Evolution of Aroma Profiles and Potential in Shine Muscat Grape Berries during Ripening

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Shine Muscat, with its excellent quality and unique Muscat aroma, is emerging as one of the most profitable table grape cultivars in Asia. However, in-depth analysis of the cultivar's aroma profile before berries are harvest-ready is lacking. The aroma components and their concentrations undergo substantial changes during berry ripening, which provides an opportunity for producing some juice/wine products with special flavour. In this study, the evolution of free and bound aroma compounds in Shine Muscat grapes was analysed from EL-35 to EL-38. The main aroma attributes of harvest-ready berries were: 'green', 'sweet', 'floral' and 'fruity'. The headspace SPME-GC-MS analysis revealed that alcohols were the most abundant free and bound compounds in Shine Muscat. Furthermore, the free forms of hexanal, β-damascenone, and (E, Z)-2,6-nonadienal determined the aroma characteristics of the grapes directly, while the bound forms of β-damascenone, (E, Z)-2,6-nonadienal, and 2-hexanol determined the aroma of Shine Muscat that cannot to be smelled directly. Taking all indicators together, we believe that picking Shine Muscat grapes at EL-37 (when a minimum TSS of 17% has been reached) maintains its fine aroma characteristics. These results lay the foundation for the further development, utilisation and study of the aroma characteristics of Shine Muscat grapes.

INTRODUCTION

Grapes are one of the most important fruit species in the world. In 2016, the area under vineyard cultivation comprised 7 453 532 ha worldwide (OIV, 2020). The global production of grapes for 2019/2020 was estimated at 23.4 million tons (USDA Foreign Agricultural Service, 2020). According to their utilisation, grape fruits are divided into table, wine and raisin grapes (Shangguan *et al*., 2020). In Asia, grape cultivation is based mainly on table grapes, and seedless and aromatic grapes are currently favoured by consumers.

In East Asia, especially China and Japan, the Shine Muscat grape has emerged as a popular table grape variety in recent years. This is owing to its unique appearance and rich aroma. The Shine Muscat grape, bred by the National Institute of Fruit Tree Science (NIFTS) in Japan, is a hybrid offspring obtained by crossing the grape varieties Akitsu-21 and Hakunan. It was introduced to China in 2009 (Wei *et al*., 2019). Shine Muscat is a Euramerican hybrid, is fresh and tasty, has large berries, and shows good resistance and adaptability to the environment. Since Shine Muscat berries do not crack easily, they are suitable for postharvest transportation and storage. Shine Muscat's excellent characteristics make it a unique table grape variety with great development potential and high economic value (Yamada & Sato, 2016).

Aroma is one of the pivotal factors in the evaluation of grape quality by consumers. Therefore, an elegant and rich aroma is especially important for table grapes. Different varieties of grapes exhibit different types and contents of aroma substances, resulting in marked differences in the flavour and typical characteristics of aromas in grapes and wines (Belda *et al*., 2017). According to the type of aroma, grapes are divided into Muscat cultivars, non-Muscat aromatic cultivars, and neutral cultivars (Wu *et al*., 2020). There are three main pathways for the biosynthesis of aroma substances in grape berries, namely the isoprene metabolism

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pathway, the fatty acid metabolism pathway, and the amino acid metabolism pathway. These produce terpenoids, C13-norisoprenoids, linear fatty alcohols, aldehydes, acids, esters, branched aliphatics and pyrazines, aromatics, as well as other aroma substances (Black *et al*., 2015; Wang, 2016). The aroma substances in grape berries exist in two forms: free and glycoside-bound compounds (Alem *et al*., 2019). The bound aroma compounds can contribute indirectly to the odour of fruits and wine through hydrolysis (Zhao *et al*., 2020). Although there are several aroma substances in grape fruits, only a few aroma components play a decisive role in the total aroma; the degree of action of each component depends on its odour threshold and actual content. Therefore, although the content of some aroma components is low, these components have a significant effect on the aroma of fruits owing to their low threshold (Belitz *et al*., 2004; Ilc *et al*. 2016). The floral aroma in the fruit is dependent on monoterpene alcohols (linalool, geraniol, citronellol and nerol) and ketones (damascenone and β-ionone). 2-Hexenal is a major contributor to the fragrance of green leaves and fruits (Yusen *et al*., 2016). The terpenoid and norisoprenoid aroma substances produced by isoprene metabolism mostly have a pleasant floral and fruity aroma. Although the content of isoprene aroma substances in grape berries is very low, these substances contribute to the aroma of grapes or wines owing to their extremely low sensory thresholds (Crupi *et al*., 2010; Baron *et al*., 2017).

Shine Muscat grape has a strong Muscat flavour (Wang *et al*., 2020). Wu *et al*. (2019) identified the differences in aroma components between Muscat grapes and two other table varieties. Matsumoto and Ikoma (2016) suggest that, after harvesting, the temperature would have a significant influence on the Muscat flavour and aroma of Shine Muscat grapes. Another study investigated five Muscat table grapevine cultivars with or without the addition of pectolytic enzyme to produce grape brandy and detected the volatile aroma compounds in it (Matijašević *et al*., 2019). Wu *et al.* (2020) described the free and bound volatile compounds in Shine Muscat grape, especially C6 and terpenes. However, the evolution of the aroma profile and potential during the ripening of Shine Muscat remains poorly studied.

The aim of this study was to explore the evolution of free and bound aroma substances in Shine Muscat berries during ripening (véraison to harvest at 20° Brix). This study had the potential to provide novel insights into the formation of aroma characteristics in Shine Muscat grapes, thus laying the foundation for further utilisation of the grapes.

MATERIALS AND METHODS

Fruit material

Berries of Shine Muscat grape from the Jintian Fruit and Vegetable Cooperative (34°27′N, 108°07′E), which is in Yangling Agricultural Demonstration Area (Shaanxi Province, China), were sampled from véraison to harvest maturity in 2017. The Shine Muscat experimental block was planted in 2010, using a V-shape shoot-positioned trellis and a cordon-trained system. Conventional viticultural management practices were applied and 40 Shine Muscat grapevines with moderate growth were sampled on sampling dates based on specific EL stages, as described by Coombe (1995), viz. EL-35 (véraison), EL-36 (berries with intermediate Brix values), EL-37 (berries not quite mature), and EL-38 (berries harvest-mature). At each sampling date, five grape clusters were randomly selected from 10 grapevines, and six grape berries per cluster were selected to obtain a total of 300 berries per sampling date. The 300 berries were divided into three subsamples of 100 berries each, to obtain three replicates for each sampling date. The berry samples were transported to the laboratory in an ice box.

Measurements of basic physical and chemical indices

The mass of each 100-berry replicate was determined using an electronic balance. Berries were crushed to obtain juice, whereafter the total soluble solids content (TSS) and titratable acidity (TA) were determined according to the methods of the OIV (2014).

Determination of aroma components

Sample preparation for aroma components

From each 100-berry replicate, 50 berries were randomly selected for determining the aroma compounds. After removal of the seeds, the berries were quickly frozen under liquid nitrogen. After adding 1.0 g of polyvinyl pyrrolidone (PVPP) and 0.5 g of d-gluconolactone, the berries were beaten in a crusher. The grape powder was extracted at 4°C for 4 h, then centrifuged at 8 000 × g at 4°C for 10 min. The supernatant was filtered through filter paper (Waterman, #1), and the clear filtrate was stored at -40°C until the extraction and analysis of aroma substances.

Extraction of free aroma compounds

The method for the extraction of free aroma substances was based on that described by Wu *et al*. (2008). Exactly 5 mL of fully defrosted grape juice and 1.00 g NaCl were mixed in a 15 mL vial, with 10 μL of 4-methyl-2-pentanol (4M2P, 1.0083 g/L) as an internal standard substance, and mixed using a magnetic agitator (1 cm). The vials were capped tightly with a polytetrafluoroethylene (PTFE)-silicon septum, heated at 40°C for 30 min on a heating platform, and agitated at 400 rpm. A pre-treated (conditioned at 270°C for 1 h) solid-phase microextraction (SPME) fibre (50/30 μm DVB/CAR/PDMS, Supelco, USA) was then inserted into the headspace, and the mixture was extracted for 30 min upon continued heating and agitation. The volatiles trapped in the fibre were subsequently desorbed by injecting the fibre into a gas chromatography (GC) injector for 8 min.

Extraction of bound aroma compounds

A solid-phase extraction column was activated with 10 mL water and 10 mL methanol (the specification was 200 mg/6 mL, Bona Ajer Technology, Tianjin, China). To elute polar substances with low molecular weight, such as sugar and acid, 2 mL of fully thawed supernatant and 5 mL of distilled water were added, whereafter 5 mL of dichloromethane was added to eliminate the interference of free aroma substances. Thereafter, 20 mL of methanol was used to elute the combined aroma substances, and this step was repeated four times to ensure complete elution. The flow rate of the whole process was maintained at 2 mL/min. Finally, the eluate was concentrated in a 50 mL centrifuge tube and evaporated to dryness under vacuum and reduced pressure at 30°C. The residue was dissolved with 10 mL of citric acid/sodium citrate buffer (0.2 M, pH 2.5, 1.63 g citric acid + 0.79 g sodium citrate + 16 g sodium chloride dissolved in 50 mL of water), and AR 2000 enzyme (100 µL) was added to the solution. To allow the free aroma to be released, the mixture was incubated at 40°C for 16 h. Simultaneously, a controlled experiment was performed by direct analysis of the obtained citric acid solution without enzymatic hydrolysis. Further steps in the process were the same as described for the extraction of free aroma substances.

Analysis of aroma compounds via GC/MS

The instruments used were the Agilent 7890 GC and Agilent 5975C (Inert MSD, USA) equipped with an autosampler and capillary column, HP-INNOWAX 60 m × 0.25 mm × 0.25 µm (J & W Scientific, USA). The carrier gas was high-purity helium with a flow rate of 1 mL/min. The inlet temperature was 250°C and the thermal analysis lasted 25 min. The column oven temperature program was as follows: holding at 50°C for 1 min, followed by heating up to 220°C at a rate of 3°C/min, and maintaining this temperature for 5 min. The ion source temperature was 230°C, and the mass spectrometry interface temperature was 280°C. The ionisation mode of mass spectrometry was EI, the ionisation energy was 70 ev, and the mass scanning range was 20 to 350 µm. The qualitative and quantitative analysis methods were based on the method of Zhang (2007).

Qualitative analysis: The spectrum of the mass spectrometry scan was obtained according to the chromatographic retention time and mass spectrometry values of the existing standards, comparing results with the NIST05 standard library and references in the related literature.

Quantitative analysis: The standard curve (peak area of the mass spectrum selective ion scan/compound concentration) was established using existing standard compounds. The aroma substances without standard compounds were quantified using the principle of similar chemical structures and similar carbon atom number.

Statistical analysis

Three biological replicates were performed on all samples. Statistical data were expressed as means ± standard deviation (SD) by using Excel and IBM SPSS Statistics 26.0 (IBM, Armonk, NY, USA) software. The one-way analysis of variance (ANOVA) with Duncan's test was used for the analysis of significant variance ($P < 0.05$). Figures were prepared using Origin 2018 (OriginLab Corporation, Northampton, MA, USA).

RESULTS AND DISCUSSION

Analysis of basic physical and chemical indicators

The changes in the TSS, total acid and berry weight of Shine Muscat grape are shown in Fig. 1. The TSS content increased from 5.3% at EL-35 to 20.4% at EL-38, which meets the required minimum TSS norm for harvest maturity. Total acid content decreased significantly, from EL-35 to EL-36 and from EL-37 to EL-38, reaching a final value of 1.57 g/L at EL-38. During the period from EL-35 to EL-37, berry weight increased gradually and reached an average of 13.11 g. However, the berry weight decreased significantly at EL-38. This is because the only source for solutes and water that

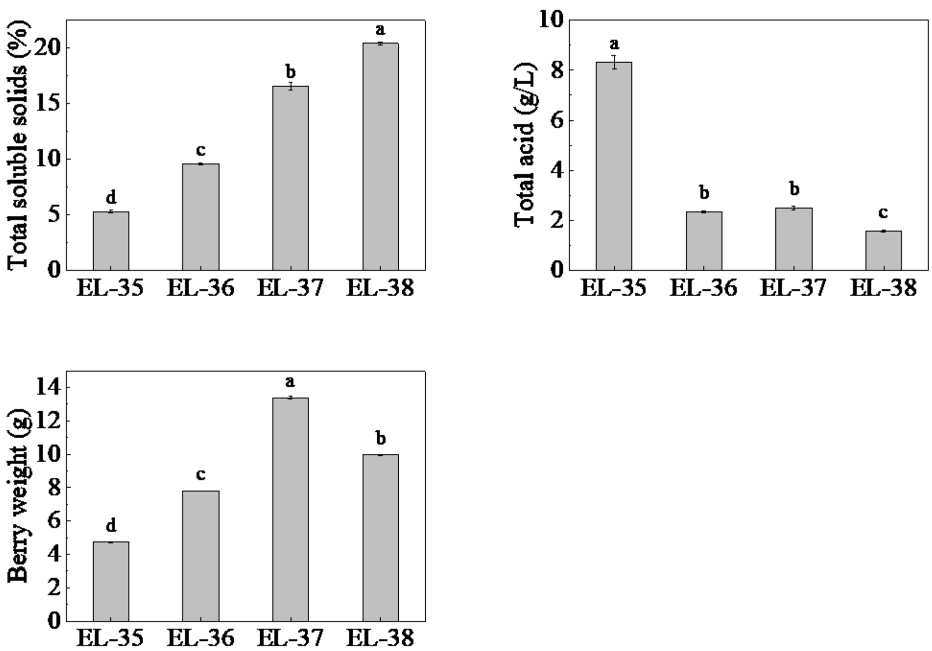

FIGURE 1
Changes in TSS, total acid and berry weight of Shine Muscat from EL-35 to EL-38 (2017 season). Data represent means ± SD ($n = 3$). Letters 'a', 'b', 'c', 'd' indicate statistical differences ($p < 0.05$, Duncan's multiple test).

enter grape berries from EL-35 to EL-37 is phloem sap, but phloem flow becomes impeded at EL-37 (maximum berry weight). Simultaneously, the continuation of grape berry transpiration leads to berry shrinkage and a concentration of solutes (McCarthy & Coombe, 1999). Berry weight reached 9.89 g, which is in line with the theoretical berry weight range of mature Shine Muscat grapes.

Analysis of aroma compounds

A total of 94 free or bound aroma compounds were detected in the Shine Muscat at different stages of ripening. According to their chemical properties, these aroma substances could be divided into nine categories: terpenoids, C13-norisoprenoids, C6 compounds, aromatic compounds, alcohols, esters, aldehydes, acids and ketones, and furans and alkenes. Figs 2 and 3 show the total contents of the free and bound above-mentioned substances. Only three aromatic compounds (benzyl alcohol, 3,4-dimethylbenzyl alcohol, methyl 3,4-di methylbenzoate), one ethyl ester (hexanoic acid, ethyl ester), and one ketone (methyl isobutyl ketone) were found in the bound form.

In Shine Muscat berries, alcohols exhibited the highest levels in both free and bound forms. This result was in accordance with a previous report (Wang *et al*., 2017), which showed that alcohols had the highest relative content in Shine Muscat grapes. Among the free forms, the levels of 2-pentanol, 1-pentanol, 1-butanol and 2-methyl-1-propanol were the highest, while the levels of the bound form of 1-butanol and 2-methyl-1-propanol were below the machine detection threshold. The level of 2-pentanol was also the highest in bound alcohols, almost five times the content of free 2-pentanol at commercial maturity. This suggests that 2-pentanol may contribute to grape aroma directly, determining the potential alcoholic, fruity and green aroma in Shine Muscat berries.

Terpenoids and C6 compounds were found in larger proportions in the free form at the commercially mature stage of Shine Muscat berries; these results are in line with the study of Wu *et al*. (2020). Terpenoids are secondary metabolites in plants synthesised from acetyl CoA. Based on the content of terpenoids, Shine Muscat grapes belong to the "rose flavour type" (> 6 mg/L terpenoids) (Mateo & Jiménez, 2000). In this study, the content of free terpenoids was above this standard and reached the maximum value at EL-38. In total, there were nine terpenoids in Shine Muscat: 3-methyl-3-buten-1-ol, linalool, cyclohexene, 3-methyl-6-(1-methylethylidene), γ-terpinene, geraniol, (R)-3,7-dimethyl-6-octen-1-ol, α-terpineol, and 3-methyl-2-buten-1-ol. The content of 3-methyl-3-buten-1-ol was the highest in the free and bound form, and it contributed to the alcoholic aroma of Shine Muscat. In a previous study, Fenoll *et al*. (2009) showed that the most abundant free compounds in Muscat grapes were linalool, geraniol, citronellol, nerol, 3,7-dimethyl-1,5-octadien-3,7-diol (diendiol I), and 3,7-dimethyl-1,7-octadien-3,6-diol (diendiol II). However, except for linalool, geraniol and citronellol (or (R)-3,7-dimethyl-6-octen-1-ol), other terpenoids were not detected in this study. Matsumoto and Ikoma (2016) quantified only linalool and nerol. The absence of nerol in this study, which is associated with the characteristic rose aroma of Shine Muscat berries, may be caused by different viticultural management practices and climatic conditions compared to previous studies. This aspect warrants further study. Wang *et al*. (2020) treated Shine Muscat berries with TDZ for

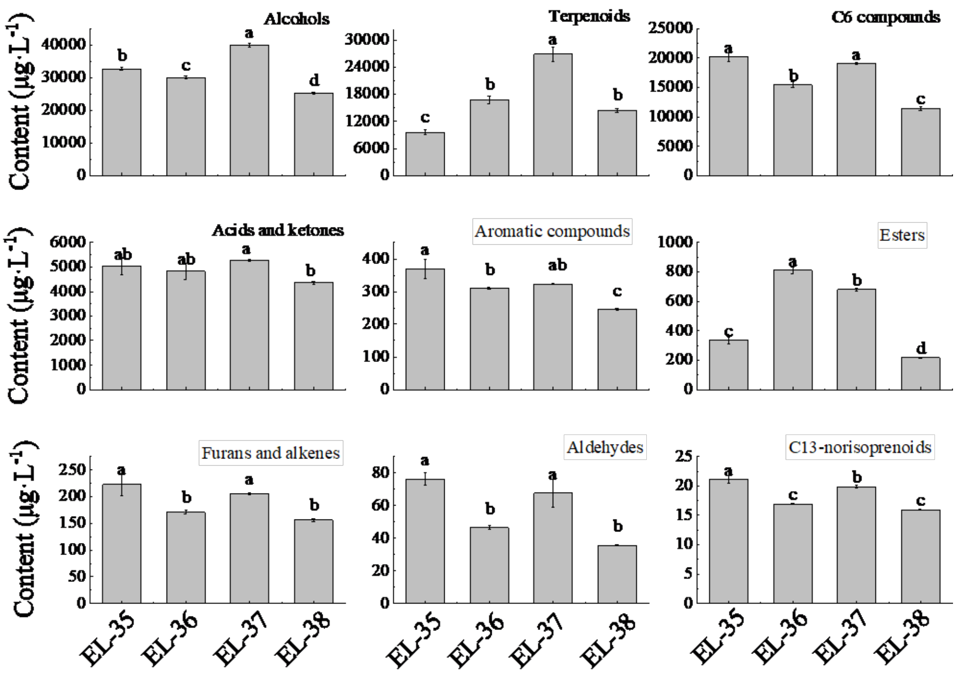

FIGURE 2

Changes in content of free aroma compounds of Shine Muscat berries from EL-35 to EL-38 (2017 season). Data represent means ± SD (n = 3). Letters 'a', 'b', 'c', 'd' indicate statistical differences ($p < 0.05$, Duncan's multiple test).

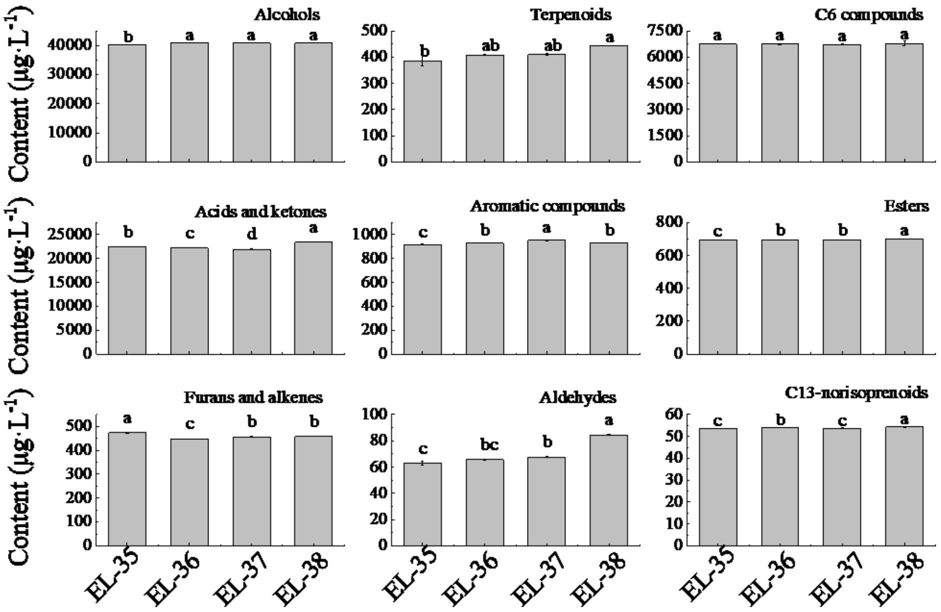

FIGURE 3
Changes in content of bound aroma compounds of Shine Muscat berries from EL-35 to EL-38 (2017 season). Data represent means ± SD (n = 3). Letters 'a', 'b', 'c', 'd' indicate statistical differences ($p < 0.05$, Duncan's multiple test).

two consecutive years, and almost all of the monoterpenes showed a significant decrease. Regulated deficit irrigation (RDI) applied after véraison can increase the concentration of key free terpenes for Gewürztraminer grapes at harvest, such as geraniol and citronellol (Kovalenko *et al*., 2020).

C6 compounds are formed by enzymatic oxidation of unsaturated lipids and are partially responsible for 'herbaceous' and 'green' aromas in grapes and wine (Hashizume & Samuta, 1997). In this study, the free C6 aroma compounds with the highest concentration was hexanal, followed by (E)-2-hexenal. These two molecules contributed to the 'green leaf' and 'fruity' aroma profiles of Shine Muscat grapes. Aubert and Chalot (2018) found that the most abundant volatile compounds in six table grape cultivars studied were (E)-2-hexenal and hexanal, which indicates that these two C6 aroma compounds play an important role in the aroma of table grapes. Bound 2-hexanol appeared to be the most abundant among the bound C6 aroma compounds and had the lowest content change in free form, which suggests that 2-hexanol is mainly stored in the bound state, thus potentially contributing to the 'alcoholic' and 'green' aroma of Shine Muscat. Matsumoto and Ikoma (2016) reported that the major aroma volatiles observed in the skin and flesh of Shine Muscat were hexanal, (E)-2-hexenal and linalool. In line with these findings, the free form of hexanal, (E)-2-hexenal, and linalool had a relatively high content in this study; based on their lower thresholds, these components might contribute directly and notably to aroma.

Carotenoids are precursors of norisoprenoids (Mendes-Pinto, 2009). Norisoprenoids can make a significant contribution to fruit aroma based on their lower thresholds, and can enhance the fruity aroma while masking the herbal aroma (Pineau *et al*., 2007). Three C13-norisoprenoids were identified in this study, namely β-damascenone, β-ionone, and 6-methyl-5-hepten-2-one. The amounts of β-damascenone and β-ionone account for 85% of the total C13-norisoprenoids in grape berries, while β-damascenone occurs mostly in the bound form (Meng *et al*., 2015). It was found that β-damascenone and β-ionone occurred mainly in the bound form in Shine Muscat (close to five times higher than in the free form), and free β-ionone was even absent at the later, mature stage. A comparison of free and bound forms of β-damascenone and 6-methyl-5-hepten-2-one revealed that the content of these two bound norisoprenoids decreased, while the content of the free forms increased, indicating that hydrolysis of bound β-damascenone and 6-methyl-5-hepten-2-one may occur during berry development. β-Ionone was stored in Shine Muscat grape mainly in the bound form, being a potential contributor to 'violet' and 'sweet fruit' aroma.

Esters can contribute to a 'fruity', 'green' and 'floral' aroma in grapes. Esters identified in Shine Muscat in this study were acetic acid and butyl ester, in both free and bound forms. Wu *et al*. (2020) reported that, after Shine Muscat grapes matured, only acetic acid and butyl ester were detected. Most of the free esters accumulated in the early stages of fruit growth, and decreased in the later stages of fruit maturity. Most of the bound esters reached their maximum at EL-38. Compared with the significant change in the total content of free esters, the total content of bound esters remained relatively stable during fruit development in Shine Muscat. The trend of change in esters recorded in this study is not the same as that reported by Wu *et al*. (2020), which is probably related to the cultivation conditions in this specific Shine Muscat production region.

Toluene, p-xylene, 1,3-dimethyl-benzene and o-xylene showed a high content of both free and bound forms. Toluene and the xylenes of the free and bound forms showed the same trend in terms of change in content, which indicates that toluene compounds have a relatively stable and conservative synthetic pathway in the Shine Muscat grape.

In total, nine aldehydes were identified: (E)-2-octenal, (Z)-2-heptenal, heptanal, (E, E)-2,4-heptadienal, (E)-2-nonenal, (E, Z)-2,6-nonadienal, octanal, nonanal and decanal. In contrast with the main aroma components of some tetraploid table grapes with a 'rose' aroma reported by Tan *et al*. (2017), the content of these free aldehydes in Shine Muscat berries in this study was relatively low and it decreased to a minimum in the later stages of fruit maturity. Unlike most bound aldehydes, which remain stable, the bound forms of (E, E)-2,4-heptadienal and octanal increased during the development stage. However, the content of bound nonanal was lower than the machine detection threshold. We anticipate that most free aldehydes (78%) accumulated during EL-35, and that individual bound aldehydes may increase in Shine Muscat during EL-38.

Acetic acid was the most important aroma compound among the free and bound acids and ketones; however, acetic acid om the free and bound form appeared to accumulate in EL-38. All furan and alkene aroma compounds occurred mainly in the bound form in Shine Muscat grapes; the content of the bound form was significantly higher than that of the free form, especially at commercial maturity.

Odour feature of Shine Muscat berries during different development stages

The concentration of aroma components is not directly related to their actual aroma contribution, but is related to their odour activity value (ratio of mass concentration to threshold value, OAV). When the OAV is ≥1, it indicates that the substance has a direct effect on the aroma of grapes. A larger OAV value indicates a greater contribution to the overall aroma (Guth, 1997).

The OAVs of the major aroma compounds found in the Shine Muscat grape, including their odour descriptions and aromatic series, are presented in Table 1. Aromatic series could be divided into seven groups based on odour descriptions: fruity, floral, alcoholic, green, sweet, fatty and chemical. For Shine Muscat as a table grape, the aroma at maturity could directly affect the market value. There were 14 free aroma compounds in Shine Muscat that had an OAV greater than 1 at maturity . They were linalool, β-damascenone, hexanal, (E)-2-hexenal, 2-hexanol, acetophenone, 1-pentanol, 1-octen-3-ol, (E)-2-octenal, heptanal, (E)-2-nonenal, (E, Z)-2,6-nonadienal, octanal, and nonanal. Among them, hexanal (OAV was 942.91), β-damascenone (168.00) and (E, Z)-2,6-nonadienal (164.00) were dominant, contributing to the 'green', 'fruity', 'canned peaches', 'dried apples', 'dried plums', 'flower' and 'cucumber-like' aromas. Although terpenoids are generally associated with a unique 'musk' smell and provide the main 'rose' aroma in Shine Muscat, in this study all terpenoids had OAVs below 1, except linalool, with OAV 7.94, contributing to 'woody', 'fruity' and 'floral' aromas. The reduction of Muscat flavour may be caused by the lower storage temperatures and the long post-harvest storage of Shine Muscat. The researchers investigated the influence of different postharvest temperatures (0°C, 2°C, 5°C and 10°C) on Muscat flavour in Shine Muscat for 12 weeks. At 0°C, the Muscat flavour decreased obviously, but at 10°C the Muscat flavour was maintained after four weeks of storage. The linalool content was much lower at 0°C than at 10°C in both the skin and the flesh (Matsumoto & Ikoma, 2016). Further investigation of the changes in Muscat flavour after harvest is needed.

There were 20 bound aroma compounds at the maturation stage exhibiting an OAV above 1. These include β-damascenone (835.40), 2-hexanol (979.92), (E)-2-nonenal (195.38), (E, Z)-2,6-nonadienal (781.50), and β-ionone (82.33), which potentially provide a rich 'fruity', 'floral' and 'green' aroma to Shine Muscat grape fruits. Thus, they can make a significant contribution to the subsequent manufacturing process of grape juice. We also found that the OAVs of all bound aroma compounds remained unchanged throughout the different stages of development.

In order to identify the characteristic aroma and changes in aroma features at different stages in Shine Muscat grapes, data processing was carried out as described by Fenoll *et al*. (2009). The logarithm based on 10 of the sum of OAVs of the aroma compounds in the same aromatic series was plotted against odour type to form the spider web diagrams of free and bound aroma compounds. As indicated in Fig. 4, the 'green' and 'fruity' aroma was the characteristic fragrance in Shine Muscat grapes in each period. Wei *et al*. (2019) also reported that the aroma components of Shine Muscat grapes were mainly associated with 'green leaf' and 'fruit' aromas. 'Floral' and 'sweet' aromas also played an important role in the free aroma of Shine Muscat berries in this study. In addition to the 'alcoholic' aroma, the other aroma characteristics were weakened at EL-38. The 'green' fragrance was the most prominent aroma feature in the bound aroma compounds of Shine Muscat berries (Fig. 4). Except for the 'fatty' aroma, other aroma series showed a broadly consistent contribution. Future studies should focus on the lack of musk smell and the increase in green fragrance after harvesting Shine Muscat grapes.

Principal component analysis (PCA) and cluster analysis

PCA graphs were produced of the free and bound aroma compounds in Shine Muscat berries at phenological stages EL-35 to EL-38. For the free aroma, as shown in Fig. 5, we found that 92.82% of the total variance could be explained by the two principal components. The first principal component (PCA1) accounted for 69.19% and the second principal component (PCA2) accounted for 23.63% of the total variability, which reflects the loadings of the free aroma functional groups in Shine Muscat grapes. The 'ester' and 'terpenoids' loadings on PCA1 were higher, at 0.73 and 0.47 respectively, while the 'ester' loading on PCA2 was 0.57. The PCA results show that, for the phenological stages from EL-35 to EL-38, changes in the aroma compound content of esters and terpenoids can better explain the differences in the total free aroma content at each stage, and the magnitude of change from EL-35 to EL-38 was greater for 'ester' and 'terpenoids'.

A cluster analysis was carried out of the content of free aroma compounds in the phenological stages from EL-35 to EL-38 in Shine Muscat berries. As shown in Fig. 6,

TABLE 1
Odour description and aroma series of aroma compounds in Shine Muscat berries (2017 season)

	Volatile compounds	Odour description	Aroma series
1	3-methyl-3-buten-1-ol	alcoholic	alcoholic
2	3,7-dimethyl-1,6-octadien-3-ol (linalool)	woody, fruity, floral	green, fruity, floral
3	cyclohexene, 3-methyl-6-(1-methylethylidene)	green, herbal, woody, pine	green
4	γ-terpinene	citrus, lemon, floral	floral, fruity
5	Geraniol	rose	floral
6	(R)-3,7-dimethyl-6-octen-1-ol	sweet flower, fruit, sweet rose	sweet, floral, fruity
7	α-terpineol	strong green fragrance with sweet, green wood	sweet, green
8	3-methyl-2-buten-1-ol	sharp, green-oily	green, fatty
9	β-damascenone	canned peaches, dried apples, dried plums, flower	fruity, sweet, floral
10	6-methyl-5-hepten-2-one	herbal，fruity	green, fruity
11	β-ionone	violet, sweet fruit	floral, sweet, fruity
12	Hexanal	green, fruity	green, fruity
13	(E)-2-hexenal	green leaf	green
14	(Z)-3-hexen-1-ol	green	green
15	1-hexanol	flower, green, cut grass	floral, green
16	(E)-2-hexen-1-ol	strong immature fruit smell, strong green aroma, accompanied by fruity, vegetable and grassy	fruity, green
17	(E)-3-hexen-1-ol	green	green
18	hexanoic acid	cheese, fatty	fatty
19	(E, E)-2,4-hexadienal	green, fruity	green, fruity
20	2-hexanol	chemical, winey, cauliflower	chemical, alcoholic, green
21	(Z)-2-hexen-1-ol	almond, grass, astringent	green, chemical
22	Toluene	special aroma	chemical
23	p-xylene	aromatic	chemical
24	1,3-dimethyl-benzene	aromatic	chemical
25	o-xylene	aromatic	chemical
26	Benzaldehyde	caramel, fruity	sweet, fruity
27	phenylethyl alcohol	rose	floral
28	Styrene	fruity	fruity
29	Acetophenone	hawthorn-like fragrance	fruity
30	o-cymene	gasoline	chemical
31	p-cymene	citrus, floral	floral, fruity
32	Benzeneacetaldehyde	floral	floral
33	Naphthalene	camphor wood smell	green
34	Phenol	paste	chemical
35	2-pentanol	alcoholic, ethery fruity, nutty, raspberry, green	alcoholic, fruity, green
36	1-pentanol	alcoholic	alcoholic
37	1-butanol	alcoholic	alcoholic
38	2-methyl-1-propanol	fusel	alcoholic

TABLE 1 (CONTINUED)

	Volatile compounds	Odour description	Aroma series
39	3-methyl-1-butanol	whiskey, malt liquor	alcoholic
40	3-pentanol	sweet, herbal, oily, nutty	sweet, green, fatty
41	3-methyl-1-pentanol	alcoholic, coconut fruit	alcoholic, fruity
42	(Z)-2-penten-1-ol	Banana	fruity
43	4-methyl-1-pentanol	almond, toasted	sweet
44	1-heptanol	aromatic, plant	floral, green
45	1-octen-3-ol	mushroom, lavender, rose, hay	green, floral
46	(E)-2-octen-1-ol	green, citrus, vegetable, oil	green, fruity, fatty
47	1-octanol	citrus, rose, sweet herbal	floral, green, fruity
48	2-ethyl-1-hexanol	herbal	green
49	2-heptanol	fruity, herbaceous	fruity, green
50	1-nonanol	rose, orange	floral, fruity
51	1-decanol	orange blossom, special light oil flavour	floral, fatty
52	acetic acid, butyl ester	strong fruit aroma, with pleasant pineapple and banana aroma after dilution	fruity
53	acetic acid, hexyl ester	fruity	fruity
54	methyl salicylate	green	green
55	octanoic acid, ethyl ester	pineapple, pear, floral	floral, fruity
56	(E)-2-octenal	fatty, green	green, fatty
57	(Z)-2-heptenal	green, oil	green, fatty
58	Heptanal	fruit	fruity
59	(E)-2-nonenal	fatty, green	fatty, green
60	(E, Z)-2,6-nonadienal	cucumber-like aroma	green
61	Octanal	fatty, fruity, orange peel, soapy, rose	fatty, fruity, floral
62	Nonanal	citrus, fatty, floral, green, tallow, rose	fruity, floral, green, fatty
63	Decanal	sweet, citrus, wax, floral	sweet, fruity, floral, fatty
64	acetic acid	acetic acid	chemical
65	cyclohexanone, 2,2,6-trimethyl-	honey, lemon	sweet, fruity
66	Furfural	sweet, bread, caramel, baking smell	sweet
67	2-furanmethanol	burnt sugar	sweet
68	5-methyl-2-furancarboxaldehyde	almond, spicy, caramel-like	sweet, chemical
69	benzyl Alcohol	mild aromatic	chemical
70	hexanoic acid, ethyl ester	fruit	fruity

the algorithm clustered EL-36 and EL-38 into a subclass, which indicates there was a high level of similarity in the total amount of free aroma compounds and the aroma composition in EL-36 and EL-38. The content of free aroma compounds in EL-35 was significantly different from that in the other three stages, which again indicates that the content of free aroma compounds was dynamic from EL-35 to EL-38. It is also evident that the relative abundance of terpenoids was higher after EL-35, and the relative abundance of esters was very small – to the point of disappearance in EL-35 and EL-38. The large change in relative abundance is the reason why esters and terpenoids are the key loading factors in the principal component analysis.

For the bound aroma, as shown in Fig. 7, 99.65% of the total variance could be explained by the two principal components. The PCA1 and PCA2 accounted for 97.45% and 2.2% of the total variability respectively. The 'aldehydes' and 'terpenoids' loadings on PCA1 were higher, at 0.51 and

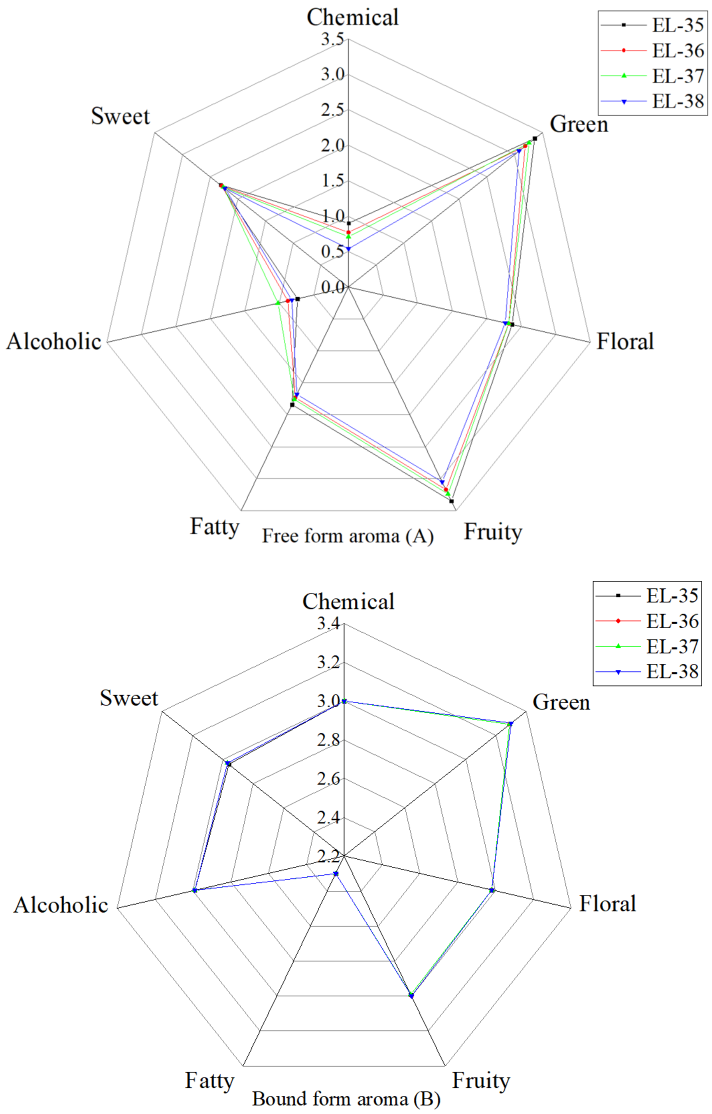

FIGURE 4

Free and bound aromatic series of sensory attributes of Shine Muscat berries according to OAVs with a logarithm based on 10 computation (2017 season). There are seven aromatic groupings: 'fruity', 'floral', 'alcoholic', 'green', 'sweet', 'fatty' and 'chemical'. There were different contributors to aroma in the Shine Muscat berries at different stages of ripening.

0.20, while the 'terpenoids' loading on PCA2 was higher, at 0.45. The PCA results show that, for the phenological stages from EL-35 to EL-38, the changes in aldehydes and terpenoids played a decisive role in the difference in the content of bound aroma compounds in each stage.

The cluster analysis of the content of bound aroma compounds from EL-35 to EL-38 is shown in Fig. 8. The algorithm clustered EL-35, EL-36 and EL-37 into a large group, which indicates that the content and components of the bound aroma were steady from EL-36 to EL-38. It also can be seen that the relative abundance of C6 compounds dropped to a minimum at EL-38.

These results show the variation in free and bound aroma in Shine Muscat berries from phenological stage EL-35 to EL-38, as well as the prominent aroma characteristics at each stage. To obtain better economic benefits, it is proposed that producers should harvest Shine Muscat at EL-37 (when a minimum TSS of 17% has been reached) to maintain its fine aroma characteristics.

CONCLUSIONS

In this work, the evolution of free and bound aroma compounds in Shine Muscat berries was studied and 94 compounds were identified. These volatile compounds play different roles in the aroma of Shine Muscat through the mutual transition between free and bound states. Among them, alcohols were the most abundant compounds in both free and bound forms. Terpenoids and C6 compounds were found in high concentrations as free aroma compounds at commercial maturity. According to OAVs, the free forms of hexanal, β-damascenone and (E, Z)-2,6-nonadienal were the main contributors to the green, fruity, floral and sweet aroma profiles of Shine Muscat grapes, with hexanal making the major contribution to green and fruity aroma.

The bound forms of β-damascenone, 2-hexanol and (E, Z)-2,6-nonadienal made a notable contribution to grape aroma based on OAVs. A green fragrance, with a fresh and sweet and pleasant fruity fragrance, represented the main aroma at every stage before Shine Muscat berries reach harvest maturity. The reasons for the absence of the key terpenoid, nerol, and the lack of musk odour remain unknown and warrant further study.

The implications of this study for the grape-producing industry are the identification of the optimum harvesting stage for Shine Muscat grape as EL-37, when TSS has reached ripeness level, acidity is appropriate, and berry weight is at its maximum. The content of terpenoids in the free form is at its maximum, and aroma sensory attribute values for 'green', 'floral' and 'fruity' are all higher in EL-37 than in EL-38. In summary, Shine Muscat at the EL-37 stage

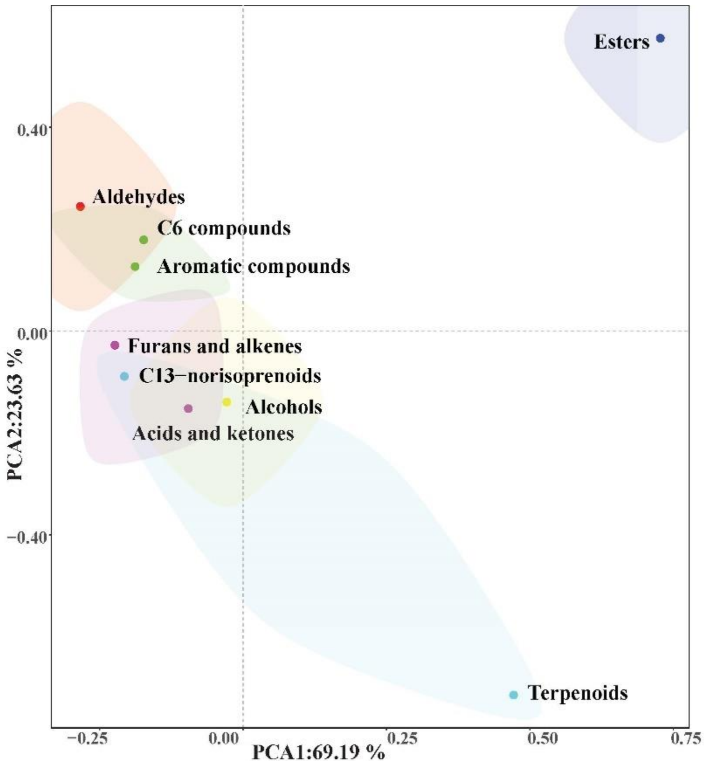

FIGURE 5
PCA of free aroma compounds in Shine Muscat berries at phenological stages EL-35 to EL-38 (2017 season).

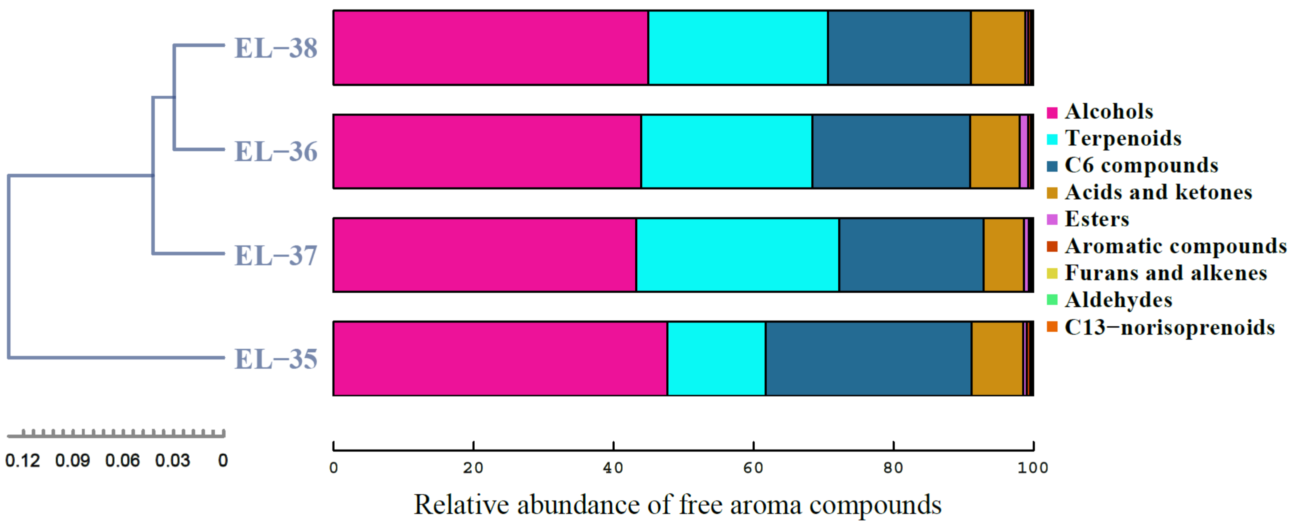

FIGURE 6
Cluster analysis of free aroma compounds in Shine Muscat berries at phenological stages EL-35 to EL-38 (2017 season).

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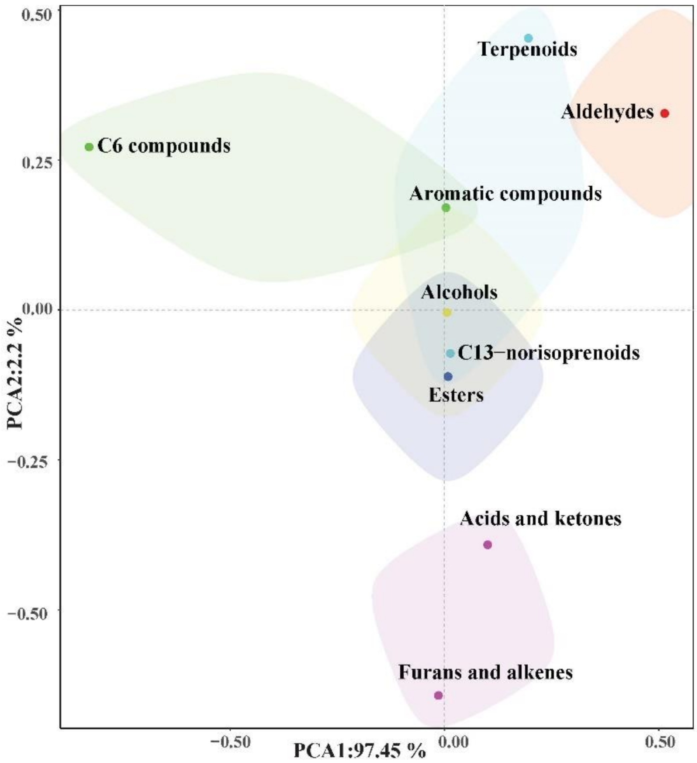

FIGURE 7
PCA of bound aroma compounds in Shine Muscat berries at phenological stages EL-35 to EL-38 (2017 season)

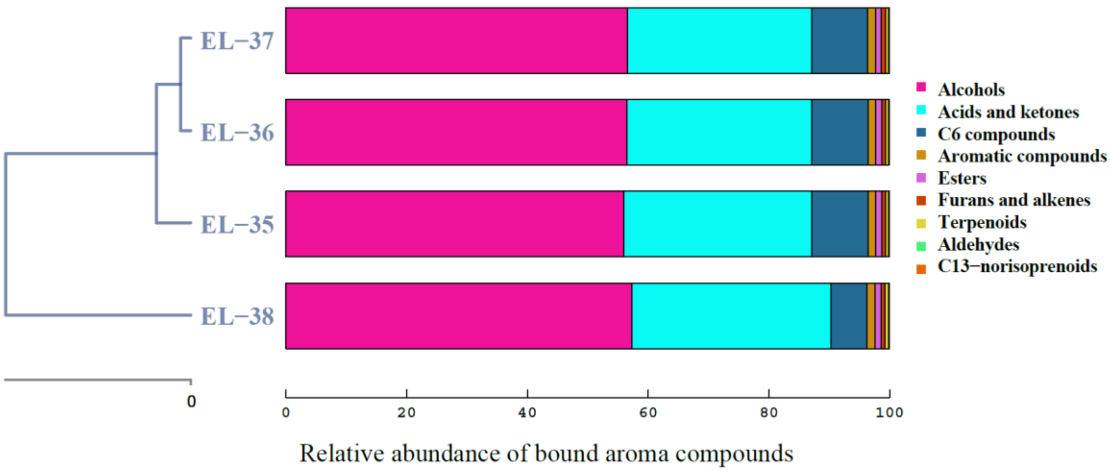

FIGURE 8
Cluster analysis of bound aroma compounds in Shine Muscat berries at phenological stages EL-35 to EL-38 (2017 season).

is more likely to be favoured by consumers.

In the future, we will continue to investigate the phenomenon of aroma loss during post-harvest storage and the effect of cultivation conditions, such as shade and regulated deficit irrigation, on aroma production in Shine Muscat grapes.

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