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Effects of Water Loss of 'Fuyu' Persimmon Fruit on Molecular Weights of Mesocarp Cell Wall Polysaccharides and Fruit Softening

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Summary

Distributions of molecular weights of cell wall fractions of persimmon 'Fuyu' fruit (Diospyros kaki Thunb.), stored under low and high humidities, were measured. The molecular weight of total sugar of water-soluble (WS) polysaccharides from alcohol-insoluble solids under both low and high humidities shifted to lower molecular mass during storage. The amount of larger sized polymers in fruits kept at low humidity was less than that at high humidity during late stage of storage. Weight-average molecular weights of pectic polysaccharides under high humidity were higher than that under low humidity condition. Coefficient between fruit firmness and the level of individual polysaccharides was higher than those between fruit firmness and weight-average molecular weight of WS, pectin or hemicellulose fraction. These results were confirmed by multiple regression analysis. Hence, fruit firmness of persimmon mesocarp is not influenced by changes in a single cell wall fraction but by all cell wall fractions, i. e., the content of cell wall polysaccharides plays a grater role in determining fruit firmness than do their molecular weights.

Key Words: cell wall, fruit firmness, molecular weight, persimmon, water loss.

Introduction

Japanese persimmons often exhibit rapid post-harvest softening that decreases their value during distribution. Persimmon fruits are exported from Japan to East Asia, albeit in small quantities. Other countries such as Israel and New Zealand are developing export markets, but the short storage life of persimmon fruit prevents the increase in export volume (Collins, 1993). Several investigations to extend the storage life of persimmon fruit had been attempted (Ben-Arie et al.,1991; Ben-Arie and Zutkhi, 1992; Grant et al., 1992; Guelfat-Reich et al., 1978; MacRae et al., 1987; Pesis et al., 1986; Tanaka et al., 1971; Tarutani, 1960, 1961). Tarutani (1960, 1961) demonstrated that polyethylene-packed 'Fuyu' fruit stored at 0℃ remained marketable for 4 months; he found that the most desirable composition for controlled atmosphere storage (CAS) for 'Fuyu' were 5-10% CO_2 and 5% O_2. Ben-Arie and Zutkhi (1992) suggested that reduced O_2 levels inhibited polyphenol oxidation that leads to the formation of brown pigments and that elevated CO_2 levels were responsible for retarding decay caused by *Alternaria alternata*. Ben-Arie and Esther (1986) demonstrated that low temperature also inhibited formation of brown pigments on the husk of pomegranate. The use of cold storage is restricted because of the high cost of electricity. Thus, it is important to clarify the mechanism of softening of persimmon fruits for improving its storing technology.

Water loss of fruit is thought to be one factor that induces fruit senescence (Xue et al., 1996). We reported previously that when persimmon fruits were stored under low and high humidity, those under low humidity condition lost more water and produced more ethylene than they did under high humidity. The amounts of arabinose (Ara) and galactose (Gal) in the pectic fraction and cellulose of mesocarp cell walls decreased significantly under low humidity (Tsuchida et al., 2003) accompanied by fruit softening because it is significantly correlated to Ara and Gal contents and cellulose content in the cell walls.

The molecular weight of cell wall polysaccharides is closely correlated with cell wall elasticity and viscosity that influence fruit firmness (Sakurai, 2002). High molecular polysaccharides increase viscosity by increasing intermolecular friction (Masuda and Yamamoto, 1985; Sakurai, 1991). Sakurai and Nevins (1997) suggested that cell wall viscosity decrease during the ripening process of avocado fruit. It is, therefore,

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important to investigate the changes in molecular weights of polysaccharides in the cell walls to clarify the mechanism in fruit softening during the ripening process. Changes in molecular weight of polysaccharides of cell walls during fruit softening have been examined in a variety of fruits. Recently degradation of xyloglucan (XG) has been reported in tomato (Sakurai and Nevins, 1993), avocado (Sakurai and Nevins, 1997), grapes (Yakushiji et al., 2001), kiwifruit (Terasaki et al., 2001) and melon (Rose et al., 1998). Changes in molecular weights of cell wall polysaccharides were not previously examined in persimmon during storage. In this report, we investigated changes in molecular weight of polysaccharides of cell wall polysaccharides of persimmon fruit stored under high and low humidities as a follow up on our former report (Tsuchida et al., 2003).

Materials and Methods

Plant materials

Fruits of Japanese persimmon 'Fuyu' (*Diospyros kaki* Thunb.) were harvested at commercial maturity and stored under low humidity (20℃, 60% RH) or high humidity (20℃, ≧ 98.5% RH). The persimmons at 60% RH were stored in an incubator (model GLMP-102, Futaba Kagaku, Ibaragi), whereas those at 98.5% RH were kept in an incubator (model LH-300-RDS, Nippon Medical and Chemical Instruments, Tokyo). The humidity was controlled by a humidifier (model NE-U12, OMRON, Kyoto).

On day (D)-0, 3, 6 and 9 of storage, fruit firmness on three fruits were recorded, and the pulp was prepared for cell wall analysis.

Fruit firmness measurement

Nine mesocarp tissues were perpendicularly sliced from three fruits with a maximum thickness exceeding 20 mm. The sliced tissue with its cut surface facing downward was placed on the moving stage of a Rheo meter (RHEOTECH, Tokyo). A small part of the top pericarp was removed for probe insertion. The stage was moved upward at a speed of 6 $cm \cdot min^{-1}$ to the fixed conical probe (3 mm in diameter); the resulting stress perceived by the probe to a depth of 15 mm was recorded. The mean firmness of a fruit was calculated as an average of three different parts of the fruit. The average values of three fruits were calculated as the average for one sample.

Cell wall fractionation

Cell wall analysis was carried out according to the method of Sakurai and Nevins (1997). A 10-g sample of diced persimmon mesocarp tissue was homogenized in 80% EtOH with a homogenizer (SMT, Tokyo) for 5 min, a polytron homogenizer (KINEMATICA, Switzerland) for 3 min, then a glass homogenizer (EYELA, Tokyo) for 5 min. The homogenate was boiled for 15 min and centrifuged for 15 min at 3000 × g. The precipitate was dispersed in Na-acetate buffer (pH 6.84, 50 mM) and heated for 1 min at 100℃. After cooling, the residue was treated for 2 hr at 37℃ with 500 units of an α-amylase (Hog pancreatic α-amylase, Boehringer Mannheim). The mixture was centrifuged for 15 min at 3000 × g. The precipitate was dispersed in distilled water, heated for 10 min at 100℃, then centrifuged (15 min, 3000 × g). This treatment was repeated three times. The combined supernatant was designated as the hot water-soluble (WS) fraction. The precipitate was dispersed in EDTA solution (50 mM, pH 6.8), heated for 15 min at 100℃, then centrifuged (15 min, 3000 × g). This treatment was repeated three times. The combined supernatant was designated as the pectic fraction. The precipitate was treated with 17.5% NaOH containing 0.02% $NaBH_4$ and centrifuged (10 min, 3000 × g). This treatment was repeated three times over a 24-hr period. The combined supernatant, neutralized with glacial acetic acid was designated as the hemicellulose fraction.

Sugar analysis

Total sugar (TS) content of WS, pectic, hemicellulose fractions was estimated by a phenol-sulfuric acid method, using glucose as a standard (Chaplin, 1986). Uronic acid (UA) of WS and pectic fractions were estimated by the *m*-hydroxydiphenyl method (Blumenkranz and Asboe-Hansen, 1973). XG content of the hemicellulose fraction was estimated by the iodine method by the following relationship; one unit of absorbency at A_{640} is equivalent to 155 $\mu g \cdot ml^{-1}$ XG (Wakabayashi et al., 1991).

Lyophilization

The WS fraction was lyophilized. Pectin and hemicellulose fractions were dialyzed for 24 hr against distilled water then lyophilized.

Gel-filtration chromatography

The lyophilized samples (equivalent to ca. 1 mg of TS) of WS, pectic and hemicellulose fractions were dissolved in 50 mM Na-phosphate buffer (pH 7.2) and introduced into an HPLC system (model LC-10, Shimadzu, Kyoto), equipped with a gel-filtration column (TSK-Gel G5000 PW, 7.5 mm × 60 cm, Tosoh, Tokyo). The samples were eluted with 50 mM Na-phosphate buffer (pH 7.2) at a flow rate of 1 $ml \cdot min^{-1}$. Fractions were collected at 0.5 min intervals. Standard dextrans of known molecular weights were run on the column for molecular calibration.

The correlation between weight-average molecular weights or content of the cell wall polysaccharides and fruit firmness

Correlation coefficients between each weight-average molecular weight or content of cell wall polysaccharides

and fruit firmness were calculated by the simple regression analysis by using spread sheet software (Excel, Microsoft, USA). Formulas to estimate fruit firmness were calculated by multiple regression from the data of weight-average molecular weight or content of all cell wall fractions by using the analysis tool of the same software; a correlation of coefficient between the estimated and measure fruit firmness was calculated.

Results

Percent water loss

Changes in the percent water loss of the whole fruit are a summary of a former report (Tsuchida et al., 2003). 'Fuyu' fruit began to lose water immediately after being placed in storage under low humidity; it became 93% of its original fresh weight on D-9. Under high humidity, water loss was barely measurable, such that on D-9, the fruits had lost only 0.7% of fresh weight.

Fruit firmness

Changes in the fruit firmness are also a summary of former report (Tsuchida et al., 2003). The fruit firmness that was 12.0 $kg \cdot cm^{-2}$ at harvest, D-0, steadily decreased under low humidity condition during storage, and finally decreased to 3.2 $kg \cdot cm^{-2}$ on D-9. Firm-

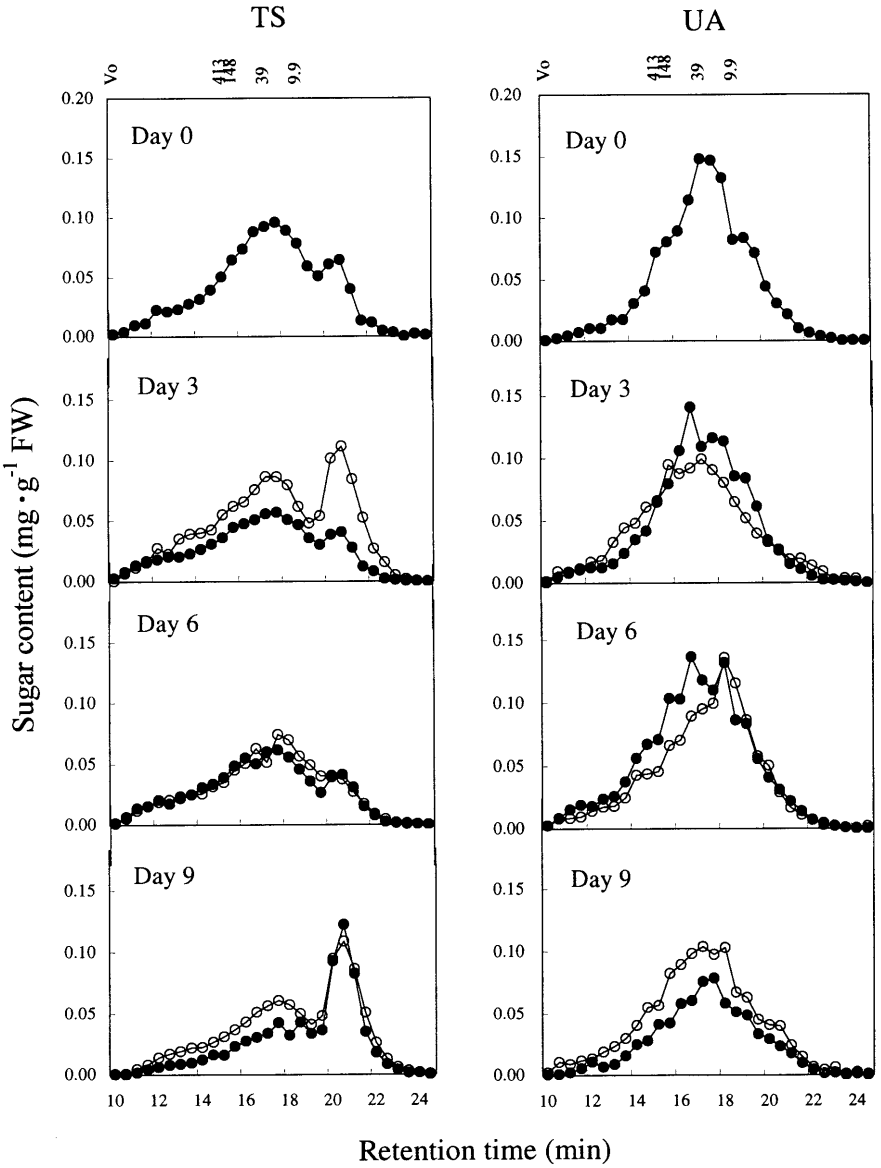

Fig. 1. Molecular distribution profile of WS fraction from mesocarp tissues of 'Fuyu' persimmon stored at 20°C under low (●) and high (○) humidity conditions. An aliquot of the lyophilized powder of WS was introduced to HPLC with a gel-filtration column. The left row of panels shows the molecular distribution of TS estimated by phenol-sulfuric acid assay, and the right panels that of UA estimated by the *m*-hydroxydiphenyl assay. Total peak area represents the amount of sugar extracted from one gFW of tissue. The column was calibrated with dextrans with known molecular mass (413, 148, 39, 9.9 kDa) as shown in the upper panel.

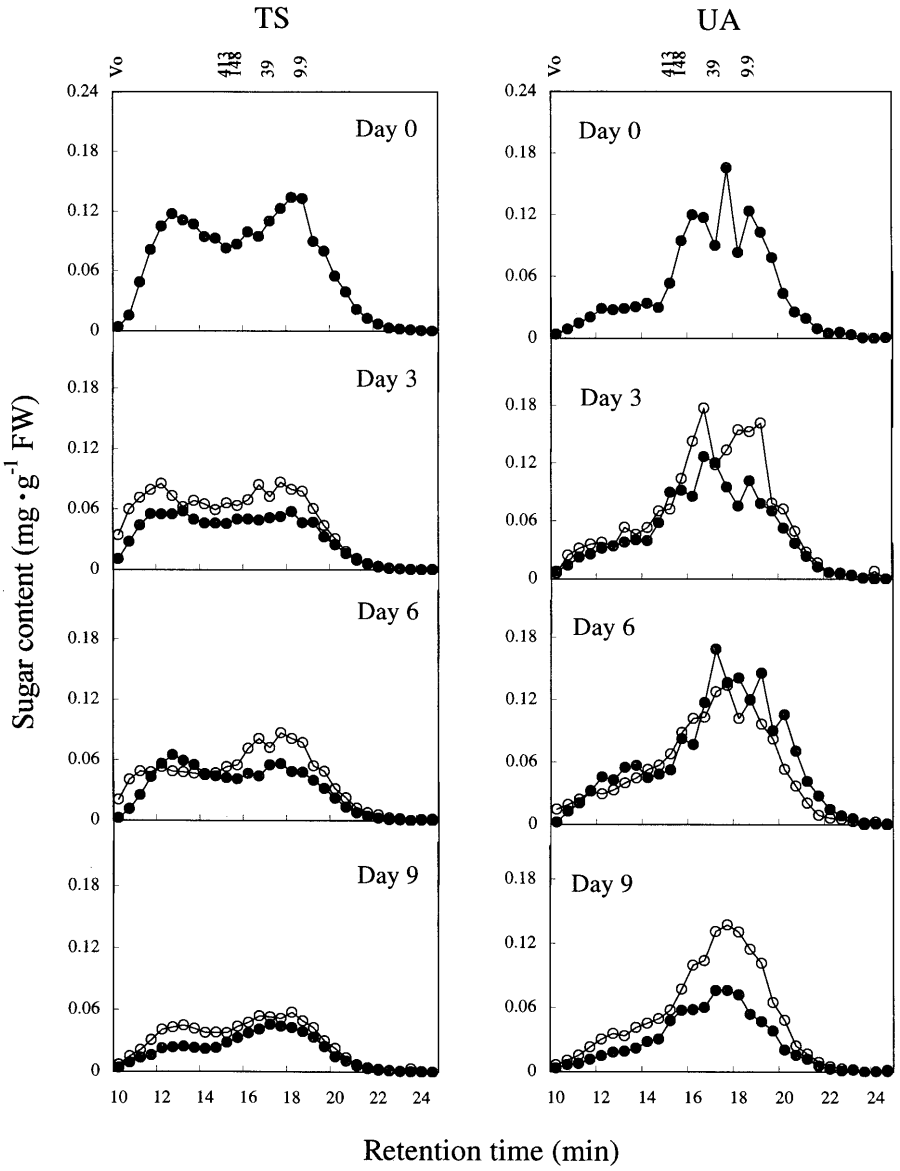

Fig. 2. Molecular distribution profile of pectic fraction from mesocarp tissues of 'Fuyu' persimmon stored at 20℃ under low (●) and high (○) humidity conditions. An aliquot of the lyophilized powder of pectin was introduced to HPLC with a gel-filtration column. The left row of panels shows the molecular distribution of TS estimated by phenol-sulfuric acid assay, and the right panels that of UA estimated by the *m*-hydroxydiphenyl assay. Total peak area represents the amount of sugar extracted from one gFW of tissue. The column was calibrated with dextrans with known molecular mass (413, 148, 39, 9.9 kDa) as shown in the upper panel.

ness under high humidity condition remained about 12.0 kg・cm^{-2} then commenced to decrease to 10.3 kg・cm^{-2} on D-6, and finally to 8.2 kg・cm^{-2} on D-9. Noticeable softening did not occur under high humidity condition until D-9.

Molecular weight distribution of cell wall polysaccharides

The molecular weight distribution of the WS fraction of persimmon tissues extracted on D-0, 3, 6 and 9 were determined by gel-filtration chromatography; the area of each chromatogram was drawn to represent the amount of TS and UA extracted from 1 g fresh weight of the fruit (Fig. 1). The left panels show the molecular distribution of TS estimated by phenol-sulfuric acid assay; the right panels are the estimated UA by the *m*-hydroxydiphenyl assay. The WS fraction always contained two major TS peaks at low mol weight (Rt 21 min) and high mol weight (Rt 17.5 min). The molecular shift to lower mass was observed under both low and high humidities on D-9. The decrease in large polymers under low humidity was significant. The elution pattern of UA of the WS fraction did not shift until D-9.

The molecular weight distribution of the pectic fraction extracted on D-0, 3, 6 and 9 and determined by gel-filtration chromatography (Fig. 2) revealed that the

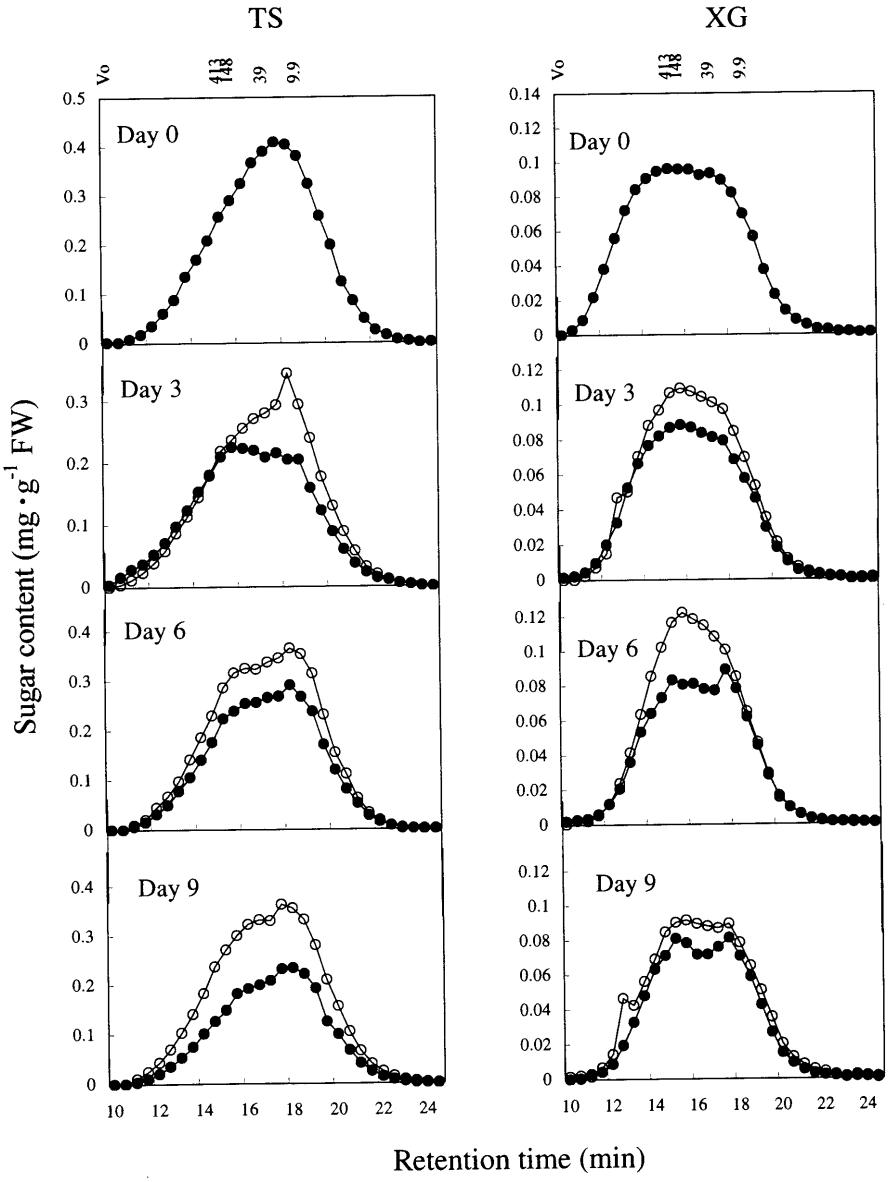

Fig. 3. Molecular distribution profile of hemicellulose fraction from mesocarp tissues of 'Fuyu' persimmon stored at 20℃ under low (●) and high (○) humidity conditions. An aliquot of the lyophilized powder of hemicellulose was introduced to HPLC with a gel-filtration column. The left row of panels shows the molecular distribution of TS estimated by phenol-sulfuric acid assay, and the right panels that of XG estimated by the iodine method. Total peak area represents the amount of sugar extracted from one gFW of tissue. The column was calibrated with dextrans with known molecular mass (413, 148, 39, 9.9 kDa) as shown in the upper panel.

pectic fraction contained two major TS peaks: a high (Rt 12.5 min) and low mol weight (Rt 18 min). The high molecular weight polysaccharides eluted near void volume (10.0-11.5 min) were more abundant under high than low humidity on D-3 and 6. The contents of TS per g fresh weight were lower under low than high humidity from D-3 to D-9 (Table 2). The distribution pattern of molecular mass of pectic UA was almost similar under low and high humidities till D-6. On D-9 the amount of polysaccharides significantly decreased under low humidity.

The molecular weight distribution of the TS and XG in the hemicelulose fraction extracted on D-0, 3, 6 and 9 and determined by gel-filtration chromatography (Fig. 3) shows that the molecular distributions of TS and XG, estimated by the iodine method, are very similar under low and high humidities till D-9. However, the total quantity of polysaccharides was consistently less under low than high humidity.

The weight- average molecular weights

The weight-average molecular weights of WS, pectic and hemicellulose fractions were calculated based on the data in Fig. 1, 2 and 3. Weight-average molecular weight in UA of WS (Fig. 4, lower panel) did not differ between storage at high and low humidities. In UA of

pectic fraction (Fig. 5, lower panel), the weight-average molecular weight remained constant during storage in both high and low humidities, whereas those of TS in WS fraction increased from D-0 to 6, and then decreased sharply on D-9 (Fig. 4, upper panel). The weight-average molecular weight of TS in the pectic fraction was consistently lower under low than high humidity, and they temporarily increased on D-3, and then decreased (Fig. 5, upper panel). The weight-average molecular weight of XG in hemicellulose fraction decreased immediately after storage under both low and high humidities (Fig. 6, lower panel).

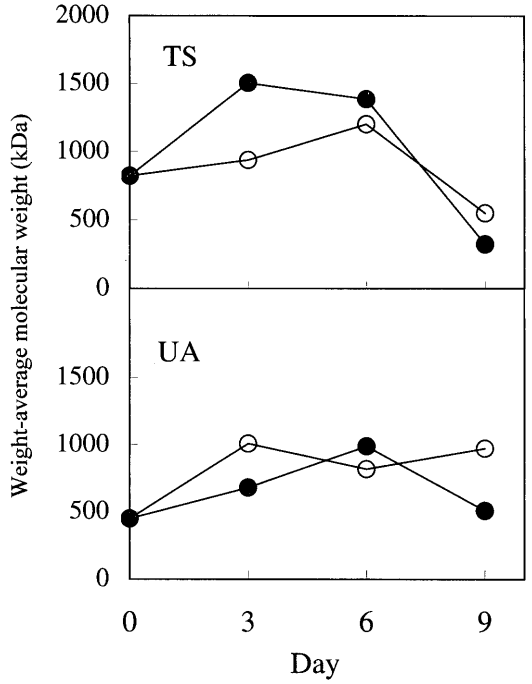

Fig. 4. Weight-average molecular weight (kDa) of TS (upper panel) and UA (lower panel) in the WS fraction. ●, low humidity; ○, high humidity. Weight-average molecular weights were calculated by the following formula; Weight-average MW (kDa)=$\Sigma(MWi \times Wi)/\Sigma wi$; *MWi*, molecular weight at *i*th fraction; *Wi*, weight at *i*th fraction.

The correlation between weight-average molecular weights or content of the cell walls and fruit firmness

A simple regression analysis, between weight-average molecular weight of TS in pectic fraction and fruit firmness revealed that low correlation between the variables existed (Table 1). However, by using a multiple regression equation, derived from the data of weight-average molecular weight of all the fractions (see below), estimated firmness (F_1, $g \cdot cm^{-2}$) was calculated,

$$F_1 = 1.3\,TS_W - 4.5\,UA_W + 4.2\,TS_P + 0.1\,UA_P - 9.1\,TS_H + 6.6\,XG_H - 939.4$$

where TS_W = TS of WS; UA_W = UA of WS; TS_P = TS of pectin; UA_P = UA of pectin; TS_H = TS of hemicellulose; XG_H = XG of hemicellulose.

The coefficients between estimated firmness and the measured fruit firmness (r^2 = 0.686: Table 1) was larger than those derived from single regression analysis.

Consequently, the coefficients between contents of individual polysaccharides (Tsuchida et al., 2003) and fruit firmness were higher than those derived from the

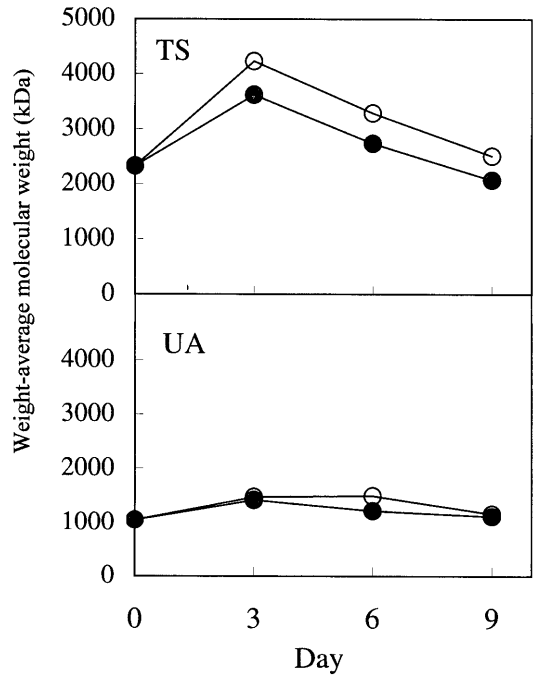

Fig. 5. Weight-average molecular weight (kDa) of TS (upper panel) and UA (lower panel) in the pectic fraction. ●, low humidity; ○, high humidity. Weight-average molecular weights were calculated as exhibited in Fig. 4.

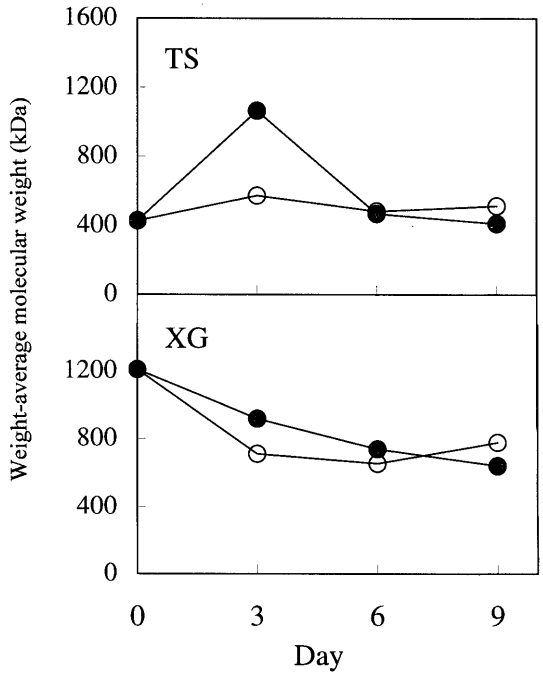

Fig. 6. Weight-average molecular weight (kDa) of TS (upper panel) and XG (lower panel) in the hemicellulose fraction. ●, low humidity; ○, high humidity. Weight-average molecular weights were calculated as exhibited in Fig. 4.

Table 1. Coefficients of determination in single and multiple regression analysis between weight-average molecular weight and fruit firmness.

WS		Pectin		Hemicellulose		All Multiple regression
TS	UA	TS	UA	TS	XG	
0.086	0.000	0.352*	0.054	0.03	0.199	0.686**

*, ** Significant at $P<0.05$ or 0.01, respectively.

Table 2. Sugar content (mg · g^{-1} FW, mean ± SE) of cell wall polysaccharides in presimmon mesocarp tissues stored under low and high humidity conditions and coefficients of determination in single and multiple regression analysis between content and fruit firmness (kg · cm^{-2}, mean ± SE).

Day	humidity	WS		Pectin		Hemicellulose		All Multiple regression	Firmness[z]
		TS	UA[z]	TS	UA[z]	TS	XG[z]		
0		1.13 ± 0.12	1.28 ± 0.16	1.95 ± 0.30	1.37 ± 0.15	4.64 ± 0.52	1.34 ± 0.14		12.0 ± 0.7
3	Low	0.77 ± 0.13	1.23 ± 0.46	0.99 ± 0.24	1.39 ± 0.13	3.00 ± 0.66	1.10 ± 0.11		10.4 ± 1.5
	High	1.32 ± 0.12	1.15 ± 0.17	1.44 ± 0.09	1.88 ± 0.44	3.62 ± 0.48	1.30 ± 0.11		12.2 ± 1.1
6	Low	0.80 ± 0.05	1.40 ± 0.59	0.91 ± 0.22	1.77 ± 0.55	3.39 ± 0.25	1.02 ± 0.14		8.6 ± 0.4
	High	0.85 ± 0.08	1.17 ± 0.03	1.22 ± 0.09	1.51 ± 0.36	4.39 ± 0.10	1.29 ± 0.14		10.3 ± 1.2
9	Low	0.75 ± 0.01	0.74 ± 0.14	0.59 ± 0.06	0.81 ± 0.07	2.63 ± 0.24	0.95 ± 0.10		3.2 ± 0.3
	High	1.00 ± 0.08	1.16 ± 0.35	0.84 ± 0.20	1.42 ± 0.36	4.34 ± 0.24	1.15 ± 0.14		8.2 ± 0.8
R^2		0.243*	0.134	0.612**	0.310*	0.118	0.602**	0.832**	

[z] Tsuchida et al. (2003).

*, ** Significant at $P<0.05$ or 0.01, respectively.

weight-average molecular weights (Table 2). Moreover, higher coefficients was obtained ($r^2 = 0.832$, Table 2) by multiple regression between measured and estimated fruit firmness (F_2, kg · cm^{-2}) calculated as follows:

$$F_2 = -2.42\,TS_W + 5.50\,UA_W + 0.95\,TS_P + 0.50UA_P - 1.18\,TS_H + 21.76\,XG_H - 17.01$$

Discussion

Weight-average molecular weight under low humidity did not significantly differ from that under high humidity except for TS of the pectic fraction (Fig. 4-6). No significant correlations existed between fruit firmness and each weight-average molecular weight of polysaccharides in cell wall fractions except for TS of the pectic fraction (Table 1).

Previously, Tsuchida et al., (2003) showed that water loss is involved in the decrease of Ara and Gal contents in pectin. In many fruit, Ara and Gal, found in the cell wall pectic polymers (Darvill et al., 1980), decreased during ripening (Ahmed and Labavitch, 1980; Bartley, 1976; Gross and Wallner, 1979; Knee, 1975; Knee et al., 1977; Yamaki et al., 1979). In persimmon fruit the loss of Ara and Gal in pectic fraction may be related to the degradation of pectic polysaccharides.

The coefficients between fruit firmness and individual polysaccharide content in cell wall fractions were higher than those between fruit firmness and weight-average molecular weight, and the coefficients between estimated firmness calculated from multiple regression and the measured fruit firmness was higher than those calculated by single regression analysis (Table 1, 2) lead us to believe that fruit firmness of persimmon mesocarp is not influenced by changes in a single cell wall fraction but by all cell wall fractions and that polysaccharide content plays a greater role in fruit softening than do their molecular weights. These results confirm our earlier report that fruit firmness is significantly correlated to contents of Ara and Gal of WS and pectic and hemicellulose fraction. Siddiqui et al. (1996) reported a similar phenomenon in apple in which the levels of ionically-associated pectin, covalently bound pectin and hemicellulose decreased while their molecular distribution was constant in softening fruit. They attributed the change to the enzymatic cleavage of linkages between hemicelluloses and pectin. In persimmons, enzymatic cleavage of linkages among cell wall fractions might also have occurred rather than degradation of polysaccharide chains.

The lower coefficient value from our multiple regression equation derived from the data of average-molecular weight to estimate of fruit firmness, indicate that TS of WS and UA of pectin may not contribute to fruit firmness. TS of hemicellulose correlated negatively with fruit firmness, suggesting polysaccharides in hemicellulose other than XG increased their average-molecular weight during softening.

In apple, increase in the WS pectin content in the cell

walls during storage has also been reported (Knee, 1978; O'Beirne et al., 1983), but our finding, nevertheless shows that XG content has a higher *r* value with fruit firmness than those of other fractions, suggesting that XG content contributes significantly to fruit softening.

The trend that weight-average molecular weight of TS in pectic fraction increased on D-3 and then decreased (Fig 5) while that of XG in hemicellulose constantly decreased (Fig 6) was also found in kiwifruit (Terasaki et al., 2001). These authors speculated that pectic fraction increased internal friction between wall polysaccharides to compensate for decreased friction between cellulose and XG by XG degradation that eventually leads to changes in the texture of fruit tissues. We also speculate that the pectic fraction in cell walls of persimmon fruit contributes to changes in fruit texture.

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カキ'富有'果実の水分損失が果実軟化と果肉細胞の分子量に与える影響

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摘　要

低湿度条件(温度20℃，湿度60%)および高湿度条件(温度20℃，湿度≧98.5%)で貯蔵したカキ'富有'果実の果肉細胞壁の分子量分布変化を調査した．熱水可溶性画分の全糖は両湿度条件で低分子化したが，高分子多糖類の量は低湿度条件では高湿度条件に比べて少なくなった．ペクチン画分の全糖の平均分子量は高湿度条件で低湿度条件に比べて常に高い値であった．果肉硬度は熱水可溶性およびペクチン画分の全糖と酸性糖，ヘミセルロース画分の全糖とキシログルカンのそれぞれの平均分子量よりも含量と相関が高かった．さらに，多糖類の平均分子量あるいは含量から重回帰分析で果肉硬度の推定値を求めると，いずれの場合も果肉硬度との間の決定係数は0.6以上の高い値であった．ただし，細胞壁多糖類の含量を独立変数とした場合のほうが平均分子量を独立変数とした場合より決定係数が大きかった．多糖類含量からの重回帰分析ではキシログルカン含量の寄与率が高かった．これらの結果より，カキ果実の果肉硬度は，特定の細胞壁画分の単独的な変化でなく，すべての画分の変化により決定されており，その寄与は分子量よりも含量の方が高いと考えられた．

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