Influence of Temperature on Berry Composition of Interspecific Hybrid Wine Grape ‘Kadainou R-1’ (\textit{Vitis} \textit{ficifolia} var. \textit{ganebu} × \textit{V. vinifera} ‘Muscat of Alexandria’)

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High temperature affects berry composition, especially titratable acidity, total soluble solids, and anthocyanin content. \textit{Vitis} \textit{ficifolia} var. \textit{ganebu}, a wild grape of sub-tropical origin with a low chilling trait, develops good coloration in its natural habitat, where daytime and nighttime temperatures are high during the berry ripening stage, while \textit{V. vinifera} ‘Muscat of Alexandria’, a table grape, has large berries and high sugar content, hence, it was hypothesized that hybridizing \textit{V. ficifolia} var. \textit{genebu} with \textit{V. vinifera} would improve the sugar content and reduce titratable acidity compared to \textit{V. ficifolia} var. \textit{ganebu} and retain berry color even under high temperature conditions. The aim of this study was to investigate the influence of temperature on the berry composition of ‘Kadainou R-1’, an interspecific hybrid wine grape derived from \textit{V. ficifolia} var. \textit{genebu} × \textit{V. vinifera} ‘Muscat of Alexandria’. Potted ‘Kadainou R-1’ vines with their ownroots were subjected to continuous temperatures of 20, 25, and 30°C in a phytotron during the berry-softening stage. Berries were harvested 15, 30, and 37 days after the vines were placed in the phytotron. Titratable acidity was lower at 30°C than at 20°C, while total soluble solids were highest at 25°C. Accumulations of glucose and fructose were higher at low rather than high temperatures. Total anthocyanin content (mg·g\textsuperscript{-1} of fresh weight) was significantly reduced at 20 and 30°C but not at 25°C. Flavonol content was highest at 25°C. The present study revealed that ‘Kadainou R-1’ grapes can produce optimum berry quality in locations where the night temperature reaches 25°C without significant loss in berry quality.

Key Words: anthocyanin, flavonols, ‘Kadainou R-1’, temperature effect, wine grape.

Introduction

High temperatures affect photosynthesis, thereby decreasing berry growth of grapes (Kliwer, 1977b; Kobayashi et al., 1965c). High temperature conditions are considered to contribute to lower total soluble solids and titratable acidity (Buttrose et al., 1971; Kobayashi et al., 1965b). High temperature also inhibits anthocyanin formation and alters anthocyanin composition (Mori et al., 2005; Mori et al., 2007; Yamane et al., 2006).

\textit{Vitis} \textit{ficifolia} var. \textit{ganebu} is native to subtropical regions of Japan and is found in the Ryukyu Islands of the Okinawa region (Nakagawa et al., 1991), where the average annual temperature is approximately 23°C. Even during the winter, the temperature averages 16°C and never drops below 10°C (National Astronomical Observatory, 2006). This species develops good coloration even in its natural habitat (Nakagawa et al., 1991), where considerably high night temperatures of more than 25°C have been reported during the berry growth and ripening stages (Japan Metrological Agency, 2007). In addition, high anthocyanin in its berry skins and a lack of chilling requirement have been reported previously (Poudel et al., 2007a, 2007b). Unlike \textit{V. ficifolia} var. \textit{ganebu}, several wine and table grapes have been reported to develop poor coloration under similar conditions (Kataoka et al., 1984; Mori et al., 2005; Naito et al., 1986; Yamane et al., 2006). Recently, global warming has been thought to inhibit the accumulation of anthocyanin, as well as flavor substances in grapes grown in temperate regions (Jones et al., 2005). Hence, it was postulated that certain traits of \textit{V. ficifolia} var. \textit{ganebu} could be useful for developing
cultivars with a low chilling trait that would produce anthocyanins without a significant reduction under higher day and night temperature conditions. To improve anthocyanin accumulation in grapes, chemical treatments, e.g., ethylene (Chervin et al., 2004) and abscisic acid (Hiratsuka et al., 2001; Jeong et al., 2004), have been studied previously. Genetic improvement by crossing with high anthocyanin accumulation species/varieties is also one possible way to improve anthocyanin accumulation. ‘Kadainou R-1’, a new wine grape cultivar, is a selection of hybridization between V. ficifolia var. ganebu and V. vinifera ‘Muscat of Alexandria’. V. ficifolia var. ganebu has small black berries and low total soluble solids (TSS), while ‘Muscat of Alexandria’, a white grape, has large berries and contains high TSS. ‘Kadainou R-1’ was also found to be rich in anthocyanin content, with a low chilling trait and short dormancy period (Poudel et al., 2007a, 2007b). ‘Kadainou R-1’ has a berry size and composition of its parents, although its adaptability and berry composition under high temperature conditions, which are the traits expected to be inherited from its female parent, are yet to be known. Hence, the aim of this study was to determine the effect of high temperatures on the composition of the ‘Kadainou R-1’ grape. Sugars and organic acid composition determine the edibility of grape berries and the final alcohol content of unfortified wine (Kliewer, 1993), while phenolic compounds determine the taste and quality of wine (Arnold and Noble, 1978); hence, we determined all these constituents in order to estimate the overall berry quality of ‘Kadainou R-1’ under different temperature treatments.

**Materials and Methods**

**Plant materials and experimental settings**

Self-rooted ‘Kadainou R-1’ vines grown in 10 L pots were used in the present experiments. Two clusters were maintained for each plant and each cluster contained approximately 100 berries. The vines were trained using the vertical trellis system and transferred to a phytotron on August 24, 2006 when the berries reached the softening stage, a high-temperature-sensitive stage (Yamane et al., 2006); this continued until final harvest (37 days after veraison). The high temperature limit for metabolic processes in grapevines is thought to be around 30°C (Coome, 1987) while night temperatures below 20°C are reported to result in inferior berry coloration (Mori et al., 2004), hence, three temperatures were maintained in the phytotron: low (20°C), intermediate (25°C), and high (30°C). The day and night temperatures were kept the same under natural sunlight conditions. Approximately 100 berries (15–25 berries from each cluster, two clusters per vine, three plants per treatment) were harvested randomly at 0, 15, 30, and 37 days after veraison (DAV) for composition analysis. The sampled berries were counted, graded and normal size berries were used for further experiments.

**Total soluble solids (TSS), titratable acidity (TA) and sugar analysis**

Approximately 10–115 berries were finger pressed to extract the juice and the juice was filtered through two layers of cheesecloth. The expressed juice was analyzed for TSS and TA. Juice extracted from 10–15 berries was taken as a replicate and the experiment was replicated at least three times. The same juice was used for TSS and TA analysis. TSS was measured as °Brix using a digital refractometer (Atago Co., Ltd., Japan), while TA was determined as tartaric acid content by titrating the juice with 0.1 N NaOH to an endpoint of pH 8.1.

For sugar analyses, five grams of flesh were taken from ten berries and homogenized (Ultra-Turrax T25; Ika-Labortechnik, Germany) with MilliQ water at 24,000 rpm for one minute. The mixture was incubated at 60°C for 30 minutes and centrifuged at 3,500 rpm for ten minutes. The supernatant was collected, and the residual tissue was re-extracted following the same procedure. The extracts were mixed and filtered through a 0.47 μm filter (Waters Co., USA). The filtrate was further purified by passing through a Sep-Pak C18 cartridge (Waters Co.). The Sep-Pak was preconditioned with 5 mL of 75% acetonitrile and washed with 5 mL of distilled water before use. The eluent was diluted to appropriate concentrations using 75% acetonitrile and was used for sugar analyses by high-performance liquid chromatography (HPLC; pump: PU 980; detector: RI-930, Jasco Inc., Tokyo, Japan). Sugar was eluted using a Shodex Asahipak NH2-504E (Showa Denko Co., Ltd., Japan) column with acetonitrile-water (75:25) at a flow rate of 1 mL·min⁻¹. The column was maintained at 30°C. Sugar constituents were identified based on their order of elution and retention time of the standard compounds; quantification was performed using the external standard method (López-Tamames et al., 1996).

**Sample preparation for anthocyanin and phenolics analyses**

The selected berries were finger-pressed to remove juice and pulp. Seeds and skins were separated and washed several times with distilled water; moisture was absorbed with blotting paper. The skin samples (1 g each) were blended for one minute at 24,000 rpm in a blender (Ultra-Turrax T25; Ika-Labortechnik) with 10 mL of acidified methanol (1:99 v/v, HCl : MeOH). The homogenate was incubated for 12 hours at 4°C in the dark before filtering with Whatman no. 1 filter paper (Whatman, UK) and centrifuging at 3,500 rpm for ten minutes. Once the extract was separated, the residual tissue was subjected to two subsequent extractions, following the same procedure, in 5 mL of acidified methanol each time. All extracts were combined, mixed thoroughly and used for anthocyanin and phenolics analyses.
Total anthocyanin content

The total anthocyanin content was measured from a calibration curve using malvidin 3,5-diglucoside as an internal standard. A 0.5 mL aliquot of the sample was diluted 10 times, and absorbance was read at 537 nm using a UV/VIS spectrophotometer (Shimadzu Co., Ltd., Japan). Results were expressed as malvidin 3,5-diglucoside equivalent (ME) against the fresh weight of the sample (mg·g⁻¹).

Procyanidin monomers (Flavan-3-ols)

The flavan-3-ols content was determined following the procedure described by Arnous et al. (2001). Briefly, a sample (0.2 mL) diluted 1:100 with MeOH was placed in a 1.5 mL Eppendorf tube (Eppendorf, Germany), and 1 mL of p-dimethylaminocinnamaldehyde (DMACA; 0.1% in 1 N HCl-MeOH) solution was added. The sample was vortexed and stood for ten minutes at room temperature. Absorbance was recorded at 640 nm. The concentration of total flavanols was determined from a calibration curve constructed by plotting absorption at 640 nm (r² = 0.9997) against known concentrations of catechin. The results are expressed as the catechin equivalent (CE) against the fresh weight of the sample (mg·g⁻¹).

Total flavonols

Total flavonols were determined following the procedure described by Mazza et al. (1999) and expressed as quercetin equivalent (QE) against fresh weight (mg·g⁻¹). Briefly, the sample was diluted with 10% ethanol. A 0.25 mL sample or standard was taken in a test tube, and 0.25 mL 0.1% HCl in 95% ethanol and 4.55 mL 2% HCl were added. The solution was thoroughly mixed and allowed to stand for approximately 15 minutes before reading absorbance at 360 nm with a spectrophotometer. Quercetin dissolved in 95% ethanol was used as the standard.

Total flavonoids

The flavonoids were determined following the procedure described by Kim et al. (2003). A 1 mL aliquot of a sample diluted 10 times was planed in a 10 mL capacity tube containing 4 mL double-distilled water. A 0.3 mL sample of 5% NaNO₂, 0.3 mL of 10% AlCl₃ and 2 mL of 1 N NaOH were added to the mixture at zero, five and six minutes, respectively. Immediately, 2.4 mL of double-distilled water was added to the reaction mixture and thoroughly mixed. Absorbance of the mixture was then measured at 510 nm versus a prepared water blank. Total flavonoid content was determined from calibration curve using catechin as the standard and expressed as the catechin equivalent (CE) mg·g⁻¹ of the fresh weight of the sample.

Total phenolic content

Total phenolics content was determined using Folin-Ciocalteu’s colorimetric assay (Singleton and Rossi, 1965). A 0.5 mL aliquot of the prepared extract was diluted five times, after which a 100 µL aliquot was taken for further analysis. The 100 µL aliquot was mixed with 1 mL phenol reagent, 1 mL 10% sodium bicarbonate, and 4 mL distilled water. The mixture was allowed to stand in the dark for one hour. Absorbance was read at 760 nm, and the total phenolic content was calculated from the calibration curve, using gallic acid as a standard. The results are expressed as the gallic acid equivalent (GAE) against the fresh weight of the samples (mg·g⁻¹).

Data analysis

Differences between means were calculated by Tukey’s test at 5% level of significance. Computations were performed using Statistical Package for the Social Sciences (SPSS) for windows (Version 13.0).

Results and Discussion

TSS increased gradually for all temperature treatments; the highest TSS was recorded at 25°C at final harvest on 37 DAV (Fig. 1). This study revealed that 25°C was the optimum temperature for the highest TSS accumulation, with a tendency for lower values at 20 and 30°C (Fig. 1). The TSS accumulation rate was faster at 30°C than at 20°C until 30 DAV and then leveled off; at 20°C, however, slower but steadily increasing TSS accumulation was observed until final harvest. Kobayashi et al. (1965a) reported that when ‘Delaware’ vines were subjected to a range of night temperatures from 15 to 35°C during the ripening stage (August to September), the highest TSS value occurred at 28°C when berries were harvested 21 days after treatment during late August. However when the berries were harvested during the first week of September 34 days after treatment, a low TSS value at the same temperature was recorded compared to at 22°C and 15°C. The results indicated that the TSS at low temperature accumulates gradually. Similar to the results mentioned in Kobayashi et al. (1965a), the present study also revealed that the...
TSS accumulation rate was higher at 30°C than at 20°C until 30 days after the onset of temperature treatments; however, TSS accumulation at both temperatures (20 and 30°C) coincided at final harvest, 37 days, after forcing (late ripening stage).

TA decreased at the fastest rate at 30°C and the lowest recorded value was under the highest temperature (Fig. 1). TA fell at higher rates at high temperature (25 and 30°C) than at low temperature (20°C). This result was in general agreement with previous findings in V. vinifera cultivars (Buttrose et al., 1971; Kobayashi et al., 1965b). Malic acid declines in berries following veraison due to cellular respiration while tartaric acid remains nearly constant (Peynaud and Maurié, 1958); hence, it is likely that the decline in TA of ‘Kadainou R-1’ grape berries at more than 25°C was attributed to increased malic acid degradation because of increased respiration.

Glucose and fructose were the major sugars in ‘Kadainou R-1’ grapes. At low (20°C) and intermediate (25°C) temperatures, both sugars increased at a faster rate than at high temperature (30°C) and leveled off at upwards of 30 DAV (Fig. 2). At 30°C, both sugars increased until harvest (Fig. 2C); however, both sugars were always lower than those recorded at 20 and 25°C (Figs. 2A and 2B). In general as the temperature increased, the accumulation rates of both sugars (glucose and fructose) decreased. This finding was in agreement with Radler (1965), who reported reduced sugar accumulation when clusters of ‘Sultana’ vines were exposed to day and night temperatures of 33°C and differed significantly according to forcing (late ripening stage). In general as the temperature increased, the accumulation rates of both sugars (glucose and fructose) decreased. This finding was in agreement with Radler (1965), who reported reduced sugar accumulation when clusters of ‘Sultana’ vines were exposed to day and night temperatures of 33°C and differed significantly according to forcing (late ripening stage).

Anthocyanin content was highest at 25°C and dropped significantly at low (20°C) or high (30°C) temperatures; however, anthocyanin content at 30°C was still higher than that at 20°C (Table 1). The remarkable characteristic of ‘Kadainou R-1’ grapes was significantly (P = 0.05) reduced anthocyanin accumulation at 20°C as compared to that at 30°C; however, both values were still lower than at 25°C. The highest anthocyanin content, at 25°C, was accompanied by the highest procyanidin monomers, a compound produced in an earlier step of the anthocyanin biosynthetic pathway. Bakhshi and Arakawa (2006) reported 24°C as the optimum temperature for anthocyanin biosynthesis in apples. Mori et al. (2004) also reported the highest anthocyanin accumulation at 25°C in ‘Kokuo’ (a bud mutation of ‘Kyoho’) grape berries compared to at very high (30°C) and cool (15°C) night temperatures. In addition, Mori et al. (2005) reported reduced anthocyanin biosynthesis in ‘Darkridge’ grape berries under high night temperatures (continuous 30°C) as compared to high day and cool night (30/15°C) temperatures. Higher temperatures (above 32°C) have also been reported to inhibit anthocyanin accumulation (Kliwer, 1977a; Kliwer and Torres, 1972). The female parent of ‘Kadainou R-1’ is V. ficifolia var. ganebu, which is native to subtropical regions; hence, it is likely that ‘Kadainou R-1’ grapes can tolerate high night temperatures to some extent, resulting in a considerable amount of anthocyanin production, even at 30°C temperature (Table 1). The anthocyanin contents of ‘Kadainou R-1’ berry skins presented in this study were higher than those of ‘Kokuo’ grapes (Mori et al., 2004) under similar night temperature conditions (25 and 30°C); however, it was hard to compare our data with other known grape cultivars due to large differences in the experimental settings and analytical procedures used in the present study and in the literature.

Total phenols and flavonols were found to be highest at 25°C and differed significantly according to

<table>
<thead>
<tr>
<th>Phenolic components (mg·g⁻¹ FW)</th>
<th>20°C</th>
<th>25°C</th>
<th>30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthocyanin</td>
<td>1.60c</td>
<td>6.00a</td>
<td>2.40b</td>
</tr>
<tr>
<td>Phenols</td>
<td>29.33c</td>
<td>49.84a</td>
<td>31.69b</td>
</tr>
<tr>
<td>Procyanidins</td>
<td>2.57b</td>
<td>10.66a</td>
<td>2.95b</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>1.31b</td>
<td>1.65a</td>
<td>1.14c</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>10.58b</td>
<td>19.10a</td>
<td>12.65b</td>
</tr>
</tbody>
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* Same letter(s) within the same row indicate no significant differences among treatments by Tukey’s test at P < 0.05 level. Data represent three replications per treatment.

**Fig. 2.** Changes in sugar constituents at 20°C (A), 25°C (B), and 30°C (C), respectively. Vertical bars represent standard error. The extract from 5 g of flesh taken from 10 berries was used as a single replicate and the experiment was repeated three times. Data are the means of three replications per treatment.
temperature treatments. The procyanidin monomer and flavonoid contents were highest at 25°C (Table 1). The highest amounts of phenolics at 25°C were attributed to the highest amounts of other metabolite products, such as flavonols and flavonoids. Although the accumulation of phenolic compounds, particularly in wine grape cultivars under different temperatures, are as important as compositional factors such as sugars and acids, the mechanisms of phenolic biosynthesis under high temperatures are poorly described in the literature. It is generally agreed that increased temperature in the plant, irrespective of heat sources such as heating or radiation, will accelerate the rate of metabolic processes in the plant, with subsequent acceleration in development and metabolite accumulation (Downey et al., 2006). In contrast, Jones (1992) reported that many metabolic processes in plants ceased or markedly reduced at high temperatures. The high temperature limit for metabolic processes in grapevines is thought to be around 30°C (Coombe, 1987). The present study revealed 25°C as the optimum temperature for phenolic/flavonol accumulation; above or below this level, accumulation fell significantly, although some metabolites were still higher at high temperature (30°C) than low temperature (20°C).

Night temperatures, particularly cool nights, are considered critical for superior berry quality with high amounts of anthocyanin formation. In the present study, day and nighttime temperatures were kept the same; a nighttime temperature of 25°C is still high compared to night temperatures in several localities in subtropical regions of Japan. Hence, it is likely that ‘Kadainou R-1’ grapes can produce optimum berry quality in places where the night temperature reaches 25°C without a significant loss in berry quality. Moreover, in climatic regions where night temperature regimes are extremely high, such as 30°C, and most grape cultivars show poor berry quality, ‘Kadainou R-1’ could be an alternative cultivar with some compromise in berry quality.

**Literature Cited**


Mori, K., S. Sugaya and H. Gemma. 2004. Changes in the coloration and activities of enzymes involved in anthocyanin


