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# **OPEN** Identification of VOCs in essential oils extracted using ultrasoundand microwave-assisted methods from sweet cherry flower

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The floral fragrance of plants is an important indicator in their evaluation. The aroma of sweet cherry flowers is mainly derived from their essential oil. In this study, based on the results of a single-factor experiment, a Box-Behnken design was adopted for ultrasound- and microwave-assisted extraction of essential oil from sweet cherry flowers of the Brooks cultivar. With the objective of extracting the maximum essential oil yield (w/w), the optimal extraction process conditions were a liquid-solid ratio of 52 mL g<sup>-1</sup>, an extraction time of 27 min, and a microwave power of 435 W. The essential oil yield was 1.23%, which was close to the theoretical prediction. The volatile organic compounds (VOCs) of the sweet cherry flowers of four cultivars (Brooks, Black Pearl, Tieton and Summit) were identified via headspace solid phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS). The results showed that a total of 155 VOCs were identified and classified in the essential oil from sweet cherry flowers of four cultivars, 65 of which were shared among the cultivars. The highest contents of VOCs were aldehydes, alcohols, ketones and esters. Ethanol, linalool, lilac alcohol, acetaldehyde, (E)-2-hexenal, benzaldehyde and dimethyl sulfide were the major volatiles, which were mainly responsible for the characteristic aroma of sweet cherry flowers. It was concluded that the VOCs of sweet cherry flowers were qualitatively similar; however, relative content differences were observed in the four cultivars. This study provides a theoretical basis for the metabolism and regulation of the VOCs of sweet cherry flowers.

Volatile organic compounds (VOCs) represent an important part of the plant metabolome and attract agronomic and biological interest due to their contribution to fruit aroma and flavor and, therefore, to fruit quality. Analysis of the composition of VOCs in plant flowers is a prerequisite for the application of their extracts<sup>1,2</sup>. The extraction/isolation techniques of VOCs include simultaneous distillation and solvent extraction (SDE), supercritical fluid extraction (SFE), hydrodistillation (HD), microwave-assisted hydrodistillation (MAHD), cold-press (CP), headspace (HS) extraction, and solid phase microextraction (SPME)<sup>3-6</sup>. Gas chromatography-mass spectrometry (GC-MS) is commonly employed to detect VOCs. Conventional extraction methods (such as SDE, HD, etc.) have problems such as a low extraction rate and weak extract activity, which restrict the development and application of plant extracts. Therefore, microwave, oscillation, and ultrasonication extraction methods have also been proposed. The purpose of using the combined ultrasound- and microwave-assisted extraction (UMAE) technique for the extraction is to alternative conventional extraction techniques, because UMAE is inexpensive, simple, rapid, green and efficient. Generally, UMAE will not affect the quality of essential oil extraction and the extraction yield was significantly increased via the UMAE method instead of conventional extraction methods<sup>7-10</sup>. e.g. UMAE was usually employed to extract essential oils, total flavonoids, dihydroquercetin etc. from seed, flower, bark, xylem of plant<sup>11-i3</sup>. As a viable extraction technique, this method combined with GC/MS or UPLC/MS can be used as a routine analysis strategy for essential oil in plant. Due to the rapid development of analytical instruments and sample preparation techniques, floral fragrance research has gradually intensified. By the detection and analysis of HS-SPME/GC-MS, the VOCs of many common plant flowers have been initially identified<sup>14</sup>.

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Many plants and flowers in nature emit aromas, and many aroma components are mixed to form a floral fragrance; different aroma components and their content differences provide their unique characteristics<sup>15</sup>. Floral fragrance is one of the important traits that are considered in evaluating ornamental plants. The fragrances of plant flowers are derived from various volatiles released from petals or flowers. Studies have shown that aroma is derived from the secondary metabolites of flowers, and major components are volatile small molecule compounds, such as terpenes, phenylpropionic acids and fatty acid derivatives<sup>16,17</sup>. During the flowering process, the types, contents and proportions of aroma components have a strong influence in attracting pollinators to forage. By volatilization, the smell of some flowers not only generates a signal to stimulate the immune response to self-heal but also has an important role in plant resistance to various stresses<sup>18</sup>. The VOCs of many plants possess obvious aromatic effects and medicinal value. The research focus on floral fragrance gives attention to the aroma of flowers with metabolomics and genomics<sup>19,20</sup> and focuses on the relationship between aroma components and regulatory mechanisms<sup>21,22</sup>.

Sweet cherry (*Prunus avium* L.), which is also known as large cherry, is a fleshy stone fruit that belongs to the genus *Prunus*, and it is grown in specific regions with temperate climates<sup>23</sup>. The color of sweet cherry fruit is bright, and the fruit is sweet, delicious, and rich in nutrients<sup>24</sup>. In southern China, the introduction and cultivation of sweet cherries has experienced problems, such as a low rate of fruit set, serious fruit drop, unstable yield and fruit malformation, which have seriously affected the development of the sweet cherry industry in the region. Some scholars have explained the effect of temperature on the flowering and fruiting of sweet cherry introduced in the southern region from the aspects of cold demand, flower bud differentiation process and rate, embryo development, and quality and fruit setting<sup>25</sup>. Lech et al.<sup>26</sup> analyzed the flowering of several sweet cherry cultivars in the climatic conditions of southern Poland. In addition, Legua et al.<sup>27</sup> investigated the bioactive and volatile compounds in sweet cherry fruits. However, related research on the VOCs of sweet cherry flowers is lacking. The aroma of sweet cherry flowers is mainly derived from its essential oil. In this experiment, sweet cherry flowers from four cultivars were utilized as the research object; their essential oils were extracted, which provide a reference for the utilization of the essential oil of sweet cherry flowers. The identification of their VOCs will provide a scientific basis for elucidating the floral fragrance formation mechanism, metabolism and regulation.

### Materials and methods

**Plant material.** The samples (blooming flowers were gathered in early April) of the sweet cherry cultivars Brooks, Black Pearl, Tieton and Summit were collected in 2020 from the Baiyi fruit tree experimental base of Guiyang Wudang at the Guizhou Institute of Fruit Tree Science (27°03'3.89"N and 106°25'47.23"E). The trees were 5 years old, and they were grafted onto "Gisela 6" root stock and planted under rain-shelter conditions in March 2015. All trees were managed with the same practical techniques. Identification of plant material was initially performed using morphological features and then confirmed by Prof. Xiang Wang at the Kaili University. A voucher specimen (No. 1033) has been deposited in the herbarium of our laboratory. The samples (well-developed flowers from the base of the shoots) from the same location were harvested from each tree. After they were washed with distilled water, the fresh samples were immediately loaded into a sampling bottle with a 50 mL-vial volume, in which the flower sample was placed. The fresh samples were tested two hours after they were transported to a laboratory.

**Extraction method.** The 5.000 g flower sample of the Brooks sweet cherry cultivar was placed in a special flask for UMAE. Methanol:chloroform:ddH<sub>2</sub>O (5:2:2, v/v/v) mixed solution was added, and the flask was placed in the UMAE apparatus. The microwave extraction power was adjusted; the ultrasonic power was set to 50 W; the extraction time was set; the filter was vacuumed the filter residue was discarded; and then the collected extracting solution was poured into a rotary evaporator. The next step was to retrieve solvents by reduced pressure distillation, and the extracts were obtained at the same time.

A simultaneous UMAE apparatus (XO-SM, Nanjing Xianou Instrument Manufacturing Co., Ltd., China) shown in Fig. 1 was applied for sample extraction.

The parameter of UMAE show in Table 1; Rotary Evaporator; model R-201D, produced by Shanghai Yike Instrument Co., Ltd. (Shanghai, China); and 7890B-5977B GC–MS, Agilent Technologies Co. Ltd. (California, USA). Methanol (CAS: 67-56-1) and chloroform (CAS: 67-66-3) were purchased from Tianjin Sayfo Technology Co., Ltd. (Tianjin, China).

**Single-factor experiment.** The purpose of this single-factor experiment was to investigate the effects of three factors (liquid-solid ratio, extraction time, and microwave power) on the yield (w/w) of sweet cherry flower essential oil (Table 2).

**Response surface methodology experiments.** For the response surface methodology and experimental design scheme, three factors were selected as independent variables, namely, the liquid–solid ratio, extraction time and microwave power, and the yield of sweet cherry flower essential oil was the response value.

**HS-SPME/GC–MS conditions.** According to the best process parameters obtained by optimizing the extraction of Brooks flower essential oil, Black Pearl, Tieton and Summit flower essential oils were extracted. After sampling, the 1 mL samples were immediately submitted to the HS-SPME/GC–MS system. Volatiles were isolated by SPME (50 °C), which included oscillation for 15 min and extraction for 30 min (250 RPM) using the CTC triad automatic sampler (Extractor head: 50/30  $\mu$ m DVB/CAR on PDMS). Gas chromatography was performed on a DBwax (30 m×0.25  $\mu$ m) to separate the volatile compounds at a constant flow of 1 mL min<sup>-1</sup> helium. The injection temperature was 260 °C. The temperatures of the column and ion source were





Model	Ultrasonic power	Ultrasonic frequency	Microwave power	Microwave frequency	Capacity	Ultrasonic probe diameter
XO-SM	0–900 W	25 kHz	0–700 W	2450 MHz	0.5-500 mL	Φ 6 mm

Table 1. Parameter of ultrasound- and microwave-assisted extracting apparatus.

	Level							
Factors	1	2	3	4	5	6		
A Liquid–solid ratio/mL g <sup>-1</sup>	10	20	30	40	50	60		
B Extraction time/min	5	10	15	20	25	30		
C Microwave power/W	100	200	300	400	500	600		

Table 2. Factors and levels in the single-factor experiment.

40 °C and 230 °C, respectively. The temperature-rise program was followed by an initial temperature of 5 °C for 5 min, 20 °C min<sup>-1</sup> with a maximum rate of 250 °C and then held constant for 2.5 min. Mass spectrometry (EI<sup>+</sup>, 70 eV) was determined by a full-scan method with a range from 200 to 400 (m  $z^{-1}$ ). The classification was referred from NIST 2014<sup>28-30</sup>.

**Statistical analyses.** Statistical analysis was performed using the SPSS Statistics Version 21.0 software (Chicago, IL, USA). Duncan's multiple comparisons test (p < 0.05) was performed to assess the statistically significant differences between the mean values (means) of three replications. Origin 9.1 (Origin Lab, Northampton, MA, USA) was employed to construct the graphs.

# **Results and discussion**

**Selection of factors and their levels by single-factor analysis.** With an extraction time of 20 min and a microwave power of 400 W, the influence of the liquid–solid ratio on yield was investigated; the results are shown in Fig. 2. As the amount of solvent increases, the yield of sweet cherry flower essential oil increases and then becomes stable. When the liquid–solid ratio is 60 mL  $g^{-1}$ , the yield approaches the maximum, perhaps because as the amount of solvent increases, the contact area between the solvent and the raw material increases, and the essential oil can be fully dissolved. However, when the amount of solvent is too high, the solvent absorbs more microwave energy, and the absorption of microwave energy by the raw material decreases, which causes a decrease in the ability of the microwave to damage raw material cells, and the yield of essential oils only slightly changes<sup>31</sup>. After comprehensive consideration, a liquid–solid ratio of 40 mL  $g^{-1}$  is selected.





	Level		
Factors	- 1	0	1
A Liquid–solid ratio/mL g <sup>-1</sup>	40	50	60
B Extraction time/min	20	25	30
C Microwave power/W	300	400	500



With a liquid–solid ratio of 40 mL  $g^{-1}$  and microwave power of 400 W, the effect of extraction time on the yield of essential oils was investigated. It can be seen from Fig. 2 that with an extension of extraction time, the yield increases and then gradually becomes stable with minimal change after 20 min. In a certain period, with an extended microwave treatment time, the raw material absorbs more microwave energy; the cell rupture is more sufficient; and the essential oil dissolves more. Thus, the yield continues to rise. However, as the microwave treatment time continues to increase, the essential oil inside the raw material is completely extracted, and further increases in the extraction time have a minimal effect on the yield<sup>32</sup>. Therefore, 20 min is an appropriate extraction time.

With a liquid–solid ratio of 40 mL g<sup>-1</sup> and an extraction time of 20 min, the influence of microwave power on yield was investigated. The results are shown in Fig. 2. As the microwave power increases, the yield gradually increases and reaches a maximum at 400 W. At a power greater than 400 W, the yield decreases slightly. A microwave extracts essential oils by desorption. With an increase in microwave power, the heating rate increases; the cell wall quickly ruptures; and a large amount of essential oils dissolves. However, when the microwave power is too high, the heat-sensitive compounds in the essential oil will oxidize and decompose, which causes a decrease in yield. The experimental results of this study are consistent with the viewpoint of Routray and Valérie<sup>33</sup>. Usually, the microwave extraction yield will increase the yield. This result may be due to the increase in molecular migration and solute dissolution rate during the heating process. However, if the temperature is too high, the energy consumption will increase, and the extraction yield will not increase significantly. Therefore, the optimum microwave power is 400 W.

**Model fitting and effect of UMAE factors on yield.** Based on the results of a single-factor experiment, three factors were selected as independent variables: the liquid–solid ratio (A), extraction time (B), and micro-wave power (C). The essential oil yield (Y) was the response value. The factors and levels are shown in Table 3. The Box–Behnken design and results are shown in Table 4. The binary polynomial regression model equation fitted by the software is:

$$Y = 1.23 + 0.023A + 0.012B + 0.045C + 0.017AB - (2.500E - 003)AC + (2.500E - 003)BC - 0.068A2 - 0.023B2 - 0.063C2$$
(1)

A variance analysis was performed using the regression model equation, and a significance analysis was performed using the model coefficients. As shown in the Table 5 model variance analysis results, the Model P-value of 0.0025 implies that the model is significant. The probability of this large "Model F-Value" is due to noise. Values of "Prob > F" less than 0.05 indicate that the model terms are significant.  $R^2 = 0.9318$  and Adj  $R^2 = 0.8442$  are near 1, which means that a 93.18% variation in the yield (w/w) of sweet cherry flower essential oil can be explained

No	A Liquid–solid ratio	B Extraction time	C Microwave power	Y Yield/% (w/w)	No	A Liquid–solid ratio	B Extraction time	C Microwave power	Y Yield/% (w/w)
1	60	25	500	1.20	10	50	25	400	1.23
2	60	25	300	1.08	11	50	30	300	1.13
3	40	20	400	1.14	12	50	20	500	1.16
4	60	30	400	1.18	13	50	25	400	1.23
5	50	20	300	1.11	14	60	20	400	1.12
6	50	25	400	1.25	15	50	25	400	1.22
7	40	25	300	1.00	16	40	25	500	1.13
8	50	25	400	1.24	17	50	30	500	1.19
9	40	30	400	1.13					

 Table 4.
 Box–Behnken experimental design and results.

Source	Sum of squares Degrees of freedom of variation		Mean square	F value	Prob>F
Model	0.065	9	7.25E-03	10.63	0.0025
A-Liquid-solid ratio	4.05E-03	1	4.05E-03	5.94	0.0449
B-Extraction time	1.25E-03	1	1.25E-03	1.83	0.2177
C-Microwave power	0.016	1	0.016	23.77	0.0018
AB	1.23E-03	1	1.23E-03	1.8	0.2219
AC	2.50E-05	1	2.50E-05	0.037	0.8535
BC	2.50E-05	1	2.50E-05	0.037	0.8535
A <sup>2</sup>	0.02	1	0.02	28.78	0.001
B <sup>2</sup>	2.28E-03	1	2.28E-03	3.34	0.1103
C <sup>2</sup>	0.017	1	0.017	24.72	0.0016
Residual	4.77E-03	7	6.81E-04		
Lack of fit	4.25E-03	3	1.42E-03	10.9	0.0215
Pure error	5.20E-04	4	1.30E-04		
Cor toal	0.07	16			
R <sup>2</sup>	0.9318				
Adj R <sup>2</sup>	0.8442				
Pred R <sup>2</sup>	0.0166				
C.V.%	2.25				

**Table 5.** Analysis of variance for the regression model. Values of "Prob > F" less than 0.05 indicate that themodel terms are significant, and values greater than 0.10 indicate that the model terms are not significant.

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by the model. The quadratic polynomial regression model employed in the experiment shows a high degree of significance, which indicates that the experimental data fit the regression mathematical model and can better predict the actual values of various indicators<sup>34</sup>.

Design Expert 8.0 software was utilized to perform quadratic multiple regression fitting of the data in Table 4. The response surface results of the quadratic regression equation are shown in Fig. 3. As shown in Fig. 3a, as the liquid–solid ratio increased, the yield appears to increase and then decrease in certain microwave power conditions. As shown in Fig. 3e, in certain liquid–solid ratio conditions, with an increase in the microwave power, the yield also appears to increase and then decrease. In this case, A, C, A<sup>2</sup>, and C<sup>2</sup> are significant model terms, and B, AB, AC, BC, and B<sup>2</sup> are nonsignificant model terms. By the response surface analysis of the regression model, with the goal of maximizing the yield of sweet cherry flower essential oil, the optimal extraction conditions for UMAE are a liquid–solid ratio of 52.06 mL g<sup>-1</sup>, an extraction time of 26.83 min, and microwave power of 435.94 W. In these extraction conditions, the regression equation model predicts the yield of sweet cherry flower essential oil to be 1.24667%. Considering the feasibility of the actual operating conditions, the extraction condition parameters were revised to a liquid–solid ratio of 52 mL g<sup>-1</sup>, an extraction time of 27 min, and microwave power of 435 W. The actual yield of essential oils measured by 3 parallel experiments is 1.23%, which is near the predicted value and indicates that the regression equation model can better simulate and predict the yield of sweet cherry flower essential oils.

**Identification of VOCs of sweet cherry flower essential oil.** The extraction of VOCs from samples is a key link in the analysis of aromatic components. In the past, solvent extraction was employed to extract aromatic compounds, but due to its defects, this method could not completely extract VOCs from the samples,



**Figure 3.** Response surface (**a**,**c**,**e**) and contours (**b**,**d**,**f**) of the effects of the liquid–solid ratio, extraction time and microwave power.

which affects the accuracy of the analysis results<sup>35–37</sup>. In this study, the HS-SPME was utilized to enrich the volatile and semivolatile components in the sample, combined with GC–MS technology to analyze, identify, and compare the aroma components of sweet cherry flower essential oil. This method offers high sensitivity and simple operation and is convenient and fast. The total ion chromatogram of the sweet cherry flower essential oils by HS-SPME/GC–MS separation analysis is shown in Fig. 4 The total ion chromatogram in four cultivars is similar, but the peak area with the same retention time is different. In this study, a total of 155 VOCs (Table 6) of 11 different chemical groups were separated and identified in the Brooks, Black Pearl, Tieton and Summit sweet cherry cultivars. Sweet cherry flower essential oils from different cultivars have different numbers and relative contents of VOCs.

The VOCs possessed by plant flowers are secondary metabolites, which have a vital role in the pollination process of plants. Lilac alcohol, dimethyl sulfide, acetaldehyde, 3-methyl-1-butanol, 3-methyl butanal, (*E*)-2-hexenal, benzaldehyde, etc. in the VOCs of sweet cherry flowers can be employed in natural floral flavors. Among them, lilac alcohol has a lilac fragrance. Dimethyl sulfide is one of the key odorants in the production of corn, tomato, potatoes, dairy product, and pineapple<sup>38</sup>. In addition, 3-methylbutanal is mainly employed to formulate various fruit flavors. Ethanol, linalool, 4-methoxy-benzaldehyde and lilac aldehyde have been shown to have certain pharmacological effects<sup>39</sup>. A large amount of ethanol is observed in the flowers of sweet cherry during the full





bloom period, which is caused by the change in carbon metabolism during the glycolysis of flowers. As the flower matures, respiration increases, and pyruvate, which is the intermediate product of glycolysis, enters the tricarboxylic acid cycle. The remaining part is converted to ethanol, which causes an accumulation of ethanol. The large amount of ethanol not only harms the flowers but also weakens the tolerance and disease resistance of the plant and increases the occurrence of decay. Linalool has many pharmacological activities, such as analgesic, sedative hypnosis, antianxiety and antitumor activities<sup>40,41</sup>. Moreover, 4-methoxy-benzaldehyde is employed in medicine as an intermediate for antihistamine drugs, such as the antibiotic hydroxyaminobenzyl penicillin. The types, contents and proportions of VOCs of sweet cherry flowers have a strong influence in attracting pollinators to forage (e.g., the noctuid moth *Hadena bicruris* relies on lilac aldehyde to find its host plant)<sup>39</sup>.

**Classification analysis of VOCs.** To identify the main aroma compounds in sweet cherry flower essential oil, the differences in the VOCs of the essential oils of the flours of the four sweet cherry cultivars were compared. The VOCs were grouped according to their chemical families as alcohols, aldehydes, esters, ether, furan, alkane, olefin, terpenes, ketones, organic acids, and other VOCs.

As shown in Fig. 5, in the Brooks, Black Pearl, Tieton, and Summit cultivars, 97 compounds, 107 compounds, 112 compounds and 111 compounds, respectively, were detected. Among the 155 VOCs, ethanol, linalool, dimethyl sulfide, acetaldehyde, (*E*)-2-hexenal, and benzaldehyde are the main compounds that comprise the aroma of the sweet cherry flower essential oil from the four cultivars.

It can be seen from Fig. 6 that the aldehyde content is the largest in the VOCs, followed by alcohols. These two substances are the main sources of sweet cherry flower aroma. Benzaldehyde was detected in four sweet cherry cultivar flowers and hyacinth, citronella, cinnamon, iris and rose. Moreover, (E)-2-hexenal has a fresh green leaf fragrance and can be used as a blending fragrance for essential oils and various floral fragrances<sup>42</sup>. Acetaldehyde is also present in the aroma of four sweet cherry cultivar flowers and naturally exists in round pomelo, pear, apple, raspberry, strawberry, pineapple, coffee, and orange juice<sup>43</sup>. After dilution, acetaldehyde has a fruity, coffee, wine, green fragrance. Benzaldehyde and benzeneacetaldehyde are important aldehyde flavor ingredients<sup>44</sup>, and benzaldehyde has the aroma of bitter almond, cherry and nut. Benzaldehyde, which is a common component of plant volatiles<sup>45</sup>, attracts many pest species. A recent study has indicated that benzaldehyde can be recognized by adult A. lucorum and can affect its behavior<sup>46,47</sup>. Benzaldehyde is produced by enzymolysis of amygdalin in flowers, and the importance of this substance has been emphasized in previous studies<sup>48</sup>. In this study, the concentrations of benzaldehyde and its derivative benzyl alcohol in the essential oil of the four sweet cherry cultivar flowers are relatively high, which shows the best fragrance of the flowers. This result confirms the importance of benzaldehyde to the sweet cherry flower aroma. Hexanal is a fatty acid that is produced under the catalytic action of lipoxygenase (LOX)<sup>49</sup>. Hexanal has the fragrance of grass and can increase the perceived intensity of fruit aroma. Linalool and phenylethyl alcohol are important alcohol flavor ingredients, among which linalool is extensively applied in cosmetic flavors and food fruit flavors with antibacterial and antiviral effects. Phenylethyl alcohol is one of the two main aroma components of rose essential oil<sup>50</sup>. The fresh, sweet smell of phenylethyl has calming and soothing effects and anti-inflammatory and antibacterial effects. Lilac alcohol isomers (lilac alcohol B and lilac alcohol C) and lilac aldehyde isomers (lilac aldehyde B and lilac aldehyde C) in sweet cherry flowers are the characteristic aroma components. Most esters can impart plant fruit fragrance. 4-Methoxybenzoic acid ethyl ester is the main ester component of sweet cherry flowers, with aromas similar to fruits and anise. Among the olefins, beta-pinene, myrcene, trans-beta-ocimene, and  $\beta$ -ocimene are the main components, and beta-pinene is only detected in the Summit cultivar<sup>51</sup>. Ocimene has a certain role in the prevention and treatment of cancer. An antidepressant experiment in mice has also shown that ocimene can effectively reduce depression traits in mice<sup>52</sup>. Ethers and ketones are relatively rare in flowers. Ketones are also typical aromatic components, and 4-hydroxy-2-butanone is typical of sweet cherry flowers. These VOCs provide the unique aromatic quality of sweet cherry flowers, which indicates that sweet cherry flowers are an important natural spice raw material, which has important scientific value and excellent development prospects.

				Relative content/%			
Peak no	Compound	Molecular formula	RT/min	Brooks	Black pearl	Tieton	Summit
1	Acetaldehyde	C <sub>2</sub> H <sub>4</sub> O	1.94	5.68	5.57	6.13	4.47
2	Dimethyl sulfide	C <sub>2</sub> H <sub>6</sub> S	2.11	5.56	5.54	5.78	5.92
3	Trimethylene oxide	C <sub>3</sub> H <sub>6</sub> O	2.29	-	1.09	-	-
4	Octane	C <sub>8</sub> H <sub>18</sub>	2.36	1.59	1.67	1.71	1.79
5	Propanal, 2-methyl-	C <sub>4</sub> H <sub>8</sub> O	2.43	2.40	2.55	2.72	3.01
6	Ethyl ether	C <sub>4</sub> H <sub>10</sub> O	2.52	-	-	0.82	-
7	2-Propenal	C <sub>3</sub> H <sub>4</sub> O	2.68	-	0.13	-	0.13
8	Cyclotrisiloxane, hexamethyl-	C <sub>6</sub> H <sub>18</sub> O <sub>3</sub> Si <sub>3</sub>	2.76	0.81	0.59	0.48	0.48
9	Butanal	C <sub>4</sub> H <sub>8</sub> O	2.97	0.14	0.15	0.10	0.12
10	CH <sub>3</sub> C(O)CH <sub>2</sub> CH <sub>2</sub> OH	C <sub>4</sub> H <sub>8</sub> O <sub>2</sub>	3.11	1.55	1.40	1.78	1.45
11	Furan, 3-methyl-	C <sub>5</sub> H <sub>6</sub> O	3.19	-	0.22	0.19	0.18
12	Butanal, 2-methyl-	C <sub>5</sub> H <sub>10</sub> O	3.45	2.21	2.73	2.55	3.38
13	Butanal, 3-methyl-	C <sub>5</sub> H <sub>10</sub> O	3.51	2.17	2.66	2.47	2.96
14	Ethanol	C <sub>2</sub> H <sub>6</sub> O	4.07	13.67	13.68	20.67	15.42
15	Cyclohexene, 1-methyl-	C <sub>6</sub> H <sub>9</sub> CH <sub>3</sub>	4.16	-	-	-	1.96
16	Heptane, 2,2,4,6,6-pentamethyl-	C <sub>12</sub> H <sub>26</sub>	4.35	-	0.71	0.70	0.56
17	2-Ethylacrolein	C <sub>5</sub> H <sub>8</sub> O	4.43	-	-	-	0.31
18	Propanoic acid, 2-methyl-, ethyl ester	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>	4.48	0.88	0.85	0.56	0.81
19	3-Pentanone	C <sub>5</sub> H <sub>10</sub> O	4.70	-	1.83	0.72	-
20	Decane	C <sub>10</sub> H <sub>22</sub>	5.34	0.11	0.04	0.17	0.04
21	trans-4-Dimethylamino-4'-methoxychalcone	C <sub>18</sub> H <sub>19</sub> NO <sub>2</sub>	5.68	0.45	-	-	-
22	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	C <sub>10</sub> H <sub>16</sub>	5.80	0.55	-	0.21	0.37
23	2-Butanol	C <sub>4</sub> H <sub>10</sub> O	6.22	0.09	_	-	0.07
24	2-Butenal. (E)-	C <sub>4</sub> H <sub>2</sub> O	6.34	1.49	0.89	1.90	0.58
25	1-Propanol	C <sub>2</sub> H <sub>0</sub> O	6.60	0.41	0.30	0.31	0.32
26	Butanoic acid. 2-methyl-, ethyl ester	C <sub>2</sub> H <sub>1</sub> (O <sub>2</sub>	6.89	0.14	0.14	0.12	0.14
27	Butanoic acid. 3-methyl-, ethyl ester	C <sub>2</sub> H <sub>1</sub> O <sub>2</sub>	7 37	0.14	0.28	0.14	0.15
28		C/H <sub>14</sub> O	7.71	2.36	2.78	2.49	2.25
29	2-Butenal. 2-methyl-	C <sub>6</sub> H <sub>12</sub> O	7 99	0.20	0.20	0.21	0.45
30	beta -Pinene	CueHuc	8.14	-	-	-	0.08
31	Tetrasiloxane. decamethyl-	CuoHaoOoSi	8.48	_	_	_	0.07
32	1-Propagol 2-methyl-	C.H.:0	8.61	0.11	0.09	0.12	0.24
33	1-Butanol. 3-methyl- acetate	C <sub>2</sub> H <sub>10</sub> O <sub>2</sub>	8.94	0.03	0.04	0.04	0.14
34	2-Pentenal (F)-	C-H-O	917	-	0.01	0.01	0.11
35	o-Yvlene	С.Н.	9.20		-	0.12	0.50
36	3-Hevenal	Сн.О	9.20		0.62	0.12	
37	alnha_Phellandrene	С. Н	9.88	0.03	-	-	
38	heta - Murcene	С. Н	10.01	0.05	0.19	0.32	0.24
30	2 Butanoic acid athyl actor (F)	Сн.О	10.01	0.40	0.17	0.32	0.24
40	2-Butenol	C H O	10.27	-	-	0.50	_
40	1 Denten 3 ol	C H O	10.57	0.10	0.09	0.11	_
41	Hentanal	CH 0	10.74	-	0.29	-	0.15
42		C H	10.90	0.09	0.10	0.00	0.15
43	Limonana	C H	10.90	0.15	0.00	0.12	-
44	Cuderenteileune desmethel	C II O S:	11.04	-	-	-	0.07
45	De decene	C <sub>10</sub> H <sub>30</sub> O <sub>5</sub> SI <sub>5</sub>	11.10	0.04	0.94	0.72	0.76
40	Dodecane	C <sub>12</sub> H <sub>26</sub>	11.21	0.25	-	0.28	0.18
4/	(2B 5D) 2 Multed 5 (area 1 ar 2 d) 2 singleter hadrone	C <sub>10</sub> H <sub>16</sub>	11.27	0.12	-	0.15	-
48	(2K,5K)-2-Metnyi-5-(prop-1-en-2-yi)-2-vinyitetranydrofuran	C <sub>10</sub> H <sub>16</sub> O	11.67	0.17	0.15	0.12	0.13
49		C <sub>6</sub> H <sub>10</sub> O	12.12	8.51	16.50	10.10	10.78
50	I-Butanoi, 5-methyl-	C <sub>5</sub> H <sub>12</sub> O	12.17	2.23	-	2.32	2.53
51	ruran, 2-pentyl-	C <sub>9</sub> H <sub>14</sub> O	12.32	0.57	1.00	0.43	1.16
52	transpeta-Ocimene	C <sub>10</sub> H <sub>16</sub>	12.42	0.68	0.30	0.55	0.41
53	Hexanoic acid, ethyl ester	$C_8H_{16}O_2$	12.58	0.09	0.18	0.17	0.12
54	(2K,55)-2-Methyl-5-(prop-1-en-2-yl)-2-vinyltetrahydrofuran	C <sub>10</sub> H <sub>16</sub> O	12.77	0.17	0.12	0.13	0.22
55 Contin 1		U <sub>10</sub> Π <sub>16</sub>	12.90	0.52	0.08	0.58	0.13
Continued							

				Relative content/%			
Peak no	Compound	Molecular formula	RT/min	Brooks	Black pearl	Tieton	Summit
56	1,3,5,7-Cyclooctatetraene	C <sub>8</sub> H <sub>8</sub>	13.08	0.03	-	0.03	0.04
57	3-Octanone	C <sub>8</sub> H <sub>16</sub> O	13.16	-	0.04	0.05	-
58	3-Buten-1-ol, 3-methyl-	C <sub>5</sub> H <sub>10</sub> O	13.27	-	-	-	0.06
59	1-Pentanol	C <sub>5</sub> H <sub>12</sub> O	13.39	-	-	-	0.12
60	Benzene, 1-methyl-3-(1-methylethyl)-	C <sub>10</sub> H <sub>14</sub>	13.42	-	0.09	0.13	-
61	Acetic acid, hexyl ester	C <sub>8</sub> H <sub>16</sub> O <sub>2</sub>	13.75	-	0.03	0.03	-
62	2-Hexanol	C <sub>6</sub> H <sub>14</sub> O	14.20	-	-	-	0.61
63	Tridecane	C <sub>13</sub> H <sub>28</sub>	14.50	-	-	0.09	-
64	cis-2-(2-Pentenyl)furan	C <sub>9</sub> H <sub>12</sub> O <sub>2</sub>	14.53	0.08	0.15	-	0.22
65	(E)-4,8-Dimethylnona-1,3,7-triene	C <sub>11</sub> H <sub>18</sub>	14.68	0.68	0.42	0.65	0.28
66	2-Heptenal, (E)-	C <sub>7</sub> H <sub>12</sub> O	15.21	-	-	0.03	0.03
67	3-Octanone, 2-methyl-	C <sub>9</sub> H <sub>18</sub> O	15.30	0.35	0.89	-	-
68	2-Penten-1-ol, (Z)-	C <sub>5</sub> H <sub>10</sub> O	15.40	-	0.06	-	-
69	2,3-Octanedione	C <sub>8</sub> H <sub>14</sub> O <sub>2</sub>	15.47	-	-	-	0.05
70	5-Hepten-2-one, 6-methyl-	C <sub>8</sub> H <sub>14</sub> O	15.66	0.08	0.06	-	0.09
71	1,6-Dioxaspiro[4.4]nonane, 2-ethyl-	C <sub>9</sub> H <sub>16</sub> O <sub>2</sub>	16.16	0.02	-	0.02	-
72	1-Hexanol	C <sub>6</sub> H <sub>14</sub> O	16.36	1.30	1.83	1.51	1.53
73	2,4,6-Octatriene, 2,6-dimethyl-, ( <i>E</i> , <i>Z</i> )-	C <sub>10</sub> H <sub>16</sub>	16.56	0.20	0.05	0.08	-
74	3-Hexen-1-ol, ( <i>E</i> )-	C <sub>6</sub> H <sub>12</sub> O	16.61	-	0.20	0.16	0.15
75	3-Hexen-1-ol	C <sub>6</sub> H <sub>12</sub> O	17.16	-	-	-	0.06
76	Nonanal	C <sub>9</sub> H <sub>18</sub> O	17.21	0.35	0.37	0.47	0.31
77	2,4-Hexadienal, (E,E)-	C <sub>6</sub> H <sub>8</sub> O	17.31	0.15	0.32	0.21	0.24
78	Furan, 2-ethyl-	C <sub>6</sub> H <sub>8</sub> O	17.43	-	0.93	0.64	0.66
79	1,3-Hexadiene, 3-ethyl-2-methyl-	C <sub>9</sub> H <sub>16</sub>	17.77	0.02	0.04	0.03	0.05
80	Furan, 3-(4-methyl-3-pentenyl)-	C <sub>10</sub> H <sub>14</sub> O	17.89	0.02	-	-	0.02
81	Sulfurous acid, cyclohexylmethyl tridecyl ester	C <sub>23</sub> H <sub>46</sub> O <sub>3</sub> S	18.09	-	-	-	0.02
82	2-Octenal, (E)-	C <sub>8</sub> H <sub>14</sub> O	18.17	0.05	-	-	0.07
83	Benzene, 1-methyl-4-(1-methylethenyl)-	C <sub>10</sub> H <sub>12</sub>	18.32	0.06	0.03	0.05	0.02
84	Octanoic acid, ethyl ester	C10H20O2	18.41	0.09	0.10	0.11	0.09
85	2.6.10-Trimethyltridecane	C10H24	18.59	0.46	0.28	0.52	0.36
86	Methional	C <sub>4</sub> H <sub>2</sub> OS	18.80	0.18	0.32	0.28	0.23
87	Acetic acid	C <sub>2</sub> H <sub>2</sub> O <sub>2</sub>	18.93	0.10	0.18	0.13	0.17
88	3-Furaldehyde	C-H.O.	19.04	0.03	-	_	-
89	Cyclotetrasiloxane. octamethyl-	C <sub>a</sub> H <sub>24</sub> O <sub>4</sub> Si <sub>4</sub>	19.31	_	0.04	_	_
90	Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate	CuaHaaO.	19.44	_	-	0.02	_
91	2.4-Hentadienal. ( <i>E.E</i> )-	C_H.O	19.84	0.08	0.10	0.09	0.13
92	2-Fthyl-1-beyanol	0/1100	19.98	-	0.05	0.03	0.05
93	Pentadecane	Cultu	20.14	_	0.03	0.03	-
94	trisilovane 1 1 1 5 5 5-bevamethyl_3_[(trimethylsilyl)ovy]_	C-HO-Si	20.11	0.08	0.11	-	
95	Renzaldehyde	C-H-O	20.10	13.04	8.49	10.05	10.03
96	2-Nonenal (F)-	Сн.О	20.57	0.34	0.45	0.31	0.41
97	Lilac aldebude B	С Н О	20.94	1 18	0.45	0.51	1.01
97	Proponoic acid	CHO	21.10	0.08	0.90	0.58	1.01
90	Lindool	C H O	21.21	5.50	3.15	4.17	3.43
100	Dimethyl sylforide	C H OS	21.41	0.12	0.10	4.17	0.10
100	2.6 Nonodianal (F.Z)	C <sub>2</sub> H <sub>6</sub> O3	22.04	0.12	0.10	0.12	0.10
101	Lilos aldabuda C	C H O	22.19	-	0.49	0.36	-
102	2 Cuclobarana 1 acataldabuda, aluba 4 dimatbul	C H O	22.22	0.99	-	0.34	0.97
105	1 Cueleb areas 1 carb and debude 266 taim athul	С. И. О.	22.95	0.05	-	-	-
104	1-Cyclonexene-1-carboxaldenyde, 2,6,6-trimethyl-	C10H16O	22.99	-	0.05	0.02	0.04
105	Dutyrolacione	$C_4 H_6 O_2$	23.07	-	-	0.03	0.02
100	Euranoi, 2-(2-etnoxyetnoxy)-	C II O	23.15	0.03	0.05	-	-
10/	Denzeneacetaldenyde	C <sub>8</sub> H <sub>8</sub> U	23.43	-	-	0.27	0.43
108	Decanoic acid, etnyi ester	$C_{12}H_{24}U_2$	23.53	0.14	0.20	0.17	0.1/
109	silanedioi, dimethyl-	C <sub>2</sub> H <sub>8</sub> O <sub>2</sub> Si	23.92	0.38	-	0.21	0.39
110	1-INONANOI	C <sub>9</sub> H <sub>20</sub> U	24.11	0.25	-	0.35	0.35
Continued							

				Relative content/%				
Peak no	Compound	Molecular formula	RT/min	Brooks	Black pearl	Tieton	Summit	
111	Benzaldehyde, 2-hydroxy-	C <sub>7</sub> H <sub>6</sub> O <sub>2</sub>	24.26	0.12	0.11	0.08	0.17	
112	Butanoic acid, 3-methyl-	C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>	24.42	-	0.16	-	-	
113	3-Pyridinecarboxaldehyde	C <sub>6</sub> H <sub>5</sub> NO	24.94	0.47	0.18	0.14	0.18	
114	1-Propanol, 3-(methylthio)-	C <sub>4</sub> H <sub>10</sub> OS	25.33	0.04	0.06	0.04	0.08	
115	Lilac alcohol B	C10H18O2	25.97	4.91	-	-	-	
116	Benzene, 1-methoxy-2-(methoxymethyl)-	$C_9H_{12}O_2$	26.18	0.06	-	0.03	-	
117	Lilac alcohol formate B	$C_{11}H_{18}O_3$	26.33	-	-	-	0.06	
118	Oxime-, methoxy-phenyl	C <sub>8</sub> H <sub>9</sub> NO <sub>2</sub>	26.55	-	2.18	1.60	1.55	
119	Lilac alcohol C	$C_{10}H_{18}O_2$	26.95	3.09	1.28	2.00	3.75	
120	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	27.24	-	-	-	0.17	
121	Tris(tert-butyldimethylsilyloxy)arsane	$C_{18}H_{45}AsO_3Si_3$	27.69	-	0.19	-	-	
122	3,5-Dimethoxytoluene	$C_9H_{12}O_2$	28.08	0.70	3.49	0.14	1.21	
123	Dodecanoic acid, ethyl ester	$C_{14}H_{28}O_2$	28.13	-	-	0.15	-	
124	Hexanoic acid	$C_{6}H_{12}O_{2}$	28.38	0.19	0.07	0.10	-	
125	Benzyl alcohol	C <sub>7</sub> H <sub>8</sub> O	28.73	-	-	-	0.03	
126	Phenylethyl alcohol	C <sub>8</sub> H <sub>10</sub> O	29.47	0.51	0.35	0.36	1.01	
127	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	C <sub>13</sub> H <sub>20</sub> O	30.05	-	0.03	-	0.02	
128	2-Cyclopenten-1-one, 3-methyl-2-(2-pentenyl)-, (Z)-	C <sub>11</sub> H <sub>16</sub> O	30.14	-	-	0.02	-	
129	Benzaldehyde, 2-methoxy-	C <sub>8</sub> H <sub>8</sub> O <sub>2</sub>	30.40	-	0.01	-	-	
130	Phenol	C <sub>6</sub> H <sub>6</sub> O	31.29	-	-	0.02	0.03	
131	Methyleugenol	$C_{11}H_{14}O_2$	31.48	-	0.04	-	0.05	
132	Benzaldehyde, 4-methoxy-	C <sub>8</sub> H <sub>8</sub> O <sub>2</sub>	31.64	1.50	0.45	0.42	0.86	
133	Benzene, 1,2,3-trimethoxy-5-methyl-	$C_{10}H_{14}O_3$	32.08	0.02	0.14	-	0.04	
134	Tetradecanoic acid, ethyl ester	$C_{16}H_{32}O_2$	32.29	0.10	0.13	0.13	0.13	
135	2-Propenoic acid, 3-phenyl-, methyl ester, (E)-	$C_{10}H_{10}O_2$	32.68	0.20	0.10	0.10	0.06	
136	Benzoic acid, 4-methoxy-, methyl ester	$C_9H_{10}O_3$	32.97	0.17	0.14	0.13	0.08	
137	Heneicosane	$C_{21}H_{44}$	33.27	0.10	0.10	0.06	0.11	
138	2-Propenoic acid, 3-phenyl-, ethyl ester, ( <i>E</i> )-	$C_{11}H_{12}O_2$	33.72	0.29	0.26	0.32	0.15	
139	Benzoic acid, 4-methoxy-, ethyl ester	$C_{10}H_{12}O_3$	33.79	1.29	0.81	0.77	0.33	
140	2,4,6-Cycloheptatrien-1-one, 2-amino-	C <sub>7</sub> H <sub>7</sub> NO	34.31	0.40	0.12	0.02	0.02	
141	1-Hexadecen-3-ol, 3,5,11,15-tetramethyl-	C <sub>20</sub> H <sub>40</sub> O	35.26	-	-	0.16	-	
142	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	35.42	-	0.02	-	0.03	
143	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	36.11	0.08	0.11	0.12	0.14	
144	3,5-Dimethoxybenzaldehyde	$C_9H_{10}O_3$	36.44	0.09	0.56	0.03	0.35	
145	Eicosane	C <sub>20</sub> H <sub>42</sub>	36.90	0.05	0.09	0.06	0.12	
146	2,7-Octadiene-1,6-diol, 2,6-dimethyl-	$C_{10}H_{18}O_2$	37.07	1.44	0.87	0.24	0.57	
147	Benzoic acid, 3,5-dimethoxy-, methyl ester	$C_{10}H_{12}O_4$	38.48	-	0.03	-	-	
148	Coumarin	$C_9H_6O_2$	39.24	-	0.09	0.04	0.54	
149	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	40.73	-	0.02	0.02	0.02	
150	Phthalic acid, isobutyl trans-hex-3-enyl ester	$C_{18}H_{24}O_4$	40.86	0.03	0.02	0.02	-	
151	Vanillin	C <sub>8</sub> H <sub>8</sub> O <sub>3</sub>	41.16	-	0.07	-	0.06	
152	1,4,7,10,13,16-Hexaoxacyclooctadecane	C <sub>12</sub> H <sub>24</sub> O <sub>6</sub>	42.47	0.01	-	-	-	
153	Dibutyl phthalate	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	42.74	0.07	-	-	-	
154	2-[2-[2-[2-[2-[2-[2-[2-(2-Methoxyethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]	C <sub>17</sub> H <sub>36</sub> O <sub>9</sub>	43.47	-	-	0.14	-	
155	2-[2-[2-[2-[2-[2-[2-(2-Methoxyethoxy)ethoxy]ethoxy[ethoxy]ethoxy[ethoxy]ethoxy[ethoxy]ethoxy[ethoxy]ethoxy[ethoxy]ethoxy[ethoxy]ethoxy[ethoxy]ethoxy[ethoxy]ethoxy[ethoxy]ethoxy[ethoxy[ethoxy]ethoxy[ethoxy[ethoxy]ethoxy[ethoxy[ethoxy]ethoxy[ethoxy[ethoxy[ethoxy[ethoxy]ethoxy[	C <sub>15</sub> H <sub>32</sub> O <sub>8</sub>	43.83	-	-	0.10	-	

**Table 6.** Volatile organic compounds identified in four sweet cherry cultivar flower essential oils by

 HS-SPME/GC–MS. *RT* retention time.

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# Conclusions

Ultrasound- and microwave-assisted extraction is an effective method that is suitable for the extraction of essential oil from sweet cherry flowers. Response surface methodology, as part of the experimental design and optimization, showed that the liquid-solid ratio and microwave power had a notable influence on the extraction yield. HS-SPME/GC–MS is an accurate, fast and effective method for determining the aromatic components of sweet cherry flower essential oil. The analysis results concluded that the detected VOCs in the Brooks, Black Pearl, Tieton and Summit sweet cherry cultivars were similar. However, significant variations in the contributions



**Figure 5.** Number of volatile organic compounds of sweet cherry flower essential oils. The vertical bars represent the standard deviation of the means (n=3). Different letters (for the same compound) indicate significant differences (p<0.05).





of these compounds to each cultivar were observed. Regardless of the cultivar, the most abundant alcohols and aldehyde compounds were ethanol and benzaldehyde, respectively. The principal volatiles were ethanol, linalool, lilac alcohol, acetaldehyde, (E)-2-hexenal, benzaldehyde and dimethyl sulfide, and their concentrations were highly dependent on each cultivar. These VOCs are the main sources of the aroma of sweet cherry flowers. Ethanol, linalool, 4-methoxy-benzaldehyde and lilac aldehyde have various biological activities. The research results provide a basis for the health benefits of sweet cherry flowers.

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### Author contributions

H.M.Z. and X.P. W. conceived the idea and designed the experiment. H.G. Y. and Q. L. performed the experiments. H.M.Z. and H. L. analyzed the data and wrote the manuscript. All authors approved the final manuscript.

# Competing interests

The authors declare no competing interests.

# Additional information

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