



## Changes in inner contents of 'Kyoho' grape berry during the growth and ripening period

T. Ban<sup>A\*</sup>, A. Nakatsuka<sup>B</sup>, K. Akaura<sup>C</sup>, S. Matsumoto<sup>B</sup>, M. Ishimaru<sup>D</sup> and H. Itamura<sup>B</sup>

<sup>A</sup>Faculty of Agriculture, Tokyo University of Agriculture and Technology, Saiwai-cho, Fuchu, Tokyo, 183-8509, Japan;

<sup>B</sup>Faculty of Life and Environmental Science, Shimane University, Nishikawatsu-cho, Matsue, Shimane, 690-8504, Japan;

<sup>C</sup>Department of Home Economics, Shimane Women's College, Hamanogi, Matsue, Shimane, 690-0044, Japan; <sup>D</sup>Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Gakuen-cho, Sakai, Osaka, 599-8531, Japan.

\*E-mail: tban@cc.tuat.ac.jp

### Abstract

The grape berry morphologically consists of epidermis, an outer wall, an inner wall and placenta. The inner contents such as soluble solids, organic acids and moisture distribution of grape berries are dramatically changed during the véraison between the growth and ripening period. However, we know little about the changes in the inner contents of the outer wall, inner wall and placenta. Our purpose of the study is to clarify the tissue specificity of the total soluble solids content, sugar composition, flesh firmness and moisture distribution of 'Kyoho' grape berry during growth and ripening period. The moisture distribution of the grape berries was analyzed with magnetic resonance imaging (MRI). The total soluble solids contents of the outer wall were higher than those of the inner wall during the investigation period. In this study, fructose, glucose and sucrose were detected in the berries. The concentrations of these sugars in the outer wall were higher than those in the inner wall; however, the components of these sugars were not different between the outer wall and inner wall. Even within the same berry, the flesh firmness and the moisture distribution were different from one part of the tissue to another. These results indicate that the growth rate of grape berry varies considerably among the different parts of the berry.

**Key words:** Flesh firmness, 'Kyoho' grape, magnetic resonance imaging, ripening, sugar contents

### Introduction

The seasonal growth pattern of grape berries shows a double sigmoidal pattern that can be divided into two major stages of growth, separated by a lag phase (Coombe, 1992). During stage I, the skin colours of berries are green and the flesh is hard, and an increase in the level of organic acids is observed, while the levels of glucose and fructose are low. Stage II is referred to as the lag phase of berry development. The lag phase is followed by a second major stage of growth called stage III. During stage III, the inner contents such as soluble solids, organic acids and moisture distribution of the grape berries are dramatically changed after the inception of berry ripening, which is called véraison.

There have been reports about the changes in the inner contents of grape berries during the growth and ripening period. Amerine (1956) showed that the titratable acidity of young berries was highest in the skin area and lowest around the seeds. Possner and Kliwer (1985) reported that the highest concentrations of sugars were found in the fruit core and the tissue below the peripheral vascular bundles after véraison. Thus, the location of compounds in the grape berries are different according to the growth stage and specific tissue. Only a few reports, however, have focused on the location of compounds within specific tissues.

Our purpose here is to clarify the tissue specificity of the total soluble solids content, sugar composition and flesh firmness of 'Kyoho' grape berry during the growth and ripening period. Berries were examined non-destructively using MRI to clarify the moisture distributions in the berries during the growth and ripening period.

### Materials and methods

Thirteen-year-old vines of 'Kyoho' grape grown at the experimental farm of Shimane University were used in 2005 and 2006. We randomly selected five clusters for monitoring the growth curve of grape berries. The diameters of five berries selected from each cluster were measured from 28 to 77 days after full bloom (DAB) in 2005 and 21 to 77 DAB in 2006, respectively. For total soluble solids content measurement, three clusters were randomly selected, and three berries were sampled from each cluster at 28, 42 (véraison), 49 and 77 DAB in 2005 (Fig. 1). The outer wall and inner wall tissue were excised from each berry with a razor blade (Fig. 2). Juice was squeezed from each tissue and analyzed with a hand refractometer (AMY-1, SHIMADZU Co., Japan). For analysis of flesh firmness of the berries, three clusters were randomly selected, and three berries were sampled from each cluster at 28, 42, 49 and 77 DAB in 2005. The flesh firmness was measured as previously described by Mori (2000) with some modification. A vertical slice (4 mm thickness) was excised with a razor blade from the equatorial part of each berry. Four points of the vertical slice were tested with a rheometer (COMPAC-100, Sun Scientific Co., Ltd., Japan) (Fig. 3). The tip angle of the probe was 30 deg., and the inserted depth was 3.9 mm. For MRI, two clusters were randomly selected, and two berries were sampled at 28, 42, 63 and 77 DAB in 2005. Magnetic resonance images of each berry were collected in a MRI system (MAGNETOM Symphony 1.5T, Siemens AG., Germany).

In 2006, three clusters were randomly selected, and five berries were sampled from each cluster at 49 (7 days after véraison),

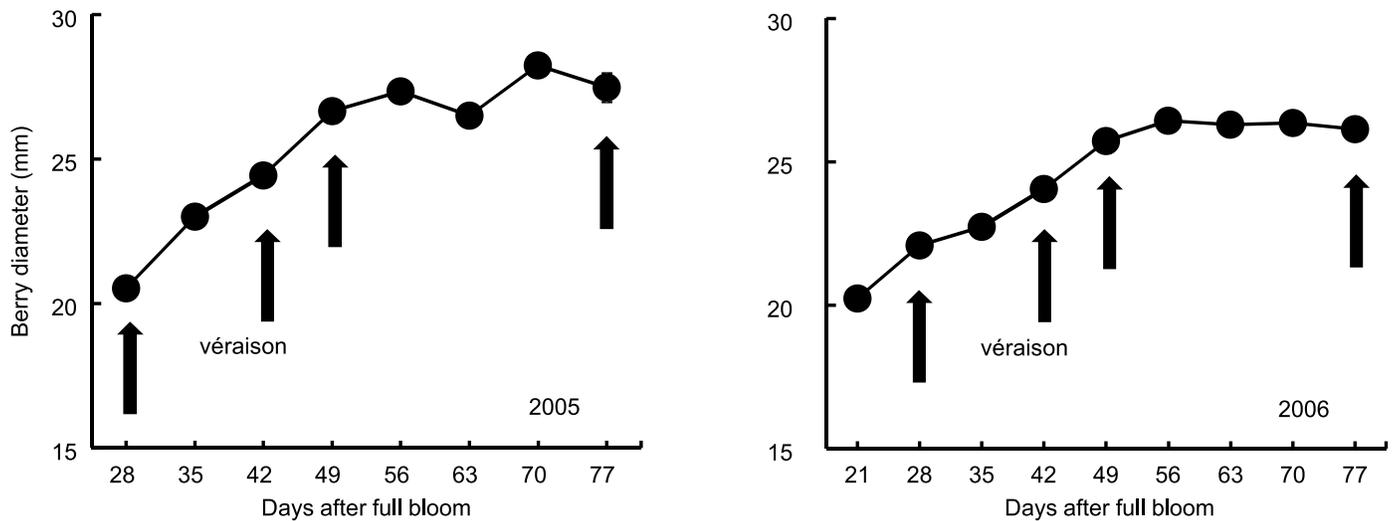


Fig. 1. Changes in diameter of 'Kyoho' grape berries during the growth and ripening period. The arrows indicate the sampling date.

63 and 77 DAB for analyzing of sugar components of the juice. Samples of the outer wall and inner wall tissue were excised with a razor blade from each berry. Juice was squeezed from each tissue and filtrated through a membrane filter (0.45  $\mu\text{m}$ , CSO45AN, Toyo Roshi Kaisya, Ltd., Japan). The juice was diluted with distilled water and subjected to HPLC analysis. Twenty microlitres of the sample was injected to the HPLC system equipped with a pump (L-2130, Hitachi High-Technologies Co., Japan), a column (Wakosil, 5NH<sub>2</sub>,  $\phi$ 4.0mm $\times$ 200mm, Wako Pure Chemical Industries, Ltd., Japan), a column oven (L-2350, Hitachi High-Technologies Co., Japan) and a refractive index detector (RID-10A, SHIMADZU Co., Japan). The column temperature was 40°C, and 80% acetonitrile was used as an eluent at a flow rate of 1 mL min<sup>-1</sup>. Each sugar was identified by the retention time and then quantified from a standard curve using an authentic sugar.

## Results

**Total soluble solids content:** During the investigation period, the total soluble solids content of the outer and inner wall samples gradually increased (Table 1). The total soluble solids contents of the outer wall were significantly higher than those of the inner wall.

**Flesh firmness:** During the investigation period, the flesh firmness gradually decreased (Table 2). At 28 and 42 DAB, the flesh firmness was highest around the seeds (Fig. 3, area III and IV) and decreased from the berry centre to the skin. However, there were no differences in flesh firmness between the areas at 49 and 77 DAB.

**Sugar composition:** In this study, fructose and glucose were detected in the juice from the outer and inner wall samples at 49, 63 and 77 DAB (Table 3). In contrast, sucrose was only detected at 77 DAB. The concentrations of these sugars gradually increased during the investigation period and were significantly higher in the outer wall than in the inner wall.

**Magnetic resonance images:** In the case of transverse sections, the areas of the outer wall, inner wall and placenta were clearly seen as regions of uniform gray signals at 28 DAB (Fig. 4A). After 42 DAB, the signals of the outer wall were lighter than those of the inner wall (Fig. 4B, C and D). This tendency was more obvious

Table 1. Changes in the total soluble solids contents of outer wall and inner wall of 'Kyoho' grape berries during the growth and ripening period

Fruit part	Days after full bloom			
	28	42	49	77
Outer wall	6.0 $\pm$ 0.4	11.0 $\pm$ 0.5	18.8 $\pm$ 0.3	25.6 $\pm$ 0.3
Inner wall	5.2 $\pm$ 0.2	9.9 $\pm$ 0.6	18.1 $\pm$ 0.3	25.2 $\pm$ 0.3
Significance	*	*	**	*

\*,\*\*Significant at  $P=0.05$  or  $0.01$ , respectively by t-test.

Table 2. Changes in the flesh firmnesses (g) of 'Kyoho' grape berries during the growth and ripening period

Fruit part <sup>z</sup>	Days after full bloom			
	28	42	49	77
I	47.6c <sup>y</sup>	14.1c	14.7 <sup>NSx</sup>	12.4 <sup>NS</sup>
II	55.4bc	24.1bc	12.1 <sup>NS</sup>	9.3 <sup>NS</sup>
III	73.2a	32.2ab	11.2 <sup>NS</sup>	8.8 <sup>NS</sup>
IV	69.4ab	39.8a	13.1 <sup>NS</sup>	13.4 <sup>NS</sup>

<sup>z</sup>Refer to Fig. 3.

<sup>y</sup>Different letters within a column indicate significance at 5% level by the Tukey's test. <sup>x</sup>Non significant.

Table 3. Changes in the concentration (mg mL<sup>-1</sup> juice) of fructose, glucose and sucrose in the outer wall and inner wall of 'Kyoho' grape berries during the growth and ripening period

Sugars	Fruit part	Days after full bloom		
		49	63	77
Fructose	Outer wall	67.64	123.00	141.74
	Inner wall	47.68	111.83	133.12
Significance		* <sup>z</sup>	*	*
Glucose	Outer wall	68.76	100.83	127.84
	Inner wall	51.79	93.35	123.11
Significance		*	*	*
Sucrose	Outer wall	ND <sup>y</sup>	ND	7.88
	Inner wall	ND	ND	6.05
Significance				*

<sup>z</sup>Significant at 5% level by t-test. <sup>y</sup>ND indicates not detected.

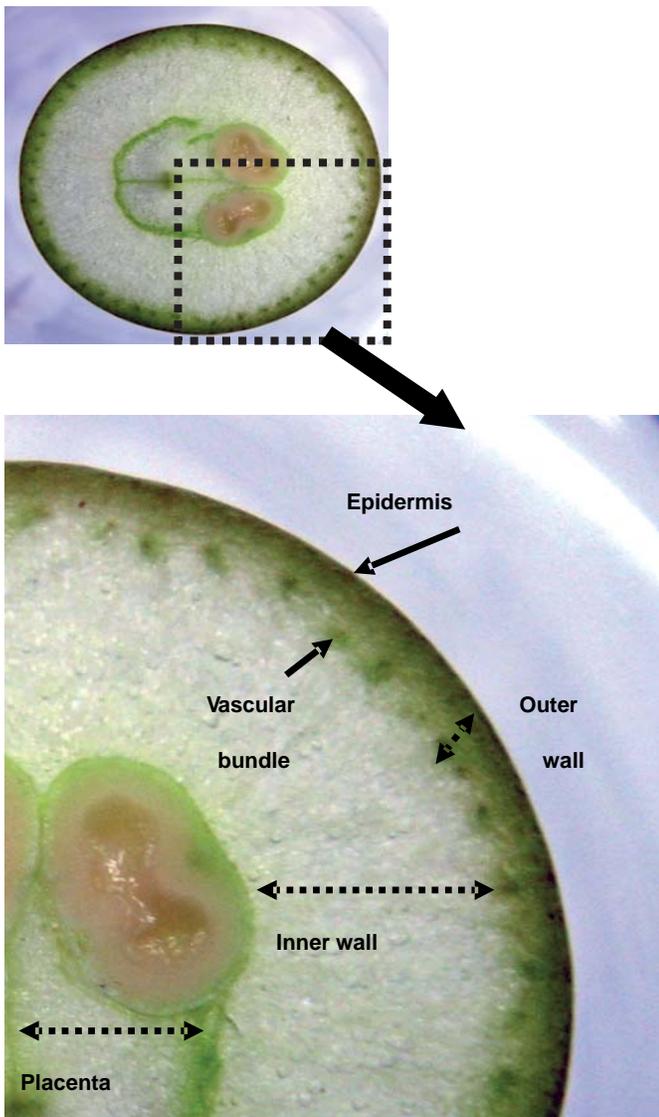


Fig. 2. Transverse section through a 'Kyoho' grape berry.

in the images at 77 DAB (Fig. 4D). At 77 DAB, black signals were clearly observed in the inner wall of a berry.

### Discussion

The developing grape berry represents a strong sink for photosynthesis products (Hale and Weaver, 1962). In the grape vine leaf, sucrose is produced as a result of photosynthesis and transported via the phloem to the berry (Swanson and Elshishiny, 1958). The photosynthesis products are mainly transported via the peripheral and central vascular systems in the berries

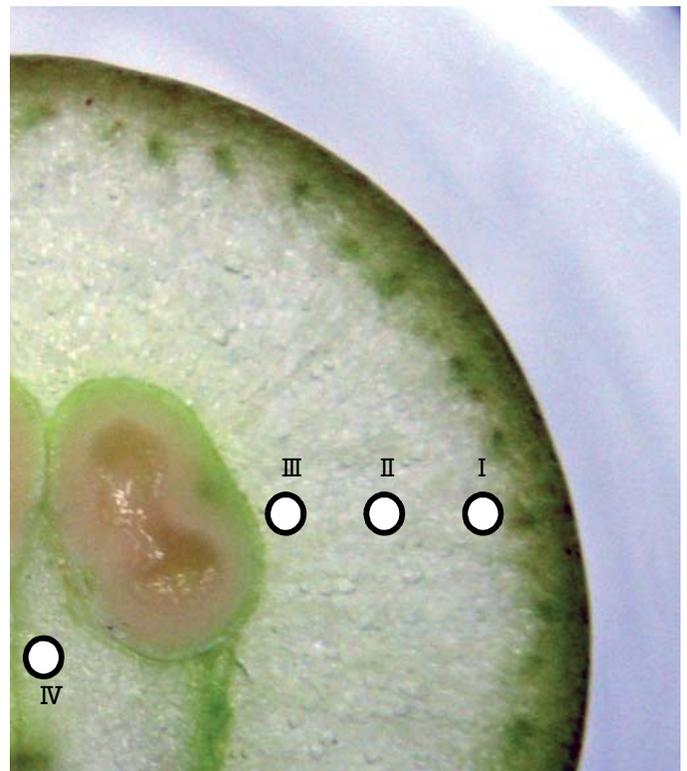


Fig. 3. The four points of a 'Kyoho' grape berry tested with a rheometer.

(Kriedemann, 1969). Possner and Kliewer (1985) discussed how these vascular systems caused the localization of sugars in grape berries. In this study, the total soluble solids, fructose, glucose and sucrose contents of the outer wall were significantly higher than those of the inner wall during the investigation period (Table 1 and 3). The peripheral vascular system of a grape berry is presented between the outer and inner walls, and the inner wall tissue is thicker than the outer wall tissue (Fig. 2). The localization of these photosynthesis products in the outer and inner wall tissue is probably correlated to the thickness of these tissues, depending on the distance from the peripheral vascular system.

The grape berry rapidly softens at the beginning of stage III, and the flesh firmness gradually decreases toward the harvesting time. To clarify this softening mechanism, researchers have mainly investigated the changes in the cell wall materials of flesh tissue (Nunan *et al.*, 2001; Yakushiji *et al.*, 2001). However, the changes in the flesh firmness of each part of a grape berry are still unknown. The results of the present study clearly show that there was a gradual decrease in flesh firmness from the core of a berry towards the skin during the growth period, whereas there

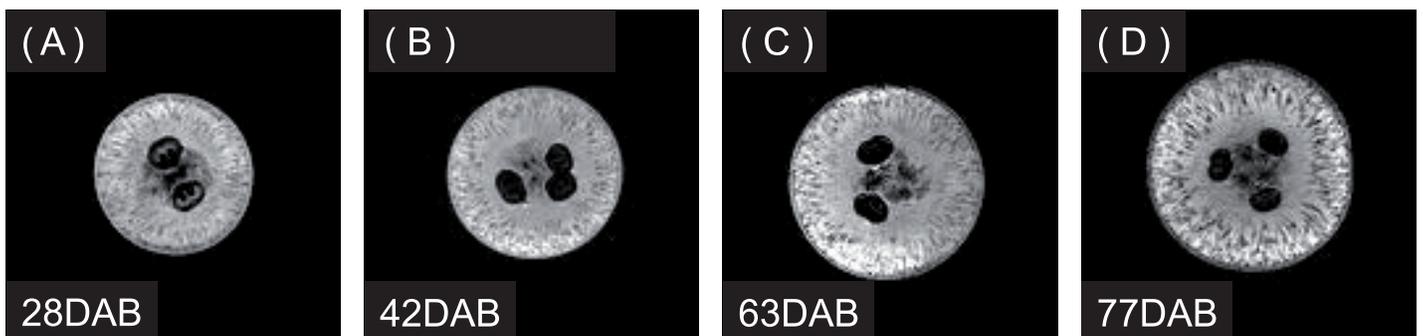


Fig. 4. Proton MR images of transverse sections through a 'Kyoho' grape berry.

were no differences in flesh firmness among the different parts of a berry after véraison (Table 2). These findings indicate that the rate of decreases in flesh firmness varies from one part of the grape to another. It is possible that the differences in flesh firmness of grape berries before véraison can be attributed to the tissue structure.

MRI is a nondestructive technique usually used in medical sciences, which examines the distribution and mobility of protons (mainly from water and fat) in living tissue (Ishida *et al.*, 1989). The concentration and state of water and fat play very important roles in plant tissues when active metabolism occurs. MRI techniques have been used to investigate the changes of physiological activities along with maturation of some fruit, such as apples (Barreiro *et al.*, 1999), citrus (Galed *et al.*, 2004), grapes (Pope *et al.*, 1993) and persimmons (Clark and MacFall, 2003). However, little is known about the changes in the water distribution of grape berries during the growth and ripening period. In this study, MR images clearly detected the differences in the distribution of water during the investigation period (Fig. 4). Even in samples from the same areas of a berry harvested at 77 DAB, the signal intensities of MR images were different from each other (Fig. 4D). These differences indicate the uneven distribution of water in a grape berry.

Ishida *et al.* (1989) analyzed tomato fruits with MRI and reported that the water was differently distributed among the tissues in the fruits. They concluded that the distribution of free water involves the places where active metabolism occurs and that low mobility resulted in slow metabolism. Since the water content of grape berry tissue do not differ among the different parts of the tissue, these results may indicate the differences in the degree of active metabolism in a berry. Further investigation is required to clarify the mechanisms of uneven water distribution in a grape berry.

In conclusion, we found that the sugar concentrations in the outer wall of a grape berry were higher than those of the inner wall. We also noted that the flesh firmness and the moisture distribution were different in each part of the grape tissue. These findings suggest that there is uneven distribution of the inner contents in a grape berry and that the growth rate of berries is quite different from one part of the tissue to another.

## Acknowledgments

The authors thank Mr. Shinichi Hino and Mr. Michio Kato of Matsue Seikyo Hospital for technical assistance with MRI experiments. This work was supported, in part, by a Grant-in-Aid (No. 17380023) from the Japan Society for the Promotion of Science.

## References

- Amerine, M.A. 1956. The maturation of wine grapes. *Wines and Vines*, 10: 27-38.
- Barreiro, P., J. Ruiz-Cabello, M.E. Fernandez-Valle and C.M. Ortiz, 1999. Mealiness assessment in apple using MRI techniques. *Magn. Reson. Imaging*, 17: 275-281.
- Clark, C.J. and J.S. Macfall, 2003. Quantitative magnetic resonance imaging of 'Fuyu' persimmon fruit during development and ripening. *Magn. Reson. Imaging*, 21: 679-685.
- Coombe, B.G. 1992. Research on development and ripening of the grape berry. *Am. J. Enol. Vitic.*, 43: 101-110.
- Galed, G., M.E. Fernandez-Valle, A. Martinez and A. Heras, 2004. Application of MRI to monitor the process of ripening and decay in citrus treated with chitosan solutions. *Magn. Reson. Imaging*, 22: 127-137.
- Hale, C.R. and R.J. Weaver, 1962. The effect of developmental stage on direction of translocation of photosynthate in *Vitis vinifera*. *Hilgardia*, 33: 89-131.
- Ishida, N., T. Kobayashi, M. Koizumi and H. Kano, 1989. <sup>1</sup>H-NMR imaging of tomato fruits. *Agr. Biol. Chem.*, 53: 2363-2367.
- Kriedemann, P.E. 1969. Sugar uptake by the grape berry: A note on the absorption pathway. *Planta*, 85: 111-117.
- Mori, T. 2000. Heritability and selection effectiveness for fruit firmness in strawberry. *J. Japan. Soc. Hort. Sci.*, 69: 90-96.
- Nunan, K.J., C. Davies, S.P. Robinson and G.B. Fincher, 2001. Expression patterns of cell wall-modifying enzymes during grape berry development. *Planta*, 214: 257-264.
- Pope, J.M., D. Jonas and R.R. Walker, 1993. Applications of NMR micro-imaging to the study of water, lipid, and carbohydrate distribution in grape berries. *Protoplasma*, 173: 177-186.
- Possner, D.R.E. and W.M. Kliever, 1985. The localisation of acids, sugars, potassium and calcium in developing grape berries. *Vitis*, 24: 229-240.
- Swanson, C.A. and E.D.H. Elshishiny, 1958. Translocation of sugars in the Concord grape. *Plant Physiol.*, 33: 33-37.
- Yakushiji, H., N. Sakurai and K. Morinaga, 2001. Changes in cell-wall polysaccharides from the mesocarp of grape berries during veraison. *Physiol. Plant.*, 111: 188-195.