Varying Levels of Sugars, Acids, Amino Acids, and Monoterpenes Contained in Muscat of Alexandria Grapes Produced in Okayama

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Sugars, acids, amino acids and monoterpenes in Muscat of Alexandria grapes were analyzed to elucidate the distribution of the fruit taste and aroma. Fruit bunches were collected from 25 commercial vineyards in Okayama Prefecture, comprising six early heated, nine normally heated, and 10 unheated greenhouses, at midharvesting time in each vineyard. Juice was squeezed out by hand and used for analyses. Total soluble solids (TSS) content in juice from 3 vineyards were lower than 16° Brix, the lowest limit for shipping. Average content of glucose was lower in early heated vines and that of fructose was lower in unheated vines than normally heated ones, although there were wide variations among vines. Average malic acid content was highest in early heated vines and by further lowest in unheated vines. However, no significant difference was found in tartaric acid content among the vines. Concentrations of alanine and proline, major amino acids enhancing sweetness, were lower in early heated vines than in normally heated ones. In unheated vines, average concentration of proline was highest, although that of glutamine, a main factor enhancing deliciousness of the juice, was lowest. Threonine, an amino acid which at higher levels decreases sweetness, was highest in normally heated vines. Linalool, a major Muscat aroma volatile substance, was detected at various levels depending on the vine. Geraniol, the second major substance of Muscat aroma, was found at significantly higher levels in early heated vines than in the other ones. The relationships between these compositions and the berry flavor were discussed.

Key words: Muscat of Alexandria grape, taste, aroma, flavor, Okayama Prefecture

Introduction

In Japan, Muscat of Alexandria grapes have been cultivated mainly in Okayama Prefecture for more than a hundred years. Muscat growers, presently estimated to be about 1500 farmers in Okayama Prefecture, have cultivated the vines in heated and unheated greenhouses. The clusters are famous for their excellent appearance and delicious taste, keeping the highest market price

among all grapes produced in Japan. Most of the clusters are purchased as presents. The marketable standards are determined by berry size, cluster appearance, and the level of total soluble solids in juice¹⁶. However, cluster appearance and berry size seem to be overvalued, ignoring the taste and aromas as shown in rating scores at competitive exhibitions¹⁷. Constituents of fruit

taste and aroma have already been analyzed, which revealed that amino acids and monoterpenes are also important components as well as sugars and acids ^{2,3,4,6,7,11,13,15)}. In this study, we compared the levels of sugars, acids, amino acids, and monoterpenes to understand the range of savor of Muscat of Alexandria grape berries produced in various heated and unheated greenhouses in Okayama Prefecture.

Materials and Methods

Vine selection and berry sampling

Muscat of Alexandria vines grown in 25 commercial greenhouses, located at Funaho, Fukutani, Ichinomiya, and Mitsu districts in Okayama Prefecture were used for this research. Six greenhouses were heated from the early December (early heating), 9 were from the late January (normally heating), and the other 10 were unheated. Vines in 23 greenhouses had been grown under a spur pruning system with 6 to 12 cordons but those in 2 greenhouses were under a cane pruning system. A vine, showing average shoot vigor and fruit-leaf ratio, was tagged in each greenhouse at veraison. Shoots, leaves, and bunches developed in about 4 to 8 m² of the canopy were investigated for their size or weight, though each vine extended to about 22 to 106 m². Three or 4 bunches with average sizes of both bunch and berry were harvested at each harvest time. After measuring the bunch weight, average 30 berries were sampled from each bunch.

Measurements of juice constituents

1) Total soluble solids (TSS) and titratable acidity (TA)

Sampled berries were squeezed using cheese-cloth to obtain juice. Using the sample juice, TSS and TA were measured by a hand refractometer (ATAGO) and by titrating the juice with 0.1 N NaOH, respectively. TSS measurements were carried out for 10 berries of each bunch. TA were measured 3 times per bunch.

2) Sugars and organic acids

Two ml of the juice samples, mixed with 1mL of 3% pentaerythritol as an internal standard, were loaded onto a column of Amberlite CG-120 (H⁺) ion-exchange resin. The column was eluted with 50 ml of water to release sugars and acids. A 2ml aliquot was freeze-dried, the residue was re-suspended in 0.5ml of pyridine, and silylated with 0.25ml of hexamethyldisilizane and 0.25ml of trimethylchlorosilane at 60 °C for 2 hours. One μ 1 aliquot was injected into a GC (SHIMADZU GC-14A) equipped with FID. The determination was replicated 3 times for each bunch.

3) Amino acids

A 0.5 ml aliquot of sample juice was added to 1 ml of water and 0.5 ml of 40% TCA, then stirred. After standing at 5 °C for 1 hour or more, the mixture was centrifuged at 7,000 rpm at 4 °C for 20 minutes. The supernatant was washed with 2 ml of diethylether 3 times to remove excess TCA. After removing the diethylether from the mixture *in vacuo*, the sample solution was filtered by a cellulose acetate membrane filter (ϕ 0.45 μ m), then applied to an automated amino acid analyzer (JEOL JLC-300). The determination was replicated 3 times for each bunch.

4) Monoterpenes

A 50 ml sample of juice was centrifuged at 7,000 rpm for 20 minutes. The supernatant was poured into a separation funnel together with $10\,\mu l$ of 2-octanol (an internal standard) and 50 ml of dichloromethane. Monoterpenes were extracted by shaking, then centrifuged again. The supernatant was dehydrated with Na₂SO₄ anhydride, and concentrated to 0.3 ml *in vacuo*. A $1\,\mu l$ of aliquot was injected into GC. The determination was carried out for each bunch.

Results

1. Crop levels and fruit size

Leaf-fruit ratio, crop weight per canopy area, and average fresh weight of bunches and berries of each test vine are shown in Table 1. Leaf-fruit

Table 1 Crop levels and fruit size of Muscat of Alexandria grape produced in 25 commercial vineyards in Okayama

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No. of	Heated ^{a)} or	Leaf/fruit	Fruit/canopy	Avg. weight (g)		
vineyard	unheated	(m^2/kg)	(kg/m^2)	Bunch	Berry	
1	Early heated	1.01	1.40	430 ± 15	12.1 ± 0.4	
2		0.69	2.23	547 ± 15	11.9 ± 0.1	
3		0.63	1.61	520 ± 16	12.9 ± 0.2	
4		0.71	1.72	473 ± 20	10.8 ± 0.5	
5		0.46	2.32	610 ± 22	12.0 ± 0.4	
6		0.57	1.92	528 ± 13	11.6 ± 0.1	
	Avg.	0.68	1.87 b ^{b)}	518.0 b	11.90	
7	Normally heated	0.78	2.01	744 ± 13	14.3 ± 0.3	
8		0.62	2.01	579 ± 37	11.1 ± 0.7	
9		0.64	2.51	646 ± 17	12.5 ± 0.1	
10		0.70	2.66	788 ± 16	13.0 ± 0.1	
11		0.52	2.91	789 ± 27	13.2 ± 0.4	
12			2.21	547 ± 23	10.2 ± 0.4	
13		0.99	1.91	618 ± 11	13.7 ± 0.2	
14		1.14	1.40	760 ± 26	11.4 ± 0.4	
15		1.43	1.29	710 ± 17	11.6 ± 0.1	
	Avg.	0.85	2.10 a	686.6 a	12.30	
16	Unheated	1.08	2.23	608 ± 18	10.5 ± 0.1	
17		0.66	2.10	715 ± 14	11.0 ± 0.1	
18		0.87	2.89	735 ± 12	10.3 ± 0.2	
19		1.03	2.01	476 ± 18	11.4 ± 0.2	
20		0.87	2.68	574 ± 17	11.6 ± 0.3	
21		0.58	3.10	601 ± 19	11.2 ± 0.3	
22		0.70	2.91	858 ± 17	13.0 ± 0.3	
23		0.63	3.13	619 ± 17	11.5 ± 0.7	
24		0.51	3.48	707 ± 40	12.3 ± 0.7	
25		0.49	3.61	700 ± 29	11.8 ± 0.5	
	Avg.	0.74	2.81 a	669.4 a	11.40	

^{a)} Early heated; vines were heated from early December to harvest bunches in early and mid-May, normally heated; vines were heated from late or early February to harvest July and August.

ratio, crop level, and bunch weight were smaller in early heated vines than those in normally heated and unheated ones. The crop levels in several unheated vines were so excessively high that they were higher than $3 \, \text{kg/m}^2$. The average berry weight was largest in normally heated vines, which exceeded 13 grams in 4 vines.

2. Sugar and acid contents

Levels of TSS, TA, and sugar and acid fractions in berry juices are shown in Table 2. Aver-

age TSS content was lower in early heated vines and higher in unheated vines. In the vines of No. 5, 14 and 23, the TSS content did not reach 16° Brix until the berries became overripe. The fructose level tended to be lower in unheated vines than early and normally heated ones, on the other hand the glucose level was lower in early heated vines. The low TSS in the three vines, mentioned above, was as a result of lower levels of both sugar fractions. TA contents were signifi-

^{b)} Significance between the 3 means of each category, p < 0.05.

Table 2 Sugar and acid contents in berry juice of Muscat of Alexandria grapes produced in 25 commercial vineyards in Okayama (Mean ± SE)

$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	No. of vineyard	Heated ^{a)} or unheated	TSS	Titratable acidity	Fructose	Glucose	Malic acid	Tartaric acid	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$			(°Brix)	(g/100mL)	(g/100 mL)				
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	1	Early heated	16.9 ± 0.4	0.43 ± 0.01	7.1 ± 1.3	7.3 ± 0.8	0.28 ± 0.06	0.13 ± 0.01	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	2		16.9 ± 0.4	0.43 ± 0.03	7.5 ± 0.6	7.1 ± 0.5	0.24 ± 0.03	0.14 ± 0.01	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	3		17.2 ± 0.4	0.37 ± 0.01	7.7 ± 0.5	8.3 ± 0.5	0.24 ± 0.03	0.12 ± 0.01	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	4		18.8 ± 0.3	0.48 ± 0.01	8.8 ± 0.2	9.5 ± 0.3	0.27 ± 0.02	0.16 ± 0.01	
Avg. 16.86 0.43 a^{10} 7.71 8.14 0.25 a 0.13 7 Normally heated 18.4 ± 0.3 0.29 ± 0.01 8.2 ± 0.5 9.1 ± 0.6 0.16 ± 0.01 0.13 ± 0.00 8 16.6 ± 0.1 0.35 ± 0.01 7.0 ± 0.3 8.2 ± 0.2 0.21 ± 0.00 0.13 ± 0.00 9 17.6 ± 0.8 0.35 ± 0.00 8.9 ± 0.4 7.9 ± 0.2 0.17 ± 0.01 0.19 ± 0.01 10 18.1 ± 0.6 0.30 ± 0.01 8.4 ± 0.5 9.0 ± 0.5 0.14 ± 0.01 0.18 ± 0.01 11 17.7 ± 0.4 0.31 ± 0.00 7.5 ± 0.5 8.7 ± 0.7 0.16 ± 0.00 0.13 ± 0.01 12 18.7 ± 0.2 0.34 ± 0.01 7.7 ± 0.5 9.0 ± 0.7 0.15 ± 0.01 0.15 ± 0.01 13 16.7 ± 0.3 0.28 ± 0.00 7.9 ± 0.2 8.8 ± 0.2 0.12 ± 0.00 0.15 ± 0.00 14 15.5 ± 0.5 0.33 ± 0.00 6.7 ± 0.4 8.2 ± 0.3 0.14 ± 0.01 0.14 ± 0.01 15 18.6 ± 0.3 0.28 ± 0.00 7.7 ± 0.1 8.5 ± 0.3	5		15.4 ± 0.0	0.43 ± 0.01					
7 Normally heated 18.4 ± 0.3 0.29 ± 0.01 8.2 ± 0.5 9.1 ± 0.6 0.16 ± 0.01 0.13 ± 0.00 8 16.6 ± 0.1 0.35 ± 0.01 7.0 ± 0.3 8.2 ± 0.2 0.21 ± 0.00 0.13 ± 0.00 9 17.6 ± 0.8 0.35 ± 0.00 8.9 ± 0.4 7.9 ± 0.2 0.17 ± 0.01 0.19 ± 0.01 10 18.1 ± 0.6 0.30 ± 0.01 8.4 ± 0.5 9.0 ± 0.5 0.14 ± 0.01 0.18 ± 0.01 11 17.7 ± 0.4 0.31 ± 0.00 7.5 ± 0.5 8.7 ± 0.7 0.16 ± 0.00 0.13 ± 0.01 12 18.7 ± 0.2 0.34 ± 0.01 7.7 ± 0.5 9.0 ± 0.7 0.15 ± 0.01 0.15 ± 0.01 13 16.7 ± 0.3 0.28 ± 0.00 7.9 ± 0.2 8.8 ± 0.2 0.12 ± 0.00 0.15 ± 0.01 14 15.5 ± 0.5 0.33 ± 0.00 6.7 ± 0.4 8.2 ± 0.3 0.14 ± 0.01 0.14 ± 0.01 15 18.6 ± 0.3 0.28 ± 0.00 7.7 ± 0.1 8.5 ± 0.3 0.09 ± 0.00 0.13 ± 0.00 Avg. 17.53 0.31 b 7.77 8.58 <td< td=""><td>6</td><td></td><td>16.1 ± 0.5</td><td>0.44 ± 0.02</td><td>7.3 ± 0.2</td><td>8.5 ± 0.3</td><td>0.24 ± 0.02</td><td>0.12 ± 0.01</td></td<>	6		16.1 ± 0.5	0.44 ± 0.02	7.3 ± 0.2	8.5 ± 0.3	0.24 ± 0.02	0.12 ± 0.01	
$\begin{array}{c} 8 \\ 9 \\ 17.6 \pm 0.8 \\ 10.35 \pm 0.01 \\ 17.6 \pm 0.8 \\ 10.35 \pm 0.00 \\ 10.35 \pm 0.01 \\ 10.35 \pm 0.$	Avg.		16.86	0.43 a ^{b)}	7.71	8.14	0.25 a	0.13	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	7	Normally heated	18.4 ± 0.3	0.29 ± 0.01	8.2 ± 0.5	9.1 ± 0.6	0.16 ± 0.01	0.13 ± 0.00	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	8		16.6 ± 0.1	0.35 ± 0.01	7.0 ± 0.3	8.2 ± 0.2	0.21 ± 0.00	0.13 ± 0.00	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	9		17.6 ± 0.8	0.35 ± 0.00	8.9 ± 0.4	7.9 ± 0.2	0.17 ± 0.01	0.19 ± 0.01	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	10		18.1 ± 0.6	0.30 ± 0.01	8.4 ± 0.5	9.0 ± 0.5	0.14 ± 0.01	0.18 ± 0.01	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	11		17.7 ± 0.4	0.31 ± 0.00	7.5 ± 0.5	8.7 ± 0.7	0.16 ± 0.00	0.13 ± 0.01	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	12		18.7 ± 0.2	0.34 ± 0.01	7.7 ± 0.5	9.0 ± 0.7	0.15 ± 0.01	0.15 ± 0.01	
15 18.6 ± 0.3 0.28 ± 0.00 7.7 ± 0.1 8.5 ± 0.3 0.09 ± 0.00 0.13 ± 0.00 Avg. 17.53 0.31 b 7.77 8.58 0.15 b 0.15 16 Unheated 18.5 ± 0.1 0.23 ± 0.02 7.6 ± 0.5 9.0 ± 0.3 0.06 ± 0.00 0.14 ± 0.00 17 18.6 ± 0.2 0.25 ± 0.01 7.1 ± 0.5 8.9 ± 0.5 0.08 ± 0.01 0.13 ± 0.01 18 18.0 ± 0.1 0.28 ± 0.00 7.4 ± 0.2 8.9 ± 0.3 0.09 ± 0.01 0.18 ± 0.01 19 18.3 ± 0.2 0.25 ± 0.00 7.6 ± 0.2 9.1 ± 0.4 0.08 ± 0.00 0.17 ± 0.01 20 17.4 ± 0.2 0.26 ± 0.00 6.4 ± 0.0 7.4 ± 0.1 0.07 ± 0.00 0.15 ± 0.00 21 16.5 ± 0.0 0.26 ± 0.01 6.9 ± 0.7 8.4 ± 0.9 0.09 ± 0.01 0.17 ± 0.01 22 17.4 ± 0.2 0.28 ± 0.00 8.3 ± 0.2 9.0 ± 0.3 0.11 ± 0.00 0.16 ± 0.00 23 15.8 ± 0.2 0.25 ± 0.01 6.9 ± 0.1 7.6 ± 0.2 0.07 ± 0.00 0.14 ± 0.00 24 18.0 ± 0.2 0.26 ± 0.01 <	13		16.7 ± 0.3	0.28 ± 0.00	7.9 ± 0.2	8.8 ± 0.2	0.12 ± 0.00	0.15 ± 0.00	
Avg. 17.53 0.31 b 7.77 8.58 0.15 b 0.15 16 Unheated 18.5 ± 0.1 0.23 ± 0.02 7.6 ± 0.5 9.0 ± 0.3 0.06 ± 0.00 0.14 ± 0.00 17 18.6 ± 0.2 0.25 ± 0.01 7.1 ± 0.5 8.9 ± 0.5 0.08 ± 0.01 0.13 ± 0.01 18 18.0 ± 0.1 0.28 ± 0.00 7.4 ± 0.2 8.9 ± 0.3 0.09 ± 0.01 0.18 ± 0.01 19 18.3 ± 0.2 0.25 ± 0.00 7.6 ± 0.2 9.1 ± 0.4 0.08 ± 0.00 0.17 ± 0.01 20 17.4 ± 0.2 0.26 ± 0.00 6.4 ± 0.0 7.4 ± 0.1 0.07 ± 0.00 0.15 ± 0.00 21 16.5 ± 0.0 0.26 ± 0.01 6.9 ± 0.7 8.4 ± 0.9 0.09 ± 0.01 0.17 ± 0.01 22 17.4 ± 0.2 0.28 ± 0.00 8.3 ± 0.2 9.0 ± 0.3 0.11 ± 0.00 0.16 ± 0.00 23 15.8 ± 0.2 0.25 ± 0.01 6.9 ± 0.1 7.6 ± 0.2 0.07 ± 0.00 0.14 ± 0.00 24 $18.0 \pm $	14		15.5 ± 0.5	0.33 ± 0.00	6.7 ± 0.4	8.2 ± 0.3	0.14 ± 0.01	0.14 ± 0.01	
16 Unheated 18.5 ± 0.1 0.23 ± 0.02 7.6 ± 0.5 9.0 ± 0.3 0.06 ± 0.00 0.14 ± 0.00 17 18.6 ± 0.2 0.25 ± 0.01 7.1 ± 0.5 8.9 ± 0.5 0.08 ± 0.01 0.13 ± 0.01 18 18.0 ± 0.1 0.28 ± 0.00 7.4 ± 0.2 8.9 ± 0.3 0.09 ± 0.01 0.18 ± 0.01 19 18.3 ± 0.2 0.25 ± 0.00 7.6 ± 0.2 9.1 ± 0.4 0.08 ± 0.00 0.17 ± 0.01 20 17.4 ± 0.2 0.26 ± 0.00 6.4 ± 0.0 7.4 ± 0.1 0.07 ± 0.00 0.15 ± 0.00 21 16.5 ± 0.0 0.26 ± 0.01 6.9 ± 0.7 8.4 ± 0.9 0.09 ± 0.01 0.17 ± 0.01 22 17.4 ± 0.2 0.28 ± 0.00 8.3 ± 0.2 9.0 ± 0.3 0.11 ± 0.00 0.16 ± 0.00 23 15.8 ± 0.2 0.25 ± 0.01 6.9 ± 0.1 7.6 ± 0.2 0.07 ± 0.00 0.14 ± 0.00 24 18.0 ± 0.2 0.26 ± 0.01 7.7 ± 0.2 8.4 ± 0.3 0.08 ± 0.00 0.15 ± 0.01 25 19.2 ± 0.5 0.25 ± 0.00 7.9 ± 0.2 8.3 ± 0.3	15		18.6 ± 0.3	0.28 ± 0.00	7.7 ± 0.1	8.5 ± 0.3	0.09 ± 0.00	0.13 ± 0.00	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Avg.		17.53	0.31 b	7.77	8.58	0.15 b	0.15	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	16	Unheated	18.5 ± 0.1	0.23 ± 0.02	7.6 ± 0.5	9.0 ± 0.3	0.06 ± 0.00	0.14 ± 0.00	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	17		18.6 ± 0.2	0.25 ± 0.01	7.1 ± 0.5	8.9 ± 0.5	0.08 ± 0.01	0.13 ± 0.01	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	18		18.0 ± 0.1	0.28 ± 0.00	7.4 ± 0.2	8.9 ± 0.3	0.09 ± 0.01	0.18 ± 0.01	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	19		18.3 ± 0.2	0.25 ± 0.00	7.6 ± 0.2	9.1 ± 0.4	0.08 ± 0.00	0.17 ± 0.01	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	20		17.4 ± 0.2	0.26 ± 0.00	6.4 ± 0.0	7.4 ± 0.1	0.07 ± 0.00	0.15 ± 0.00	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	21		16.5 ± 0.0	0.26 ± 0.01	6.9 ± 0.7	8.4 ± 0.9	0.09 ± 0.01	0.17 ± 0.01	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	22		17.4 ± 0.2	0.28 ± 0.00	8.3 ± 0.2	9.0 ± 0.3	0.11 ± 0.00	0.16 ± 0.00	
$25 \hspace{1.5cm} 19.2 \pm 0.5 \hspace{0.25cm} 0.25 \pm 0.00 \hspace{0.25cm} 7.9 \pm 0.2 \hspace{0.25cm} 8.3 \pm 0.3 \hspace{0.25cm} 0.06 \pm 0.00 \hspace{0.25cm} 0.14 \pm 0.00$	23		15.8 ± 0.2	0.25 ± 0.01	6.9 ± 0.1	7.6 ± 0.2	0.07 ± 0.00	0.14 ± 0.00	
	24		18.0 ± 0.2	0.26 ± 0.01	7.7 ± 0.2	8.4 ± 0.3	0.08 ± 0.00	0.15 ± 0.01	
Avg. 17.78 0.26 c 7.38 8.50 0.08 c 0.15	25		19.2 ± 0.5	0.25 ± 0.00	7.9 ± 0.2	8.3 ± 0.3	0.06 ± 0.00	0.14 ± 0.00	
	Avg.		17.78	0.26 c	7.38	8.50	0.08 c	0.15	

a) Refer to Table 1.

cantly higher in early heated vines and lower in unheated vines. These differences were mainly dependent on the levels of malic acid.

3. Amino acids

Amino acid contents in harvested berry juice are shown in Table 3. Arginine was the most abundant amino acid in all test vines, followed by alanine, γ -butyric acid, proline, etc. In comparing among the heating categories, the levels of arginine, alanine, glutamine, and threonine were

highest in normally heated vines. The levels of γ -butyric acid and proline were highest in unheated vines, though that of glutamine was lowest. There was no significant difference in averaged total amino acid content between normally heated and unheated vines. In contrast to these, the total content of amino acids was much lower in early heated vines, especially in the No. 5 vine, than normally heated and unheated vines, mainly due to lower contents of arginine, alanine, γ -

b) Refer to Table 1.

Table 3 Amino acid levels (mmol/ml) in berry juice of Muscat of Alexandria grapes produced in 25 commercial vineyards in Okayama.

No. of vineyard	Heated ^{a)} or unheated	ALA	ARG	GABA	GLN	GLU	ILEU	LEU	PRO	SER	THR	OTHERS	TOTAL
1	Early heated	1.3	6.5	1.4	0.59	0.46	0.08	0.20	0.81	0.34	0.34	1.1	13.8
2		1.4	10.9	1.1	1.27	0.55	0.12	0.24	0.76	0.36	0.37	1.6	18.5
3		2.9	10.6	2.7	1.68	0.88	0.12	0.27	1.52	0.57	0.59	1.7	23.0
4		1.2	7.3	1.4	0.64	0.52	0.09	0.21	0.57	0.37	0.34	1.3	13.9
5		1.1	5.8	1.3	0.46	0.39	0.08	0.16	0.50	0.32	0.31	1.0	10.5
6		1.4	7.1	1.5	0.55	0.37	0.09	0.19	0.55	0.32	0.27	1.2	13.6
Avg.		1.6 b ^{b)}	8.0	1.6	0.87 a	0.53	0.10	0.21 b	0.79 b	0.38	0.37 b	1.3 c	15.6
7	Normally heated	2.1	8.4	1.8	0.60	0.37	0.10	0.25	1.61	0.40	0.42	1.4	17.5
8		2.5	8.2	1.8	1.58	0.82	0.09	0.18	1.36	0.58	0.54	1.8	19.4
9		1.8	9.2	1.0	1.03	0.54	0.10	0.18	0.59	0.48	0.47	1.8	17.3
10		2.8	9.2	2.1	1.10	0.58	0.10	0.22	1.11	0.46	0.46	1.5	19.5
11		2.2	9.5	2.0	1.27	0.56	0.12	0.25	1.39	0.43	0.62	1.6	21.8
12		1.8	9.2	1.4	0.55	0.43	0.12	0.24	0.99	0.43	0.48	1.6	17.1
13		3.0	11.3	2.0	0.99	0.76	0.14	0.34	1.44	0.48	0.56	2.1	23.1
14		3.1	8.6	1.8	1.57	0.75	0.06	0.14	0.69	0.37	0.33	1.3	18.8
15		2.3	9.8	1.7	0.40	0.53	0.14	0.29	1.19	0.46	0.47	1.8	19.0
Avg.		2.4 a	9.3	1.7	1.01 a	0.59	0.11	0.23 b	1.15 b	0.45	0.48 a	1.7 b	19.3
16	Unheated	1.4	5.9	1.7	0.20	0.54	0.10	0.21	1.48	0.34	0.31	1.3	13.7
17		3.4	9.0	2.6	0.58	0.80	0.16	0.35	2.55	0.64	0.50	2.5	23.3
18		1.6	8.8	1.7	0.44	0.75	0.10	0.25	1.64	0.38	0.35	2.0	18.4
19		1.6	9.4	1.7	0.33	0.58	0.13	0.28	1.71	0.39	0.36	2.0	18.6
20		1.7	10.5	1.8	0.42	0.51	0.14	0.35	2.33	0.42	0.41	2.3	21.0
21		2.0	8.2	2.7	0.33	0.37	0.12	0.29	2.03	0.40	0.36	1.9	18.9
22		2.4	9.9	2.3	0.49	0.51	0.12	0.30	2.19	0.53	0.39	2.3	21.7
23		1.9	8.7	1.6	0.37	0.53	0.10	0.26	1.87	0.38	0.30	1.7	18.0
24		2.2	8.7	2.7	0.32	0.45	0.12	0.27	1.65	0.45	0.39	2.0	19.3
25		1.3	6.6	1.6	0.28	0.38	0.11	0.27	1.68	0.35	0.29	1.7	14.2
Avg.		2.0 ab	8.6	2.0	0.38 b	0.54	0.12	0.28 a	1.91 a	0.43	0.37 b	2.0 a	18.7

a) Refer to Table 1.

butyric acid, proline, and other minor fractions.

4. Monoterpenes

Levels of 3 major monoterpenes, linalool, geraniol, and nerol, are shown in Fig. 1. Wide variations in the levels of each monoterpene were observed among heating categories. It can be noted, however, that the levels of geraniol and nerol were significantly higher in early heated vines than normally heated and unheated vines.

Discussion

Analytical data on chemical properties of soil, size of vines, shoots, and bunches in vineyards employed for this research project have been already published¹²⁾. In this report, chemical compositions of harvested fruit are mainly shown to discuss the variation in fruit flavor of Muscat of Alexandria grapes produced in Okayama Prefecture.

Flavor of grape berries is influenced by many

b) Refer to Table 1.

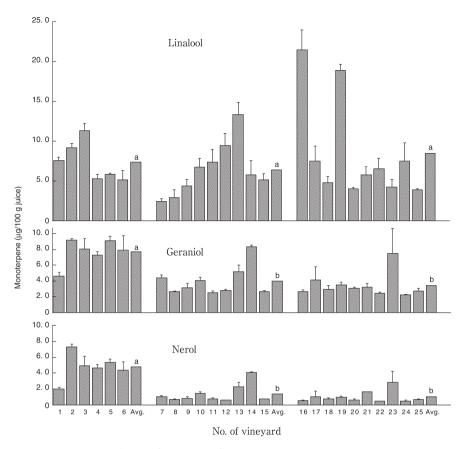


Fig. 1 Monoterpene levels in berry juice of Muscat of Alexandria grapes produced in 25 commercial vineyards in Okayama. Vineyards No. 1-6 are early heated, No. 7-15 normally heated, and No. 16-25 unheated. Vertical bars represent SE. Means of each monoterpene level were compared among 3 vineyard categories by DMRT at p < 0.05.

factors including taste, aroma, texture, juiciness, etc. Among these the most prominent may be the sweetness and sourness of the juice, that are mainly dominated by the levels of sugars and free acids, respectively. In this study, bunches were sampled when more than half of them had been harvested from each vine. In most vines, except for the vines No. 5, 14, and 23, juice TSS had reached around 17° Brix or higher, which indicates that the bunches in most vines were ripening normally. However, In the three vines TSS levels did not reach 16° Brix even at the over ripe stage. The reason why the berries in their vines did not ripen normally is unclear, since neither leaf-fruit ratio nor crop load per unit canopy area were abnormal. It is a serious problem that such substandard bunches are shipped commercially.

Considerable differences are found in the levels of fructose and glucose, the major sugar constituents in grape berries. The low fructose levels in berries of most unheated vines may be a result of high crop load levels or low leaf/fruit ratios as observed in No. 18, 20, 21, 23, 24, and 25 vines. Kliewer et al.8,9,10) carried out a vast scale of research on crop levels of grapevines affecting fruit growth and quality. They concluded that leaf-fruit ratio needed to produce high quality berries fell in the range between 0.8-1.2 m²/kg in most grape cultivars. For Muscat of Alexandria grapes, leaf-fruit ratios of 0.8-0.9 m²/kg and fruitcanopy area ratios of around $2.1 \,\mathrm{kg/m^2}$ are recommended16). Values of leaf-fruit ratio and fruit-canopy area ratio, obtained in this study, are mostly insufficient and excessive, respectively. Berries containing low levels of fructose must have a bland taste because sweetness of fructose is higher than that of glucose⁵⁾.

It is highly interesting that significantly high levels of malic acid were detected in berries from early heated vines in contrast to the low levels in those from unheated ones. Malic acid is metabolized as a respiratory substrate during fruit maturation and the rate is largely dependent on temperature conditions. Fruit ripens during April and May in early heated vines and during August and September in unheated ones. Furthermore, early heated bunches are apt to be shipped early for obtaining high market prices. The different levels in malic acid found between heating categories will probably have a significant effect on juice sourness of Muscat of Alexandria grapes.

Amino acids in fruit juice influence the taste in a complicated manner. It is generally known that alanine and proline increase the sweetness of the fruit taste, glutamine and glutamic acid deepen the delicious taste, and arginine gives the bitter taste. However, Jia et al.7, who tested the effects of amino acid concentrations on the taste of peach juice, reported that arginine, asparagine, and serine increase the sweetness of the juice, but they increase bitterness at very high concentrations under high N applications. Hirano et al.3) reported that arginine, histidine, leucine, valine, phenylalanine, and isoleucine reduce the sourness and/or increase the sweetness of Muscat of Alexandria berry juice, though they taste bitter in each water solution. They also noted that threonine reduces the sweetness of the juice at a concentration higher than 500 nmol/mL. In this study the low concentrations of alanine and proline in most early heated vines may make their taste unsavory, as well as the higher level of malic acid compared to those in normally heated and unheated ones. Berries in most normally heated vines contained high levels of alanine and glutamine, which improves the taste. However, some of the vines, No. 8, 11, and 13, contained threonine at higher than 0.5 mmol/mL, which may reduce the sweetness as noted by Hirano *et al.*⁴). On the other hand, unheated vines contained a high level of proline but low levels of glutamine. These facts, added to the low levels of malic acid, result in a sweet but flat taste compared to those from normally heated ones.

Amino acid levels in fruits usually coincide with N fertilizer application level 2,4,6,7,15). However, there was no significant relationship between juice amino acid levels and soil N levels. For examples, amino acid level was significantly lower in early heated vines than the other vines, though both HNO₃- and NH₄OH-N levels in soil were much higher in early heated vineyards 12). Furthermore, shoot development by means of shoot length, leaf size, and lateral shoot size, were significantly smaller than the other vines. From these results, it is suggested that the low concentrations of amino acids in early heated vines may be due to the vine physiology such as inactive N absorption by roots and/or translocation of nitrogenous compounds into shoots and bunches.

Aromatic substances evolved from berries and juices are highly attractive factors in Muscat of Alexandria grapes^{1,3,11,13,14)}. However, Hirano et al. reported that linalool, giving floral smell, extracted from Muscat of Alexandria berry juice increased rapidly from 30 days after veraison until late September, then decreased. Furthermore, in our previous work, we noted that linalool, geraniol, and nerol evolving from the berries increased until 2 days after sampling, then decreased rapidly¹¹⁾. These facts suggest that monoterpene levels in grape berries are critically unstable at the ripening stage, which may be the main reason for the wide variations of linalool levels in our current study. However, it is most interesting that higher levels of geraniol and nerol were analyzed in most early heated vines than in most of the other vines. These facts also suggest the specific or abnormal physiology in early heated vines or berries, though the mechanisms producing such big differences are unknown. Geraniol and nerol give a rosy and light floral smell, respectively. Berries harvested from early heated vines are thought to have a somewhat strong smell with a rosy aroma compared to those from normally heated and unheated vines. The significance of high linalool levels detected from berries of No. 16, 19, and 13 vines in relation to human perception must be investigated.

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岡山産のマスカット・オブ・アレキサンドリア果実に含まれる 糖、酸、アミノ酸、モノテルペン含量のばらつき

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岡山県で生産されるマスカット・オブ・アレキサンドリア果実の味のばらつきを検討するために、ガラス 室またはビニールハウス25園(早期加温6園,普通加温9園,無加温10園)で収穫された果房について、果 汁に含まれる糖、酸、アミノ酸、モノテルペン含量を分析した、各樹の収穫盛期に果実をサンプリングした が、各作型の1園ずつでは可溶性固形物含量 (TSS) が最低基準の16°付近に達していなかった。これらを除 くと、早期加温樹ではグルコース含量が特に低いものがあり、無加温樹ではフラクトース濃度が低い傾向で あった。リンゴ酸濃度は早期加温樹で高く、無加温樹で低かったが、酒石酸濃度には作型や樹による差が少 なかった。アミノ酸濃度には樹による差が大きかったが、早期加温樹では全アミノ酸、アラニン、 γ -アミノ 酪酸、プロリンが他の作型よりも低い傾向であった。また、無加温樹ではプロリンは他の作型の樹よりも高 い樹が多かったが、グルタミンは著しく低かった。モノテルペンの内、マスカット香の主成分であるリナロ ール含量は、樹による差が非常に大きかったが、第2主要成分であるゲラニオールは早期加温樹で高かった。 これらの果汁成分の相違が果実の味に及ぼす影響について考察した.