Biochemical Differences in Cell Wall of Cherry Fruit between Soft and Crisp Fruit

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The cell wall differences between crisp and soft cherry fruits are reported. The penetrometric measurements are correlated with the physiological stage of fruits but not with the sensory analysis at maturity. The major difference lies in the degree of polymerization of pectin side chains. A high degree of polymerization produces a rigid cell wall with numerous bonds between the polymers of crisp fruits: the cells present regular forms. On the contrary, the soft fruits possess fewer interactions between polymers, and consequently, the cells present irregular forms.

Keywords: *Cherry; pectin; softening; turnover*

INTRODUCTION

Most fruit softens during ripening, and this is a major quality attribute that often dictates shelf life. The texture change during the ripening of fruits involves the cell wall degradation, which consists of a dissolution of the pectin-rich middle lamella region (Pilnik and Voragen, 1970; Bartley and Knee, 1982; Leshem et al., 1986). At a biochemical level, the important modifications that can be observed during ripening are a loss of neutral sugars (predominantly galactose) (Woodward, 1972; Knee, 1973; Wallner and Bloom, 1977; Yamaki et al., 1979; Gross and Sams, 1984), an increase in pectin solubility (Davignon, 1961), and a progressive depolymerization of the pectins (Knee et al., 1977; Knee and Bartley, 1981; Huber, 1984; Smith et al., 1990).

However, genetically transformed tomatoes containing antisense polygalacturonase still ripen and soften without pectin depolymerization (Smith et al., 1988), so the concept that polygalacturonase is the primary cause for fruit softening is in question. In addition, it has been shown, using banana (Wade et al., 1992) or cherry (Batisse et al., 1994), that softening does not result from polygalacturonase activity or pectin depolymerization but from cell wall polymer association changes (Mitcham et al., 1989).

Further studies are necessary to advance our understanding of softening and changes in texture of fruit during development and ripening. In addition, the organoleptic qualities of fruit can change with agricultural conditions or storage.

In this paper, we report on the comparison of the cell wall composition and structure between crisp and soft fruits, of the same variety, produced by two orchards.

MATERIALS AND METHODS

Plant Material. Cherries (*Prunus avium* L. Bigarreau Burlat) were harvested from the 39th to the 65th day after anthesis in two orchards producing fruits of different organoleptic qualities as evaluated by sensory analysis at maturity. The fruit flesh was pulverized in liquid nitrogen and stored at −20 °C before the analysis. Twenty physicochemical criteria usually used to characterize changes occurring during fruit ripening were studied to localize the principal physiological stages of fruits (Fils-Lycaon et al., 1988). The mature-green stage was determined to be 46 days after anthesis for the crisp fruits and 41 days after anthesis for the soft fruits. The difference was due to agroclimatic conditions. After the mature-green stage, the rates of physiological evolution were similar between the two kinds of fruits, so we can make a comparison of the fruits from the two orchards between mature-green and postmaturity stages. It is important to note that stages marked by days after anthesis were not directly comparable.

Firmness Measurement. *Sensorial Analysis.* A sensorial analysis of firmness was carried out at the laboratory between the mature cherries of the two orchards. We have employed a triangle test in which the tasters were asked to state whether one of the samples differs from the other two presented.

Penetrometric Analysis. Firmness was measured by deformation, under constant load of 400*g*, with a penetrometer according to the method of Duprat et al. (1986) on all cherries of one randomly chosen batch.

Alcohol-Insoluble Residue Preparation. Alcohol-insoluble residue (AIR) was prepared using the Barbier and Thibault method (1982). Cherry powder was put for 2 min in boiling pure ethanol (5 mL/g of cherry powder). After filtration, the residue was successively washed with 85% ethanol, pure ethanol, then pure acetone. The AIR was dried for 24 h at 40 °C.

Cell Wall Polymers Extraction. Cell wall polymers were extracted from the AIR as described by Saulnier et al. (1988). Six fractions were obtained by successive extractions with water [water-soluble pectins (WSP)] and 1% ammonium oxalate at room temperature (23 °C) [oxalate-soluble pectins (OSP)], 0.05 M hydrochloric acid at 85 °C [hydrochloric acid soluble pectins or protopectins (HSP)], 0.05 M sodium hydroxide at 4 °C (OHSP) and 4 M sodium hydroxide (COHSP) at room temperature (23 °C). All of the fractions were dialyzed (size exclusion, 24 Å) and freeze-dried before analysis. The residues of insoluble material obtained after these different extractions were washed with distilled water and freeze-dried. For more accuracy, see Saulnier et al. (1988).

Neutral Sugar Analysis. Pectic fractions were hydrolyzed by trifluoroacetic acid according to the method of Quemener and Thibault (1990). The neutral sugars obtained were separated by thin layer chromatography (Batisse et al., 1992). Chromatography was performed on plastic sheets precoated with silica gel (Merck) which had previously been impregnated with phosphate buffer (0.2 M, pH 6.8). Chromatograms were developed with acetonitrile−amyl alcohol−water (60:20:20, v/v). Three successive developments were performed, over 9,

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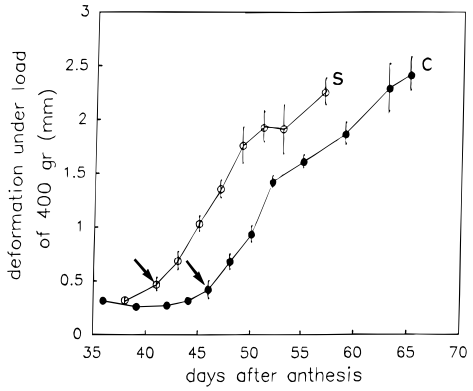

Figure 1. Evolution of deformation under constant load of 400*g* for soft and crisp fruits during ripening. The arrows mark the mature-green stages. Bars are standard deviations based on 10 measurements. S, soft fruit; C, crisp fruit.

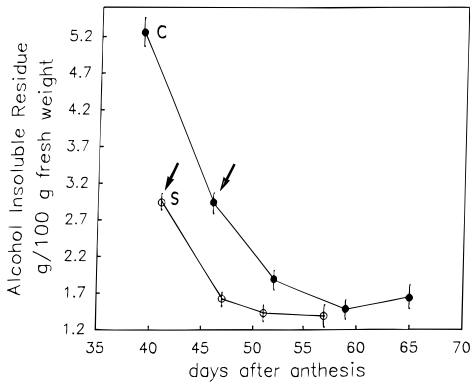

Figure 2. Evolution of the proportion of alcohol-insoluble residue for soft and crisp fruits during ripening. The arrows mark the mature-green stages. Bars are standard deviations based on 10 measurements. S, soft fruit; C, crisp fruit.

12, and 15 cm with full drying of the plates between each. The spots were revealed by uniform spraying with a solution of *N*-(1-naphthyl)ethylenediamine dihydrochloride (NEDIAC) and quantified by spectrodensitometry (spectrodensitometer Camag, Merck, Nogent S/Marne) (Batisse et al., 1992).

Acidic Sugar Analysis. Uronic acid was determined according to the *m*-hydroxydiphenyl (mHDP) colorimetric method of Blumenkrantz and Asboe-Hansen (1973), using D-galacturonic acid as the standard. This method was automated in Integral (Alliance Instrument, Cergy Pontoise) by Prioult (1992).

Protein Analysis. Proteins were estimated in cell wall fractions according to the Kjeldahl method using a coefficient of 6.25.

Study of the Size of Pectic Fractions. The size of pectic fractions was studied by high-pressure size exclusion chromatography (HPSEC), according to the procedure of A. Baron and P. Massiot (private communication, 1991). The pectic fractions were dissolved in 0.4 M acetic acid−sodium acetate buffer (pH 3.5) to a final concentration of 2 mg/mL. The resulting solution was filtered before 20 μL was injected into the HPSEC system, using Supelco TSK PWXL G2500-G3000 and G4000 columns connected in sequence. The mobile phase, which was filtered and deaerated before used, was 0.4 M sodium acetate buffer (pH 3.5) at 35 °C. The flow rate was 0.8 mL/min. The HPSEC system was calibrated with dextran weight markers.

Photonic Microscopy. Material for photonic microscopy was fixed in 4% glutaraldehyde with 0.1 M phosphate buffer (pH 7.5) and then washed in the same buffer. The material was dehydrated in an ethanol series and embedded in historesin (Reichert-Jung, Germany). Sections were cut at 3 μm, stained with a multiple staining solution (0.1% Ladd solution), and then observed by photonic microscope (Optiphot 2, Nikon, Japan).

RESULTS AND DISCUSSION

Firmness Measurements. During the increasing phase, the fruit deformation is constant (Figure 1). The end of this phase, called mature-green stage, is characterized by the beginning of stone lignification, observed for the first harvest of soft fruits and between the fourth and fifth harvests for crisp fruits. After the mature-green stage, the fruit deformation increases and does not shown any differences between the fruit of the two orchards.

The cherries of Venasque were more crisp than the cherries of Pernes for each of the eight panelists; therefore, the firmness measurements by penetrometric analysis were not correlated with the crispness of fruits evaluated by sensory analysis.

AIR Quantity. The proportion of AIR decreases from 5.2 (green stage) to 1.5 g/100 g of FW (postmaturity stage) (Figure 2). During ripening, the proportions of AIR for the fruits of the two orchards were similar at every physiological stages. At maturity and postmaturity, the proportion of AIR of cherry fruit was approximatively 1.5 g/100 g of FW. This result is in accordance with those of Fils-Lycaon and Buret (1990). The comparison between the AIR proportion of different vegetables at maturity [apple = 2% FW (Renard, 1989) carrot = 2.4% FW (Voragen et al., 1983)] or during ripening for the same fruit suggests that firmness and AIR proportion are correlated. However, this hypothesis did not explain the textural differences between the fruits of the two orchards at maturity, so the AIR proportion was not sufficient to determine the fruit texture. The proportion of AIR for 100 g of FW decreases during ripening (Figure 2), but its quantity increases in the fruit (mature-green = 0.12 mg/fruit, maturity = 0.135, and postmaturity = 0.16 mg/fruit). These results, in accordance with those of Mitcham et al. (1989), suggest a permanent synthesis of AIR.

Table 1. Composition[a] of Soft and Crisp Fruit Alcohol-Insoluble Residues

	mature-green		postmaturity	
	soft	crisp	soft	crisp
GalA	28.5 ± 0.8	31.4 ± 0.8	28 ± 0.3	21.1 ± 0.7
NS	29 ± 1.6	28.5 ± 1.14	26.4 ± 1.6	32.4 ± 1.29
Cell	13 ± 0.1	13.6 ± 0.1	11.6 ± 0.3	12.2 ± 0.2
Prot	14.5 ± 0.5	12.9 ± 0.6	24.6 ± 0.3	18.2 ± 0.5
Gal	5.8 ± 0.23	7 ± 0.28	6.2 ± 0.23	6.6 ± 0.27
Ara	11 ± 0.3	11 ± 0.33	11.2 ± 0.33	11.8 ± 0.36
Glc	3 ± 0.12	2.2 ± 0.09	1.6 ± 0.1	3 ± 0.11
Man	1.6 ± 0.09	1.2 ± 0.07	1.6 ± 0.1	2.2 ± 0.13
Xyl	4.4 ± 0.15	3.4 ± 0.11	2.8 ± 0.1	4.8 ± 0.16
Rha	3.2 ± 0.09	3.8 ± 0.15	3 ± 0.09	4 ± 0.12

[a] Data are given in percent of dry weight of AIR. Measurements were done in triplicate, and standard deviations are indicated. GalA, galacturonic acids; NS, neutral sugars; Cell, cellulose; Prot, proteins; Gal, galactose; Ara, arabinose; Glc, noncellulosic glucose; Man, mannose; Xyl, xylose; Rha, rhamnose.

AIR Composition. Table 1 presents the AIR composition of the fruits of the two orchards at two development stages (mature-green and postmaturity).

At the mature-green stage, the fruit AIR compositions are similar for the two orchards. However, the crisp fruits present more galacturonic acid (31.4%) and less proteins (12.9%) than the soft fruits (GalA = 28.5%, Prot = 14.5%). The proportions of the sums of neutral sugars are similar for the two orchards. Nevertheless, the proportion of individual sugars reveals more galactose and rhamnose units for the crisp fruits and more glucose, mannose, and xylose units for the soft fruits.

During maturity, the two kinds of fruits show differential changes in their wall components. Only cellulose proportions present similar changes in both types of fruits with a decrease during ripening. Numerous authors found that the cellulosic glucan level remains constant or perhaps decreases slightly during softening for apples (Bartley, 1976), tomatoes (Gross and Wallner, 1979), and pears (Ahmed and Labavitch, 1980).

The proportion of galacturonic acid does not vary for the soft fruits, but the quantity of AIR increases in the fruit. These results suggest a permanent synthesis of the galacturonic acid for the soft fruit. Mitcham et al. (1989) have shown a synthesis of pectic, hemicellulosic, and cellulosic wall fractions during development and ripening of the tomato fruits. In contrast, crisp fruits present a decrease in proportion (from 31.4 to 21.4% AIR) and quantity (from 37.05 to 28.48 mg/fruit) of galacturonic acids resulting from a degradation of galacturonan. Cherry fruits do not possess endo-PG activity, so this degradation needs an exo-PG activity. The exo-PG presents the predominant pectolytic enzyme in pear (Pressey and Avants, 1976), banana (Markovic et al., 1975), and clingstone peach (Pressey and Avants, 1978). Chan et al. (1981) suggested that an exo-PG might be of major importance in the hydrolysis of pectins in ripening papaya.

Total neutral sugars and proteins changes are similar between the fruits of the two orchards. The two kinds of fruits present a synthesis of neutral sugars and proteins during ripening, but with different speeds. The neutral sugars proportion of the soft fruits decreases but their quantity increases (because the quantity of AIR by drupe increases). The rate of synthesis for the neutral sugars is greater for the crisp fruits than for the soft fruits. The rate of synthesis of proteins is greater for the soft fruits than for the crisp fruits. The possible role of synthetic processes in the alteration of the texture of fruit has rarely been considered; nevertheless, in this case, we observe clearly a difference in the synthetic processes of cell wall between soft and crisp fruits.

At postmaturity, the value of Gal A/NS was very different between the two kinds of fruits (0.65 for crisp fruits and 1.06 for soft fruits). The cell wall structure of crisp fruits is richer in neutral sugars, and the pectins could have a very branched structure. The degree of association with other polymers may be more important. The cell wall of soft fruits contains less neutral sugars and presents fewer interactions between the different kinds of polymers.

These results show a relation between the sensory analysis of texture and the value of Gal A/NS.

The study of different neutral sugars reveals some differences in the neutral sugar constitution for hemicelluloses (glucose, mannose, and xylose). The hemicellulose quantity of the soft fruit decreases (or remains constant), in contrast with that of the crisp fruits.

Our results suggest a different turnover involving the synthesis speed or the biochemical pathway for the hemicellulosic fractions of the two kinds of fruits.

The rhamnose content varies weakly, and the crisp fruits are richer than the soft fruits, so the pectins of the crisp fruits could have more “pectic elbows”.

The arabinose content increases significatively for the crisp fruits. These results suggest a greater arabinan side chain concentration in the pectin of the crisp fruits and, so, an important possibility of association between these polymers. These interpretations are in accordance

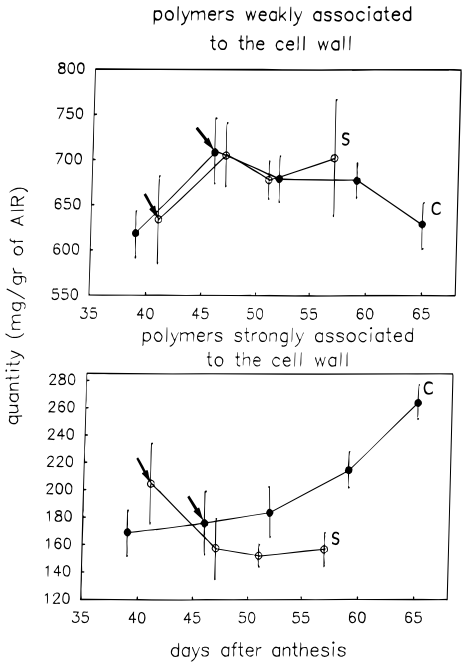

Figure 3. Evolution of the proportion of weakly and strongly associated polymers in the cell wall, for soft and crisp fruits during ripening. The arrows mark the mature-green stages. Bars are standard deviations based on three measurements. S, soft fruit; C, crisp fruit.

with those found by Barrett and Northcote (1965), who propose an attachment of arabinan to the rhamnose residues in the polygalacturonic acid chains.

The galactose percentage does not vary in the same way in the fruits of the two orchards. It increases in the soft fruit and decreases in the crisp fruit. The galactan turnover seems to be different between the fruits of the two orchards.

In ripening apples, galactosyl residues of protopectins decreased by about 70% (Knee, 1973). Similarly in tomatoes (Gross and Wallner, 1979), loss of galactose was the dominant change in neutral sugars, but in pears (Ahmed and Labavitch, 1980) and strawberries (Neal, 1965), the change was smaller. Our results are not in accordance with those previously quoted, but our results give total cell wall galactose and not only pectin galactose.

In conclusion, at the mature-green stage these compositions are similar. The cell wall turnover seems to be different between the two kinds of fruits during ripening. These results give evidence that the cell wall is responsible for the texture. The two kinds of fruits present different compositions of cell walls at postmaturity; more neutral sugars and, consequently, more possibility of associations between the different cell wall polymers for the crisp fruits.

AIR Structure. *General Evolution of the Cell Wall.* After the mature-green stage, for the crisp fruit the polymers weakly associated to the cell wall (WSP-OSP-HSP-OHSP) decrease and the polymers strongly associated (COHSP-R) increase (Figure 3). In contrast, for the soft fruits, the situation is the inverse. This process leads to different degrees of association between cell wall polymers.

Changes in Neutral Sugars during the Cell Wall Evolution. Our previous study has shown an absence of arabinan solubilization in water- and oxalate-soluble fractions (Batisse et al., 1994). Table 2 presents the changes in neutral sugars of the other fractions, be-

Table 2. Changes[a] in Neutral Sugars Content of the Different Fractions between Mature-Green and Postmaturity Stage of the Two Kinds of Fruits

	HSP	OHSP	COHSP	R	total
			Neutral Sugars		
CF					
MG	47.04 ± 1.8	44.4 ± 4	18.02 ± 0.72	33.45 ± 1.67	95.87
PM	36 ± 1.8	11.2 ± 0.78	32.64 ± 0.98	63.77 ± 2.55	107.61
SF					
MG	44.71 ± 2.2	19.35 ± 1.9	41.53 ± 2.07	37.24 ± 1.49	98.12
PM	44.44 ± 2.6	20.68 ± 1.8	35.52 ± 2.13	14.47 ± 0.86	70.67
			Arabinose		
CF					
MG	23.52 ± 0.7	18.5 ± 0.77	3.3 ± 0.21	5.42 ± 0.38	
PM	24.48 ± 0.8	3.54 ± 0.25	7.09 ± 0.44	11.12 ± 0.61	
SF					
MG	23.18 ± 0.7	7.99 ± 0.56	4.74 ± 0.34	7.28 ± 0.45	
PM	27.47 ± 0.8	8.90 ± 0.67	4.55 ± 0.38	3.88 ± 0.28	

[a] Data are given in mg/fraction. Measurements were done in triplicate and standard deviations are indicated. Stages examined: MG, mature-green stage; PM, postmaturity stage. HSP, acid-soluble pectins; OHSP, base-soluble fractions; COHSP, concentrated base-soluble fractions; R, extraction residues; total, OHSP + COHSP + R; CF, crisp fruit; SF, soft fruit.

tween mature-green and postmaturity stages for the fruits of the two orchards. The crisp fruits present a decrease in protopectin neutral sugars found in the HSP and OHSP fractions and an increase in neutral sugars found in the COHSP and R fractions.

On the contrary, for the soft fruits, the protopectin neutral sugars do not vary in HSP and OHSP fractions and the neutral sugars of COHSP and R fractions decrease. The sum of the neutral sugars, present in the OHSP, COHSP, and R fractions, increases between the mature-green and the postmaturity stages of the crisp fruits. It decreases strongly in the soft fruits.

The second part of Table 2 shows changes in arabinose (major neutral sugar of pectin side chains) in these fractions. The crisp fruits, in contrast to the soft fruits, present an increase in the quantity of arabinose in the fractions strongly associated to the cell wall framework.

These results show a different evolution of protopectin side chains. These ramifications remain hooked on the cell wall framework (probably on the hemicelluloses) for the crisp fruits. In a contrary way, the ramifications of soft fruit pectins are solubilized with the polygalacturonic acids.

Changes in Molecular Size of the Pectic Fractions. In HPSEC, the protopectin fractions (HSP) of the crisp fruits present three families of polymers at about 100 000, 20 000, and 1000 Da. These polymers do not depolymerize during ripening (Batisse et al., 1994). Protopectin fractions of the soft fruits present similar chromatograms and evolutions. Textural differences between the two kinds of fruits were not due to a depolymerization of pectins.

Structural Differences in the Mesocarp of the Crisp and Soft Fruits. Figure 4 presents a representative organization of the mesocarp of the two kinds of fruits at the green stage. The crisp fruit mesocarp reveals thick cell walls and numerous large spaces between cells. In a contrary way, the soft fruit mesocarp presents cells larger than the crisp fruits, distorted, with thin undulated cell walls and few spaces between cells.

These two kinds of tissues present differences in their structural aspect from the green stage. These differences are not revealed by the penetrometric measurements. These results corroborate the hypothesis of structural difference in the cell wall of the two kinds of green fruits.

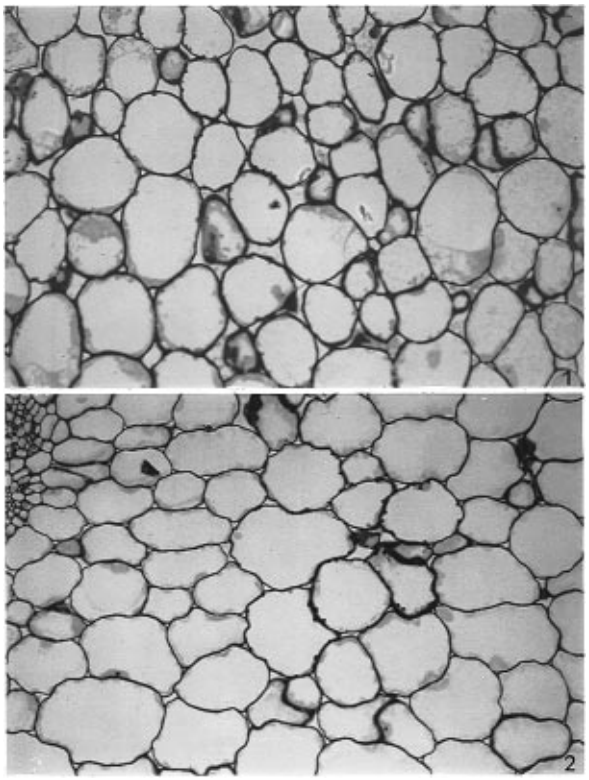

Figure 4. Mesocarp of the crisp (top) and soft (bottom) fruits at the green stage stained by Ladd solution. ×207. (The figure is reproduced here at 50% of the original size.)

At maturity, the general organization becomes similar, but the cell wall evolution shows great difference. This different evolution was confirmed by an electronic microscopy analysis (data not shown).

Our next paper will present a fine study by electron microscopy of the cell wall organization during ripening and the structural differences between the crisp and soft fruits.

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