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Differences in Levels of Pectic Substances and Firmness in Fruit From Six Sweet Cherry Genotypes

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Abstract

The composition of sweet cherry fruit cell-walls relative to firmness was investigated for six cultivars and selections based on previous firmness data and harvest date at Summerland, B.C. Among the cultivars and selections, 'Merpet' had the lowest fruit firmness (early ripening), followed by 13S-27-17, 'Celeste', 13S-34-50, 'Lapins', and 'Sweetheart' (late ripening). In general, the firmer fruit cultivars and selections had a greater concentration of alcohol insoluble residues (AIR) (mg / g fruit) and more total AIR per fruit. Softer fruit genotypes had a greater amount of polymers weakly associated with the cell wall (e.g., the water soluble fraction). However, softer fruits had less of the highly esterified and tightly bound pectins (e.g., Na_2CO_3 soluble fraction). Total amount of hemicellulosic neutral sugars and uronic acids were not related to fruit firmness. Xyloglucan in hemicellulose fractions appeared to be less in softer fruits but the relationship was not consistent across all cultivars and selections.

Introduction

In the sweet cherry industry, fruit firmness is one of the most important factors for fruit quality, since firm fruits are desired for fresh eating, handling, marketing and shipping. The best cherry described by the market is firm overall and has crisp flesh.

The softening that occurs during the ripening of many fruits is presumably the result of enzymatic modifications in cell wall polysaccharides (10, 13). Pectic substances are the major component of the primary cell walls and of the middle lamella in fruit tissues. They are polyuronides composed mainly of 1, 4-linked α-galacturonic acid with neutral sugars as side chains. Softening is due, at least in part, to the depolymerization of polygalacturonic acid residues, which are the backbone of pectin (20). It has been demonstrated that the high levels of polygalacturonase (PG) mRNA accumulation, PG activity and depolymerization of pectic materials occurs in ripening tomato and many other fruits (4, 6). However, molecular genetic approaches have revealed that the loss of firmness which accompanies ripening cannot be explained by modification of cell wall pectin alone (11, 22, 23). When PG antisense RNA is introduced into a normal tomato plant, transgenic tomato fruits with as little as 1% of the original fruit specific PG activity levels soften normally (23). In addition to pectin degradation, the loss of cell wall structure and fruit firmness may be from the modification of cross-linking polymers that bond sugars and the polygalacturonic acid backbone (19).

In addition to changes in pectin polymers, modification of hemicellulosic components occurs during ripening of nonclimacteric hot pepper (12) and climateric apple (15, 16). Hemicellulosic polysaccharides are a main component of cell walls. The modification of tightly bound hemicellulose, specifically xyloglucan, was one of the earliest events in a sequence of cell wall disassembly in ripening melon fruit (22). The depolymerization of xyloglucan has been observed in several ripening fruits (2).

The general objective of our work on cherries is to better understand the genotype effect on biochemical and structural

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features in the cell walls of cherry fruit in relation to firmness. In this paper, we studied the compositional differences in fruit cell wall components for six sweet cherry genotypes with differing harvest date and fruit firmness.

Materials and Methods

Plant material

Six sweet cherry cultivars and selections ('Merpet', 'Celeste', 13S-27-17, 'Lapins' 13S-34-50, and 'Sweetheart') were chosen based on harvest season and relative firmness (Fig. 1). 'Merpet' and 'Celeste' were chosen as early; 13S-27-17 and 'Lapins' as mid; and 13S-34-50 and 'Sweetheart' as late ripening genotypes for this study.

Fruit quality measurement

At harvest, the general fruit quality was measured as follows. Twenty-five fruit from each genotype were used to determine average cherry weight. Pools of five fruit replicated four times were tested for total soluble solids concentration (SSC) (ABBE Mark II digital refractometer), titratable acidity (TA) (719S Titrino, Metrohm Ltd., Switzerland), and pH. Fifty fruit were tested for fruit firmness by both durometer (Shore Instrument, Jamaica, N.Y.) and FirmTech (BioWorks, Stillwater, Okla.). The durometer provides a dimensionless measure of firmness, from 0 to 100 (the higher the number, the firmer the fruit). The FirmTech measures the amount of force required to compress the fruit a constant distance. For the FirmTech measurement, the force thresholds were set at 250/25 g (Max/Min). The load cell was preloaded to 1 kg and its speed was set at 10 $mm \cdot sec^{-1}$.

Preparation of alcohol insoluble residues (AIR)

Approximately 50g of fruit flesh was frozen in liquid nitrogen, pulverized with a freezer mill (Spex CertiPrep, Metuchen, NJ) and stored at -25°C. The cherry powder was homogenized for 2 min in boiling 95% EtOH (5 mL/g cherry powder), and then refluxed at 84°C for 30 min to inactivate pectin hydrolase activity. The suspension was then filtered through Miracloth (Calbiochem, Ca), and insoluble material was washed, in succession, with boiling 95% EtOH, 80% acetone and 100% acetone until it was completely decolorized. The residues were air-dried at 30°C, and stored in a dessicator, yielding an AIR fraction.

Cell wall polymers extraction

Cell wall polymers were extracted from the AIR fraction as described by Rose et al. (21). Five sub-fractions were obtained by successive extraction with: H_2O; 50 mM CDTA and 50 mM $NaC_2H_3O_2$ at pH 6.5; 50mM Na_2CO_3 and 20mM $NaBH_4$; 4% KOH; and 24% KOH. All fractions were dialyzed (Mr cut off 4.5kD) against dH_2O for 24 hr and lyophilized for further analysis.

Assay for uronic acids, total neutral sugar, and xyloglucan

Aliquots of each sub-fraction were assayed for uronic acids by the m-hydroxydiphenyl (mHDP) method (3) and for neutral sugar by the anthrone method (7). Xyloglucan was estimated in aliquots of fractions by adding I-KI solution and measuring the amount of iodine:xyloglucan complex at A_{640} (17).

Results and Discussion

The wide range of fruit firmness among our genotypes grown at Summerland, B. C. is presented in Fig. 1. Fruit firmness was directly related to genotype and to the ripening time of these genotypes. Most late-ripening cultivars and selections were firm or very firm whereas the very early cultivars were generally softer. Among the cultivars and selections, 'Merpet' (the earliest ripening genotype in our study) had the lowest fruit firmness, followed by 13S-27-17, 'Celeste', 13S-34-50, 'Lapins', and 'Sweetheart' (the latest ripening genotype in our study) in both Durometer and Firm Tech measurement in 2000 (Table 1). The firmness measurements from both the Durometer and Firm Tech were consistent in this study in spite of unreliability reported with variability associated with

Table 1. General sweet cherry fruit quality characteristics, and AIR[Z] content of six genotypes (3 different groups of ripening time) in 2000.

Ripening time	Genotypes	Harvest date in 2000	Firmness (Durometer)	Firmness (FirmTech)	AIR	Fruit wt. (g)	SSC (%)	TA[Y] (%)	pH
Early	Merpet	Jul. 05	56.1 a[X]	96.1 a	1.08 a	9.9 a	19.6 b	12.9 b	3.79 a
	Celeste	Jul. 05	74.3 b	202.4 b	1.35 b	10.8 a	17.1 a	8.9 a	3.54 a
Medium	13S-27-17	Jul. 17	71.0 a	157.6 a	1.26 a	10.6 a	16.7 a	14.8 b	3.46 a
	Lapins	Jul. 17	76.6 b	251.8 b	1.38 b	12.4 b	15.4 a	11.1 a	3.82 b
Late	13S-34-50	Jul. 24	76.8 a	244.4 a	1.35 a	12.4 a	18.4 a	12.2 a	3.74 a
	Sweetheart	Aug. 01	79.6 b	275.1 b	1.46 b	11.3 a	18.5 a	13.7 a	3.81 b

[Z]Alcohol Insoluble Residue (g/100g fresh wt.)
[Y]Titratable acidity expressed as malic acid.
[X]Lower case letters indicate mean separation between genotypes within a ripening time by Student's *t* test, $P = 0.05$.

Durometer (5). The correlations between the fruit firmness and other quality characters, which were commonly used commercially to gauge maturity and selection value, were determined. There was no significant correlation between fruit firmness and other quality characters in this study (data not shown). Other studies have shown that increased firmness of sweet cherry fruit was associated with higher level of SSC with the same genotype (9). This inconsistency may be due to genetic variance of fruit firmness and SSC in sweet cherry.

The proportion of AIR ranged from 1.07 to 1.45 g/100g fresh weight (Table 1). The firmest, latest ripening genotype 'Sweetheart' had the highest concentration of AIR. The results also confirmed that the firmer cherry fruit genotypes (when similar ripening genotypes were compared) had a greater concentration of AIR, as has been reported (1,8). Firmer cherry fruit genotypes may synthesize fruit wall materials at much later stages of ripening than softer fruit genotypes.

Autolytically inactive crude cell wall extracts, termed AIR, were subjected to sequential chemical extraction and assayed for both uronic acids and total neutral sugars content. The water-soluble fraction is typically thought to include polymeric material that has been solubilized from the cell wall. Non-covalent bound pectic substances held in the wall by Ca^{2+}, ionic, and steric interactions were solubilized in 50 mM CDTA and may include lamella pectic substances (21). The soft fruit genotypes had higher amounts of water and CDTA soluble pectins than firm fruit genotype when two similar ripening genotypes were compared (Table 2). Na_2CO_3-soluble fractions are generally considered to be enriched for covalently bound pectins. The Na_2CO_3-soluble pectin was higher in firmer genotypes than in softer genotypes. In general, softer fruit genotypes had greater amounts of polymers weakly associated to the cell wall (e.g. water soluble fraction). However, softer fruit genotypes had lower amounts of a highly esterified population of the tightly bound pectins (e.g. Na_2CO_3 soluble fraction). The results

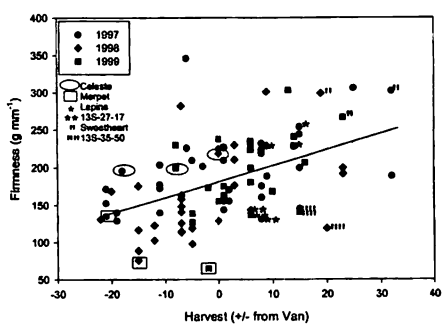

Figure 1. Fruit firmness and harvest dates of 34 sweet cherry genotypes from 1997 to 1999 at Pacific Agri-Food Research Centre, summerland, BC (six cultivars and selelctions which were used for this study are marked) (R^2 - 0.4610, P - 0.05).

Table 2. Uronic acids (*ug mg $^{-1}$ AIR^{Z}*) and total neutral sugars (*ug mg $^{-1}$ $^{\Delta}$AIR^{Z}*) from the cell wall-polymer fraction of six sweet cherry genotypes.

Ripening time	Genotype	Uronic acids in Alcohol insoluble residues (% total)^{Y}					
		Water-soluble	CDTA-soluble	Na_2CO_3-soluble	4% KOH-soluble	24% KOH-soluble	Residue
Early	Merpet	111.3 a^{X}(*53.4*)	13.4 a (*6.4*)	70.9 b(*34.0*)	7.4 a(*3.5*)	3.3 a(*1.6*)	2.1 a(*1.0*)
	Celeste	65.9 b(*39.0*)	6.8 b(*4.0*)	81.6 a(*48.3*)	7.6 a(*4.5*)	3.5 a(*2.1*)	3.2 a(*1.9*)
Medium	13S-27-17	85.9 a(*46.6*)	11.5 a(*6.2*)	72.7 b(*39.5*)	8.2 a(*4.4*)	3.4 a(*1.8*)	2.5 a(*1.4*)
	Lapins	74,6 b(*38.7*)	7.1 b(*3.7*)	95.3 a(*49.5*)	8.8 a(*4.6*)	3.7 a(*1.9*)	3.2 a(*1.7*)
Late	13S-34-50	73.2 a(*41.9*)	6.2 a(*3.5*)	80.1 b(*45.8*)	8.8 a(*5.0*)	3.5 a(*2.0*)	2.8 a(*1.6*)
	Sweetheart	68.5 b(*35.9*)	6.7 a(*3.5*)	100.8 a(*52.8*)	8.8 a(*4.6*)	3.4 a(*1.8*)	2.4 a(*1.3*)
		Total neutral sugars in Alcohol insoluble residues (% total)^{Y}					
Early	Merpet	131.3 a(*38.7*)	23.2 a(*6.8*)	57.5 b(*17.0*)	33.5 a(*9.9*)	71.2 a(*21.1*)	22.1 a(*6.5*)
	Celeste	81.8 b(*27.4*)	13.4 b(*4.5*)	70.2 a(*23.5*)	41.6 a(*13.9*)	66.0 a(*22.1*)	25.4 a(*8.5*)
Medium	13S-27-17	87.7 a(*30.0*)	17.7 a(*6.1*)	65.2 b(*22.3*)	34.1 b(*11.7*)	70.8 a(*24.2*)	16.3 b(*5.6*)
	Lapins	79.7 b(*24.0*)	14.7 b(*4.4*)	78.7 a(*23.7*)	48.0 a(*14.4*)	75.2 a(*22.6*)	36.2 a(*10.9*)
Late	13S-34-50	71.7 a(*20.4*)	13.4 a(*3.8*)	91.8 a(*26.1*)	61.3 a(*17.4*)	85.2 a(*24.2*)	28.5 a (*8.1*)
	Sweetheart	69.4 a(*19.9*)	14.2 a(*4.1*)	94.3 a(*27.1*)	68.3 a(*19.6*)	74.8 b(*21.5*)	26.9 a(*7.7*)

^{Z}Alcohol insoluble residues.
^{Y}Data in parenthesis are expressed in percent of uronic acids and total neutral sugars in total alcohol insoluble residues.
^{X}Lower case letters indicate mean separation between genotypes within a ripening time by Student's *t* test, $P = 0.05$.

of this study suggest that the composition of pectic polymers varies with genotype. The fruit of late, firmer genotypes had a great degree of ionically and covalently linked pectins. Whether differences are due to synthesis or degradation of pectic polymers during sweet cherry fruit development and ripening remains unknown.

Polymers extracted with 4% KOH usually contain a high proportion of hemicellulose polysaccharides, which are extensively hydrogen-bonded to the cellulose fibrils. However, 24% KOH is necessary to extract hemicellulose-rich polymers that are tightly bound to the cell wall, and to cellulose microfibrils in particular. The 4% KOH, 24% KOH and residue sub-factions together contained only minor quantities of uronic acids (Table 2). In contrast to uronic acids, the percentage of neutral sugars was relatively high in both 4% KOH and 24% KOH fractions. The level of total hemicellulosic neutral sugars and uronic acids was not correlated ($P \leq 0.05$) to with fruit firmness.

Xyloglucan in the hemicellulose fractions was generally lower in softer fruit genotypes but it was not consistent across all genotypes (data not shown). Endo β-1, 4-glucanase (C_x-Cellulase), shown to function in the depolymerization of xyloglucan (14), would seem a likely candidate to participate in softening-related cell wall metabolism. The amount of xyloglucan remained constant during tissue softening in ripening fruits of tomato, but the relative molecular weight of xyloglucan decreased (18). Further, it has been suggested that the depolymerization of xyloglucan may play an important role in regulating early events in melon fruit softening (21). In sweet cherry, it is possible that the modification of xyloglucan in th early ripening stage, and variable turnover rates, or transglycosylation on different genotypes may mask any net changes of xyloglucan depolymerization as it does in ripening tomato (18).

In conclusion, fruit firmness in sweet cherry was affected by genotype, related to

fruit maturation periods. The early ripening genotypes were relatively softer than late ripening genotypes. Also, fruit firmness was related to AIR concentration, but not to fruit weight, SSC, TA and pH. AIR concentrations were higher in firmer fruits in general. The amounts of polymers weakly associated to the cell wall were higher in softer genotypes, whereas the amount of tightly bound pectins were higher in firmer fruit genotypes. However, the fruit firmness was not completely related to the level of total hemicellulosic polymers.

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