COMPARISON OF CHANGES IN BERRY FIRMNESS AND CELL WALL COMPONENTS DURING RIPENING AMONG GRAPE CULTIVARS

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ABSTRACT

In this work, we investigated the developmental changes in cell-wall polysaccharides associated with the physiological properties of pericarp and mesocarp tissues of grape berries during ripening, and aimed to clarify the mechanisms involved in the softening process. The firmness of fruits, ethanol insoluble solids content, changes in pectins content, and hemicellulose and cellulose content were studied. The results showed that the changes in pectic fractions occurred dramatically in the sub-epidermal layer (pericarp), and the amount of water soluble pectin (WSP) increased greatly during berry ripening in the three cultivars tested, while the changes were not significant in mesocarp and/or other pectic polysaccharide fractions. Moreover, the hemicellulose content did not change markedly from stage 1 to stage 3, and decreased significantly to stage 4 in all cultivars, while the cellulose content decreased markedly during ripening in all cultivars analyzed, both in pericarp and mesocarp tissues.

Keywords: Cell wall, cultivars, grape, pericarp and mesocarp, ripening, softening.

So sánh sự biến đổi độ cứng quả và thành phần thành tế bào trong quá trình chín giữa một số giống nho

TÓM TẮT

Chúng tôi nghiên cứu những biến đổi phát triển trong các polysaccharides thành tế bào kết hợp với những đặc tính sinh lý của mô tế bào lớp vỏ trong và lóp thịt quả trong quá trình chín của quả Nho để làm rõ các cơ chế liên quan trong quá trình mềm hóa. Ở lớp vỏ quả trong, những thay đổi của phần pectic đã xảy ra đáng kể, hàm lượng pectin hòa tan trong nước của cả ba giống nho tăng mạnh trong quá trình chín. Trong lớp thịt quả, sự thay đổi của hàm lượng pectin hòa tan trong nước và các phần pectic khác không đáng kể trong quá trình chín của cả ba giống nho. Hàm lượng hemicelluloses hầu như không thay đổi rõ rệt từ giai đoạn 1 đến giai đoạn 3 và giảm đáng kể đến giai đoạn 4 ở tất cả các giống. Hàm lượng cellulose trong cả lớp vỏ quả trong và lớp thịt quả giảm đi rõ rệt trong quá trình chín ở cả ba giống nho phân tích.

Từ khóa: Giống, nho, lớp vỏ trong và lớp thịt quả, mềm hóa, quá trình chín, thành tế bào.

1. INTRODUCTION

The grape berry is a non-climacteric fruit that exhibits a double-sigmoidal growth curve characteristic of berry fruits (Coombe, 1976). There are many factors that contribute to and influence the quality of grapes, and one of these important factors is the optimal time for harvest. The signal announcing the beginning of the harvest period is the ripening process of grapes on the vine. Ripening marks the completion of the development of the fruit and the commencement of senescence, and it is normally an irreversible event. Ripening is the

result of a complex series of changes, many of them probably occurring independently of one another. Ripening fruits undergo many physicochemical changes after harvest that determine the quality of the fruit purchased by the consumer (Wills *et al.*, 1998). In grape berry composition, the most dramatic changes occur during the ripening phase. Berries switch from a status where they are small, hard, and acidic, with little sugar, to a status where they are larger, softer, sweeter, less acidic, and strongly flavoured and coloured. The flavour that builds in grapes is mostly the result of the acid/sugar balance, and the synthesis of flavour and aromatic compounds, or precursors, taking place at this time. The development of these characteristics will largely determine the quality of the final product (Boss and Davies, 2001; Conde *et al.*, 2007).

One of the most notable changes during fruit ripening is softening, which is related to biochemical alterations at the cell wall, middle lamella, and membrane levels. Therefore, softening is an important part of the ripening process in most fruits, and it is widely recognized that changes in cell walls accompany fruit softening. Gross changes in wall composition may not always occur, and indeed more subtle structural modifications of constituent polysaccharides are often observed during softening (Brady, 1987; Fischer and Bennett, 1991).

Modifications of cell wall components might also be expected in ripening grape berries, but little is known about cell wall composition in grapes during ripening or of the mechanism of softening in this fruit (Coombe, 1976). The grape berry is somewhat unusual in that it softens at the same time as it expands during the second growth, or ripening, phase. The onset of the second growth phase is referred to as "veraison," which is a viticultural term that describes the point at which a number of developmental events are initiated, including the accumulation of sugars, a decrease in organic acids, colour development, berry expansion, and softening (Coombe, 1973).

Wills *et al.* (1998) reported that the largest quantitative change associated with ripening is usually the breakdown of carbohydrate polymers, especially the near total conversion of starch to sugar. This alters both the taste and texture of the produce. Even with non-climacteric fruits, the accumulation of sugar is associated with the development of optimum eating quality, although the sugar may be derived from sap imported into the fruit rather than from the breakdown of the fruit's starch reserves.

In grapevines, pectic polysaccharides from mature grape berries have been mainly studied in terms of their composition and structure in wine and juice (Saulnier and Thibault, 1987; Saulnier *et al.*, 1988). As part of the ripening process in grapes, the molecular mass, solubility, and degree of substitution of individual cell-wall polysaccharides may be modified during veraison. Actually, the pectin solubility of the grape mesocarp has been shown to change as the berry ripens after veraison (Silacci and Morrison, 1990). In mature grape berries, cellulose and polygalacturonans were the major constituents that accounted for 30-40% by weight of the polysaccharide components of the walls (Nunan *et al.*, 1997). Nunan *et al.* (1998) also reported that no major changes in cell-wall polysaccharide composition occurred during softening of the ripening grape berries, but that a significant modification of a specific polysaccharide component, such as type I arabinogalactan, was observed. However, little is known about changes in molecular mass distribution and degradation of xyloglucans during berry softening. In this study, we investigated the developmental changes in cell-wall polysaccharides associated with physiological properties of pericarp and mesocarp tissues of grape berries during ripening, and aimed to clarify the mechanisms involved in the softening process.

2. MATERIALS AND METHODS

2.1. Plant material

Grape (*Vitis* spp.) fruits were obtained from the experimental orchard at Chungnam ARS,

located in Yesan, Korea. Fruits were harvested at different stages according to the external colouration degree, firmness, and days after full blossom (DAFB): stage 1, stage 2, stage 3, and stage 4 in 'Campbell Early', 'Kyoho', and 'Sheridan' cultivars. Harvested grape berries were rinsed thoroughly with water and stored at -80°C until the cell-wall analysis could be performed. The remaining berries were used for the fruit quality test.

'Campbell Early': stage 1: 60 days after full blossom, stage 2: 70 days after full blossom, stage 3: 80 days after full blossom, and stage 4: 90 days after full blossom

'Kyoho': stage 1: 70 days after full blossom, stage 2: 80 days after full blossom, stage 3: 90 days after full blossom, and stage 4: 100 days after full blossom

'Sheridan': stage 1: 90 days after full blossom, stage 2: 100 days after full blossom, stage 3: 110 days after full blossom, and stage 4: 120 days after full blossom

2.2. Methods

2.2.1. Determination of firmness

Firmness was measured with the SUN RHEO METER COMPAC-100 (CR-100D, SUN SCIENTIFIC CO., LTD.) and with a flat-tipped probe (0.8 cm diameter). The cross-head speed of the Rheometer was 100 $mm.min^{-1}$, and the driving depths were 8 mm. Values were expressed in Newtons (N).

2.2.2. Isolation of cell wall polysaccharides

During thawing of the frozen berries, the skin and seeds were removed, and pericarp and mesocarp cell walls were isolated as described by Nunan *et al.* (1997). Skin and seeds were removed manually, and the remaining pericarp and mesocarp tissue was homogenized in 4 volumes of absolute ethanol using a household blender. 20 g samples of fresh grape were homogenized in 80 ml of EtOH 100% and boiled at 90-95°C for 20 minutes. After waiting for cooling, the homogenate was filtered with GF/C filter paper (Whatman, USA) and washed with EtOH 80% to remove soluble sugars. Total sugars and simple sugars were determined from the filtrate. The retained cell wall residues were stirred into 100 ml of chloroform:methanol (1:1, v/v) for 30 minutes. The homogenate was filtered with GF/C filter paper and then washed three times with about 120 ml of 100% acetone. Finally, the remaining solids were considered to be ethanol insoluble solids (EIS), and were dried in an oven at 38°C and stored over silica gel in a vacuum desiccator.

2.3. Sequential extraction of cell wall polymers

Polyuronides were isolated according to the methods of Maclachlan and Brady (1994) and Rose *et al.* (1998) with partial modifications for the discarded starch fraction. 100 mg samples of dry EIS were homogenized in 40 ml of DMSO (90%) and shaken in a shaker for 12 hours at room temperature. The homogenate was filtered, and washed three times with 10 ml of water. The filtrates were pooled and labeled for starch. The residue was then resuspended in 40 ml of water (containing 0.02% Na-azide) and shaken for 12 hours at room temperature. The homogenate was filtered and washed three times with 10 ml of water. The residue was then resuspended in 40 ml of 50 mM CDTA (containing 50 mM Na-acetate pH 6.5) and shaken for 12 hours at room temperature. The homogenate was filtered and washed three times with 10 ml of water. The residue was then resuspended in 40 ml of 50 mM $Na_2CO_3$and 20mM Na BH_4 and shaken for 12 hours at room temperature. The homogenate was filtered and washed three times with 10 ml of water. The resulting pooled filtrates were regarded as water-, CDTA-, and Na_2CO_3 soluble polyuronides, respectively. After the extractions for pectins, the residue was then resuspended in 40ml of 4% KOH and 24% KOH (containing 0.1% $NaBH_4$) and shaken for 24 hours at room temperature. The homogenate was filtered and washed three times with 10 ml of water. The filtrates were pooled and labeled 4% KOH and

24% KOH soluble fractions, respectively. The final volume of all fractions was 50 ml. The last residue was washed three times with about 100 ml of 80% EtOH and three times with 100 ml of 100% Acetone. Finally, the remaining solid was considered to be cellulose after drying at 45°C for 2 days.

Quantification of pectins was estimated as uronic acid by the *m*-hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen, 1973) using galacturonic acid as a standard. The quantification of water soluble pectin was calculated as the total of the starch fraction and water soluble fraction. Quantification of hemicelluloses was estimated as glucose using a phenol-sulfuric acid method (Dubois *et al.*, 1956) using glucose as a standard. Quantification of cellulose was estimated by weighing the amount of the last dry residue.

2.4. Experimental design and statistics analysis

The entire experiment was completed with three replications for each type of analysis except for testing firmness in which 10 replications were completed. All of the data were analyzed using ANOVA, and the means were compared by the LSD test at a significance level of 5%. All analyses were performed with the IRRISTAT software package v. 5.0 for Windows (IRRISTAT, Version 5.0.20050701).

3. RESULTS AND DISCUSSION

3.1. Berry firmness

There was a decrease in berry firmness throughout maturation and ripening stages in all cultivars. This decrease due to ripening involved physical changes. Physical changes included a decrease in firmnessand an altered texture that resulted from changes in the pectic substances binding cells together making them less firmly cemented (Brady, 1987). In our case, the firmness contents decreased rapidly from stage 1 to stage 3, and changed little from stage 3 to stage 4 in all cultivars that were investigated (Fig. 1).

3.2. Ethanol insoluble solids

The ethanol insoluble solids (EIS), which reflect the principal constituents of cell walls and may be partially associated with each other and with some phenolic compounds, were analyzed. The results showed that the EIS content increased in all cultivars during ripening but only in sub-epidermal tissue (pericarp). There were no significant changes (in 'Kyoho') or decreased ('Campbell Early' and 'Sheridan') EIS content in mesocarp tissue during ripening. In general, EIS contents were higher in the pericarp tissue than in the mesocarp tissue (Fig. 2). The increase of EIS content in the pericarp tissue may be related to the increase of dry matter content during berry ripening and/or the physical effects of water depletion in the epidermal tissue.

3.3. Pectins

Pectin constituents in fruits and vegetables can be extracted from EIS. Experimental analysis of the changes in the molecular masses of pectins during ripening typically involves fractionating them into several classes based on different solvents that are used to extract them from the wall. A typical sequential extraction generates water soluble pectin, chelator soluble pectin (e.g. CDTA soluble pectin), and Na_2CO_3 soluble pectin (Rose *et al.*, 1998). These subsets are generally described as corresponding to pectins that are freely soluble in the apoplast, ionically associated with the wall, or linked into the wall by covalent bonds, respectively.

A common observation is that ripening-related increases in water soluble pectin (WSP) are parallel to equivalent decreases in the amounts of pectins in the wall-associated fractions (Rose *et al.*, 1998). Our results below also agree with Rose *et al.* (1998), especially the increase of WSP during ripening in the sub-epidermal layer (pericarp) of the three cultivars tested.

Pectin consists mainly of uronic acid (UA) and in our case, the content of UA in the three kinds of pectin was measured in the three cultivars during ripening. The Na_2CO_3 soluble pectin content had the highest UA levels, followed by the CDTA soluble pectin content,

and the water soluble pectin content had the lowest levels in all cultivars, in both in pericarp and mesocarp tissues. In general, the changes in the pectic fractions occurred dramatically in the sub-epidermal layer (pericarp) and the amount of water soluble pectin (WSP) increased greatly during berry ripening in all three cultivars while the changes were not significant in the mesocarp or other pectic polysaccharide fractions (Fig. 3).

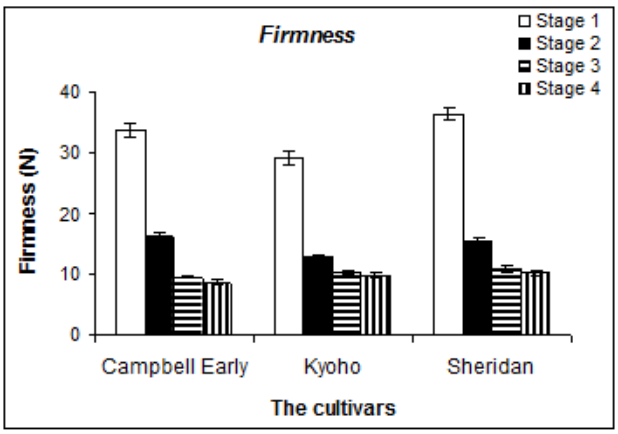

Figure 1. Changes in firmness during berry ripening in three grape cultivars

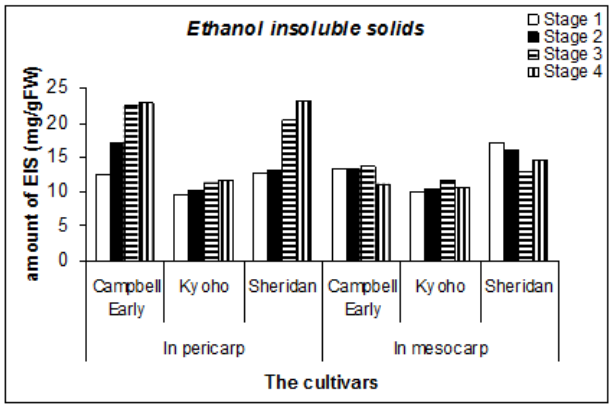

Figure 2. Changes in EIS during berry ripening of three grape cultivars

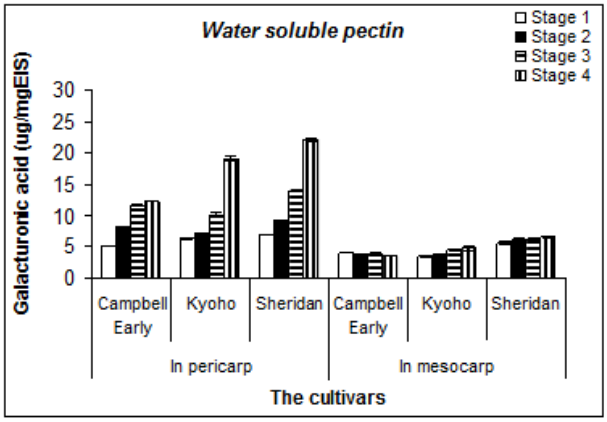

Figure 3. Changes in WSP during berry ripening of three grape cultivars

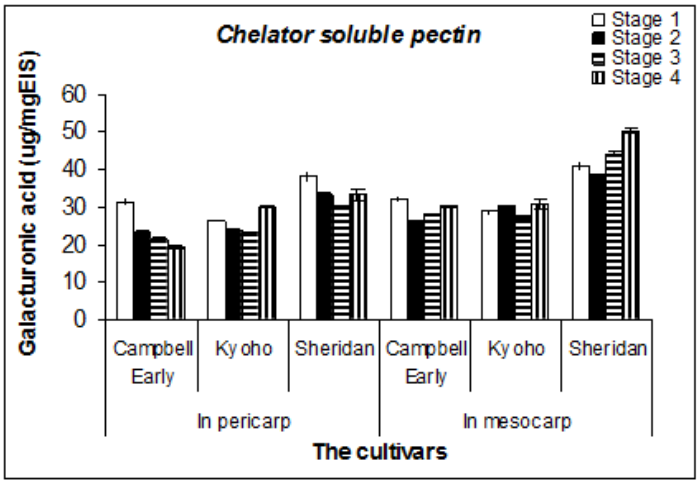

Figure 4. Changes in CDTA-SP during berry ripening of three grape cultivars

3.3.1. Water soluble pectin (WSP)

The amount of water soluble pectin in the three cultivars increased greatly in the pericarp layer during berry ripening while the changes in the mesocarp were not significant (Fig. 3). It is uncertain whether or not these changes in the pericarp were related to the activation of cell wall hydrolases. In general, the degradation of pectin is catalyzed by two groups of enzymes, polygalacturonase (PG) and pectin methyl esterase (PE). One study found that an increase in PG activity during ripening accompanied an increase in WSP and fruit softening (Eskin, 1990 cited from Pressey *et al.*, 1971) indicating that these UA containing compounds are actively synthesized. Rose (2003) also reported that the enzyme polygalacturonase hydrolyses the α-1,4-D-galacturonan backbone of pectic polysaccharides, and PG activity has long been known to increase substantially in many species of ripening fruit, concomitant with polyuronide depolymerization. The author also concluded that the role of PG in fruit softening is still open to debate. Undoubtedly, the enzymes catalyze substantial depolymerization and solubilization of a subset of wall polyuronides in many ripening fruits, but there is an apparent restriction of PG action by a range of possible factors, and the relationship between PG, pectin depolymerization and solubilization, and specific textural changes is considerably more complex than originally conceived.

3.3.2. CDTA soluble pectin (CDTA-SP)

The amount of CDTA-SP decreased slightly during ripening of 'Campbell Early' berries in the pericarp tissue. For 'Kyoho' and 'Sheridan' cultivars, the contents of CDTA-SP changed temporarily, but did not follow a decreasing trend during ripening. In the mesocarp tissue, the amount of CDTA-SP also changed insignificantly and there was not a clear pattern in the cultivars during ripening (Fig.4). In general, there were no clear differences in the contents CDTA-SP of the cultivars both in pericarp and mesocarp during ripening.

3.3.3. Na_2CO_3 soluble pectin (Na_2CO_3-SP)

The content of Na_2CO_3-SP in the pericarp tissue during and after stage 2 was lower than stage 1 in 'Campbell Early' and 'Sheridan' berries. However, for the 'Kyoho' cultivar, a significant increase of UA content after stage 2 was observed. There were also no significant changes from stage 1 to stage 3 in mesocarp tissue but the content of Na_2CO_3-SP decreased in stage 4 in all cultivars analyzed (Fig. 5).

Altogether, these results showed that differences in cell wall metabolism of pectin could not be clearly measured in the stages analyzed, except for water soluble pectin content in the pericarp tissue.

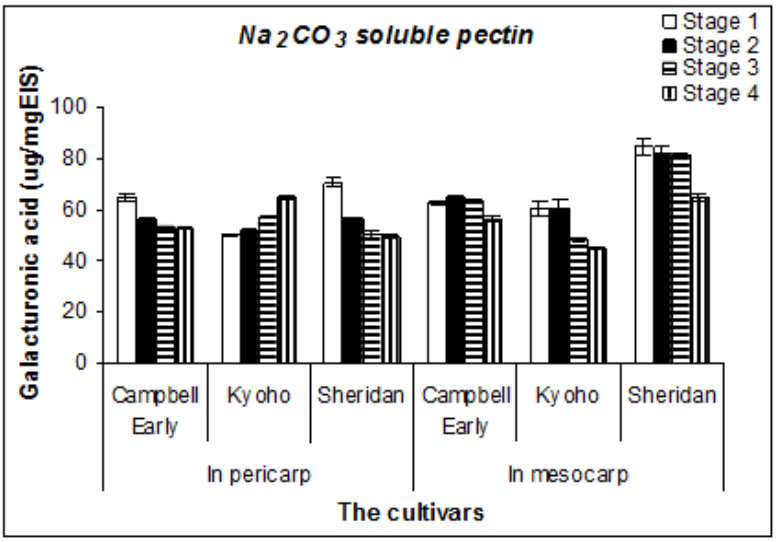

Figure. 5. Changes in Na_2CO_3-SP during berry ripening of three grape cultivars

Table 1. Hemicellulose and cellulose content during berry ripening in three grape cultivars

Stages	4% KOH soluble fraction (μg Glucose/mgEIS)			24% KOH soluble fraction (μg Glucose/mgEIS)			Cellulose fraction (μg Glucose/mgEIS)		
	Campbell Early	Kyoho	Sheridan	Campbell Early	Kyoho	Sheridan	Campbell Early	Kyoho	Sheridan
Sub-epidermal layer (pericarp)									
1	9.14 a[z]	12.18 a	12.30 a	39.76 a	31.05 a	38.49 a	141.00 a	150.00 a	153.00 a
2	8.17 a	10.71 b	9.92 a	38.51 a	30.76 a	36.30 ab	138.00 a	144.00 a	142.00 ab
3	8.12 a	10.64 b	9.62 a	35.69 b	29.81 a	35.03 ab	118.00 b	141.00 a	138.00 ab
4	6.30 b	8.72 c	6.46 b	29.69 c	24.66 b	33.23 b	107.00 bc	136.00 ab	128.00 b
Mesocarp									
1	3.38 c	2.98 b	3.65 c	37.60 a	28.31 a	36.01 a	198.00 a	125.00 a	154.00 a
2	5.60 b	3.36 b	5.61 b	29.76 b	24.66 b	34.94 a	132.00 b	109.00 b	137.00 b
3	8.17 a	4.15 a	8.97 a	24.79 c	24.03 b	34.89 a	105.00 c	102.00 b	130.00 b
4	3.34 c	3.65 ab	3.69 c	24.04 c	20.59 c	33.71 a	101.00 c	99.00 b	126.00 b

Note: Values are the means of three replicate extracts.

[z] Different letters within the same column on each fruit organ show a significant difference by Tukey-Kramer's LSD test at the 5% level.

3.4. Hemicelluloses and cellulose

In our study, the hemicellulose content was lower than the cellulose content in all the cultivars analyzed in both pericarp and mesocarp tissues, and both polysaccharides consisted mainly of glucose. The content of glucose in the two kinds of hemicelluloses (4% KOH soluble fraction and 24% KOH soluble fraction) was calculated during ripening inthe three cultivars (Table 1).

The hemicellulose content did not change markedly from stage 1 to stage 3 and decreased significantly at stage 4 in all cultivars, with one exception. In the 4% KOH soluble fraction in mesocarp tissue, the content increased gradually from stage 1 to stage 3 and then

significantly decreased at stage 4. The cellulose content decreased markedly during ripening in all cultivars analyzed, both in the pericarp and mesocarp tissues (Table 1).

In general, the hemicellulose and cellulose content decreased during ripening, especially the cellulose content, which decreased rapidly in both the pericarp and mesocarp layers. These decreases strongly correlated with the process of grape berry softening during ripening in the three cultivars analyzed. The measurements of berry firmness and cell-wall polysaccharides in all cultivars strongly suggest that berry softening at veraison is caused by the constant decrease of cellulose and hemicelluloses. Nunan *et al.* (1998) reported that cellulose and xyloglucan levels decrease on a fresh weight basis after veraison, but both cellulose and xyloglucan content at a molar percentage basis changed little after veraison in "Muscat Gold Blanco" grapes.

4. CONCLUSIONS

This study was designed to look at the changes in cell-wall polysaccharide properties in grape berries during the ripening process. In the three grape cultivars utilized in this work, no clear correlation could be established between fruit firmness and EIS content in the different ripening stages and grape cultivars. The amount of water soluble pectin (WSP) increased dramatically in the sub-epidermal layer (pericarp) during berry ripening in all three cultivars, but the changes in mesocarp tissue and other pectic polysaccharide fractionswere not significant. The content of hemicelluloses decreased at the last stage in all cultivars, and the cellulose content decreased during ripening in all cultivars, both in pericarp and mesocarp tissues. These results indicate that the changes in cell-wall polysaccharides during berry ripening occurred mainly in sub-epidermal (pericarp) tissues. Our conclusions do not exclude the possibility that berry softening also involves depolymerization of cellulose molecules and changes in structural proteins (Nunan *et al.*, 1998), which we did not investigate in the present study.

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