

[Research Note]

Effect of Additional GA₃ Application 10–15 Days after Full Bloom on Berry Skin Fracture and Flesh Firmness at Harvest Time in Shine Muscat Grapes

Koji OIDA¹, Motoko MATSUI^{2*}, Shoi OHBA²,
Yukari MURAMOTO³, Kimiko OHTANI² and Hino MOTOSUGI⁴

¹Tango Agriculture Research Division, Kyoto Prefectural Agriculture, Forestry and Fisheries Technology Center,
488 Kurobe, Yasaka-cho, Kyotango-shi 627-0142, Japan

²Graduate School of Life and Environmental Science, Kyoto Prefectural University,
1-5 Shimogamohangi-cho, Sakyo-ku, Kyoto-shi 606-8522, Japan

³Division of Applied Life Sciences, Kyoto Prefectural University,
1-5 Shimogamohangi-cho, Sakyo-ku, Kyoto-shi 606-8522, Japan

⁴University Farm, Faculty of Life and Environmental Sciences, Kyoto Prefectural University,
74 Kitainayazmaohji, Seika-cho, Soraku-gun 619-0244, Japan

In Japan, many cultivars of table grapes are consumed by removing the grape berry's skin and eating only the flesh. 'Shine Muscat' (*Vitis labruscana* Bailey × *V. vinifera* L.) seedless grapes are obtained by applications of gibberellic acid (GA₃) at 25 ppm 0–3 days after full bloom (DAFB). Their size is increased by applications of GA₃ at 10–15 DAFB. These grapes are eaten whole with the skin by Japanese consumers. To research the relationship between the application of GA₃ and the palatability of the unpeeled grape berries, we studied the effects of the second application of GA₃ at different concentrations on the palatability of the unpeeled berry. Our findings revealed that the second application of GA₃ at 25 ppm increased the berry size and made the berries crisper compared to the use of GA₃ at 12.5 ppm and those without the second application of GA₃ at 10 or 15 DAFB. There were no differences in fruit composition or texture between the second application of GA₃ at 10 or 15 DAFB. In the instrumental texture analysis and sensory evaluation, 25-ppm GA₃-treated berries were easier to eat with the skin intact than those without the second application of GA₃ at 10 or 15 DAFB. We concluded that compared with the berries not treated with GA₃ at 10 or 15 DAFB, the second application of GA₃ at 25 ppm at 10–15 DAFB improved the berry texture such that the flesh was crisp, and the berry was easy to eat with the skin.

Keywords: gibberellic acid, plant growth regulators, table grape, texture analysis

Introduction

Grapes, one of the world's largest fruit crops with approx. 74 million tons produced each year, are one of the

most diffused fruits (International Organisation of Vine and Wine, 2016). In Japan, there is considerable rainfall during the berry growing season, and *V. labruscana* cultivars have been grown primarily as table grapes (Sato, Yamada 2003). Almost all Japanese peel the skin from these grapes and eat only the flesh. Sato et al. (1997) had

* Corresponding author. Email: motokomh@kpu.ac.jp
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classified the texture of many *V. labruscana* cultivars' berries as 'tough' or 'tender,' and Sato et al. (2004) described 'tough' as firm but difficult to masticate, and 'tender' as easily breakable but soft. The skin of many *V. labruscana* cultivars' berries is thick.

Japan's National Institute of Fruit Tree Science developed and released the table grape cultivar 'Shine Muscat' (*V. labruscana* Bailey × *V. vinifera* L.), which has crisp flesh texture, muscat flavor, a high concentration of soluble solids, and low acidity (Yamada et al. 2008). Shine Muscat grape berries have been eaten whole by Japanese consumers, without peeling the skin. There is an increasing demand by Japanese consumers for table grapes that are not only seedless but can also be eaten whole.

Sensory characteristics such as skin friability, skin thickness, and crispness have been proposed for the descriptive profiling of commercial table grape cultivars (Cliff et al. 1996). For table grapes, the berry texture depends on numerous factors including the cultivar, ripening stage, environmental variation, treatment with plant growth regulators, and cultural practices (Cefola et al. 2011; Mochida et al. 2014; Río Segade et al. 2013; Rolle et al. 2013; Zsófi et al. 2014). To meet consumer needs, cultivation techniques need to be established to improve the flesh texture and palatability of unpeeled grapes.

The plant growth regulator gibberellic acid (GA_3) has been used extensively to increase the berry set and size in genetically seedless cultivars (Shiozaki et al. 1998; Weaver, McCune 1959a, b) and induce seedlessness in seeded cultivars (Itakura et al. 1965; Kishi, Tazaki 1960). Nagata and Kurihara (1982) reported that there were varietal differences in response to GA_3 application; other studies have reported that the effect of GA_3 application varied at different berry growth stages (Ishikawa, Baba 2004; Mochida, Kurahashi 2010). Sato et al. (2004) reported that GA_3 application made berries' flesh firmer and improved the flesh texture.

In many Japanese table grape cultivars, GA_3 is applied twice for the production of seedless berries. The first application of GA_3 at full bloom to 3 days after full bloom (DAFB) induces seedlessness, and the second application of GA_3 at 10–15 DAFB increases the berry size for some

diploid and tetraploid table grape cultivars including Shine Muscat (Kyowa Hakko Bio Co. 2016). Although GA_3 has been commonly used to improve berry size, there are few studies regarding the use of GA_3 to improve the palatability of Shine Muscat unpeeled grape berries.

We conducted the present study to investigate the effects of the second GA_3 application at 10–15 DAFB on the palatability of unpeeled Shine Muscat berries. We evaluated the effects of the second GA_3 application on fruit composition and texture by conducting an instrumental texture analysis and sensory evaluation of the berries.

Materials and Methods

This study was performed at the Tango Agricultural Research Division, Kyoto Prefectural Agriculture, Forestry and Fisheries Technology Center, Japan (35° 40' 27.4" N; 135° 05' 50.0" E) in 2014. This study was conducted using a grapevine in which 9-year-old Shine Muscat was grafted on Teleki-kober 5BB rootstock trained with a spur-pruned cordon (one-side 6-m primary scaffold branch); all current canes were pruned to a few basal buds in winter. For rain protection, the grapevines were planted in a plastic house, the sides of which were not covered.

Before bloom initiation, the shoots were reduced to 36 shoots per 3-m primary scaffold branch and were trained on trellis wires. The flower clusters were thinned to one cluster per shoot and were trimmed to leave 4 cm at the top of each flower cluster (Fig. 1). At the same time, the

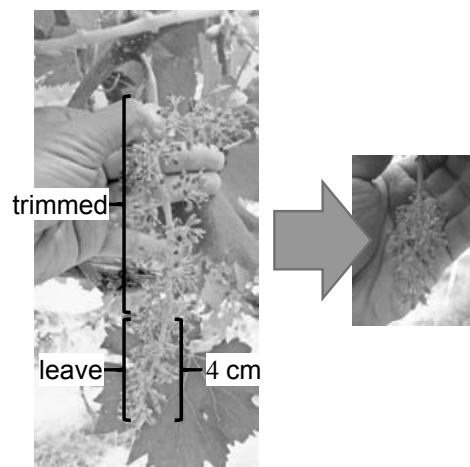


Fig. 1. The trimmed flower cluster. The flower clusters were trimmed to leave 4 cm at the top.



Fig. 2. Dipping a flower cluster in GA₃ solution.

shoot length was restricted with 10 primary leaves by pinching off shoot-tips. To induce seedlessness, the flower clusters were sprayed with a 200-ppm streptomycin solution at the early flowering period. One day after full bloom, the flower clusters were dipped in 25 ppm GA₃ solution (Gibberellin-Kyowa-Powder, Kyowa Hakko Bio Co., Tokyo) added with 2 ppm forchlorfenuron (Fulmet-Liquid, Kyowa Hakko Bio Co., Tokyo) (Fig. 2). Once berry setting was confirmed, the fruit clusters were reduced to 20 clusters per 3-m primary scaffold branch, and the berries were thinned to approx. 40 berries per fruit cluster.

We then tested four treatments for the secondary GA₃ application: (i) At 10 DAFB (10d), dipping the fruit clusters in 25-ppm GA₃ solution (10d-25); (ii) At 15 DAFB, dipping fruit clusters in 25-ppm GA₃ solution (15d-25); (iii) At 10 DAFB, dipping fruit clusters in 12.5-ppm GA₃ solution (10d-12.5); and (iv) no GA₃ applied (untreated). The experimental design was randomized in one grapevine. After the second GA₃ application, fruit clusters were bagged with commercially available paper bags (Grape 20 (white), Kobayashi Bag MFG Co., Nagano, Japan) to protect the fruit clusters from diseases as Japanese growers do normally. Thereafter, the lateral shoots were removed leaving only the first leaf, until harvest.

Instrumental texture analysis

The physical properties of the berries were measured using a creep meter (RE-2-3305B, Yamaden Co., Tokyo) with an automatic analyzer (CA-3305, Yamaden Co.,

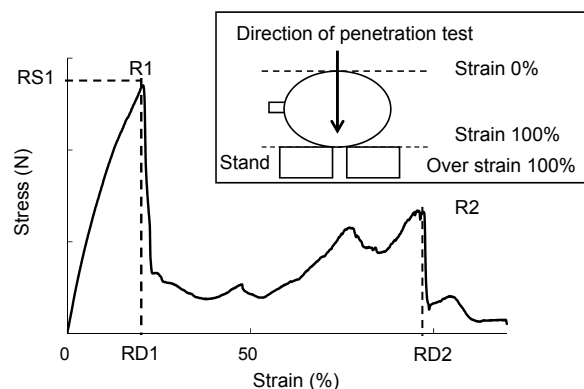


Fig. 3. Example of texture analysis of a whole grape berry using a creep meter (Oida et al. 2017). R1: First fracture point (the plunger penetrated the upper skin). RS1: First fracture stress (stress of the first fracture point). RD1: First fracture strain (strain of the first fracture strain). R2: Second fracture point (the plunger penetrated the bottom skin). RD2: Second fracture strain (strain of the second fracture strain). The flesh firmness was determined as the average stress from the first fracture peak to the second fracture peak when the plunger penetrated sample flesh.

Tokyo). Four fruit clusters that were in full bloom on June 2, 2014 were randomly harvested from each treatment group on September 9 2014, and eight berries were collected from the middle of each cluster. A whole berry was placed on a stand with a hole in the center. Each berry was penetrated at the equator by a plastic plunger with a 5-mm dia. at a penetration rate of 1 mm·s⁻¹.

The point of the first stress peak, when the plunger penetrated the upper skin of the sample berry, was recorded on the stress as the first fracture stress (RS1) and on the strain as the first fracture strain (RD1). Sato et al. (1997) defined ‘crisp’ as high maximum force (N) and low deformation at the first major peak (m) indicating firm and easy breakdown of the berry. In this evaluation method, when a berry had a high RS1 and a low RD1, the texture was described as ‘crisp.’ Conversely, a high RD1 indicated that the berry texture was ‘tough.’ The strain value that was obtained when the plunger penetrated the lower skin of the sample berry was recorded as the second fracture strain (RD2). If a berry had a high RD2, it was judged to be difficult to eat with the skin intact. The flesh firmness was determined as the average stress from the first fracture peak to the second fracture peak as the plunger penetrated the sample flesh.

Fruit composition

Four fruit clusters that were full bloom on June 2, 2014 were randomly collected from each treatment group on September 3, 2014. Ten berry samples were randomly collected from each cluster and used to determine the berry weight, and the juice was used to determine the soluble solid content (SSC) and titratable acidity (TA). The SSC was determined using a refractometer (Master- α , Atago Co., Tokyo). The TA was determined by titrating 10 mL of the juice with 0.1 N NaOH.

Sensory tests

Sensory tests were performed to investigate the effects of the GA₃ applications on berry taste and texture. The tests were performed by a total of 15 panelists in two sessions: September 2 (eight panelists who were staff of the berry tree section of Tango Agricultural Research Division) and September 5 (seven panelists who were university faculty members and students), 2014. Berry samples that were full bloom on June 3, 2014 were collected in the morning of the test days.

Before the test, sampled berries of a moderate size and skin color were selected for each treatment. In the tests, 10d-25 was used as the standard. The panelists first consumed a standard whole berry with the skin, followed by a berry from a test treatment. The panelists compared the berries from these two treatments and assigned a score of

–3 (very bad) to +3 (very good) points to the test treatment. The evaluation items were taste, Muscat flavor, and texture (crispness, skin thinness during mastication, and total texture evaluation considering the palatability of unpeeled berry) (Table 1).

Statistical analysis

All statistical analyses were performed using the statistical analysis software for Windows (Ekuseru-Toukei 2012, Social Survey Research Information Co., Tokyo). The fruit composition and texture analysis data are expressed in units of fruit clusters (n=4) and not per berry. The Tukey test was used to establish statistical differences between means for texture properties and fruit composition. The Steel-Dwass test was used to establish statistical differences between means for the sensory test.

Results

Instrumental texture analysis

The RS1 analysis results are shown in Figure 4. While there was no significant difference among treatments, the RS1 of the untreated berries tended to be lower than that of the 10d-25- and 15d-25-treated berries. The RD1 analysis results are shown in Figure 5; the higher the GA₃ concentration, the lower the RD1. The RD1 of the 10d-25- and 15d-25-treated berries were significantly lower than that of the untreated berries. The RD2 analysis results

Table 1. The evaluation items of sensory test and score

Evaluation item	Score						
	-3	-2	-1	0	+1	+2	+3
Taste	very poor	moderately poor	slightly poor	same as standard	slightly good	moderately good	very good
Muscat flavor	very less flavor	moderately less flavor	slightly less flavor	same as standard	slightly more flavor	moderately more flavor	very much flavor
Crispness	very non-crisp or tough	moderately non-crisp or tough	slightly non-crisp or tough	same as standard	slightly crisp	moderately crisp	very crisp
Skin thinness during mastication	very thick	moderately thick	slightly thick	same as standard	slightly thin	moderately thin	very thin
Total texture evaluation considering the edibility with the skin	very bad	moderately bad	slightly bad	same as standard	slightly good	moderately good	very good

are shown in Figure 6. The RD2 values of the 10d-25- and 15d-25-treated berries were <100% and were lower than those of the other treatments. The RD2 values of the 10d-12.5-treated berries were >100% but were lower than those of the untreated berries.

The flesh firmness results are shown in Figure 7. The

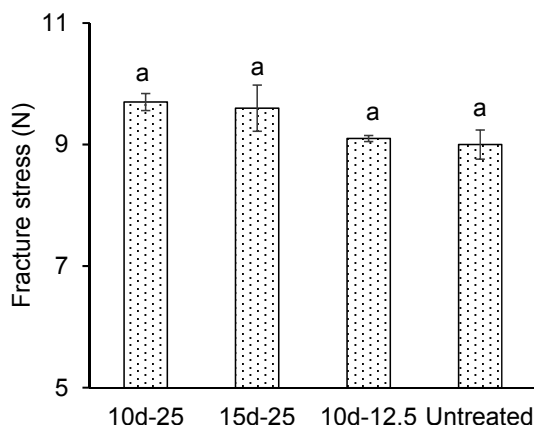


Fig. 4. Effect of the second GA₃ application on the first fracture stress (RS1) of a whole berry (n=4). Vertical bars represent SE. Different letters within the same column indicate significant differences according to Tukey test ($p<0.05$). 10d-25: Dipping fruit clusters in 25 ppm GA₃ solution at 10 DAFB. 15d-25: Dipping fruit clusters in 25 ppm GA₃ solution at 15 DAFB. 10d-12.5: Dipping fruit clusters in 12.5 ppm GA₃ solution at 10 DAFB. Untreated: no GA₃ applied at 10 to 15 DAFB. DAFB: days after full bloom. GA₃: gibberellic acid.

flesh firmness of the 10d-25- and 15d-25-treated berries was significantly firmer than that of the berries in the other treatment groups. The flesh firmness of the 10d-12.5-treated berries was slightly higher than that of the untreated berries.

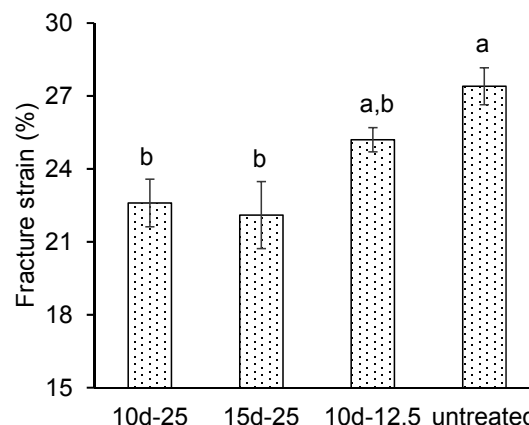


Fig. 5. Effect of the second GA₃ application on the first fracture strain (RD1) of a whole berry (n=4). Four treatments as shown in Fig. 4 are performed. Vertical bars: SE. Different letters within the same column indicate significant differences according to Tukey test ($p<0.05$).

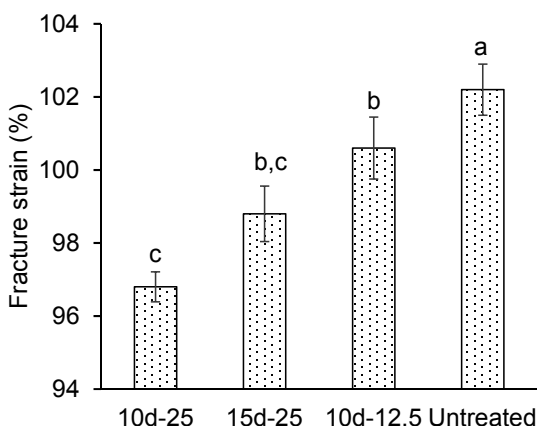


Fig. 6. Effect of the second GA₃ application on the second fracture strain (RD2) of a whole berry (n=4). Four treatments as shown in Fig. 4 are performed. Vertical bars: SE. Different letters within the same column indicate significant differences according to Tukey test ($p<0.05$).

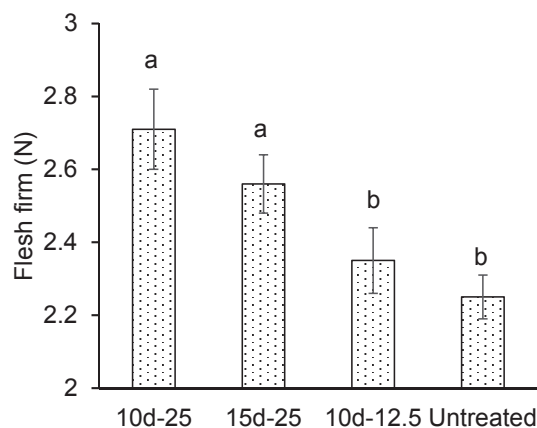


Fig. 7. Effect the second GA₃ application on the flesh firmness (n=4). Four treatments as shown in Fig. 4 are performed. Vertical bars: SE. Different letters within the same column indicate significant differences according to Tukey test ($p<0.05$).

Fruit composition

The effects of the second GA₃ application on the berry weight, SSC, and TA are shown in Table 2. The berry weight of the untreated berries was significantly lower than that of the berries in the other treatment groups. The weight of the 10d-12.5 berries was slightly lower than that of the 10d-25 treatment group. There was no significant difference in berry weight between the 10d-25 and 15d-25 treatments. However, the SSC of the untreated berries was significantly higher than that of the berries in the other treatment groups; the SSC values of the 10d-25, 15d-25, and 10d-12.5 treatment groups were almost the same. There were no significant differences in TA among any of the treatment groups.

Table 2. Effect of the second GA application of berry weight, soluble solids concentration (SSC), and titratable acidity (TA)

Treatment	Berry weight (g)	SSC (Brix°)	TA (%)
10d-25	14.9 a ^z	19.3 b	0.22 a
15d-25	14.8 a	18.9 b	0.22 a
10d-12.5	14.1 a	19.1 b	0.23 a
Untreated	11.5 b	20.4 a	0.21 a

Four treatments as shown in Fig. 4 are performed.

^zDifferent letters within the same column indicate significant differences according to Tukey test ($p < 0.05$) ($n = 4$).

Sensory tests

Table 3 summarizes the results of the panel evaluation. The evaluations of taste and Muscat flavor did not differ significantly among the treatments. The scores of crispness in berries of the 10d-12.5 treatment group and the untreated group were significantly lower than those of the berries in the 10d-25 group. Similarly, the scores of skin thinness during the mastication of berries in the 10d-12.5 and untreated groups were significantly lower than for those in the 10d-25 group. Thus, the total texture evaluation score of the untreated berries was the lowest.

Discussion

There were no significant differences in fruit composition or texture between the second GA₃ application at different growth stages (10 or 15 DAFB) in the Shine Muscat cultivar (Tables 2 and 3). Both of the second GA₃ applications increased the berry size relative to the berries that were not given the second GA₃ application (Table 2). The SSC in the untreated berries was higher than that in the berries of the other treatment groups. The smaller size of the untreated berries compared to the berries of the other treatment groups suggests that not only the GA₃ but also the berry size affected the SSC.

In the instrumental texture analysis, higher RS1 and lower RD1 values were recorded for the berries treated with 25 ppm GA₃ compared to the untreated berries (Figs. 4 and 5). The physical properties of the berries treated with 12.5 ppm GA₃ were between those treated with 25

Table 3. Effect of the second GA₃ application on sensory test score of a whole berry with the skin

Treatment	Taste	Muscat flavor	Crisp	Skin thinness during mastication	Total texture evaluation considering the edibility with the skin
10d-25 ^z	0.00 a ^y	0.00 a	0.00 a	0.00 a	0.00 a
15d-25	-0.27 a	0.00 a	-0.13 ab	-0.33 ab	-0.27 a
10d-12.5	-0.80 a	-0.27 a	-1.07 bc	-1.07 bc	-1.20 b
Untreated	0.27 a	0.47 a	-1.20 c	-1.20 c	-1.33 b

Four treatments as shown in Fig. 4 are performed. The scores of each attributes among the treatments were compared with the 10d-25 treatment as a standard ($n = 15$).

^zAs standards, all 10d-25 scores were set to 0.00.

^yDifferent letters within the same column indicate significant differences according to Steel-Dwass test ($p < 0.05$).

ppm GA₃ and the untreated berries (Figs. 5-7). This finding indicates that the second GA₃ application made the berries crisper than the berries that were not treated with a second GA₃ application. These results suggested that the higher the GA₃ concentration, the greater the effect of the GA₃ application.

Conversely, the absence of the second GA₃ application left the berries tough. Sato et al. (2004) reported that GA₃ application effectively increased the flesh firmness of Shine Muscat grape berries; this is consistent with the results of the present study. In our sensory evaluation, the crispness scores of the berries treated with 25 ppm GA₃ were higher than those of the untreated berries (Table 3). The scores of the 12.5 ppm GA₃-treated berries were similar to those of the untreated berries. During the measurement of RD2 for the 25 ppm GA₃-treated berries, we observed that before the plunger touched the bottom skin of the berry, the skin was torn by the pressure exerted upon the flesh by the plunger. This behavior was not observed in the untreated berries; in these the bottom skin of the berry was directly penetrated by the plunger. The tearing of the bottom skin as described for the 25 ppm GA₃-treated berries resulted in RD2 values <100% (Fig. 6). Matsui found that the RD2 of an easily eaten whole berry with the skin intact was <100% (unpubl. observation). As the RD2 values of most 25 ppm GA₃-treated berries were <100%, the whole berry with the skin was evaluated as being friable and easy to eat. However, the RD2 of most of the untreated berries was >100%, so these berries were evaluated as being difficult to eat with the skin intact. In the sensory evaluation, the skin thinness during mastication and the total texture evaluation scores of the 25 ppm GA₃-treated berries were higher than those of the 12.5 ppm GA₃-treated and untreated berries (Table 3). Thus, the results of the sensory evaluation largely agreed with the physical properties determined through the mechanical analysis. This finding indicated that the GA₃ application at 10 or 15 DAFB improved the texture and palatability of unpeeled Shine Muscat grapes.

In the sensory test, five panelists reported that the skin separated more readily from the flesh in the untreated berries compared to the berries from the other treatment

groups; this led to swallowing of only the flesh of the untreated berries with the skin retained in the mouth. Skin separation from flesh might affect the palatability of unpeeled grapes, and this is consistent with the results of the present study (Yamada et al. 2008). In white peaches, it was observed that an abscission layer appeared, and it became easier to peel off the skin as the fruit matured (Oguma et al. 1992). Sato et al. (2004) suggested that a GA₃ application affects the cell wall composition in berries. The second GA₃ application may affect the adhesion of the skin to the flesh.

We did not investigate the strength of the berry skin in the present study, and thus it could not be determined whether the second GA₃ application affected skin strength. Our results suggest that the palatability of unpeeled berries was affected by not only skin strength (Ayabe et al. 2009) but also by crispness and skin friability. The second GA₃ application of 25 ppm GA₃ did not spoil the palatability of unpeeled Shine Muscat berry.

Our findings showed that the second GA₃ application of 25 ppm GA₃ improved the berry texture, and all of these grapes were eaten by the panelists without peeling the skin. The effects of GA₃ on skin strength and adhesion between the flesh and skin remain to be investigated.

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ブドウ“シャインマスカット”における無核果生産のための 2回目ジベレリン処理が果粒の皮ごと食べやすさに及ぼす影響

笈田幸治¹・松井元子^{2*}・大場将生²・村元由佳利³・大谷貴美子²・本杉日野⁴

¹京都府農林水産技術センター農林センター丹後特産部 〒627-0142 京丹後市弥栄町488

²京都府立大学院生命環境科学研究科 〒606-8522 京都市左京区下鴨半木町1-5

³京都府立学生命環境学部 〒606-8522 京都市左京区下鴨半木町1-5

⁴京都府立大学生命環境科学研究科附属精華農場 〒619-0244 相楽郡精華町北稻八間大路74

摘 要

日本において“巨峰”，“デラウェア”などの栽培されているブドウ品種の果粒の多くは，皮を剥いてから食べられる．“シャインマスカット”は皮ごと食べるブドウとして普及しているが，皮ごと食べやすさは栽培条件によって変化する．そこで，満開10日後から15日後に果粒肥大を目的として処理される無核果生産体系の2回目ジベレリン処理（GA₃）の処理時期，処理の有無，または処理濃度が果粒の皮

ごと食べやすさに及ぼす影響について検討した．処理時期が満開10日後と満開15日後では果実品質およびテクスチャーに差はなかった．一方で，GA₃濃度を25 ppmで処理した果房では，12.5 ppmで処理した果房および無処理果房と比較して果粒が大きくなり，クリスプ感が増大した．物性分析と官能評価によって，満開10日後から15日後に25 ppmでGA₃を果房に処理することで，果肉が硬くなり，皮ごと食べやすくなることが明らかとなった．