 were higher (286.88µg/g) than volatile contents in root (129.85 µg/g) and leaf sheath (41.1µg/g); while in Jiu Xing 22 the root contained highest contents of the total volatiles (59.49 µg/g), followed by leaf sheath (47.92 µg/g) and leaf (27.68 µg/g). Ethers were found in higher concentrations for leaf sheath, leaf and root for Chinese chive varieties Fu Jiu Bao F1 (178.06, 64.25 and 13.61µg/g) and Jiu Xing 22 (26.37, 13.7 and 25.17µg/g), respectively. Furfuryl and furan derivatives were determined in Fu Jiu Bao F1 (8.5, 20.62 and 2.9µg/g) but not in Jiu Xing 22 (0.7, 0 and 1.29µg/g) in leaf sheath, leaf and roots, respectively. Acids in leaf of variety Fu Jiu Bao were 48.55µg/g against 3.38µg/g in Jiu Xing 22. At this growth stage (Seedling stage), ethers showed more consistency in concentration in all the studied organs in both the varieties, followed by heterocyclic polymers, aldehydes and esters. Apart from the variation in concentration, both the varieties showed quite different status of volatile concentrations.

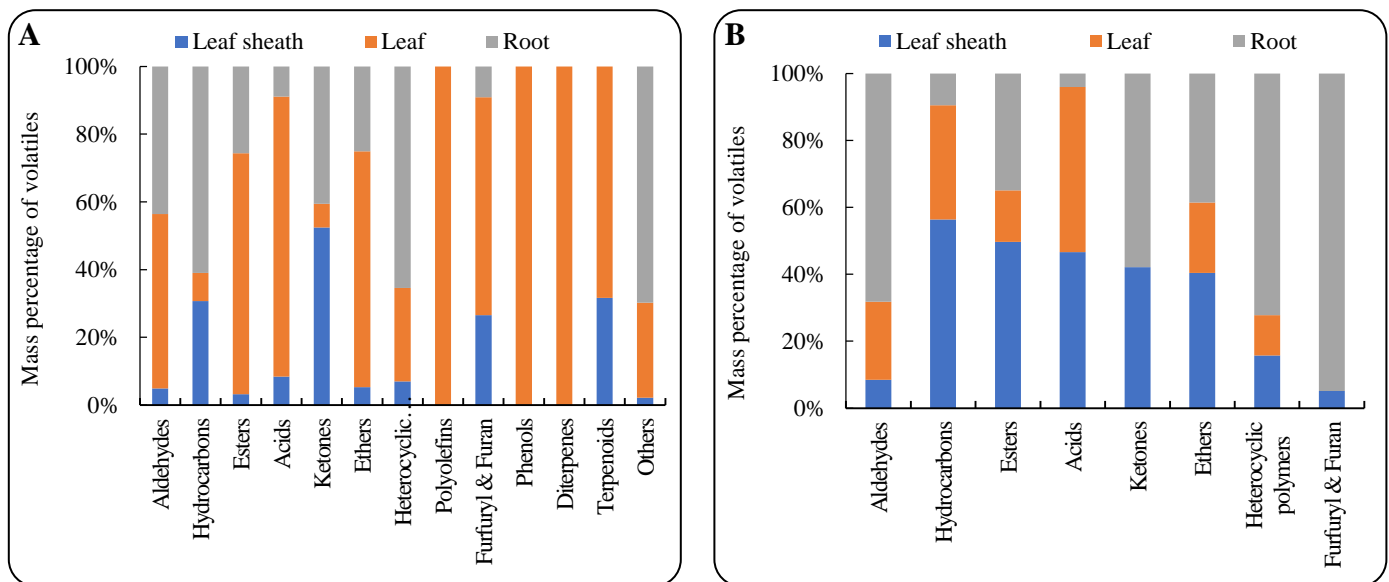


Figure 2. Volatile content (µg/g) of different classes of substances in different organs of Chinese chive varieties Fu Jiu Bao F1 (A) and Jiu Xing 22 (B) at seedling stage.

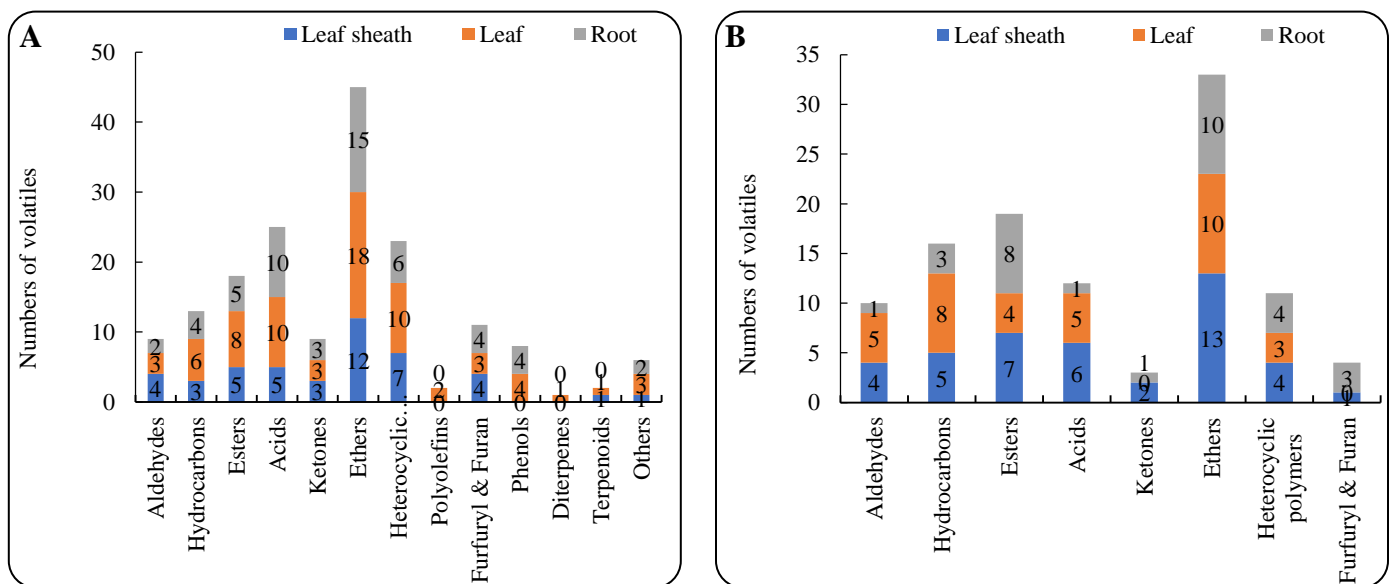


Figure 3. Number of substances in different volatile classes in various plant organs of Chinese chive varieties Fu Jiu Bao F1 (A) and Jiu Xing 22 (B) at seedling stage.

### 30-days after transplanting (DAT) stage

At 30 DAT stage (Figure 4, 5) the volatile contents in leaf sheath, leaf and root in variety Fu Jiu Bao F1 were to the extent of 322.95, 492.54 and 20.979  $\mu\text{g/g}$ , respectively while in variety Jiu Xing 22 the total volatile contents were 44.91, 27.63 and 663.18  $\mu\text{g/g}$ , respectively. At 30 days after transplanting stage, variety Fu Jiu Bao F1 contained a total of 58, 72 and 50 volatile compounds in leaf sheath, leaf and root; while variety Jiu Xing 22 contained 42, 38 and 43 total number of volatiles in leaf sheath, leaf and root, respectively.

In variety Fu Jiu Bao F1, ethers content was higher in leaf (237.03  $\mu\text{g/g}$ ) but in variety Jiu Xing 22, total ether contents were higher in root (229.54  $\mu\text{g/g}$ ). Similarly, in variety Fu Jiu Bao F1 the Hydrocarbons in leaf sheath, leaf and root were 76.02, 63.11 and 87.06  $\mu\text{g/g}$  against 11.13, 4.82 and 7.34  $\mu\text{g/g}$  in variety Jiu Xing 22, respectively. Ethers in variety Fu Jiu Bao F1 in leaf sheath, leaf and root were 95.75, 237.03 and 36.14  $\mu\text{g/g}$  against 9.11, 0.32 and 229.54  $\mu\text{g/g}$  in variety Jiu Xing 22, respectively. The aldehydes were higher in leaf sheath of variety Fu Jiu Bao F1 (69.28  $\mu\text{g/g}$ ) but in variety Jiu Xing 22 the aldehydes were in greater content in root (19.23  $\mu\text{g/g}$ ). The contents of Heterocyclic polymers were higher in leaf sheath (29.88  $\mu\text{g/g}$ ) and leaf (53.89  $\mu\text{g/g}$ ), but in variety Jiu Xing 22 it was higher in root (148.76  $\mu\text{g/g}$ ).

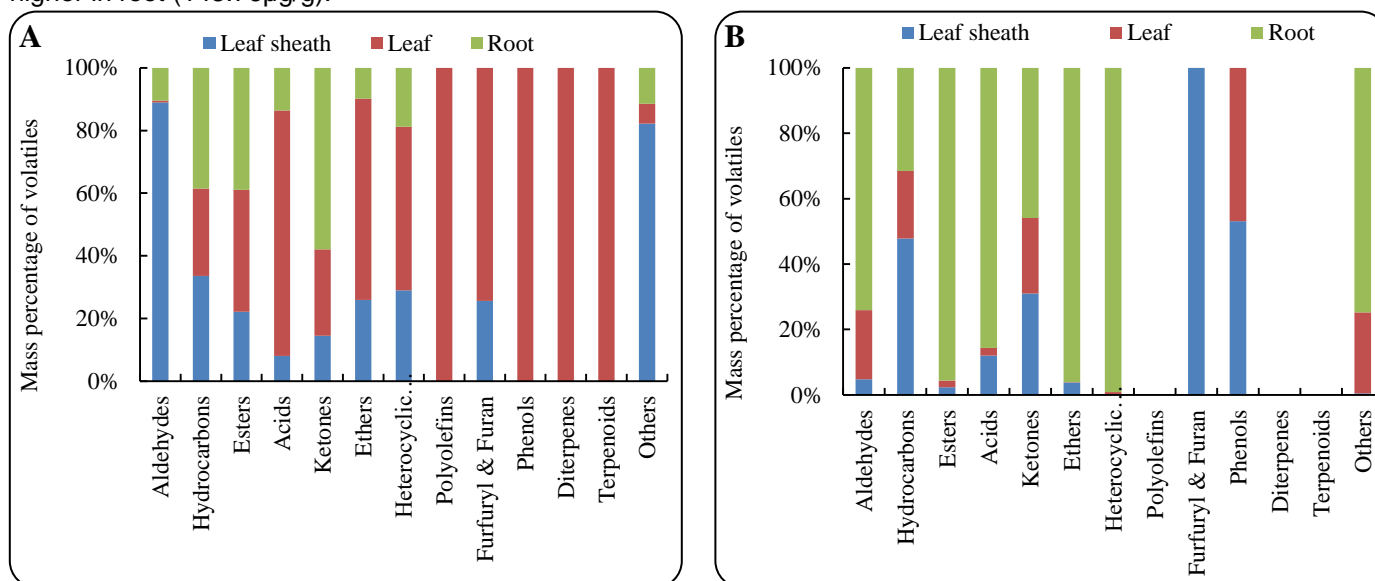


Figure 4. Volatile content ( $\mu\text{g/g}$ ) of different classes of substances in different organs of Chinese chive varieties Fu Jiu Bao F1 (A) and Jiu Xing 22 (B) at 30 days after transplanting stage.

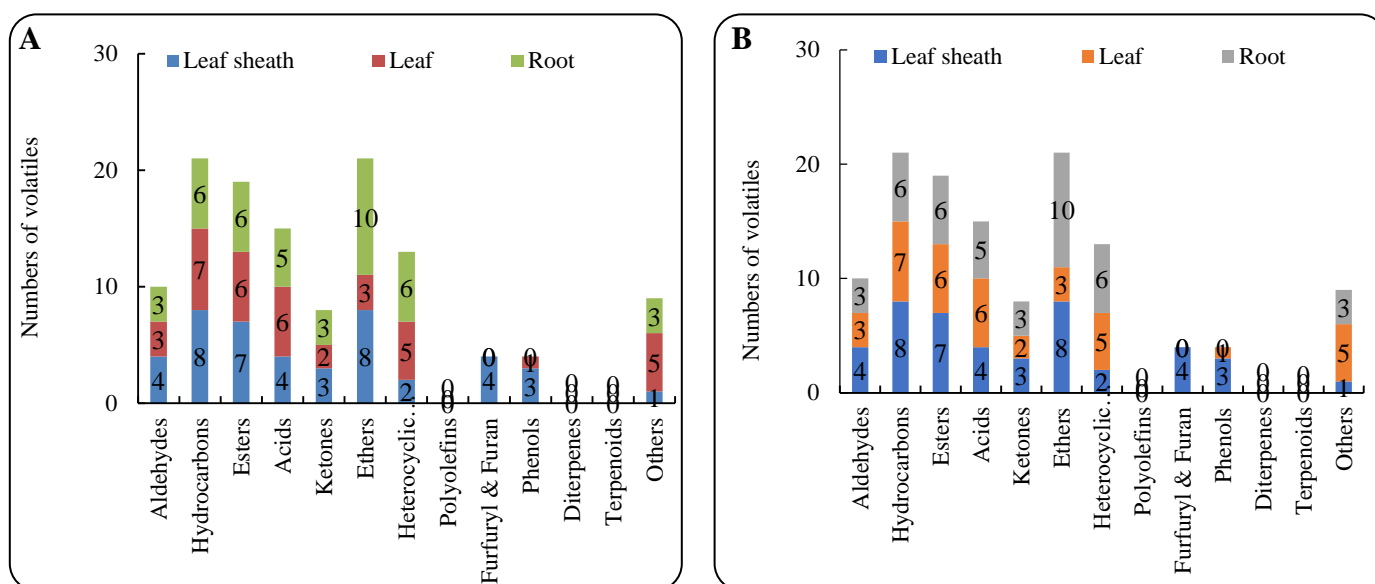


Figure 5. Number of substances in different volatile classes in various plant organs of Chinese chive varieties Fu Jiu Bao F1 (A) and Jiu Xing 22 (B) at 30 days after transplanting stage.

### Vegetative harvesting stage

At stage-III (Vegetative harvesting stage), the plant samples of Chinese chive varieties Fu Jiu Bao F1 and Jiu Xing 22 from leaf sheath, leaf and root were analysed for total number of contents of volatile compounds and results are illustrated in Figure 6, 7. At vegetative harvesting stage, Aldehydes, Hydrocarbons, Esters, Acids, Ketones, Ethers, Heterocyclic polymers, Furfuryl & Furan derivatives and Phenols were determined in the samples collected from different plant organs of Chinese chive. The total volatile contents in leaf sheath, leaf and root in variety Fu Jiu Bao F1 were to the extent of 115.29, 499.36 and 50.33µg/g, respectively while in variety Jiu Xing 22 the total volatile contents were 21.56, 789.72 and 16.07µg/g, respectively. At vegetative harvesting stage, variety Fu Jiu Bao F1 contained a total of 47, 28 and 35 volatile compounds in leaf sheath, leaf and root; while variety Jiu Xing 22 contained 18, 35 and 49 total number of volatiles in leaf sheath, leaf and root, respectively.

In variety Fu Jiu Bao F1, ethers content was higher in leaf (183.25µg/g); and similarly it was also higher in variety Jiu Xing 22 137.35µg/g) in leaf. In varieties Fu Jiu Bao F1 and Jiu Xing 22, the total volatile contents were as follows: Aldehydes (5.3, 29.71µg/g), Hydrocarbons (1.16, 171.76µg/g), Esters (185.87, 104.13µg/g), Acids (77.08, 138.11µg/g), Ethers (183.25, 137.35µg/g), Furfuryl & Furan derivatives (15.86, 29.71 µg/g) and Phenols (27.9, 171.76µg/g), respectively. Apart from the varietal influence, at vegetative harvesting stage, the volatile contents were remarkably higher in leaves (499.36, 789.72µg/g) in Fu Jiu Bao F1 and Jiu Xing 22, respectively. In varieties Fu Jiu Bao F1 and Jiu Xing the total volatiles were higher (115.29, 21.56µg/g) in leaf sheath samples as compared to root samples (50.33m 16.07µg/g), respectively.

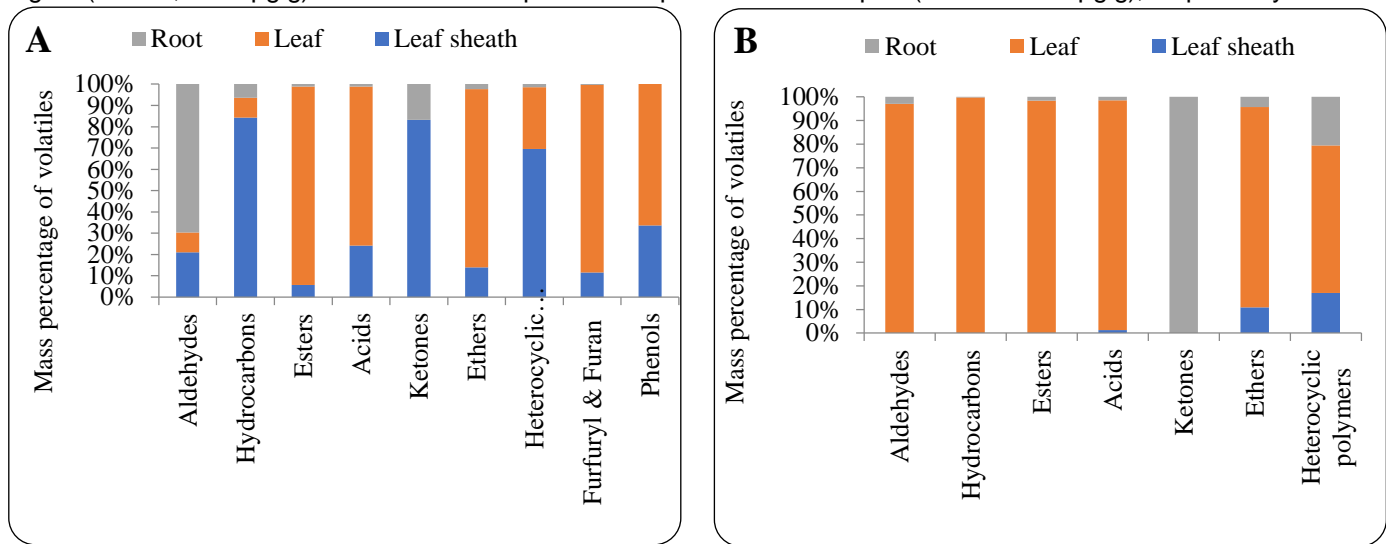


Figure 6. Volatile content (µg/g) of different classes of substances in different organs of Chinese chive varieties Fu Jiu Bao F1 (A) and Jiu Xing 22 (B) at vegetative harvesting stage.

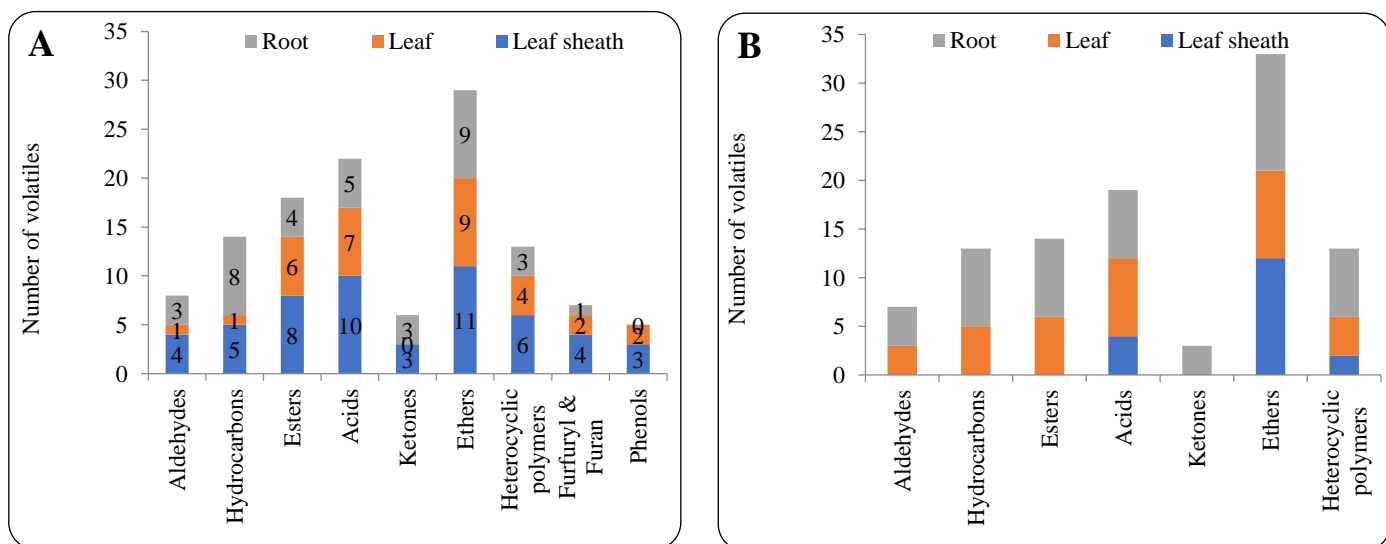


Figure 7. Number of substances in different volatile classes in various plant organs of Chinese chive varieties Fu Jiu Bao F1 (A) and Jiu Xing 22(B) at vegetative harvesting stage.

## Flowering stage

At flowering stage (Figure 8, 9), the plant contained volatiles belonging to different classes such as: aldehydes, hydrocarbons, esters, acids, ketones, ethers heterocyclic polymers, polyolefins, furfuryl and furan, phenols, diterpenes and terpenoids. The total volatile contents in leaf sheath, leaf, root, floral axis, mature flower and inflorescence in variety Fu Jiu Bao F1 were to the extent of 20.684, 316.48, 22.01, 21.62, 530.6 and 310.24 $\mu\text{g/g}$ , respectively while in variety Jiu Xing 22 the total volatile contents were 283.4, 24.29, 64.98, 9.589, 39.85 and 37.35 $\mu\text{g/g}$ , respectively. At flowering stage, variety Fu Jiu Bao F1 contained a total of 45, 57, 33, 43, 51 and 37 volatile compounds in leaf sheath, leaf, root, floral axis, mature flower and inflorescence, respectively; while variety Jiu Xing 22 contained 48, 31, 32, 44, 65 and 40 total number of volatiles in leaf sheath, leaf, root, floral axis, mature flower and inflorescence, respectively. In comparative analysis of total volatiles in two varieties indicated that the total volatile contents in leaf sheath in Fu Jiu Bao F1 and Jiu Xing 22 were 20.684 and 283.4 $\mu\text{g/g}$ , leaf 316.48 and 24.29 $\mu\text{g/g}$ , root 22.01 and 64.98 $\mu\text{g/g}$ , floral axis 21.62 and 9.589 $\mu\text{g/g}$ , mature flower 530.6 and 39.85 $\mu\text{g/g}$ , inflorescence 310.24 and 37.35 $\mu\text{g/g}$ , respectively. At flowering stage, among organs, the highest total volatile contents were determined in mature flower samples (530.6  $\mu\text{g/g}$ ), followed by leaf (316.48 $\mu\text{g/g}$ ) and inflorescence (310.24  $\mu\text{g/g}$ ) in variety Fu Jiu Bao F1; while in variety Jiu Xing 22, the highest total volatile contents were observed in leaf sheath (283.4 $\mu\text{g/g}$ ), followed by root samples (64.98 $\mu\text{g/g}$ ) and mature flower (39.85 $\mu\text{g/g}$ ).

Among volatile classes, mature flower contained higher contents of aldehydes (130.41 $\mu\text{g/g}$ ), hydrocarbons (224.53 $\mu\text{g/g}$ ), esters (38.55 $\mu\text{g/g}$ ), acids (28.17 $\mu\text{g/g}$ ), ethers (97.42 $\mu\text{g/g}$ ); while ethers were also highest in leaf (184.1 $\mu\text{g/g}$ ) and inflorescence (278.01  $\mu\text{g/g}$ ) in variety Fu Jiu Bao F1. In variety Jiu Xing 22, only leaf sheath contained exceptionally higher contents of volatiles of different classes including aldehydes (42.24 $\mu\text{g/g}$ ), hydrocarbons (46.82 $\mu\text{g/g}$ ), esters (7.53 $\mu\text{g/g}$ ), acids (10.93  $\mu\text{g/g}$ ), ketones (41.29 $\mu\text{g/g}$ ), ethers (80.41 $\mu\text{g/g}$ ), heterocyclic polymers (27.24 $\mu\text{g/g}$ ), polyolefins (23.4 $\mu\text{g/g}$ ) and phenols (43.41 $\mu\text{g/g}$ ). Ethers were also relatively higher in inflorescence (28.32 $\mu\text{g/g}$ ), root (24.01 $\mu\text{g/g}$ ) and leaf (14.8 $\mu\text{g/g}$ ) in variety Jiu Xing 22. The results showed that number of volatiles and the total volatile contents were greater in Jiu Xing 22 than the companion variety Fu Jiu Bao F1. Fu Jiu Bao F1 showed consistency in all the organs but higher in leaves and mature flower; while in case of Jiu Xing 22, the total volatiles were higher leaf sheath. Hence, at the flowering, stage leaf and mature flower was at their best to be utilized in variety Fu Jiu Bao F1; while variety Jiu Xing 22, leaf sheath was the best organ to use at flowering stage.

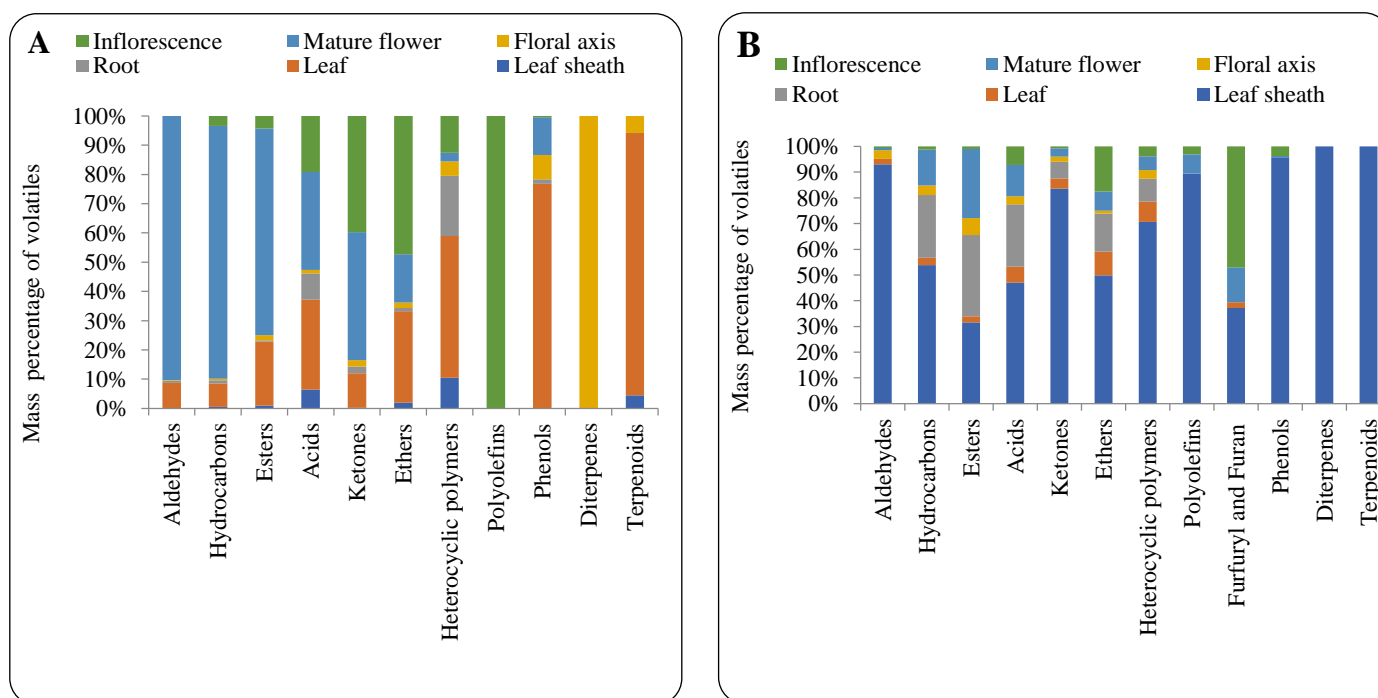


Figure 8. Volatile content ( $\mu\text{g/g}$ ) of different classes of substances in different organs of Chinese chive varieties Fu Jiu Bao F1 (A) and Jiu Xing 22 (B) at flowering stage.

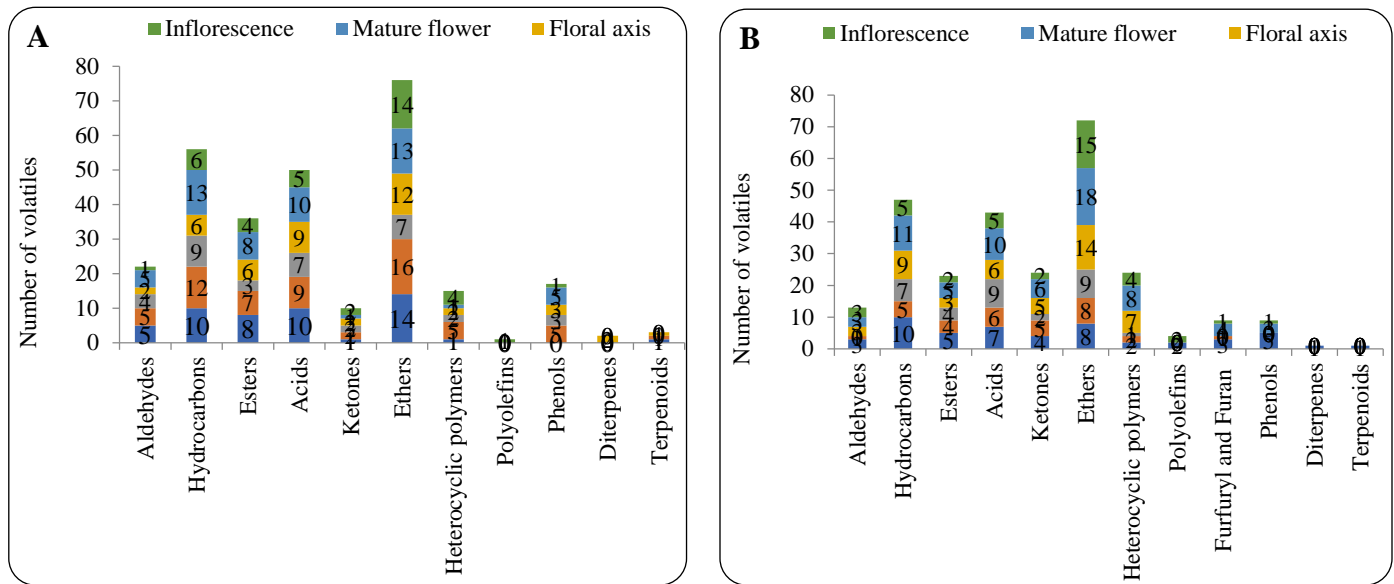


Figure 9. Number of substances in different volatile classes in various plant organs of Chinese chive varieties Fu Jiu Bao F1 (A) and Jiu Xing 22(B) at flowering stage.

**Fruiting stage**

At fruiting stage (Figure 10, 11), the plant contained volatiles belonging to different classes such as: aldehydes, hydrocarbons, esters, acids, ketones, ethers, heterocyclic polymers, Furfuryl and Furan, phenols and terpenoids. The total volatile contents in leaf sheath, leaf, root, floral axis, mature flower and inflorescence in variety Fu Jiu Bao F1 were to the extent of 16.02, 10.08, 34.57, 21.18, 7.42 and 252.16µg/g, respectively while in variety Jiu Xing 22 the total volatile contents were 977.2, 18.18, 28.05, 23.09, 137.21 and 20.11µg/g, respectively. At fruiting stage, variety Fu Jiu Bao F1 contained a total of 45, 49, 51, 52, 25 and 15 volatile compounds in leaf sheath, leaf, root, floral axis, mature flower and inflorescence, respectively; while variety Jiu Xing 22 contained 62, 38, 40, 66, 22 and 10 total number of volatiles in leaf sheath, leaf, root, floral axis, mature flower and inflorescence, respectively.

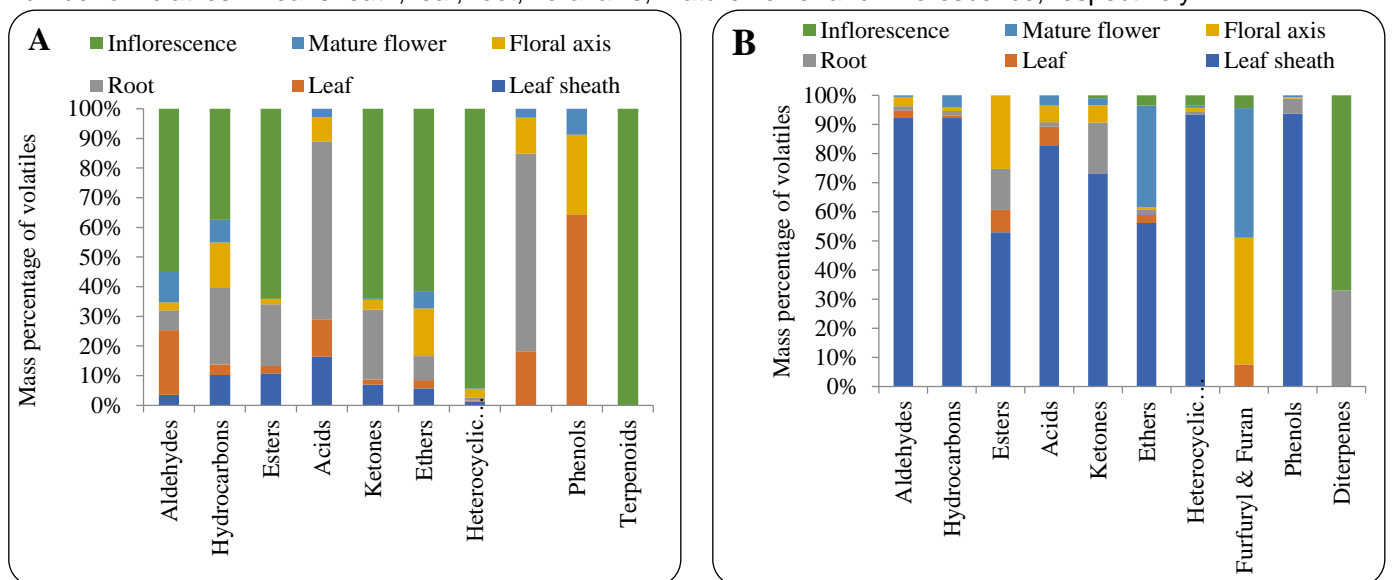


Fig. 10 Volatile content (µg/g) of different classes of substances in different organs of Chinese chive varieties Fu Jiu Bao F1 (A) and Jiu Xing 22 (B) at fruiting stage.

In volatiles comparison in two varieties indicated that the total volatile contents in leaf sheath in Fu Jiu Bao F1 and Jiu Xing 22 were 16.02 and 977.2µg/g, leaf 10.08 and 18.18µg/g, root 34.57 and 28.05µg/g, floral axis 21.18 and 23.09µg/g, mature flower 7.42 and 137.21µg/g, inflorescence 252.16 and 20.11µg/g, respectively. At fruiting stage, among organs, the highest total volatile contents in variety Fu Jiu Bao F1 were determined in inflorescence (252.16µg/g); while in variety Jiu

Xing 22, the highest total volatile contents were observed in leaf sheath (977.2 $\mu\text{g/g}$ ), followed by mature flower (137.21  $\mu\text{g/g}$ ). At this stage, among volatile classes, inflorescence samples contained higher contents of aldehydes esters (26.86 $\mu\text{g/g}$ ), ethers (43.85 $\mu\text{g/g}$ ) and Heterocyclic polymers (144.8 $\mu\text{g/g}$ ) in variety Fu Jiu Bao F1. In variety Jiu Xing 22, leaf sheath contained exceptionally higher contents of volatiles of different classes including Aldehydes (80.12  $\mu\text{g/g}$ ), Hydrocarbons (298.78 $\mu\text{g/g}$ ), Acids (31.89 $\mu\text{g/g}$ ), Ethers (183.91  $\mu\text{g/g}$ ), Heterocyclic polymers (192 $\mu\text{g/g}$ ) and Phenols (175.8 $\mu\text{g/g}$ ). Ethers were also relatively higher in mature flower samples (114.3 $\mu\text{g/g}$ ) in variety Jiu Xing 22. An uneven variation between varieties and their organs was found for volatile contents in different plant organs at fruiting stage; and leaf sheath of Jiu Xing 22 contained extraordinarily higher total volatile content and there was no match of other companion variety for this organ and similar situation was noted for mature flower.

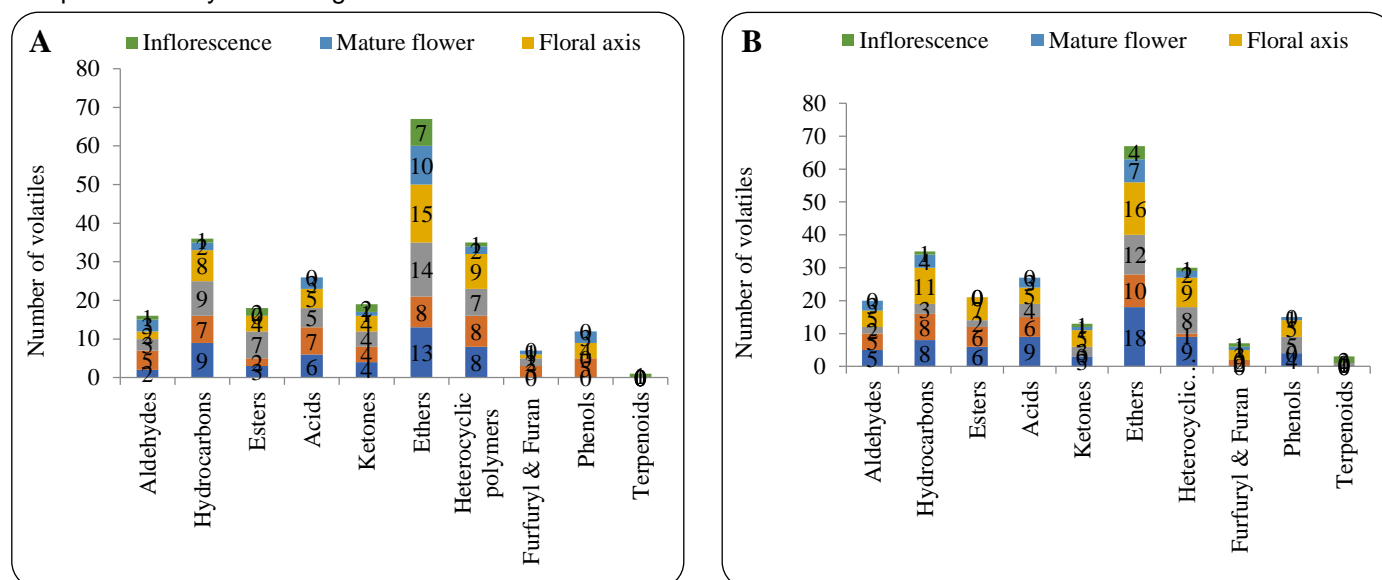


Figure 11. Number of substances in different volatile classes in various plant organs of Chinese chive varieties Fu Jiu Bao F1 (A) and Jiu Xing 22(B) at fruiting stage.

## E-nose analysis

### Seedling stage

#### Radial chart analysis

The sensors used in E-nose analysis are distributed as W1C, W5S, W3C, W6S, W5C, W1S, W1W, W2S, W2W and W3S for flavor of two Chinese chive varieties (Fu Jiu Bao F1 [A] and Jiu Xing 22 [B]) at seedling stage. Variety Fu Jiu Bao F1 showed superiority in volatile contents than Jiu Xing 22 and characterized aromatic, designated in broad-alcohol and sulph-chlor groups. The distinctive flavor constituents in Chinese chive cultivars are controlled by specific biologically active sulfur-organic components. Variety Fu Jiu Bao F1 has aromatic and organic sulfur compounds and their contents were higher than variety Jiu Xing 22. The fingerprint profiles of two Chinese chives varieties show dissimilarity, and W5S was the largest sensor, followed by W2W. Fig 12 concludes that Fu Jiu Bao F1 was stronger in overall flavor characteristics than Jiu Xing 22 cultivar based on the results of leaf sheath, leaf and root samples at seedling stage.

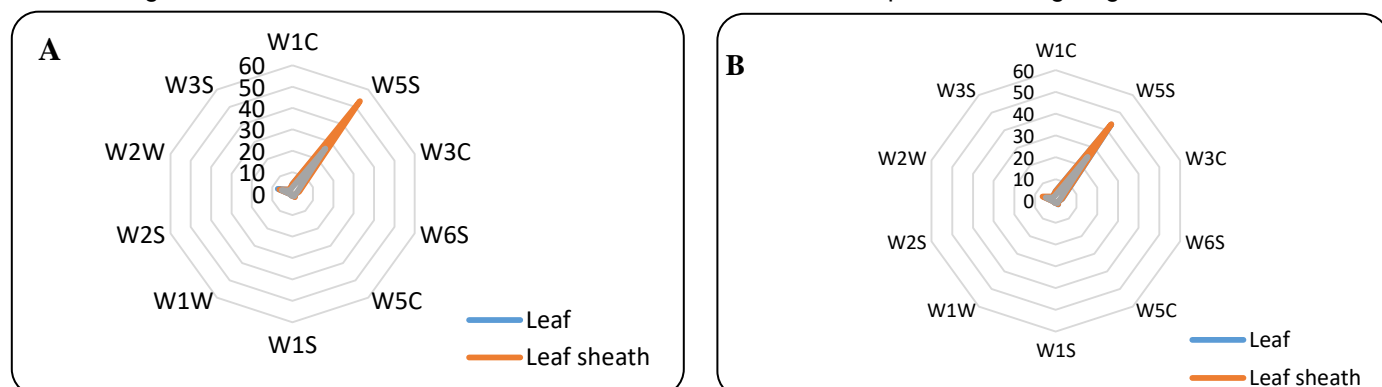


Figure 12. Flavor radar chart analysis of different organs at seedling stage of Chinese chive variety Fu Jiu Bao F1 (A) and Jiu Xing 22 (B)

**PCA analysis**

As a clustering method of analysis of the dataset, the PCA is derived from a two-dimensional PCA illustrated as figures 13, 14, where at seedling stage of Chinese chives cultivars different plant organs and ten sensors develop the corresponding groups of volatiles. The sum of the first two principal components reached 100%, out of which the main axis-1 characterized 99.9% of the total variance, while main axis-2 characterized 0.1% of the entire variance. It suggests that the order difference of various Chinese chives cultivars can be highly distinguished. The sum of these two components is greater than 90%, so we distinguished them according to different plant organs at seedling stage. The first principal component can be divided into four categories.

Fig. 14 is the PCA of odor data of electronic nose showing a diagram of the PCA analysis of plant organs at seedling stage with sensors W5S, W1S, W3C, W1C, and W2W as primary sensors. It indicates that the farther each sensor was from the origin of the coordinate, the greater was the rate of contribution; and W5S had a more effective contribution rate. To increase recognition of the sensor, more sensors were added to distinguish the mixed types.

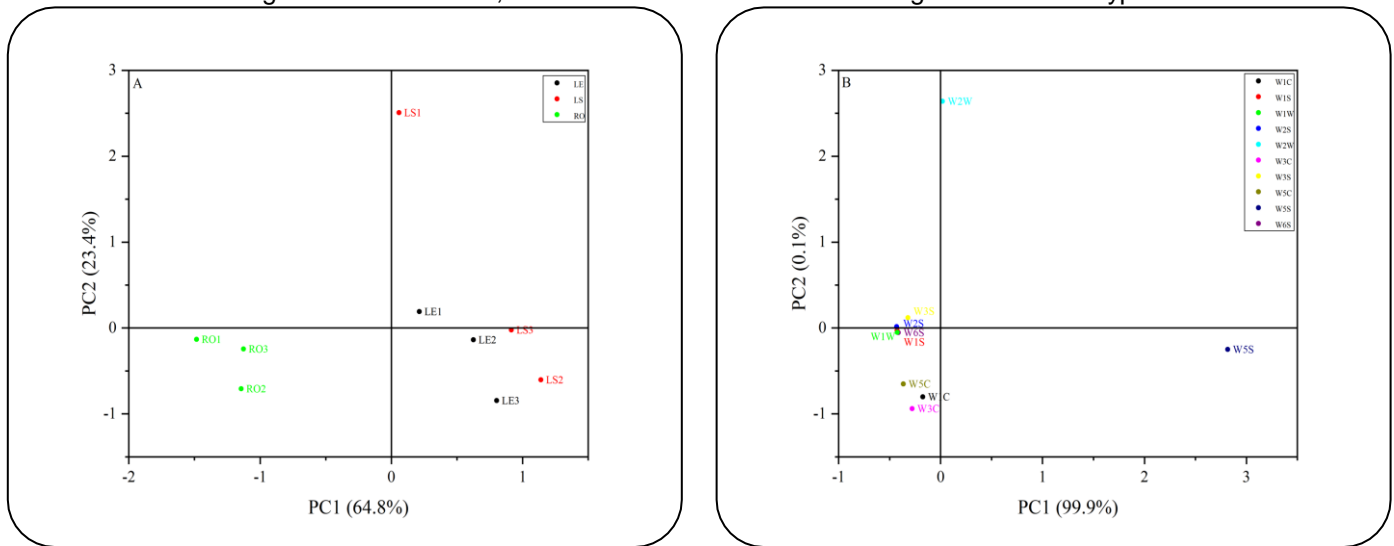


Figure 13. PCA of flavor at seedling stage in different organs of Chinese chive varieties Fu Jiu Bao F1 (A) and Jiu Xing 22 (B)

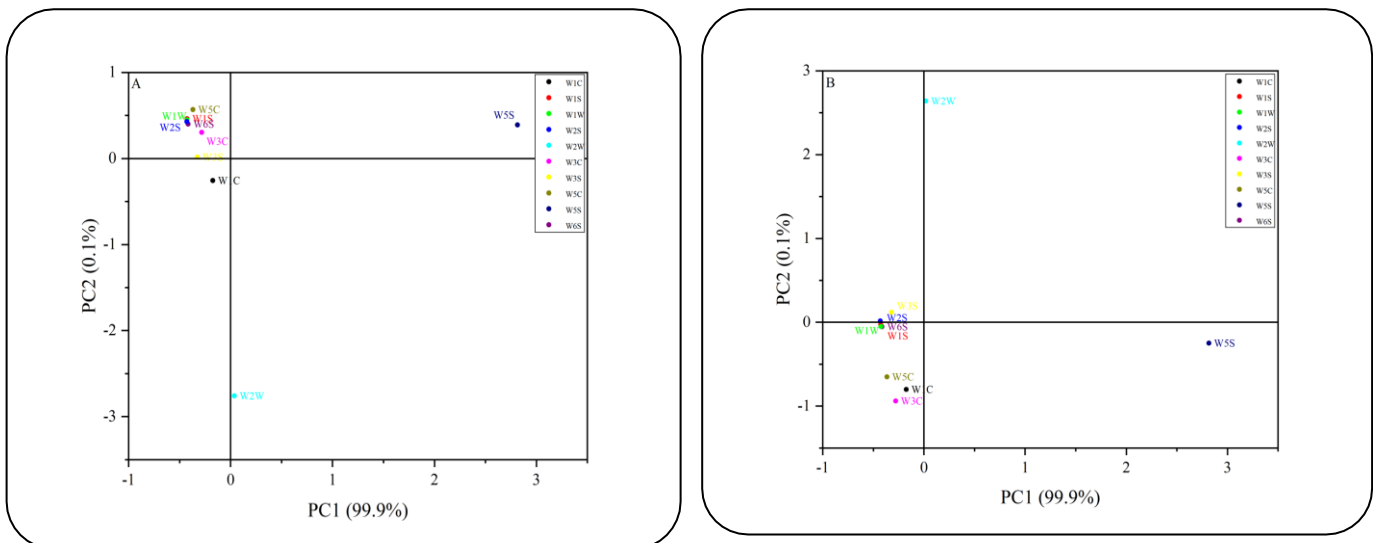


Figure 14. PCA of sensor at seedling stage in different organs of Chinese chive varieties Fu Jiu Bao F1 (A) and Jiu Xing 22 (B)

**30-days after transplanting stage**

**Radar chart analysis**

After 30 days of transplanting stage, the leaf sheath, leaf and root samples of Chinese chive varieties Fu Jiu Bao F1 [A] and Jiu Xing 22 (B) were subjected to E-nose analysis for flavor at this stage. Variety Fu Jiu Bao F1 remained consistent in

volatile contents in leaf sheath and root samples; but Jiu Xing 22 showed superiority in volatile contents in roots. Fig. 15 indicated that variety Fu Jiu Bao F1 was superior in texture and characterized aromatic, in broad-alcohol and sulph-chlor groups. The fingerprint profiles of two Chinese chives varieties showed no similarity, but in both varieties W5S was the largest sensor. It was concluded that Fu Jiu Bao F1 was stronger in overall flavor characteristics than Jiu Xing 22 cultivar based on the results of leaf sheath, leaf and root samples at 30 days of transplanting stage.

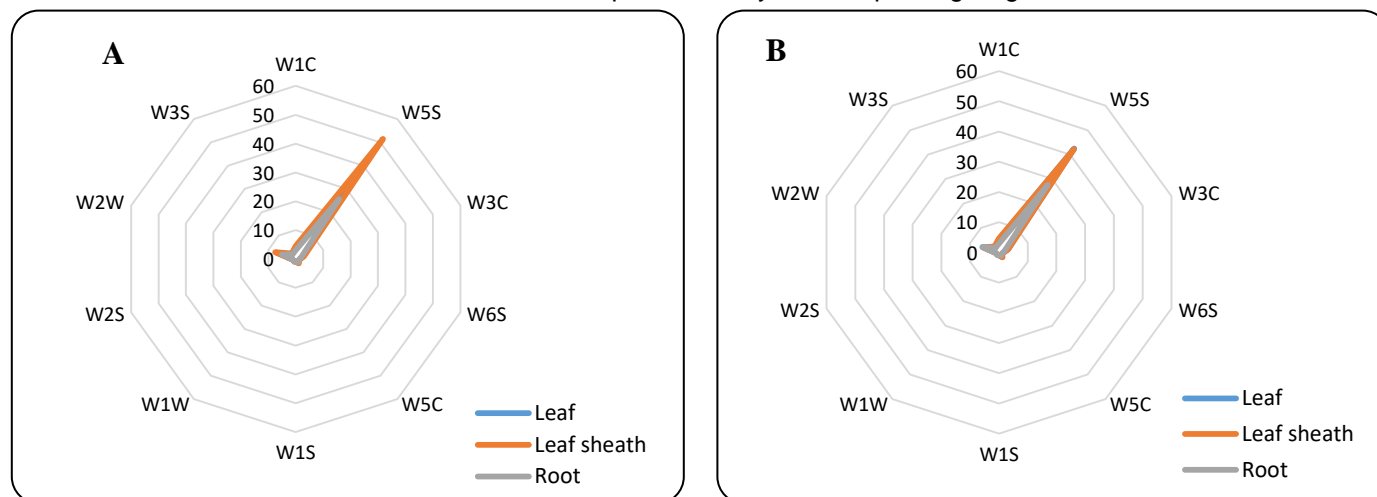


Figure 15. Flavor radar chart analysis of different organs at 30 days after transplanting stage of Chinese chive variety Fu Jiu Bao F1 (A) and Jiu Xing 22 (B).

### PCA analysis

Figure 16 and 17 are revealing the variance in volatile contents in different organs of Chinese chive varieties at 30 days after transplanting stage using ten sensors developing the corresponding groups of volatiles. The sum of the first two principal components reached 82.7%, out of which the main axis-1 characterized 59.4% of the total variance, while main axis-2 characterized 23.3% of the entire variance. It suggests that the order difference of various Chinese chives cultivars cannot be highly distinguished. The sum of these two components was not greater than 90%, so we cannot distinguish them according different plant organs at 30 days after transplanting stage. Fig. 4-21 is the PCA of odor data of e-nose showing a diagram of the PCA analysis of plant organs at 30 days after transplanting stage with sensors W5S, W1S, W3C, W1C, and W2W as primary sensors. It suggests that the farther each sensor was from the origin of the coordinate, the greater was the rate of contribution; and W5S was a larger sensor with more effective contribution rate.

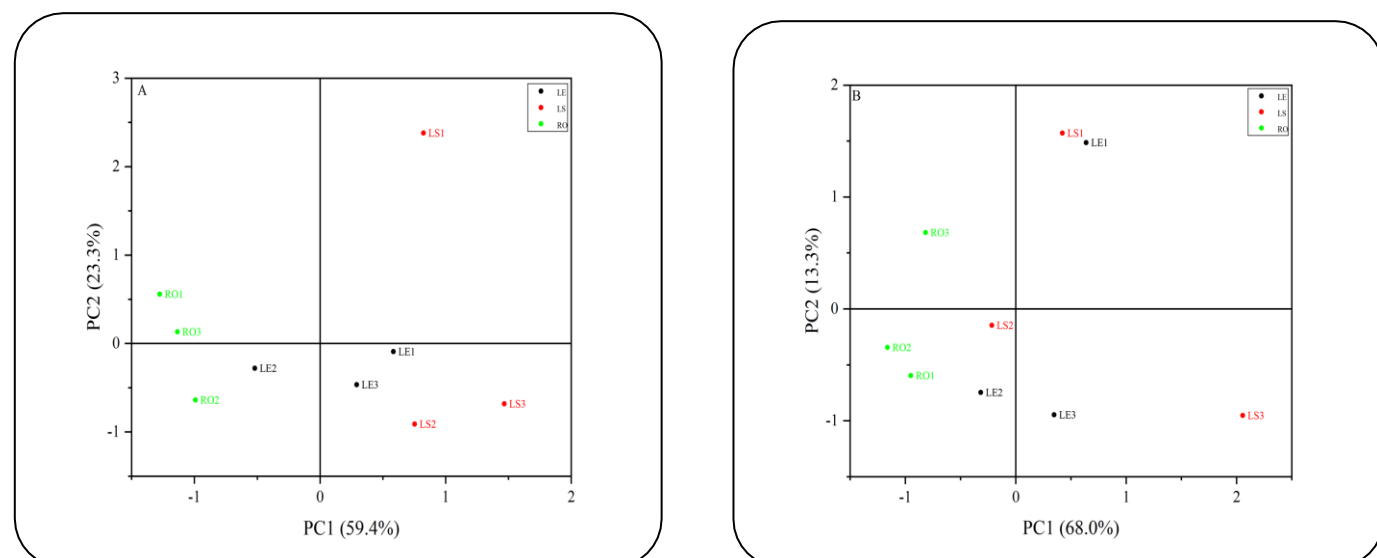


Figure 16. PCA of flavor at 30 days after transplanting stage in different organs of Chinese chive varieties Fu Jiu Bao F1 (A) and Jiu Xing 22 (B).

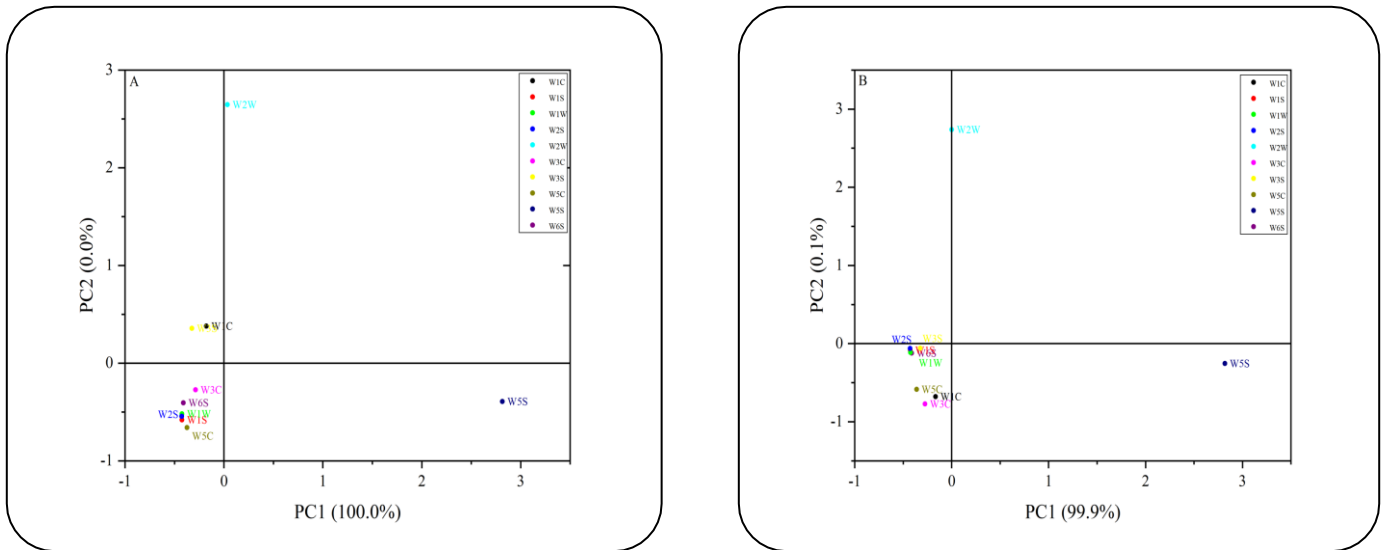


Figure 17. PCA of sensors at 30 days after transplanting stage in different organs of Chinese chive varieties Fu Jiu Bao F1 (A) and Jiu Xing 22 (B)

**Vegetative harvesting stage  
Radar chart analysis**

The radar chart for sensing the texture of the samples obtained from leaf sheath, leaf and root at vegetative harvesting stage of Chinese chive varieties Fu Jiu Bao F1 (A) and Jiu Xing 22 (B) indicated that Fu Jiu Bao F1 showed consistency in compounds in leaf sheath and root while in both varieties compounds concentration was markedly higher in leaf samples. Fig. 4-23 revealed that variety Fu Jiu Bao F1 has texture superiority to Jiu Xing 22 and characterized as aromatic as and stronger in overall flavor characteristics than Jiu Xing 22 cultivar based on the results of leaf sheath, leaf and root samples at vegetative harvesting stage of Chinese chive. The radar chart also revealed that W5S was the largest sensor followed by W2W (Figure 18).

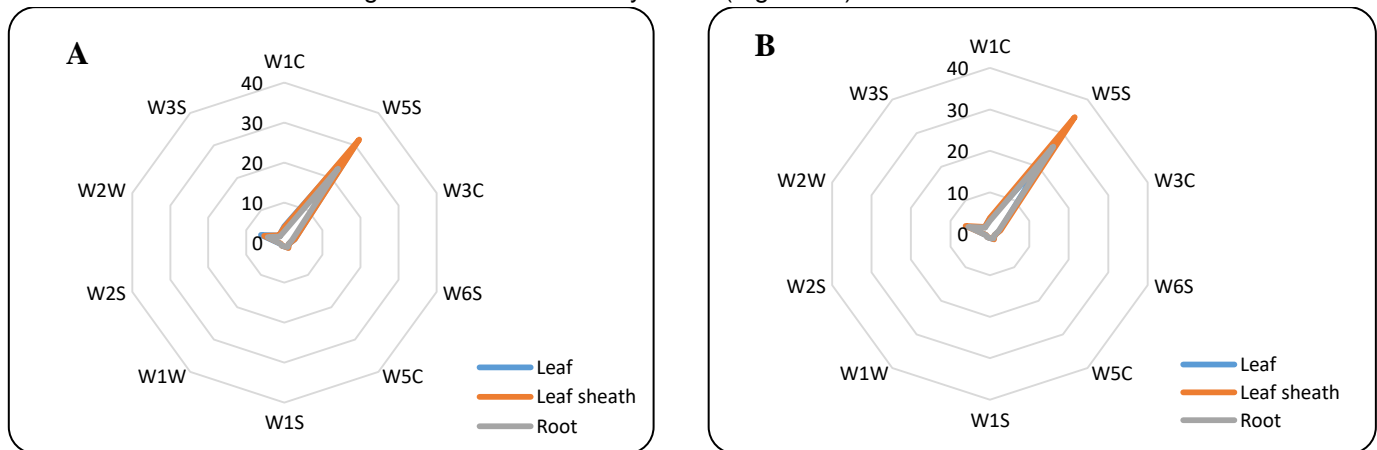


Figure 18. Flavor radar chart analysis of different organs at vegetative harvesting stage of Chinese chive variety Fu Jiu Bao F1 (A) and Jiu Xing 22 (B).

**PCA analysis**

Fig. 19, 20 depicted the difference in volatile contents in leaf sheath, leaf and root of Chinese chive varieties (Fu Jiu Bao F1 and Jiu Xing 22) at vegetative harvesting stage and ten sensors that develop corresponding groups of volatiles. The sum of the first two principal components reached 86.9%, out of which the main axis-1 characterized 64.2% of the total variance, while main axis-2 characterized 22.7% of the total variance. It indicated that the order difference of two Chinese chives cultivars cannot be highly distinguished. The sum of these two components was not greater than 90%, so we cannot distinguish them according different plant organs at vegetative harvesting stage. Fig. 4-24 is the PCA of odor data of e-nose showing a diagram of the PCA analysis of plant organs at vegetative harvesting stage with sensors W5S, W1S, W3C, W1C, and W2W as primary sensors; which pointed out

that farther each sensor was from the origin of the coordinate, the greater was the rate of contribution; and W5S was an effective and larger sensor with more greater contribution rate.

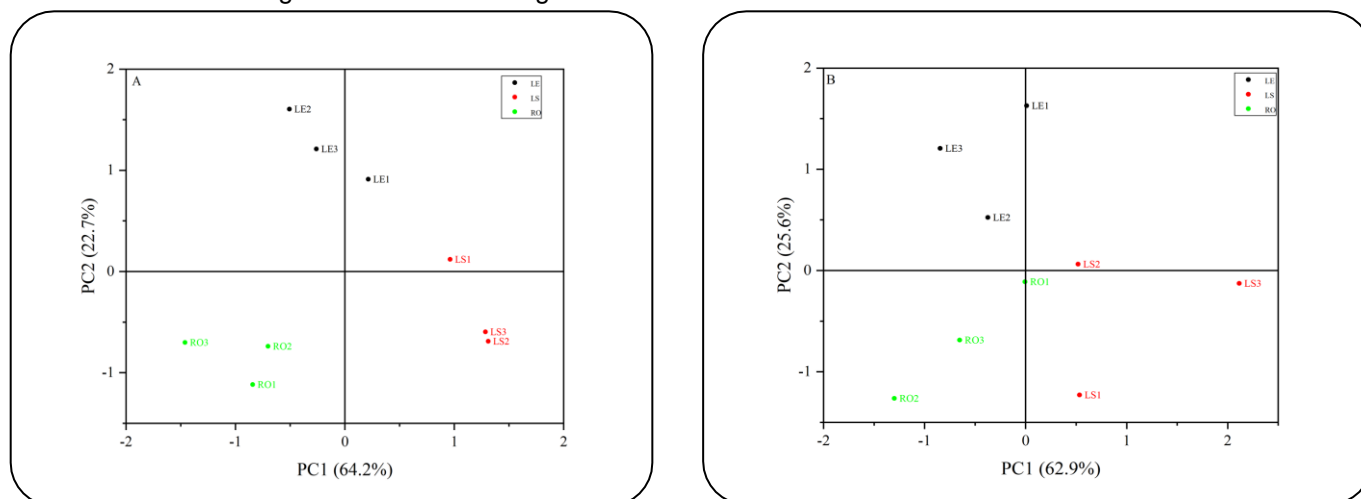


Figure 19. PCA of flavor at vegetative harvesting stage in different organs of Chinese chive varieties Fu Jiu Bao F1 (A) and Jiu Xing 22 (B).

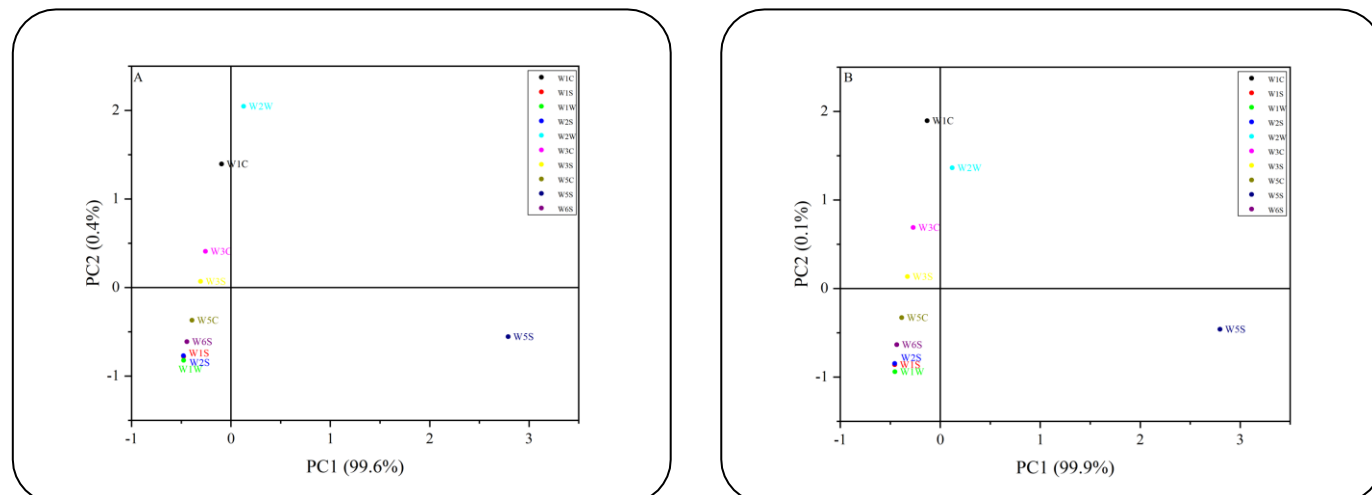


Figure 20. PCA of sensors at vegetative harvesting stage in different organs of Chinese chive varieties Fu Jiu Bao F1 (A) and Jiu Xing 22 (B).

## Flowering stage

### Radar chart analysis

At flowering stage the samples were taken from leaf sheath, leaf, root, floral axis, mature flower and inflorescence and these samples were subjected to E-Nose analysis. The radar chart results revealed that variety Fu Jiu Bao F1 was better in flavor characteristics as indicated from the contents of compounds in leaf, mature flower and inflorescence; while variety Jiu Xing 22 showed superiority for flavor characteristics in leaf sheath and root. However, Fu Jiu Bao F1 showed consistency in compounds in all the organs. Figure 21 revealed that variety Fu Jiu Bao F1 has greater consistency for flavor characteristics over Jiu Xing 22 and this variety can be considered for stronger overall flavor characteristics based on the results of leaf sheath, leaf, root, floral axis, mature flower and inflorescence samples at flowering stage of Chinese chive. The radar chart revealed that W5S was the largest sensor for sample characterization for volatiles in Chinese chive.

### PCA analysis

Figures 22, 23 portrayed the variance in volatile contents in leaf sheath, leaf and root of Chinese chive varieties Fu Jiu Bao F1 and Jiu Xing 22 at flowering stage and ten sensors that develop corresponding groups of volatiles. The sum of the first two principal components reached 89.2%, out of which the main axis-1 characterized 71.8% of the total variance, while main axis-2 characterized 17.4% of the total variance. It indicated that the order difference of

two Chinese chives cultivars can be distinguished. The sum of these two components was nearly 90%, so we can distinguish minimally them according different plant organs at flowering stage. The PCA of odor data of e-nose showing a diagram of the PCA analysis of plant organs at flowering stage with sensors W5S, W1S, W3C, W1C, and W2W as primary sensors; which indicated that farther each sensor was from the origin of the coordinate, the greater was the rate of contribution; and hence W5S was an effective sensor with more effective contribution rate to detect volatiles in plant organs at flowering stage.

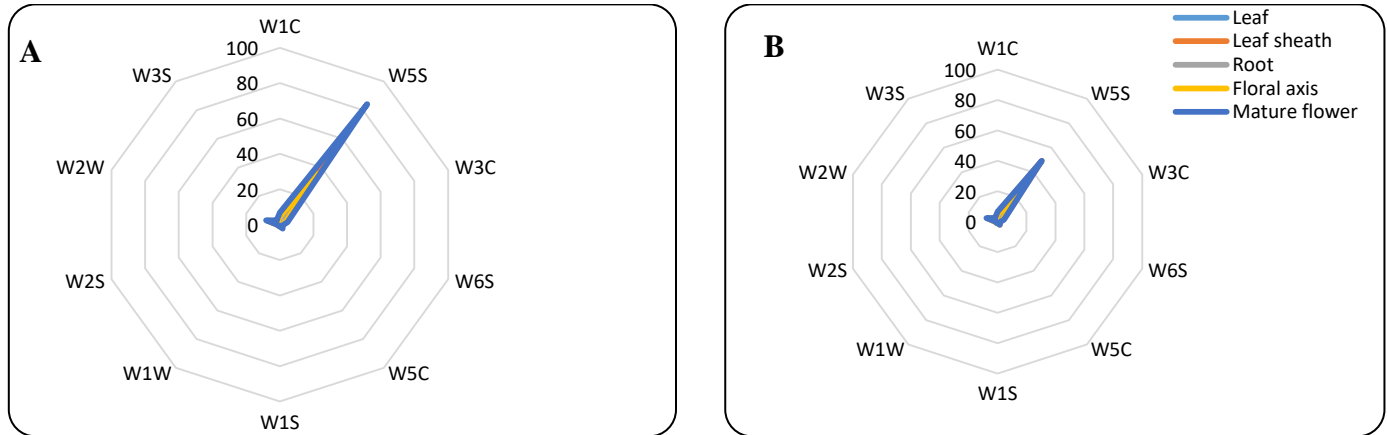


Figure 21. Flavor radar chart analysis of different organs at flowering stage of Chinese chive variety Fu Jiu Bao F1 (A) and Jiu Xing 22 (B).

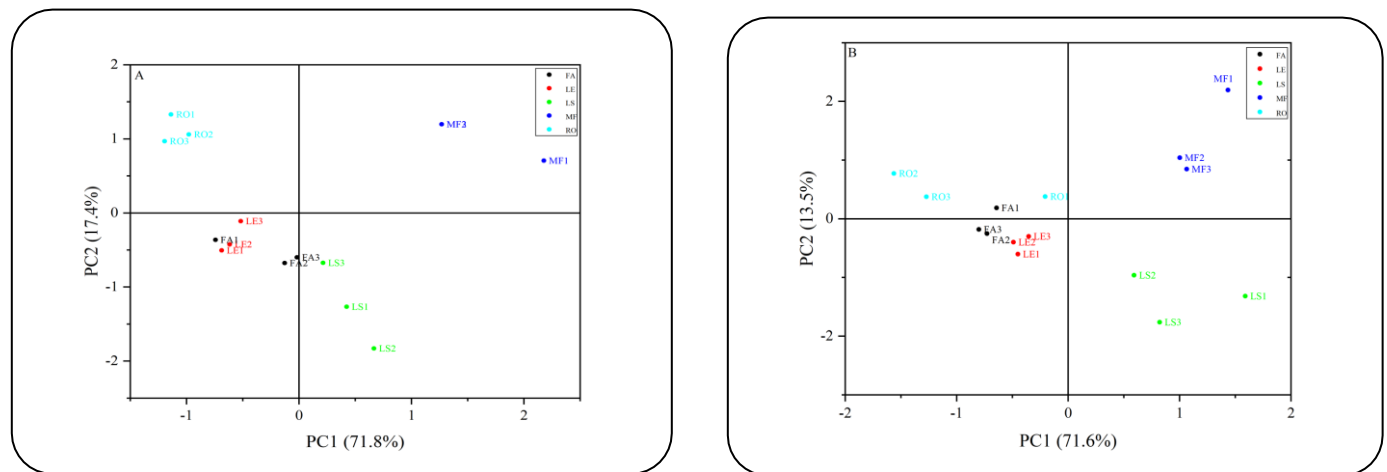


Figure 22. PCA of flavor at flowering stage in different organs of Chinese chive varieties Fu Jiu Bao F1 (A) and Jiu Xing 22 (B)

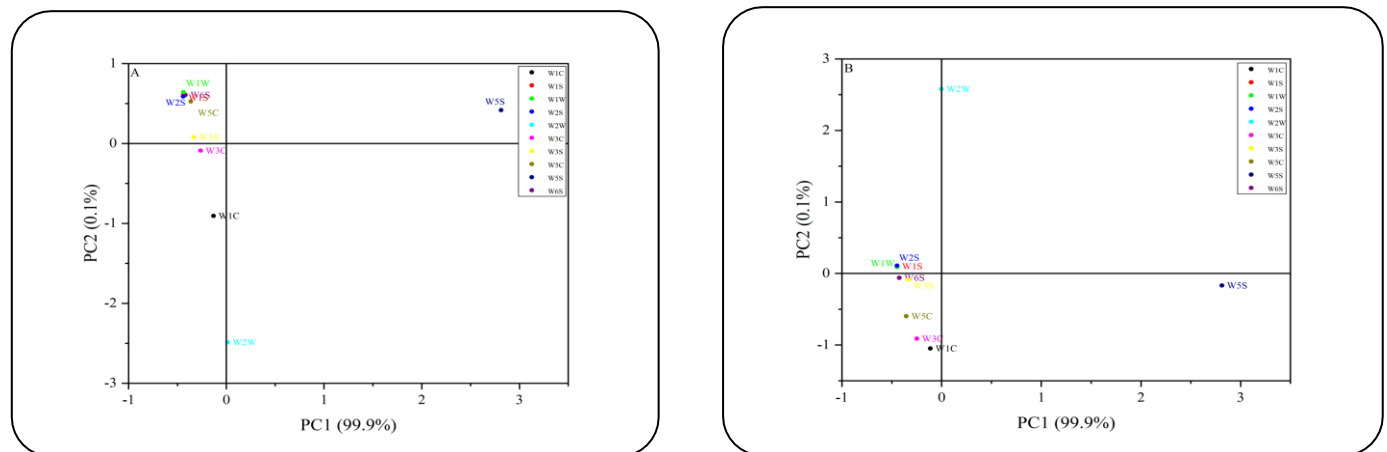


Figure 23. PCA of sensors at flowering stage in different organs of Chinese chive varieties Fu Jiu Bao F1 (A) and Jiu Xing 22 (B).

## Fruiting stage

### Radar chart analysis

The radar chart showing the results of e-nose analysis of Chinese chive for flavor quality at fruiting stage indicated that Chinese chive varieties Fu Jiu Bao F1 and Jiu Xing 22 showed dissimilarity in volatile compounds in leaf sheath, leaf, root, floral axis, mature flower and seed. However, variety Jiu Xing 22 was better in flavor characteristics as indicated from the contents of compounds in leaf and mature flower; and consistency was noted for volatile contents in other organs as well. The variety Fu Jiu Bao Jiu showed superiority only in the volatile contents in seed samples. Figure 24 portrayed that variety Jiu Xing 22 at fruiting stage has greater consistency for flavor characteristics than Fu Jiu Bao F1 and this variety can be considered for stronger overall flavor characteristics based on the results of different plant organs. The radar chart revealed that W5S was the largest sensor for sample characterization for sensing flavor and aromatic characteristics in Chinese chive.

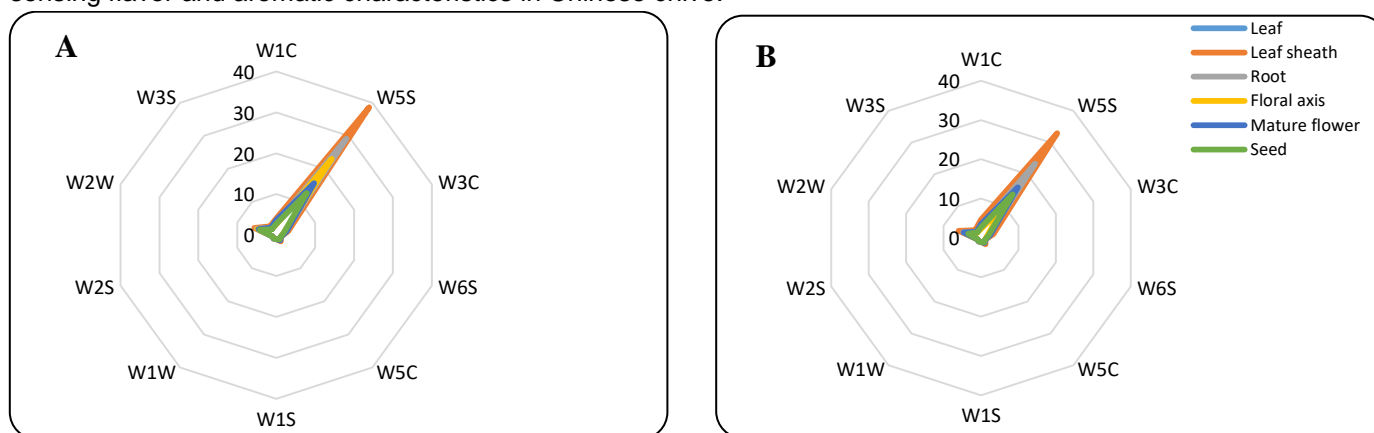


Figure 24. Flavor radar chart analysis of different organs at fruiting stage of Chinese chive variety Fu Jiu Bao F1 (A) and Jiu Xing 22 (B).

### PCA analysis

Figures 25, 26 reveal the difference in volatile contents in different plant organs of Chinese chive varieties Fu Jiu Bao F1 and Jiu Xing 22 at fruiting stage and ten sensors that develop corresponding groups of compounds. The sum of the first two principal components reached 88.3%, out of which the main axis-1 characterized 65.9% of the total variance, while main axis-2 characterized 22.4% of the total variance; indicating that the order difference of two Chinese chives cultivars can be distinguished at low level. The sum of these two components was nearly 90%, so we can distinguish them according different plant organs at fruiting stage. Fig. 26 is the PCA of odor data of e-nose showing a diagram of the PCA analysis of plant organs at fruiting stage with sensors W5S, W1S, W3C, W1C, and W2W as primary sensors; which indicated that farther each sensor was from the origin of the coordinate, the greater was the rate of contribution; and hence W5S was an effective sensor with more effective contribution rate to detect volatiles in plant organs at fruiting stage. To increase recognition of the sensor, more sensors were added to distinguish the mixed types.

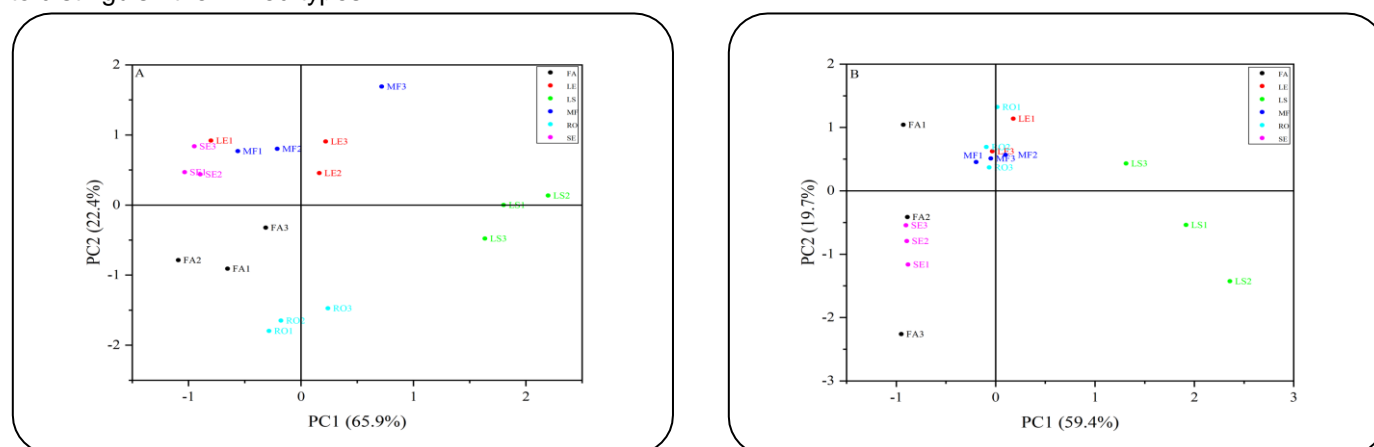


Figure 25. PCA of flavor at fruiting stage in different organs of Chinese chive varieties Fu Jiu Bao F1 (A) and Jiu Xing 22 (B)



were detected; whereas, in root samples at this stage, no new compound was detected in variety Fu Jiu Bao F1. In roots of variety Jiu Xing 22, 03 new compounds were detected. At harvesting stage, the varieties and plant organs varied significantly ( $P < 0.01$ ) for volatile contents and such deviation was exceptional in leaves of two varieties. The roots of variety Jiu Xing 22 surpassed variety Fu Jiu Bao F1 in total volatile contents; and the total volatile contents were markedly greater in leaves as compared to roots regardless of varieties. At this stage, cultivar Fu Jiu Bao F1 recorded 30 novel compounds in leaves. In roots of varieties Fu Jiu Bao F1 and Jiu Xing 22 no novel compounds were detected. The organosulfur compounds affect functional qualities of fruits and vegetables (Cinara *et al.*, 2012); these compounds are responsible for antimicrobial and antioxidant activities (Chen *et al.*, 2016); where flavonoids possess strong antibacterial and antioxidant activity Chope *et al.* (2011) determined that the intensity and quality of the volatiles is affected by the varieties and growth stage in different plant parts in *alliums*.

E-Nose analysis revealed that 10 sensors developed corresponding groups of volatiles for two Chinese chive varieties to characterize at different plant growth stages in different plant organs. The analysis reveals that distinguishing volatiles were found scattered as aromatic, broad range, sulfur organic, hydrogen, arom-aliph, broad methane, broad alcohol, and methane aliph. The results suggested that the order difference of varieties and plant organs at different growth stages can be highly distinguished. The sum of these two components in both the varieties distinguishes them according to several growth stages and plant organs. Similar results have been found by several past workers. Kang *et al.* (2012) mentioned that volatile is promising when its concentration is higher. Kim *et al.* (2008) have suggested that  $>1\mu\text{g/g}$  volatile concentration is minimally useful, but depends on the quality of the substance. Gao *et al.* (2018) proved that scatter diagram (Radar) are effective in find potential volatile substances for aromatic, texture and flavor purposes in Chinese chives cultivars.

## CONCLUSION

Fu Jiu Bao F1 leaves contained higher volatile organic compounds than roots and leaf sheath during vegetative development (seedling, 30 days after transplanting, and harvesting). Jiu Xing 22 root volatiles exceeded leaf sheath and leaves. Maximum volatile chemical levels were seen in mature Fu Jiu Bao F1 flowers (530.6 $\mu\text{g/g}$ ), followed by leaves, inflorescence, leaf sheaths, and roots (3.90% and 4.15%, respectively) while flowering. Jiu Xing 22 leaf sheath is highly volatile. Fu Jiu Bao F1 exhibited the highest volatile chemical content in seeds (252.16 $\mu\text{g/g}$ ) during fruiting, whereas other organs had lower amounts (7.42-34.57 $\mu\text{g/g}$ ). Leaf sheath has most Jiuxing 22 volatiles. Unlike other Chinese chive cultivars, Fu Jiu Bao F1 transported volatiles from vegetative to reproductive organs. Jiu Xing 22 showed higher volatile intensity and quality in leaf sheath, inflorescence stalks, and mature flowers than Fu Jiu Bao F1, although both had identical volatile contents in blooming leaves. Significant changes ( $P < 0.01$ ) in volatile intensity were observed in both leaf sheath and seed during fruiting. Jiu Xing 22 outperformed Fu Jiu Bao F1 in seed and leaf sheath chemicals. Leaf sheath and seeds had substantially greater volatile concentrations than floral axis, leaves, and roots, regardless of variety. Volatile concentrations in leaves and roots varied substantially after 30 days of transplanting, although sulfur-containing compounds in leaf sheath did not. E-Nose identified aromatic, broad range, sulphur organic, hydrogen, arom-aliph, broad methane, broad alcohol, and methane aliph, although plant component and stage may affect flavour, fragrance, and other features.

## AUTHOR CONTRIBUTIONS

All authors contributed equally to this research.

## COMPETING OF INTEREST

The authors declare no competing interests.

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