Seasonal Trends of Titratable Acids, Tannins and Polyphenolic Compounds, and Cell Wall Constituents in Oriental Pear Fruit (*Pyrus serotina*, Rehd)

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Thirteen *Pyrus serotina* clones selected for differences in fruit size and texture were analyzed for titratable acidity, tannins and polyphenols, starch, and cell wall fractions. In clones which were relatively high in acid content at maturity, organic acids increased to a maximum level during the growing season and decreased as the fruits matured. A high correlation exists between the maximum amount accumulated and the acidity at harvest. Seasonal trends between total tannins and polyphenol content and lignin content in the cell wall were nearly parallel. Comparison between Loewenthal's volumetric method and Pro's colorimetric method indicated that the composition of the tannin complex changed with maturity. Except for one clone, a straight-line relationship was observed between lignin and cell wall content on a fresh weight basis as the fruits approached harvest.

The quantity of stone cells and their aggregates materially affects the eating quality of oriental Sand pears, *Pyrus serotina*. One factor which influences the texture of the fruit is the rootstock upon which commercial varieties are grafted. When seedlings of *P. serotina* are used for rootstocks, a physiological disorder known as Yuzuhada (Hayashi and Wakisaka, 1957) often occurs in which the fruits fail to soften at maturity. Hayashi and Wakisaka (1957) showed that the severity of Yuzuhada symptoms is accentuated by drought conditions, which seemingly induce abnormal lignin and suberin formation. A similar disorder known as black-end of Bartlett also occurs on the same stocks (Ryugo and Davis, 1968).

Crist and Batjer (1931) reported that the stone cell aggregates decreased in size as pears approached maturity, but Smith (1935) found that the total cell wall content together with lignin gradually increased up to harvest. Smith (1935) postulated from an inverse relationship between the alcohol-soluble fraction and the synthesis of cell wall materials that sugars were built up and converted to lignified tissue. Archbold (1932), finding a correlation between the disappearance of organic acids and the rapid synthesis of cell wall material in the apple, proposed that acids were intermediates in this pathway. In a broad sense both these proposals are correct, in that the pathways of lignin, tannins, and polyphenols stem from glucose via a common intermediate, shikimic acid (Freudenberg, 1960). This study was undertaken to establish the seasonal trends for the compounds associated with cell wall metabolism in the hope that once these basic patterns are determined, the information could facilitate the diagnosis of rootstock effects with greater confidence.

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MATERIALS AND METHODS

Own-rooted seedlings of *P. serotina* were selected for this study for their differences in the texture and size of fruits. These clones are identified in Table I by the row and tree numbers of the orchard in which rootstock suckers were gathered and propagated.

Sampling. Beginning after June drop, samples consisting of at least 15 fruits were harvested periodically until the fruits were mature. Early in the season, entire fruits without stems and ovules were preserved at −10° C. in 50-gram lots. As the fruits increased in size, thin slices were cut from each fruit and diced. Upon randomizing the diced material, replicate 50-gram samples were preserved in a freezer until analyzed.

Titratable Acidity. Duplicate samples were well homogenized with 200 ml. of water in a Waring Blendor.

Table I. Seasonal Changes in Tannins and Polyphenolic Compounds, Titratable Acidity, Alcohol-Insoluble Substances, and AIS in Selected *P. serotina* Clones

Clone	Sampling Date 5/20	6/17	7/11	8/1	8/21	9/15	10/2	10/23
Coggeshall								
4-6	5.20[a]	3.65	2.85	2.70	1.17	0.81	0.79	0.55
	5.55[b]	4.42	4.81	6.28	3.88	4.12	4.52	3.88
	17.7[c]	26.2	21.5	16.6	9.2	8.0	7.0	6.4
9-25	1.84[a]	1.41	0.95	0.57	0.41	0.37		
	3.24[b]	3.34	3.63	2.70	2.90	2.36		
	16.6[c]	17.10	11.80	8.0	5.6	4.2		
Elliot 5-22	3.44[a]	2.24	1.94	1.26	1.00	0.81	0.76	
	4.42[b]	2.85	3.54	3.78	2.36	2.11	2.26	
	27.7[c]	34.8	31.9	22.6	20.4	16.6	16.2	
Harley								
5-6	4.96[a]	8.39	10.18	4.60	1.88	1.62		
	6.53[b]	12.32	15.47	15.17	14.44	9.77		
	31.5[c]	25.0	20.9	14.7	11.3	9.0		
6-10	2.37[a]	1.61	1.10	0.76	0.50	0.46		
	4.12[b]	3.63	3.73	1.91	2.60	2.70		
	28.3[c]	27.0	20.2	13.6	10.8	8.0		
11-1	3.95[a]	2.86	1.19	0.61	0.50			
	5.55[b]	6.58	7.07	7.12	6.68			
	16.7[c]	17.0	9.4	6.2	4.8			
13-7	2.08[a]	1.57	1.71	1.21	0.85	1.03	0.57	0.67
	4.32[b]	3.14	3.43	3.78	2.80	3.49	3.29	3.14
	28.1[c]	34.0	32.2	23.6	19.2	13.6	12.2	11.2
15-7	2.44[a]	2.32	2.66	2.03	1.77	1.00	1.01	
	4.62[b]	4.51	6.28	7.17	6.53	5.01	5.60	
	31.10[c]	35.1	31.3	23.9	19.6	15.2	12.2	
Staten	2.27[a]	2.48	1.48	1.56				
Island	6.38[b]	7.51	6.97	7.56				
9-7	17.80[c]	14.3	9.9	7.7				
Toepelman	4.38[a]	3.21	1.94	1.18	1.03	0.74	0.86	
11-17	5.55[b]	7.56	7.12	5.74	4.37	3.44	3.44	
	27.60[c]	22.7	16.2	10.7	8.1	7.2	6.8	
Wilcox	1.79[a]	1.31	0.80	0.70	0.60	0.51	0.55	0.45
2-41	4.12[b]	2.90	2.70	1.57	1.08	0.98	1.13	1.18
	21.80[c]	19.3	12.6	8.2	6.3	4.8	4.2	4.0
4-26	5.18[a]	3.53	2.71	2.48	1.07	0.98	0.71	0.52
	5.55[b]	4.76	6.19	6.58	4.86	4.37	4.12	5.17
	31.40[c]	33.7	30.2	22.2	16.6	15.2	11.4	10.8
5-22	2.75[a]	3.56	3.21	1.31	0.94	0.73		
	5.65[b]	11.67	9.23	10.46	8.89	8.99		
	20.2[c]	15.2	8.3	6.1	4.6	3.4		

[a] Mg. tannin and polyphenolic compounds as tannic acid per 100 g. of fruits.
[b] Meq. of acid per 100 g. of fruit neutralized to pH 8.0.
[c] Per cent alcohol-insoluble substances.

A 200-gram aliquot of the slurry was weighed immediately into a beaker and titrated with 0.20*N* NaOH at 0.5 pH intervals from the initial pH to pH 10.0. The titratable acidity is expressed as milliequivalents of acid per 100 grams of fruit neutralized to pH 8.0.

Tannin and Total Polyphenol. Samples were blended with methanol and analyzed for this class of compounds using both the Loewenthal and Pro methods as described by Smit *et al.* (1955). In the former, the tannin extract was analyzed volumetrically using $KMnO_4$ as an oxidant and indigo carmine as indicator. In the Pro method, the tannin content was determined colorimetrically using the Folin-Denis reagent. In both methods appropriate blanks were subtracted. The method of extracting tannins was modified by substituting 2.2*N* H_2SO_4 for an ion exchange resin as a means of liberating the phenolic compounds from the lead complex.

Alcohol-Insoluble Substances and Estimate of Cell Wall. The methanol-insoluble substance dried at 70° C. was designated as AIS. To estimate the amount of cell wall, CW, 1 gram of preboiled AIS was incubated 48 hours with amylase (0.5% Clarase 900) buffered at pH 5.0. Preliminary tests revealed a slight increase in reducing power of the supernatant at the end of 48 hours over the 24-hour incubation period, but the residue showed no further loss of dry matter and gave a negative

test for starch with KI-I_2 solution. At the end of the incubation period, the Clarase-insoluble residue designated as CW was filtered, dried, and weighed. Since protein content is neglected, the values are higher than the sum of cellulose, hemicellulose, lignin, and pectic substances which usually make up the cell wall.

Lignin Determination. This cell wall fraction was determined by a method developed by Noll *et al.* and described by Hagglund (1951). One gram of AIS was moistened overnight in 5 to 8 ml. of dimethylaniline, to which 25 to 35 ml. of 78% H_2SO_4 were added with constant stirring. Acid hydrolysis proceeded for 24 hours, after which the mixture was poured into 200 ml. of hot water and boiled for 5 minutes to complete the hydrolysis. The insoluble lignin was filtered, dried at 105° C., and weighed. Preliminary tests indicated that the lignin fraction reached a minimum value in 24 hours, after which a slight increase was noted. Besides the hydrolysis period, the values of lignin obtained depended upon the amount of AIS analyzed and acid concentration, as reported by Noll *et al.* (Hagglund, 1951).

Histochemical Tests. Slices of tissues were immersed in a solution containing 0.1% ferrous sulfate and 0.5% Rochelle salt to detect tannins and polyphenols; 3% H_2O_2 and a mixture of 3% H_2O_2 and guaiacol to detect catalase and peroxidase activities, respectively; and 1% phloroglucinol–3*N* HCl to detect lignified cells.

RESULTS AND DISCUSSION

The seasonal changes in titratable acidity of these selected oriental pear fruits follow a pattern similar to other fruit species—namely, the peach and apple (Archbold, 1932; Ryugo and Davis, 1958). Pear clones that are relatively rich in acid at maturity showed an increase after the initial sample, proportional to the degree of acidity. In those that were low in acidity when mature the level decreased from the initial to the last sample, an occasional sample showing slight fluctuations from the trend (Table I). In spite of these fluctuations and relatively long sampling intervals, a high positive correlation, $r = 0.88$, was found when the seasonal maximum in acid content of a clone was plotted against its acid content at harvest (Figure 1). Thus, those acidic at harvest accumulate more organic acids during the growth process than those not as acidic. A similar correlation was obtained in peaches and nectarines (Ryugo and Davis, 1958).

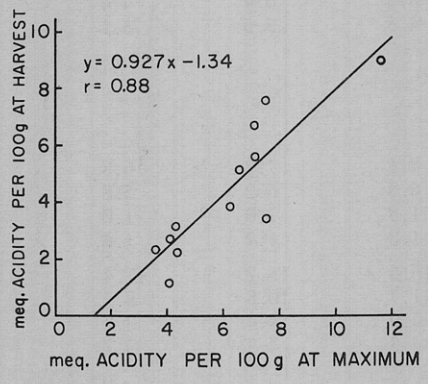

Figure 1. Correlation between titratable acidity at seasonal maximum and at harvest for fruits of selected *Pyrus serotina* clones

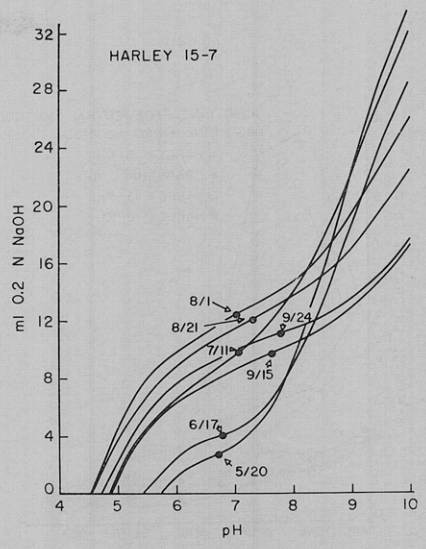

Figure 2. Seasonal changes in buffering capacity and shift in inflection points of a *Pyrus serotina* clone

Titration curves of seasonal samples collected from Harley 15–7 are shown in Figure 2. The inflection point on the curves gradually shifted from pH 6.7 in the initial sample to pH 7.8 at harvest. This shift in the maximum buffer point and the fluctuations in buffering capacity (Δ base per Δ pH) of the juice suggest that changes were occurring in the equilibria among the several acids, and/or the salt-acid ratio of individual acids, as the fruits matured.

Figure 3 shows sections of immature and mature fruits treated with a solution of ferrous sulfate and Rochelle salt. In most clones, the tannins and polyphenols are localized about the vascular bundles, core line, and epi-

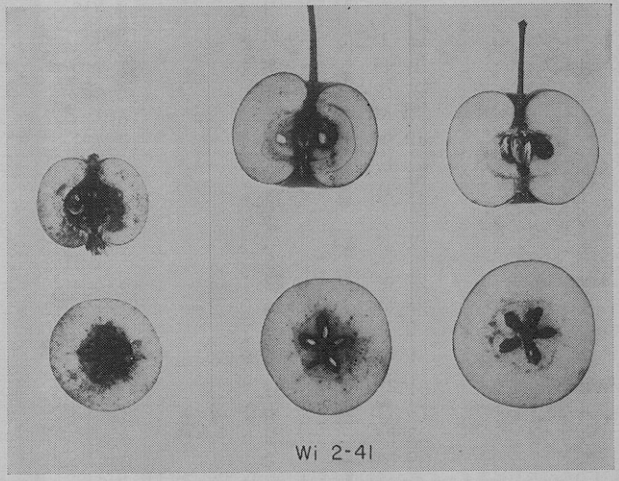

Figure 3. Sections of immature and mature oriental pears treated with ferrous sulfate–Rochelle salt solution to develop tannin and phenolic reactions

Sampling dates (left to right). July 3, September 14, October 8

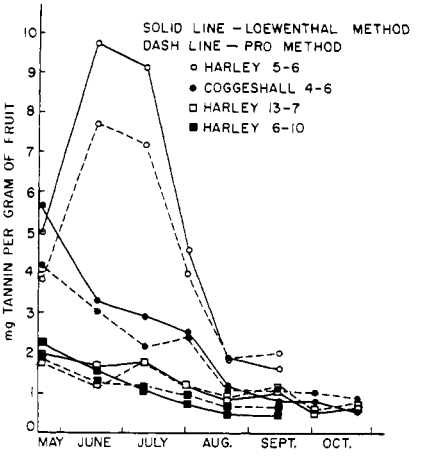

Figure 4. Comparisons between Loewenthal and Pro methods for determining tannins and total polyphenols

dermal layers where the stone cell aggregates are most abundant. Small isolated groups of parenchyma cells in green fruits also contained tannins. Reeve (1959) made similar observations on immature peaches in which the endocarp prior to lignification gave an intense nitroso reaction, as did parenchyma cells in the mesocarp and epidermis. These observations point out the nonspecificity of the tests within this class of compounds.

The reduction in the intensity of the iron-tannin color reaction with maturation shown in Figure 3 was confirmed by chemical analyses (Table I). Except for three clones which showed increases after the initial sample, tannin content decreased gradually from May until the fruits were harvested. Comparisons between the Loewenthal and Pro methods (Figure 4) show that the former yielded higher values than the latter early in the season, but as the fruits matured the situation was reversed. Smit *et al.* (1955) found that these methods gave different values depending upon the compound being analyzed. These analyses indicate that the make-up of the tannin complex may have been changing from one predominant type or group to another as the fruits matured.

During the early stages of fruit development when the tannin and polyphenolic contents are relatively high, cell wall, including lignin, is synthesized very rapidly (Table II).

Table II. Seasonal Changes in Per Cent Composition of Fruits of Selected *P. serotina* Clones on Fresh Weight Basis

	Sampling Date							
Clone	**5/20**	**6/17**	**7/11**	**8/1**	**8/21**	**9/15**	**10/2**	**10/23**
Elliott 5–22	27.7[a]	34.8	31.9	22.6	20.2	16.6	16.2	
	25.0[b]	32.7	29.8	19.8	16.4	13.5	13.1	
	2.7[c]	2.1	2.1	2.8	3.8	3.1	3.1	
	8.9[d]	11.7	9.9	6.2	5.0	3.8	3.6	
Harley								
5–6	31.5[a]	25.0	20.9	14.7	11.3	9.0		
	29.5[b]	22.5	15.2	8.9	7.6	7.2		
	2.0[c]	2.5	5.7	5.8	3.7	1.8		
	10.8[d]	8.2	5.9	3.0	2.9	3.1		
13–7	28.1[a]	34.0	32.3	23.6	19.2	13.6	12.2	11.2
	26.0[b]	32.8	29.4	20.6	16.6	11.6	10.6	9.1
	2.1[c]	1.2	2.9	3.0	2.6	2.0	1.6	2.1
	8.8[d]	11.1	10.4	7.0	5.4	3.6	3.2	2.5
15–7	31.1[a]	35.1	31.3	23.9	19.6	15.2	12.2	
	27.6[b]	30.9	24.6	15.9	11.1	8.8	7.8	
	3.5[c]	4.2	6.7	8.0	8.5	6.4	4.4	
	10.9[d]	14.2	10.4	6.3	4.5	3.5	3.1	
Staten	17.8[a]	14.3	9.9	7.7				
Island	15.4[b]	11.9	6.2	5.7				
9–7	2.4[c]	2.4	3.7	2.0				
	4.9[d]	3.2	2.2	1.9				
Toepelman	27.6[a]	22.7	16.2	10.7	8.1	7.2	6.8	
11–17	25.5[b]	21.0	12.9	8.4	6.3	6.2	5.8	
	2.1[c]	1.7	3.3	2.3	1.8	1.0	1.0	
	8.8[d]	6.9	3.8	2.6	1.8	1.7	1.6	
Wilcox	31.4[a]	33.7	30.2	22.2	16.6	15.2	11.4	10.8
4–26	29.2[b]	30.1	25.8	16.6	11.7	10.8	8.2	7.4
	2.2[c]	3.6	4.4	5.6	4.9	4.4	3.2	3.4
	9.6[d]	8.1	6.4	5.5	4.3	4.3	3.7	2.9

[a] Alcohol-insoluble substances.
[b] Cell wall fraction insoluble in Clarase 900.
[c] AIS fraction soluble in Clarase 900.
[d] Lignin.

As the fruits increase in size and the proportion of cell wall decreases, the level of phenolic constituents shows a concurrent decrease. Hillis and Swain (1957) observed a similar relationship between leucoanthocyanin levels in exposed and shaded plum leaves and the initiation of lignin synthesis in the endocarp. In all clones starch accumulation began after the cell wall content passed its maximum value. This may be supporting evidence that in the carbohydrate economy of plants, starch is accumulated only after a priority for structural and growth requirements are satisfied, as was observed in bearing peach branches (Ryugo and Davis, 1959).

Lignin deposition in the wood of conifers is mediated by hydrolysis of coniferin by β-glucosidase to coniferol and glucose (Freudenberg, 1960). Coniferol is then peroxidatively polymerized to lignin in the cell wall. In this study, immersing young pear sections in solutions of indican (indoxyl-β-D-glucoside) failed to disclose β-glucosidase activity. However, similar tests using H_2O_2 and guaiacol, singly and together, disclosed that both catalase and peroxidase are omnipresent but their activities are much higher about the stone cell aggregates in which lignin synthesis is occurring rapidly. These qualitative tests support the findings on the peach endocarp, in which an excellent correlation was found between the rate of lignin synthesis and catalase and peroxidase activities (Ryugo, 1969). Thus, part of the tannin and polyphenolic complex associated with stone cell aggregates shown in Figure 3 might be aglycone precursor(s) of lignin. This would explain the difference found between Loewenthal and Pro methods. The phenolic domposition could well be changing from one which was predominantly lignin precursors early in the season to one dominated by other types—e.g., leucoanthocyanins as the fruits matured.

In most varieties, the maximum lignin content on a dry weight basis was attained early in the season, while a few showed peaks later in mid-season (Table II). On a fresh weight basis, the trends of lignin content were nearly parallel to those of cell walls in all varieties from the earliest to the latest samples. These results expand the findings of Smith (1935) in Kieffer pears and Wagner apples. The lignin content is plotted against the cell wall content for each sampling date (Figure 5). With the exceptions of samples taken early in the season from Wilcox 4–26 (not shown in Figure 5), a linear relationship was found between the two variables. The linearity or straight-line relationship between these variables which are independent of time, nevertheless, reveals that as the cell wall content decreased with maturity, its composition with respect to lignin remained constant. The similarity in the

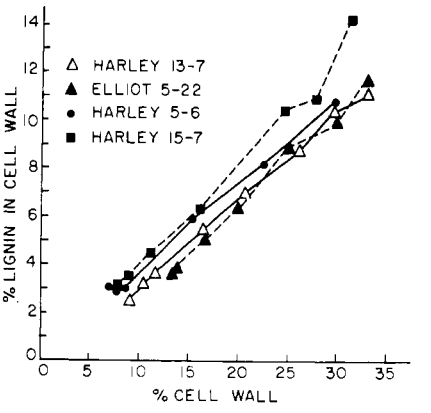

Figure 5. Linear relationship between lignin and cell wall contents of *Pyrus serotina* fruits on fresh weight basis

slopes of the curves among the different varieties indicates that clones which have a relatively high lignin content early in the developmental stages tend to remain so until harvest and vice versa.

Based on an observation by Hayashi and Wakisaka (1957) that pears afflicted with Yuzuhada disease contain a higher density of stone cell aggregates than normal fruits, one might predict that the lignin–cell wall curve of abnormal pears would deviate from those in Figure 5—that is, either the intercept values or the slope of the curves could be shifted by the influence of the rootstock.

LITERATURE CITED

Archbold, H. K., *Ann. Botany* **46,** 407 (1932).
Crist, J. W., Batjer, L. P., *Mich. Agr. Exptl. Sta. Tech. Bull.* **113** (1931).
Freudenberg, K., *Holz Roh Werkstoff* **18,** 282 (1960).
Hagglund, E., "Chemistry of Wood," Academic Press, New York, 1951.
Hayashi, S., Wakisaka, I., *J. Hort. Assoc. Japan* **26,** 178 (1957).
Hillis, W. E., Swain, T., *Nature* **179,** 586 (1957).
Reeve, R. M., *Am. J. Botany* **46,** 210 (1959).
Ryugo, K., unpublished data, 1969.
Ryugo, K., Davis, L. D., *Hort. Sci.* **3,** 15 (1968).
Ryugo, K., Davis, L. D., *Proc. Am. Soc. Hort. Sci.* **72,** 106 (1958).
Ryugo, K., Davis, L. D., *Proc. Am. Soc. Hort. Sci.* **74,** 130 (1959).
Smit, C. J. B., Joslyn, M. A., Lukton, A., *Anal. Chem.* **27,** 1159 (1955).
Smith, W. W., *Plant Physiol.* **10,** 587 (1935).

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