Changes in Biochemical and Volatile Flavor Compounds of Shine Muscat at Different Ripening Stages

Kyeong-Ok Choi, Dong Hoon Lee, Seo Jun Park, Dongjun Im, Youn Young Hur and Su Jin Kim*

Fruit Research Division, National Institute of Horticultural and Herbal Science, Wanju 55365, Korea; koi1786@naver.com (K.-O.C.); chocho90@korea.kr (D.H.L.); grapepark@korea.kr (S.J.P.); neulbobung@korea.kr (D.I.); yyhurst6@korea.kr (Y.Y.H.)

* Correspondence: himssem@korea.kr; Tel./Fax: +82-63-238-6750

Received: 27 July 2020; Accepted: 13 August 2020; Published: 14 August 2020

Abstract: Changes in the biochemistry and flavor of Shine Muscat grapes at different ripening stages (RS) were analyzed to identify factors affecting these characteristics. The yellowness index values were 45.1, 49.4, and 50.2 in the ripening stage 1 (RS1), ripening stage 2 (RS2), and ripening stage 3 (RS3) groups, respectively, representing the different ripening stages. The yellowness of the grape berries tended to increase with ripening due to the gradual breakdown of chlorophylls and the evolution of carotenoids. The total content of monoterpenes, on the other hand, was approximately two-fold higher at RS3 than RS1 and RS2. Moreover, linalool was the most abundant compound contributing to the total content of monoterpenes. The highest correlation was observed between the linalool content and °Brix/acid ratio (r = 0.9981), followed by the monoterpene content and °Brix/acid ratio (r = 0.9933). These findings indicate that changes in the contents of linalool and its oxidized forms may be used as a quality index and an indicator of the timing of harvest for Shine Muscat grapes.

Keywords: linalool; ripening; Shine Muscat; volatile flavor compounds; °Brix/acid ratio

1. Introduction

The composition and distribution of volatile flavor compounds are important characteristics that contribute to the quality of grape berries and wine. The flavors of grape berries and wine can be characterized by complex families of volatile compounds, including terpenes, esters, C6-aldehydes and alcohols, and methoxypyrazines, which contribute to fruity, floral, green leafy, and green capsicum flavors, respectively [1]. The development of flavor compounds in grape berries is likely to be affected by various factors, including grape cultivars, growing conditions, and climate [2]. The flavor intensity and aromatic profiles of grape berries are primarily affected by the concentrations and odor thresholds of volatile flavor compounds and their interactions with other compounds [1]. It has been reported that grape berries develop in two successive growth stages: berry formation and ripening [3]. The first stage is characterized by the formation of berry and seed embryos, followed by rapid cell division. During the second stages, berries become softer and start to change color. Furthermore, various secondary metabolites, including volatile aroma compounds, are developed in the berries during this stage [4]. Thus, the changes in biochemical and volatile flavor compounds of grape berries during the second stage are more closely related to consumer’s sensory perception than those of the first growth stage. For this reason, the degree of ripening is a crucial factor influencing the development and distribution of volatile compounds, and is closely related to consumer’s preferences for grape berries.

In recent years, a new grape cultivar called Shine Muscat (Vitis labruscana Bailey × Vitis vinifera L.), developed in Japan in the late 1980s and commercialized in the early 2000s [5], has been gaining...
attention as a table grape in Korea. This cultivar has attracted consumer attention due to its unique taste, namely, a strong muscat flavor with excellent sweetness and not much sourness, despite its high price. The muscat flavor of Shine Muscat is the most important attribute in determining consumer preferences. Monoterpene compounds are associated with the distinctive taste and flavor of Muscat grape varieties [6]. In particular, terpenic alcohols, including linalool, geraniol, nerol, citronellol, and α-terpineol, are known to be the major aroma compounds in Muscat grape varieties [1], and their chemical structures are represented in Figure 1.

Thus, the development and alteration of flavor compounds in Muscat grape varieties during berry ripening has been widely investigated [1,7]. Wilson et al. [6] investigated the alterations in volatile monoterpene compounds of Muscat of Alexandria during berry ripening and they found that only the concentration of free linalool increased with ripening in parallel to its glycoside, and Ribereau-Gayon et al. [8] compared the concentrations of monoterpene compounds among various Muscat grape varieties, such as Muscat of Alexandria, Muscat de Frontignan, Muscat Saint-Vallier, Italia, and Muscat Hamburg, and they found that linalool and geraniol were the most important aroma compounds in Muscat grape varieties. In addition, Bordiga et al. [9] evaluated the alteration of flavor compounds in Muscat-based wines (Asti Spumante and Moscato d’Asti), and they showed the changes of linalool, β-damascenone, ethyl hexanoate, and ethyl octanoate levels in both two Muscat-based wines. However, to the best of our knowledge, the flavor compound profiles of Shine Muscat grapes during ripening have not yet been extensively investigated. Although several relevant studies have been conducted in China and Japan [10–12], no such studies have been performed in Korea.

Increases in consumer demand for Shine Muscat grapes and higher prices have led many grape growers to harvest and market immature berries, thereby decreasing the quality of Shine Muscat grape and thus consumer demand. The objective of this study was to analyze changes in the biochemistry and flavor in Shine Muscat grapes at different ripening stages to identify factors that affect the flavor characteristics of this cultivar. Grape berries were classified into three groups depending on their color in order to analyze the sugar content, titratable acidity and pigments at different ripening stages. Furthermore, the volatile flavor compound profiles were evaluated by SPME/GC/MS.

2. Materials and Methods

2.1. Reagents

Analytical standard grade 1-dodecanol, 1-hexanol, hexanal, benzaldehyde, geraniol, linalool, nerol, terpinen-4-ol, α-phellandrene, α-terpineol, β-myrcene, β-ocimene, 4-nonanol, and C8–C20...
alkane standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of analytical reagent grade.

2.2. Grape Samples

Shine Muscat grape cultivated in the orchards at the National Institute of Horticultural and Herbal Science (Ansung, Korea) were used to evaluate the volatile aroma compounds. Grape berries were harvested at 85, 95, and 105 days after full bloom (DAFB) on late September 2019 and classified into three groups depending on their DAFB and color: ripening stage 1 (RS1, 85 DAFB), ripening stage 2 (RS2, 95 DAFB), and ripening stage 3 (RS3, 105 DAFB) (Figure 2), where the numbers indicate the degree of ripening. All grape samples were stored at −70 °C prior to analysis.

2.3. Sugar Content, Acidity, Color, and Pigments

The total sugar content in the extracts of the grape berries was measured using a digital refractometer (Palete Digital Refractometer PR-32 Alpha; ATAGO Co. Ltd., Tokyo, Japan). Twenty berries from each group were crushed to collect free flowing juice. After filtering the juice, the total soluble solid content was measured at the constant temperature of 2 °C. The total sugar content was expressed as °Bx. The titratable acidity was determined using an automatic titrator (TitroLine Easy; SI Analytics GmbH, Mainz, Germany). Briefly, 5 mL juice was mixed with 20 mL distilled water. Then, 0.1 M NaOH standard solution was titrated until the pH of the sample solution reached 8.2. The titratable acidity was expressed as tartaric acid equivalent and calculated using the following Equation:

$$\text{Titratable acidity (\%)} = \frac{\text{mL NaOH} \times 0.1 \times \text{Mw. tartaric acid}}{S \times 10}$$

(1)

The °Brix/acid ratio is known to be a better attribute to describe the consumers acceptability, such as sweetness, sourness, and flavor attributes, as compared with individual °Brix or acidity value [13]. Thus, the °Brix/acid ratio was calculated by dividing the °Brix value of the grape juice samples by the titratable acidity (°Brix/° acidicity). For color measurement, the color values L, a, and b of twenty randomly selected berries were measured using a colorimeter (DP-400; Konica Minolta, Tokyo, Japan). Standard illuminant C was used as a reference. The hunter color indices L, a, and b were converted into their corresponding X, Y, and Y values. The yellowness index (YI) of the berries was calculated using the equation below [14].

$$\text{YI} = 100(1.28X - 1.06Z)/Y$$

(2)
The concentrations of pigments, including chlorophyll a and b (Chl\textsubscript{a} and Chl\textsubscript{b}, respectively) and total carotenoids (x+c) (C\textsubscript{x+c}), were estimated by spectrophotometry [15]. For the extraction of pigments, 0.15 g of grape skin was added to 1.5 mL of 0.1% CaCO\textsubscript{3} in acetone solution, vortexed, and centrifuged at 4500 rpm for 5 min. The supernatant was isolated from the sediment. Residual pigments were further extracted using fresh extraction solution. The resulting supernatants were merged and filtered using a 0.2 \( \mu \)m membrane filter. The absorbances of the extracted pigment samples were recorded at 661.6 nm, 644.8 nm, and 470 nm on a microplate reader (Multiscan GO; Thermo Fisher Scientific, Waltham, MA, USA). The whole experimental process was performed under dim light to prevent pigment loss. The concentrations of Chl\textsubscript{a}, Chl\textsubscript{b}, and C\textsubscript{x+c} were calculated using the equations below [15]:

\[
\text{Chlorophyll a (Chl}_a) = 11.24\text{Abs}_{661.6} - 2.04\text{Abs}_{644.8} \tag{3}
\]

\[
\text{Chlorophyll b (Chl}_b) = 20.13\text{Abs}_{644.8} - 4.19\text{Abs}_{661.6} \tag{4}
\]

\[
\text{Carotenoids (x+c) (C}_{x+c} = (1000\text{Abs}_{470} - 1.09\text{Chl}_a - 63.14\text{Chl}_b)/214 \tag{5}
\]

2.4. Volatile Flavor Compounds

An SPME fiber coated with divinylbenzene/carboxen/polydimethylsiloxane (50/30 \( \mu \)m DVB/CAR/PDMS; Supelco, Bellefonte, PA, USA) was used for the analysis of volatile flavor compounds. Frozen grape berries were quickly sliced using a razor blade and ground in an electric blade grinder. Then, 20 g of the homogenized sample was centrifuged at 4500 rpm for 10 min. The resulting supernatant was filtered under vacuum. For SPME fiber adsorption, 2 mL of the grape juice was transferred into a headspace vial with NaCl to improve the volatility of flavor compounds. The sample vial was heated to 70 °C for 30 min on a heating block (HB-48P; DAIHAN Scientific, Wonju, Korea) to bring the sample solution to temperature equilibrium. Then, the SPME fiber was introduced into the sample vial headspace. After adsorption for 30 min, the fiber was removed and injected into the GC injection port for desorption. A gas chromatograph (6890N; Agilent Technologies, Santa Clara, CA, USA) equipped with an Agilent 5975 series mass selective detector was used for the analysis of volatile flavor compounds. The volatile compounds were separated on an HP-INOWAX capillary column (30 mm \times 0.32 mm \times 0.25 \( \mu \)m; Agilent Technologies). Purified helium was used as a carrier gas at a constant flow rate of 2 mL/min. The analytes were thermally desorbed at 250 °C for 10 min in splitless mode. The column temperature was initially held at 40 °C for 5 min, increased to 220 °C at a rate of 5 °C/min, and lastly held at 250 °C for 5 min. The injector and source temperatures were set at 250 °C and 230 °C, respectively. The mass detector was operated in positive ion electron impact ionization mode at 70 eV with a scan range of 50–700 m/z. Volatile compounds were identified by comparing the retention times with those of authentic standards. The volatile compounds without their corresponding standards were tentatively identified by comparing their mass spectra with those from NIST 11 library and retention indices with those from the literature [16]. The concentrations of volatile flavor compounds were estimated using a 4-nonanol calibration curve. The calibration curve was constructed in the concentration range 1–4 \( \mu \)g/mL.

2.5. Statistical Analysis

The experimental results were statistically analyzed using SPSS (ver. 20, IBM, Armonk, NY, USA). Analysis of variance (ANOVA) was applied, followed by Duncan’s multiple range tests for mean comparisons. Pearson’s correlation coefficients (r) for the relationships between the biochemical properties and flavor compounds concentrations were estimated using OriginLab Pro software (Origin Lab Corporation, Northampton, MA, USA). Differences were considered to be significant at \( p < 0.05 \).
3. Results and Discussion

3.1. Sugar Content, Acidity, Color and Pigments

The $a$ value, indicating the level of redness or greenness, showed no significant difference between the RS1 and RS2 groups (−9.64 and −10.04, respectively) (Table 1). On the other hand, the $a$ value of the RS3 group was significantly lower (−6.06) than those of the other groups (Table 1). The reduced $a$ value in the RS3 group indicates that the greenness of the grape skin faded as ripening progressed. The $b$ value, which is responsible for the level of yellowness or blueness, was significantly higher in the RS2 and RS3 groups compared to RS1. The $b$ value was found to be slightly higher in the RS2 group compared to the RS3 group (22.50 and 20.44, respectively), but no significant differences between them (Table 1). The higher $b$ values in the RS2 and RS3 groups indicate that the berries in these groups were more yellowish than in the RS1 group. For a more comprehensive indication, the entire yellowness was expressed as yellowness index (YI). The YI values were 45.1, 49.4, and 50.2 for the RS1, RS2, and RS3 groups, respectively (Table 1). The YI values of the RS2 and RS3 groups were significantly higher than that of the RS1 group. The compact green color of grape berries changes during the period of veraison or ripening. In particular, white varieties gradually become transparent, and eventually golden. The grape berries of the RS1, RS2, and RS3 groups were dark green, pale green, and yellowish green, respectively (Figure 2). Changes in the skin color of the grape berries were regarded as a good indicator of the degree of ripening [17].

### Table 1. The change in skin color of Shine Muscat grape berries at different ripening stages.

| Group | Skin Color | L    | $a$     | $b$     | $^2$ YI |
|-------|------------|------|---------|---------|---------|
|       |            |      |         |         |         |
| 1 RS1 | $38.18 \pm 1.71^b$ | $−9.64 \pm 0.88^b$ | $18.18 \pm 1.68^b$ | $45.1 \pm 2.3^b$ |
| RS2   | $48.35 \pm 1.69^a$ | $−10.04 \pm 0.17^b$ | $22.50 \pm 0.66^a$ | $49.4 \pm 0.4^a$ |
| RS3   | $47.13 \pm 1.84^a$ | $−6.06 \pm 0.9^a$ | $20.44 \pm 1.05^a$ | $50.2 \pm 1.2^a$ |

$^1$ RS: ripening stage, $^2$ YI: yellowness index. Different letters in the same column indicate a statistically significant difference ($p < 0.05$).

During the period of veraison and ripening, changes in the skin color are biochemically associated with the metabolism of chlorophylls and carotenoids [18]. In particular, chlorophylls are known to be a major pigment determining the skin color of white grape varieties, including Shine Muscat [19]. The chlorophyll $a$ (Chl $a$), chlorophyll $b$ (Chl $b$), and total chlorophyll contents tended to decrease as ripening progressed (Figure 3). The total carotenoid content showed a similar tendency as that of chlorophyll. This result is in agreement with the results reported by Razungles et al. [20], who observed decreases in the β-carotene and lutein contents in three grape varieties during ripening.
The total sugar content had a tendency to increase significantly as ripening progressed (Figure 4). Glucose and fructose are the most abundant sugars in grapes, rapidly accumulating in the vacuoles of grape berries during ripening [21]. On the other hand, titratable acidity had a tendency to decrease as ripening progressed, though there was no significant difference between the RS1 and RS2 groups (Figure 2). The titratable acidity, however, showed a marked decrease in the RS3 group. This decrease in titratable acidity led to the low °Brix/acid ratio value of the RS3 group. The titratable acidity of grape berries decreases from 2.0~1.5% at veraison to 0.7~0.5% at maturity, depending on the grape varieties [22]. Based on the biochemical changes analyzed in this study, it was assumed that the RS1 group represents the onset of ripening stage, RS2 group the initial or medial ripening stage, and RS3 the final ripening stage or maturity.

Figure 3. The change of pigments concentrations in Shine Muscat grape berries at different ripening stages: Chl a, Chlorophyll a; Chl b, Chlorophyll b; Total Chl, total Chlorophyll; and C<sub>c+</sub>, total carotenoids. RS1, ripening stage 1; RS2, ripening stage 2; and RS3, ripening stage 3. All data represent mean ± SD. Different letters in the same category indicate a statistically significant difference (p < 0.05).

Figure 4. Plot of total sugar content (top), titratable acidity (middle), and °Brix/acid ratio (bottom) of Shine Muscat grape berry extracts at different ripening stages: RS1, ripening stage 1; RS2, ripening stage 2; and RS3, ripening stage 3. All data represent mean ± SD. Different letters in the same plot layer indicate a statistically significant difference (p < 0.05).
3.2. Free Volatile Aroma Compounds

The variation of volatile flavor compounds in grape berries is closely related to the biochemical changes of berries [11]. The total ion chromatograms of free volatile flavor compounds and their profile are presented in Figure 5 and Table 2. The concentration of total alcohols had a tendency to decrease as ripening progressed. The concentration of most alcohols also tended to decrease, while that of 1-dodecanol tended to increase as ripening progressed. The concentration of 1-hexanol showed a decreasing trend as ripening progressed. C6-compounds, such as C6-aldehydes (hexanals and hexenals), alcohols (hexanols and hexenols), and esters (hexyl acetates), are synthesized via complex enzymatic reactions, namely, the lipoxygenase pathway, during ripening [23]. In addition, Wu et al. [11] found that (Z)-hexenal, a C6-aldehyde, could be converted into (E)-2-hexenal and (E)-2-hexenol and (E)-2-hexenyl acetate. However, our results showed a constant decrease of C6-alcohols during ripening, which was inconsistent with the results of previous works [11,23,24]. Different C6 compound profiles are observed depending on the grape varieties and their cultivation regions [2]. The concentration of C6-alcohols, including 1-hexanol, showed different trends depending on the cultivation regions. A decrease in C6-alcohols concentration in grapes was related to differences in the activity of enzymes involved in the lipoxygenase pathway and in a reduction of unsaturated fatty acids, which are precursors of C6-compounds during ripening. Thus, decreasing 1-hexanol observed in this study may be associated with reduced lipoxygenase and alcohol dehydrogenase activity, since their activity reached a maximum during the second week after veraison [25]. The decrease in aldehydes may also be related to the activity of these enzymes. The decrease in C6-alcohols and aldehydes during ripening is favorable to the consumer’s sensory perception, since their herbaceous characteristics give an unpleasant odor [11]. The variation of volatile C13-norisoprenoids is related to the degradation of carotenoids, which are precursors of volatile C13-norisoprenoids [26]. In this study, damascenone and 2-methyl-β-ionone were identified. Damascenone was identified in the RS2 and RS3 groups (Table 2), while 2-methyl-β-ionone was identified in the RS1 and RS2 groups. The concentration of total C13-norisoprenoids was the highest in the RS2 group, followed by RS3 and RS1, in agreement with previous studies [11,24]. In regards to carotenoid degradation (Figure 3), the rapid reduction of the total carotenoid concentration in the RS1 group seemed to correspond to a rapid increase in the concentration of damascenone and 2-methyl-β-ionone.

Table 2. Volatile aroma compound profiles of Shine Muscat grape berries at different ripening stages.

| Group   | Volatile Compounds | RS1 Concentration (µg/kg) | RS2 Concentration (µg/kg) | RS3 Concentration (µg/kg) |
|---------|--------------------|---------------------------|---------------------------|---------------------------|
| Alcohols| 1-Dodecanol        | 4.6 (1.6) b               | 9.3 (2.7) a               | 11.2 (0.4) a              |
|         | 1-Hexanol          | 9.8 (2.0) a              | 2.8 (0.5) b              | 0.2 (0.1) c              |
|         | 1-Octen-3-ol       | 1.5 (0.2)               | -                        | -                        |
|         | 2-Ethyl-1-hexanol  | 1.5 (0.3) a              | 0.7 (0.1) b              | -                        |
|         | 3-Methyl-1-butanol | 8.8 (0.8) a              | 3.1 (0.3) b              | -                        |
|         | Subtotal           | 26.0                    | 15.9                     | 11.4                     |
| Aldehydes| 3-Methylbutanal   | 8.9 (2.4)               | -                        | -                        |
|         | Hexanal            | 9.1 (2.2) a              | 10.9 (1.5) a             | 0.2 (0.1) b              |
|         | Pentanal           | -                        | 1.2 (0.1)               | -                        |
|         | Benzaldehyde       | 1.1 (0.1)               | -                        | -                        |
|         | Phenyl acetaldehyde| 0.9 (0.3) a              | 1.2 (0.2) a              | -                        |
|         | Subtotal           | 19.9                    | 13.3                     | 0.2                      |
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Table 2. Cont.

| Group               | Volatile Compounds          | Concentration (µg/kg) | RS1     | RS2     | RS3     |
|---------------------|----------------------------|-----------------------|---------|---------|---------|
|                     |                            |                       | 1       | 2       | 3       |
| C13-norisoprenoids  | Damascenone                |                       | -       | 6.5(1.8)| 1.3(0.3)|
|                     | 2-Methyl-β-ionone          |                       | 0.2(0.04)| 0.4(0.1)| -       |
|                     | Subtotal                   |                       | 0.2     | 6.9     | 1.3     |
| Esters              | Ethyl octanoate            |                       | -       | 1(0.4)  | -       |
|                     | Subtotal                   |                       | 0       | 1.0     | 0       |
|                     | Geraniol                   |                       | -       | 1.5(0.2)| 1.4(0.2)|
|                     | Hotrienol                  |                       | -       | -       | 3.4(1.0)|
|                     | Limonene                   |                       | 2.8(0.3)| 1.5(0.1)| 5.5(1.3)|
|                     | Linalool                   |                       | 76.3(8.7)| 78.7(13.8)| 181.5(4.5)|
|                     | Linalyl oxide              |                       | -       | -       | 1.5(0.3)|
|                     | Linalyl acetate            |                       | -       | -       | 20.4(4.6)|
| Monoterpenes        | Nerol                      |                       | 1.7(0.3)| -       | 3.3(0.2)|
|                     | α-Terpinene                |                       | -       | 0.2(0.02)| -       |
|                     | Rose oxide                 |                       | 0.3(0.1)| 0.3(0.02)| -       |
|                     | Terpinen-4-ol              |                       | -       | -       | 0.2(0.1)|
|                     | Sabinene                   |                       | 0.4(0.1)| -       | -       |
|                     | α-Phellandrene             |                       | -       | -       | -       |
|                     | α-Terpineol                |                       | 0.9(0.3)| 2.3(0.7)| 19.4(3.9)|
|                     | β-Citronellol              |                       | 1.8(0.5)| -       | -       |
|                     | β-Myrcene                  |                       | 5.3(0.6)| 0.9(0.1)| -       |
|                     | β-Ocimene                  |                       | -       | -       | 8.1(2.8)|
|                     | γ-Terpinene                |                       | 1.3(0.2)| 0.5(0.1)| 0.6(0.1)|
|                     | Subtotal                   |                       | 90.8    | 85.87   | 247.65  |
|                     | Total                      |                       | 136.9   | 123.0   | 260.5   |

1 RS: Ripening stage. Different letters in the same row indicate a statistically significant difference (p < 0.05).

Monoterpenes are a major volatile constituent of Muscat grape varieties [6]. The importance of monoterpenes in the flavor characteristics of Muscat grape varieties has been emphasized in previous studies [6,27], and many experimental attempts have been made to elucidate the effects of their composition and concentration on the flavor intensity and aromatic profile of Muscat grape varieties [7,28,29]. Monoterpenes, in particular linalool, geraniol, nerol, citronellol, and α-terpineol, are a major constituent of the floral and fruity flavors of Muscat grape varieties [1]. A total of 17 different monoterpenes have been identified (Table 2). The concentrations of linalool and its oxidized forms (linalyl oxide and linalyl acetate) were found to be much higher than that of the other monoterpene compounds in all experimental groups. The concentrations of linalool were 76.3 and 78.8 µg/kg in the RS1 and RS2 groups, respectively, and increased rapidly to 181.5 µg/kg in the RS3 group (Figure 6). The occurrence of linalyl oxide and linalyl acetate in the RS3 group seemed to result from the oxidation of linalool [11]. The two oxidized forms of linalool were assumed to be found in ripe grapes, only because of their higher oxidation levels compared to linalool [6].
Figure 5. Total ion chromatograms of Shine Muscat grape berry extracts at different ripening stages: RS1, ripening stage 1; RS2, ripening stage 2; and RS3, ripening stage 3. The increasing numbers behind the abbreviation RS indicate the degree of ripening. (1) 3-Methyl butanal, (2) Pentanal, (3) Sabinene, (4) Hexanal, (5) \( \alpha \)-Phellandrene, (6) \( \alpha \)-Terpinene, (7) Limonene, (8) \( \beta \)-Myrcene, (9) \( \gamma \)-Terpinene, (10) 3-Methyl-1-butanol, (11) \( \beta \)-Ocimene, (12) 1-Hexanol, (13) Rose oxide, (14) Ethyl octanoate, (15) Linalool oxide, (16) 1-Octen-3-ol, (17) 2-Ethyl-1-hexanol, (18) Benzaldehyde, (19) Linalool, (20) Linalyl acetate, (21) Terpinen-4-ol, (22) Hotrienol, (23) Phenyl acetaldehyde, (24) \( \alpha \)-Terpineol, (25) \( \beta \)-Citronellol, (26) Damascenone, (27) Geraniol, (28) Nerol, (29) 1-Dodecanol, and (30) 2-Methyl-\( \beta \)-ionone. Peaks without a number correspond to the contaminants from the column and septa bleedings.

Geraniol and nerol, which are geometrical isomers and \( \beta \)-Citronellol, were observed at very low concentrations. The highest concentration of geraniol was observed in Muscat grape varieties before veraison, after which the concentration decreased to low levels before disappearing at maturity [6]. In this study, geraniol was found at very low concentrations in the RS2 and RS3 groups (1.5 and 1.4 \( \mu \)g/kg, respectively) (Table 2). Nerol was observed in the RS1 and RS3 groups (1.7 and 3.3 \( \mu \)g/kg, respectively), while \( \beta \)-Citronellol was only observed in the RS1 group (1.8 \( \mu \)g/kg) (Table 2). The low concentration or disappearance of these two isomers and \( \beta \)-Citronellol in the experimental groups was associated with the ripening degree of the berries, since all berries entered the ripening stage after the onset of veraison, regardless of group. The presence of hotrienol in the RS3 group may be derived from the thermal degradation of dienediol-1 during analysis [6]. The total concentration of monoterpene compounds was 136.9, 123.0, and 260.5 \( \mu \)g/kg in the RS1, RS2, and RS3 groups, respectively (Table 2).

As mentioned in the introduction, the major volatile compounds composing the muscat flavor are linalool, geraniol, nerol, citronellol, and \( \alpha \)-terpineol. Our volatile aroma profile result also indicated that the linalool was the most abundant volatile compound, possibly having a strong influence on the muscat flavor of Shine Muscat grape. Furthermore, Fenoll et al. [30] reported that cis-rose oxide, linalool, citral, and geraniol were the only volatile monoterpenes present above their odor activity value (OAV) in the Muscat Hamburg grape berries during the period of ripening. Thus, regarding the relative concentrations of those monoterpenes compounds detected in Shine Muscat berries, the change in the linalool concentration could be a good indicator for determining the ripening degree and timing of harvests of Shine Muscat grape.
Figure 6. Dual axes bar and scatter overlay plots for (a) the monoterpenes content and °Brix/acid ratio and (b) the linalool content and °Brix/acid ratio of Shine Muscat grape berry extracts at different ripening stages: RS1, ripening stage 1; RS2, ripening stage 2; and RS3, ripening stage 3; TTC, total monoterpenes content. The increasing numbers behind the abbreviation RS indicate the degree of ripening. All data represent mean ± SD.

Nevertheless, using the linalool concentration as a quality index for Shine Muscat grape is challenging because it requires a special equipment, i.e., gas chromatograph to determine the concentration of linalool in the grape berries. Thus, in the economic aspect, an easier way to evaluate the quality of Shine Muscat grape, in particular flavor acceptability, is necessary. The determination of sugar content and acidity of grape berries is much easier than that of volatile aroma compounds profile. For this reason, in this study, the correlations of the °Brix/acid ratio with total monoterpenes and linalool content were determined. The highest correlation was observed between the linalool content and °Brix/acid ratio \( r = 0.9981 \), followed by the monoterpenes content and °Brix/acid ratio \( r = 0.9933 \) (Figure 6). The linalool and monoterpenes content and °Brix/acid ratio were found to increase suddenly in the RS1 group (Figure 6). The balance of sugar and acidity partially corresponded to the development of flavor compounds in table grapes [31]. Further study on the investigation of the relationship between linalool content and °Brix/acid ratio accompanied with a sensory evaluation is
necessary to provide more scientific and reliable evidence on their relationships to evaluate the quality of Shine Muscat grape.

4. Conclusions

Changes in the biochemistry and flavor of Shine Muscat grapes at different ripening stages were evaluated. The total sugar content was found to increase as ripening progressed. However, this was accompanied by a decrease in the titratable acidity and the chlorophyll and carotenoid contents. Linalool was found to be the most abundant free monoterpene in the Shine Muscat grapes, altering the total content of monoterpenes during ripening. Moreover, the balance between sugar and acidity was found to be correlated with the linalool content ($r = 0.9981$). This result indicates that the concentration of linalool and its oxidized forms is responsible for the muscat flavor of Shine Muscat grapes. Therefore, changes in the contents of linalool and its oxidized forms could be used as a quality index for Shine Muscat grape, as well as to determine the timing of harvests. Furthermore, a $\$\text{Brix}/\text{acid}$ ratio could be an indirect quality index for Shine Muscat grape alternate to the linalool content.

**Author Contributions:** K.-O.C.: Study design, Methodology, Investigation, Writing—original draft(review and editing; D.H.L.: Study conception and design, Methodology; S.J.P.: Study conception and design, Methodology; D.I.: Study conception and design, Methodology, Writing—original draft(review; Y.Y.H.: Study conception and design, Methodology, Writing—original draft(review and editing. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was carried out with the support of “Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ01343101) Rural Development Administration, Republic of Korea.

**Conflicts of Interest:** The authors declare no conflict of interest.

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