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THE COURSE OF STONE CELL FORMATION IN PEAR FRUITS

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(WITH EIGHT FIGURES)

Introduction

CRIST and BATJER'S (7) analyses of the isolated grit cell clusters of pear fruits show these structures to be approximately three-fourths lignocellulose, of which about a third is lignin. This quantitative relationship was consistent enough in the several samples tested to justify quantitative estimates of the grit cell content of the fruits by lignocellulose determinations. Their histological studies revealed cell wall thickenings 20 days after blossom fall, and chemical analyses indicated lignification occurring two or three days after blossoming.

The accumulation of lignocellulose as a percentage of the dry weight proceeded rapidly for about four weeks, reaching a concentration of half the fruit's dry weight, then began sharply to decrease until at harvest time it amounted to about a fifth of the dry weight. During the period of lignification there occurred a steady decline in the relative amounts of alcohol extractable material, which reached a minimum at the time the lignocellulose reached a maximum. At this point, indicated by a decrease in the percentage of lignified tissue, began the accumulation of alcohol extractable material which continued for the rest of the growing season.

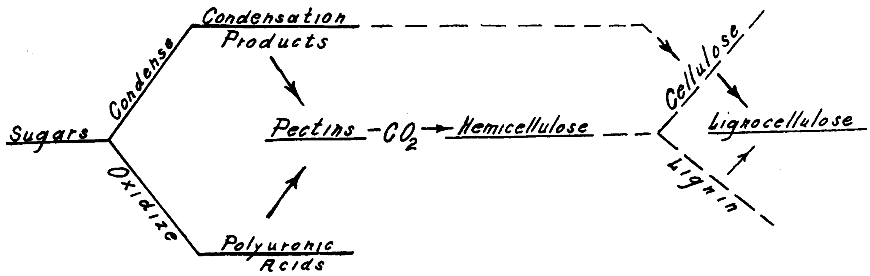
How perfectly the relative amount of alcohol extractable substances can be a reciprocal of the amounts of lignocellulose, in the two varieties of pears studied, appears in figure 21 of CRIST and BATJER'S report. For example, the Kieffer fruits on May 18 had an alcohol extractable content of 55 per cent. of the dry weight, which dropped to nearly 25 per cent. in the latter part of June, and then increased steadily, reaching a concentration of 55 per cent. again by September 2. The alcohol extract is composed largely of sugars. The lignocellulose started with a concentration of about 25 per cent., reached a maximum the last of June of over 55 per cent. and then dropped to 25 per cent. by September.

This same interrelation of these materials was found in the Bartlett pear, which in comparison with the Kieffer is less "gritty" and shows a lower percentage of lignocellulose, coupled with a higher percentage of alcohol extractable substances.

It is also interesting that the "conversion point," so to speak, in this variety is about a week earlier, at which time the relative amounts of these materials correspond closely with those of the Kieffer. The regularity of this typical change in relative concentrations for all varieties of pear fruits studied, grown under different cultural conditions, in widely separated localities, for several unlike seasons, establishes it as a fundamental basic phenomenon and as the inherent order of these changes in growing pear fruits.

In general, the chemical changes incident to cell wall formation are in the direction of lignification. So far as the author is aware, the literature presents no clear cut evidence of a reversal in this order. The suggestion of CRIST and BATJER, however, that such a reversion occurs incident to the development of grit cells in the pear, lignocellulose being transformed to sugars, makes desirable a further study of the chemical changes occurring in these structures.

The results of studies of several investigators suggest a theoretical course of events in the process of lignification of plant tissues which may be indicated by the following diagram:



It is known that the first products of photosynthesis, translocated as monosaccharides or disaccharides, are sugars (**9, 21, 29**) and that the end products in the lignification of plant tissues are lignocelluloses (**11, 16, 17**). The course the sugars follow and the changes they undergo to reach this final stage is a point of fundamental concern. Many investigators have sought, chiefly on woody material, a clue to this process (**3, 5, 8, 10, 11, 21**). That the sugars condense to form polysaccharides, there is no doubt (**25, 27**). That they oxidize to form sugar acids known as polyuronic acids is supported by the results of certain research (**25, 27**).

The polyuronic acids, galacturonic, glucuronic, and others, are of particular interest because they combine with certain condensation products (arabinose, galactose) (**12, 13, 15**) to form pectins, and also, they seem to be a part of the hemicelluloses (**5, 24**).

Change of pectin to hemicellulose was fairly well established by CANDLIN and SCHRYVER (**5**). In their investigations on chemical changes taking place in cell wall substances during lignification, they group the substances accompanying cellulose in cell walls into three classes: pectins, hemicelluloses, and lignins. They were able to decarboxylate pectins with the formation of hemicelluloses which resembled in all respects the hemicelluloses isolated directly from timbers. Their results indicate that decarboxylation takes place when plant tissues lignify. They were unable, however, to establish a direct connection between pectins and lignins.

The aim of this investigation was to seek additional evidence of movement to right as indicated by the foregoing diagram (*i.e.,* from sugars through pectins, etc., to lignocellulose), to examine the possibility of a reversal of the direction of movement, and also to further the objective of relieving pear fruits generally, those of the Kieffer in particular, from the burden of grittiness in quality.

Technical methods

Sampling.—The material used for lignocellulose determinations was sampled as described by CRIST and BATJER (**7**); that is, transverse segments were cut from the center of each fruit, the loculi of the carpels removed, and the segments dried in an electric oven at 65° C. For carbohydrate and pectin analyses similar portions of the fruits were taken. These were finely ground in a meat grinder and thoroughly mixed. Small amounts were placed in weighing bottles for dry weight determinations, made at 95° C. Samples consisting of 25 grams of this material were quickly weighed and dropped into mason jars containing boiling 95 per cent. alcohol of sufficient volume to give a final concentration, including the moisture of the sample, of 80 per cent. alcohol. Boiling was continued for 10 minutes, after which the jars were sealed and stored pending analysis. Reductions obtained in similar samples boiled 10, 30, and 60 minutes indicate that complete extraction of the reducing substances was obtained by boiling 10 minutes.

Small amounts of calcium carbonate were added to the first samples to neutralize the acids, but because of the small amounts of acids present and the short period of heating, it seemed that hydrolysis would be negligible. ARCHBOLD (**2**) in a report on work with apples states: "No difference was found in the estimated amounts of sugar in untreated solutions compared with solutions treated with calcium carbonate during the hot extraction or with ammonia during both cold and hot extractions. Hydrolysis during alcohol extraction is therefore presumed to be negligible.'' The first season's study showed that the calcium carbonate interfered with the pectin determinations by neutralizing the weak acid used in extracting total pectins. Therefore it was omitted in subsequent samples.

Analysis.—Benzene extractions, alcohol extractions, water extractions, and alkali extractions were made and the cellulose and lignin determinations secured as described by CRIST and BATJER.

Total sugars, sucrose, dextrins, starch, and hemicellulose were determined as outlined by the committee on chemical methods of the American Society of Plant Physiologists (**31**).

Pectins as calcium pectate were determined by the method established by CARRÉ and HAYNES (**6**) and employed by APPLEMAN and CONRAD (**1**).

During the growing seasons of 1930 and 1931, reducing substances were determined by the modified SHAFFER-HARTMANN titration method (**30, 33**). The sugar solutions obtained by taking up the alcohol extracts in water, being quite free of coloring matter, were used directly to avoid loss of sugars which might be thrown down in the clearing process (**4**).

Investigations by PHILLIPS (**28**) indicate that with certain materials the SHAFFER-HARTMANN method gives high values. He cites SULLIVAN (**34**) as finding that iodine liberated in the presence of plant extracts may be absorbed by some constituent of the extract, such as phlorizin, causing an error in the determination of the reducing copper. To test this possibility, reductions were determined on the water solutions of the alcohol extracts of the 1933 samples after being reduced at 80° C. for 30 minutes, first by the modified SHAFFER-HARTMANN titration method in which titration was carried out in the presence of the plant extract; and second, by the volumetric thiosulphate method (**17**) in which the cuprous oxide is separated by means of an asbestos mat in a Gooch crucible and titrated free of the plant extracts. To check further on this point, the filtrate from which the cuprous oxide had been separated was titrated at once by the modified SHAFFER-HARTMANN method, with the results presented in tables I and II.

These data show clearly that with these extracts the modified SHAFFER-HARTMANN method gives values greater than the amounts of copper reduced warrant. Indications are that this difference is due to some substance in the plant extract which does not reduce the copper, but probably behaves as suggested by SULLIVAN (**34**). This substance, whatever its nature, seems to be present in larger amounts in extracts from the earlier samples, which are more highly colored and contain larger proportions of skin to flesh of the fruit.

This test was repeated on cleared solutions taken from apple tissue. Apparently some of the reducing substances were removed by clearing as the amounts of reduced copper were slightly lower. The greatest loss was in

TABLE I

AVERAGE CC. OF 0.1 N SODIUM THIOSULPHATE SOLUTION REQUIRED TO TITRATE REDUCTION OF FEHLING'S SOLUTION SECURED WITH 50 CC. OF THE ALCOHOL SOLUBLE EXTRACT OF APPLE FRUITS

DATE OF SAMPLING*	UNCLEARED SOLUTIONS							CLEARED SOLUTIONS WITH 0.5 GRAMS OF NEUTRAL LEAD ACETATE PER 100 CC.			
	FIRST TEST			SECOND TEST							
	TITRATION IN PRESENCE OF PLANT EXTRACT BY SHAFFER-HARTMANN METHOD	REDUCED COPPER SEPARATED FROM PLANT EXTRACT AND TITRATED BY VOLUMETRIC THIOSULPHATE METHOD	DIFFERENCE BETWEEN THE TWO METHODS	TITRATION IN PRESENCE OF PLANT EXTRACT BY SHAFFER-HARTMANN METHOD	REDUCED COPPER SEPARATED FROM PLANT EXTRACT AND TITRATED BY VOLUMETRIC THIOSULPHATE METHOD	SHAFFER-HARTMANN TITRATION OF FILTRATE FROM VOLUMETRIC THIOSULPHATE METHOD	COPPER TITRATION PLUS FILTRATE TITRATION	TITRATION IN PRESENCE OF PLANT EXTRACT BY SHAFFER-HARTMANN METHOD	REDUCED COPPER SEPARATED FROM PLANT EXTRACT AND TITRATED BY VOLUMETRIC THIOSULPHATE METHOD	SHAFFER-HARTMANN TITRATION OF FILTRATE FROM VOLUMETRIC THIOSULPHATE METHOD	COPPER TITRATION PLUS FILTRATE TITRATION
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
May 27				3.4	1.0	2.4	3.4	1.9	0.3	1.5	1.8
June 4	4.7	2.4	2.3	4.8	2.4	2.4	4.8	3.2	2.0	1.0	3.0
10	4.5	3.0	1.5	4.8	3.0	1.7	4.7	3.5	2.6	0.7	3.5
16	6.9	5.7	1.2	6.9	5.3	1.6	6.9	5.7	5.1	0.8	5.9
24	7.9	6.7	1.2	8.0	6.8	1.0	7.0	6.0	6.2	0.4	6.6
30	8.2	7.7	0.5	8.3	7.7	0.7	8.4	7.6	7.4	0.5	7.9
July 7	8.4	8.1	0.3	8.7	8.3	0.6	8.9	8.4	8.0	0.4	8.4
14	9.5	9.5	......	9.9	9.4	0.5	9.9	9.6	9.2	0.4	9.6
25	10.7	9.7	1.0	11.2	10.7	0.6	11.3	11.5	11.3	0.2	11.5
Aug. 9	11.8	11.8	......	11.7	11.4	0.4	11.8	11.0	10.7	0.4	11.1
Sept. 9	14.5	14.3	0.2	14.6	14.0	0.4	14.4	13.9	13.6	0.2	13.8
Oct. 9	13.3	13.5	0.3	13.8	13.4	0.3	13.7	13.4	13.1	0.2	13.3

* 80 grams fresh weight for each sample.

the SHAFFER-HARTMANN titrations and shows that about half of this unknown material was taken from the solutions by clearing. It would seem from these facts that this substance is associated with the skin or pigments in both apple and pear fruits.

Reducing substances in the 1933 samples were determined by the volumetric thiosulphate method, as described (**17**), except that reduction was carried out at 80° C. for 30 minutes by means of a hot water bath.

TABLE II

AVERAGE CC. OF 0.1 N SODIUM THIOSULPHATE SOLUTION REQUIRED TO TITRATE REDUCTIONS OF FEHLING'S SOLUTION SECURED WITH 50 CC. OF THE ALCOHOL SOLUBLE EXTRACT OF PEAR FRUITS

DATE OF SAMPLING*	TITRATION IN PRESENCE OF PLANT EXTRACT BY SHAFFER-HARTMANN METHOD	REDUCED COPPER SEPARATED FROM PLANT EXTRACT AND TITRATED BY VOLUMETRIC THIOSULPHATE METHOD	FILTRATE FROM VOLUMETRIC THIOSULPHATE METHOD TITRATED BY SHAFFER-HARTMANN METHOD	COPPER TITRATION PLUS FILTRATION TITRATION
	cc.	*cc.*	*cc.*	*cc.*
May 20	1.6	0.3	0.6	0.9
27	3.2	1.5	1.5	3.0
June 4	3.7	2.2	0.9	3.1
10	3.1	2.3	0.9	3.2
16	4.1	2.9	0.6	3.5
24	3.8	3.0	0.4	3.4
30	3.7	3.0	0.2	3.2
July 7	5.2	4.6	0.2	4.8
14	6.3	5.4	0.3	5.7
25	7.3	6.3	0.3	6.6
August 9	9.6	8.7	0.1	8.8
September 9	15.8	14.7	0.1	14.8
October 9	16.7	16.3	......	16.3
Novemebr 4	19.0	18.6	0.2	18.8

* 80 grams fresh weight per sample.

Hardness of fruits.—Hardness of the fruits was obtained on each sampling date by means of a pressure tester (**20**). A plunger of 4/16 inch diameter was substituted for the regulation 5/16 inch one, to permit readings on the early hard fruits. The pressures thus obtained may be converted, approximately, over to 5/16 plunger values, by the factors 1.42 when skin is removed and 1.35 when skin is not removed. These factors were derived by determining the pressure for each plunger on the same fruits. As the ratio varies a little, being greater with the softer fruits, these factors are only approximate, but serve to give an estimate of the hardness of the early fruits.

Procedure and results

During the growing season of 1930, samples for chemical analysis were taken periodically of Kieffer and Bartlett pears and Wagener apples from vigorous, productive trees.

Changes in the amount of lignocellulose, total sugars, reducing sugars, total pectins and soluble pectins, as percentages of the dry weight were determined. Figure 1 compares graphically the changes in lignocellulose and total sugars in the three fruits studied.

A more detailed story was desired of the changes occurring in the Kieffer fruits. Consequently in 1931 fruits of Kieffer pear were sampled every third day from June 2 until July 17 and less frequently thereafter, as long as any fruits remained on the tree. As many as 1200 fruits were required to furnish enough material for a single sample on the earlier dates and a minimum of 25 fruits was used in each sample.

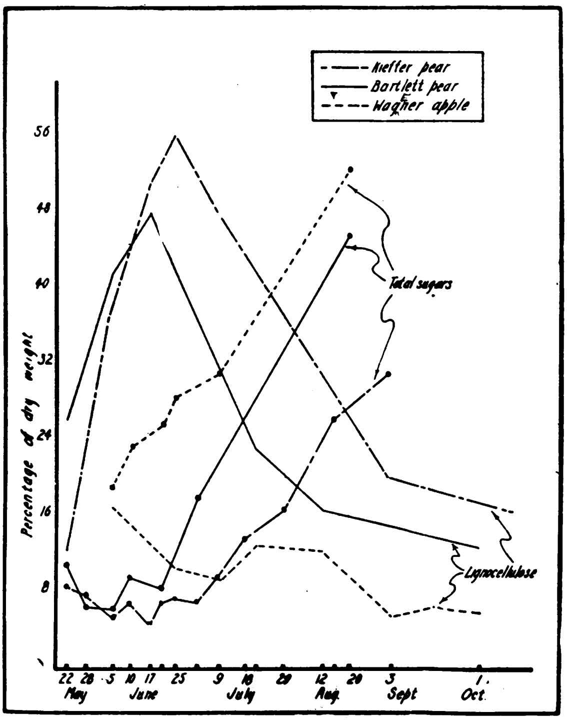


FIG. 1. Seasonal course of total sugars and lignocellulose as percentage of dry weight in fruits of different "grittiness," 1930.

Quantitative determinations of the constituents of the Kieffer pear fruits secured during the growing season of 1931 are presented in table III and graphically in figure 2. The changes in lignocellulose, sugars, and pectins are similar to those secured in 1930.

Changes in relative amounts of lignocellulose in the Kieffer (fig. 1 and 2), consisting of a very rapid accumulation during the first four weeks after fruit setting followed by a less rapid decrease, are identical with those found by CRIST and BATJER (7). The accumulation of lignocellulose is accompanied by a decrease in the amounts of total and reducing sugars, until

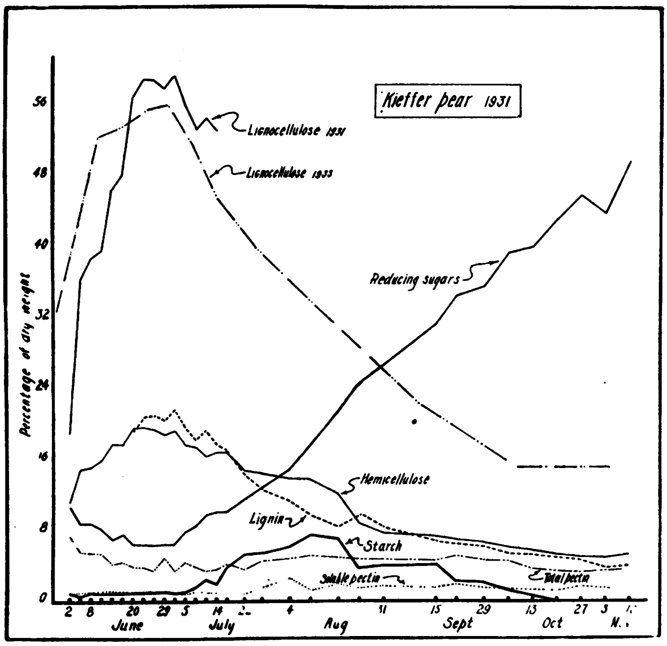


FIG. 2. Seasonal course as percentage of dry weight of several constituents of Kieffer pear fruit, 1931.

a few days before the peak of concentration of lignocellulose is reached. The sugar curves then indicate a piling up of sugars coincident with the decrease in lignocellulose. The difference between total and reducing sugars is reported as sucrose and persists in relatively small amounts. The curve for sugar concentrations could almost be a reciprocal of the curve for lignocellulose both in value and direction. The percentage of total pectins decreases during the early growing season, while soluble pectins increase. After the first part of July, both show a slight gradual increase. The difference between total and soluble pectins is reported as protopectin (**6**), which goes over to soluble pectins during the life of the pear.

COMPARISON OF CHANGES IN THE APPLE AND PEAR FRUITS

That "grit cells" are composed chiefly of lignocellulose, and that their formation is a result of lignification which may be measured quantitatively by lignocellulose determinations has been established by CRIST and BATJER.

As would be expected from their findings, the percentage of lignified tissue does not increase in the "grit cell"-free Wagener apple fruits (fig. 1). Although lignocellulose starts at a concentration equal to that in the pear, it decreases continuously throughout the season, except for a short

TABLE III

PERCENTAGE COMPOSITION OF KIEFFER PEAR FRUITS, 1931, DRY WEIGHT BASIS

DATE OF SAMPLING	MOISTURE	DRY WT. OF FRUIT	DRY WT. OF ALC. EXT.	REDUCING SUGARS	DEXTRINS	STARCH	HEMICELLULOSE	RESIDUE*	LIGNIN	PECTIN	TOTAL PECTIN
	%	%	%	%	%	%	%	%	%	%	%
June 2	84.87	15.13	51.67	10.63	0.84	0.76	11.14	19.07		0.75	7.30
5	84.27	15.73	42.82	8.46	0.61	0.54	14.91	36.20		0.99	5.61
8	83.54	16.46	43.25	8.62	0.54	0.77	15.01	39.10		0.75	5.52
11	83.43	17.57	42.19	8.12	0.64	0.67	15.93	39.79		0.87	5.42
14	81.23	18.77	34.62	6.92	0.62	0.74	17.82	46.22		0.92	4.08
17	78.92	21.08	34.83	7.44	0.80	0.66	17.64	48.15		0.74	4.21
20	78.26	21.74	32.58	6.20	0.42	0.63	19.59	41.74	19.20	1.07	3.95
23	77.63	22.37	29.96	6.25	0.83	0.75	19.63	40.22	20.70	1.10	3.85
26	77.58	22.42	30.50	6.19	0.71	0.66	19.51	41.62	20.80	1.10	3.68
29	77.53	22.47	31.64	6.36	1.00	0.85	18.82	42.87	20.30	0.85	4.75
July 2	76.83	23.17	29.77	6.38	1.07	0.75	19.23	42.90	21.28	0.71	3.68
5	75.85	24.15	33.28	7.94	1.80	0.87	17.75	42.98	19.80	0.71	4.30
8	76.34	23.66	34.79	8.45	.95	1.62	17.50	40.01	18.36	1.19	3.89
11	76.55	23.45	36.67	9.56	1.07	2.02	16.30	38.68	19.24	1.05	3.58
14	77.96	22.04	37.60	10.08	1.44	1.72	16.79	37.96	17.86	1.03	3.64
17	78.60	21.40	37.80	10.00	1.16	3.85	16.98	35.80	17.36	1.00	4.11
22	76.54	23.46	39.53	11.30	1.14	5.19	14.78	32.74	14.45	0.65	3.79
27	77.00	23.00	44.69	12.60	1.60	5.40	14.60	28.97	12.40	1.61	4.34
August 4	78.00	22.00	49.42	14.72	1.75	6.10	13.97	26.66	11.05	2.68	4.62
10	79.57	20.43	54.76	17.79	2.05	7.61	13.77	24.20	9.81	1.27	5.08
18	79.77	20.23	59.86	21.38	1.76	7.06	12.12	21.14	8.36	1.97	5.03
24	80.00	20.00	63.53	24.55	1.26	3.82	8.88	19.14	9.82	1.40	4.61
31	80.36	19.64	64.73	26.73	1.80	4.00	7.80	17.53	8.53	1.80	4.73
September 15	80.91	19.09	71.83	31.40	1.32	4.12	7.65	14.27	6.86	1.61	4.69
21	82.59	17.41	71.90	34.73	1.44	2.11	7.08	14.18	6.54	1.94	5.15
29	82.58	17.42	76.23	35.87	0.99	2.10	6.53	11.97	6.05	1.60	4.58
October 6	82.65	17.35	80.23	39.59	0.93	1.14	5.94	10.79	5.44	1.47	4.41
13	82.18	17.82	80.61	40.17	0.69	0.39	5.79	10.65	5.27	1.42	3.73
20	82.26	17.74	84.72	43.29	0.66	trace	5.38	9.72	4.67	1.26	3.67
27	82.16	17.84	86.49	46.07	0.61	trace	5.03	9.37	4.34	1.76	3.91
November 3	82.62	17.38	87.27	44.76	0.87	0.13	4.85	9.42	3.96	1.62	3.85
10	84.00	17.00	98.85	49.87	0.62	0.25	5.37	10.13	4.22	1.35	3.88

* Fraction of sample remaining after carbohydrate extraction.

period in July. Accumulation of sugars is not delayed as in the pear fruits, but proceeds at a uniform rate from the very start.

Changes in hemicelluloses in the Kieffer pear (fig. 2) follow closely changes in lignin. Although the changes are of a different character in the apple, this relation of hemicellulose to lignin seems to hold, as WIDDOWSON (**37**) also shows a rapid decrease in the percentage of hemicellulose in the early life of Bramley's seedling apple, followed by a less rapid decline during the remainder of the growing season. Changes in starch concentration found in the apple by WIDDOWSON (**37**) and TETLEY (**35**) have the same character as those found in the Kieffer pear.

Changes in the Bartlett pear are similar to those in the Kieffer, but with a general shortening of the whole process. Lignocellulose curves start at a higher concentration, reach a minimum earlier in the season and fall to a lower level than those for the Kieffer (fig. 1). Although total sugars show a decrease for the first two weeks after fruit set, they are less pronounced than in the Kieffer and accumulation of sugars starts about 10 days earlier (fig. 1). The greater amount of total pectins in the Bartlett seems to be due to a greater quantity of protopectin. The character of the pectin changes is much like that found in Kieffer.

Inspection of figures 1 and 2 reveals a critical "point of change" in the pear fruits where those constituents which have been accumulating decrease suddenly, and those materials which have been decreasing, begin to accumulate. This "conversion point" occurs about ten days earlier in the Bartlett fruit than in the Kieffer.

In the Wagener apple there is a rapid accumulation of sugars, showing a steady increase in sucrose, and a gradual decrease in lignocellulose from the very earliest sampling. As in the pear fruits, protopectin goes over to soluble pectin early in the season. The increase in both total and soluble pectins during the last part of the growing season distinguishes pectin changes in the apple qualitatively from those in the pear.

ONSLOW (**27**) and CRIST and BATJER show lignin to account consistently for about one-third of the lignocellulose. These data coincide with their findings and, as would be expected, lignin changes are qualitatively the same as lignocellulose (fig. 2).

Hemicellulose changes, presented graphically in figure 2, are almost identical with lignin changes both in amounts and direction, except for the period of starch concentration from July 17 to August 24. During this time hemicelluloses do not decrease as rapidly as does the lignin and they maintain a difference of about 4 per cent. of the dry weight. It may be significant that this over-rapid decrease in lignin, and slowing of the hemicellulose decline, coincide closely with the high concentration of starch.

TABLE IV

PERCENTAGE COMPOSITION OF KIEFFER PEAR FRUITS IN STORAGE AT 33° F., DRY WEIGHT BASIS

	MOISTURE	DRY WT. OF FRUIT	DRY WT. OF ALC. EXT.	REDUCING SUGARS	DEXTRIN	STARCH	HEMI-CELLULOSES	RESIDUE*	LIGNIN	PECTIN	TOTAL PECTIN
Harvested and placed in storage September 29, 1931											
	%	%	%	%	%	%	%	%	%	%	%
Sept. 29	82.30	17.70		37.92	0.99	0.67	6.39	14.07	6.67	2.32	4.76
Oct. 13	82.42	17.58		38.81	0.86	0.31	6.34	14.99	6.84	2.50	4.64
Nov. 26	82.70	17.30		38.84	0.73	0.45	6.76	15.77	7.29	1.79	5.82
Dec. 17	83.31	16.69		39.98	0.89	0.34	6.04	16.19	7.73	2.07	5.05
Jan. 7	83.13	16.87		40.02	1.30	0.35	6.82	15.52	7.51	1.98	5.88
" 21	84.37	15.63		41.59	1.36	0.31	6.50	16.89	7.72	2.73	6.77
Feb. 11	84.90	15.10		43.97	1.08	0.42	6.42	16.85	7.53	3.19	5.56
" 25	84.30	15.70		42.47	1.24	0.98	5.57	15.75	7.16	3.48	5.32
Harvested and placed in storage October 13, 1931											
Oct. 13	82.44	17.56	79.72	42.14	0.79	0.22	5.55	12.41	5.33	2.22	4.55
Nov. 26	82.54	17.46	83.61	44.26	0.75	0.28	5.77	12.91	4.93	1.65	4.17
Dec. 17	83.00	17.00	85.29	44.70	0.78	0.24	5.84	12.91	6.25	1.81	4.70
Jan. 7	83.10	16.90	85.02	45.20	....	0.32	5.39	13.64	6.44	1.46	4.73
" 21	84.21	15.79	85.54	45.22	1.16	0.39	6.48	15.52	7.16	2.00	....
Feb. 11	83.60	16.40	83.92	45.85	1.16	0.87	5.60	14.31	7.12	2.53	5.70
Feb. 25	83.57	16.43	84.50	45.74	0.90	0.35	5.49	13.64	6.09	2.73	4.54
Harvested and placed in storage October 27, 1931											
Oct. 27	82.40	17.60		45.79	0.68	0.18	4.90	11.87	5.13	1.30	3.88
Nov. 26	82.74	17.26		46.16	0.63	0.28	5.04	11.30	5.36	1.01	4.00
Dec. 17	83.44	16.56		48.26	0.62	0.28	5.89	12.43	5.51	1.87	4.00
Jan. 7	83.72	16.28		48.69	1.11	0.46	5.40	12.20	5.77	1.62	5.52
" 21	84.16	15.84		50.75	0.90	0.22	4.74	12.11	5.19	2.27	5.15
Feb. 11	84.22	15.78		49.10	0.80	0.68	4.08	12.53	5.47	2.10	5.06
" 25	83.78	16.22		47.59	0.81	0.63	4.53	12.75	5.48	2.51	4.26

* Fraction of sample remaining after carbohydrate extraction.

Starch remains insignificant (less than 1 per cent.) until early July when it begins to accumulate rapidly, reaching a concentration of 5 per cent. This high concentration is maintained until the middle of August, after which a uniform decline occurs and starch again becomes insignificant about the middle of October.

Dextrins and soluble starches do not become important at any time. They do, however, follow the general trend of the starches, with concentrations varying from 0.5 to 1.5 per cent. of the dry weight.

Carbohydrate residue curves are similar to lignocellulose curves. Analyses show this residue to be composed almost entirely of lignocellulose.

Rivière and Bailhache (**32**) report that ripening, as measured by the sugar content, is progressive from the stem end to the calyx end in the three varieties of pears studied; namely, Beurré Hardy, Angouleme, and Comice. If this is true in the Kieffer pear, sampling which includes the whole fruit would be more representative of the sugar content than that taking only the mid-section. For this reason, and to permit the expression of the various constituents in absolute quantities per fruit, the samples taken in 1933 of Kieffer pear friuts and Wagener apple friuts from the college orchard comprised whole fruits from which the loculi of the carpels with their contents were removed. The average weights and volumes of the fruits were determined at each sampling.

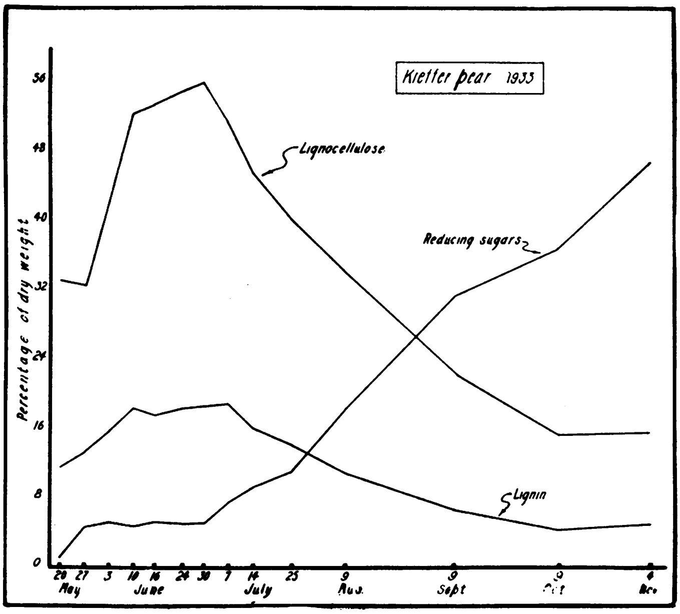


Fig. 3. Seasonal course of reducing sugars, lignocellulose, and lignin as percentage of dry weight. Kieffer pear fruit, 1933.

The findings secured in 1933 are presented graphically in figures 3 and 4 on a dry weight basis and in figures 5 and 6 as absolute amounts of

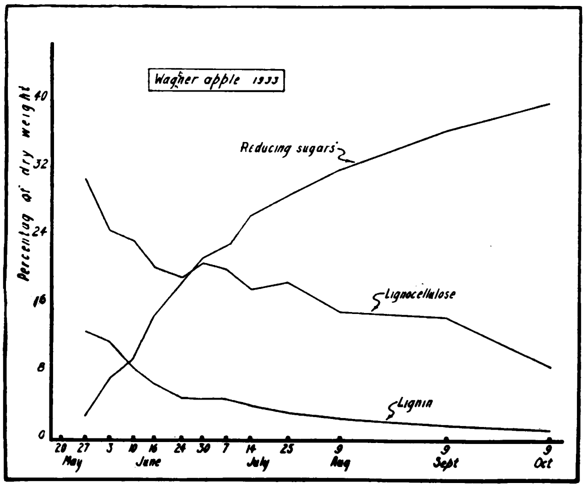


FIG. 4. Seasonal course of reducing sugars, lignocellulose, and lignin as percentage of dry weight. Wagener apple fruit, 1933.

the constituents per fruit. On a dry weight basis the findings are in accord with those of 1930 and 1931. On an absolute amount per fruit basis, an entirely different picture of the changes in the constituents is obtained.

Inspection of figures 3 and 5 reveals the deceptiveness of expressions on the basis of percentage of dry weight. None of the constituents of the

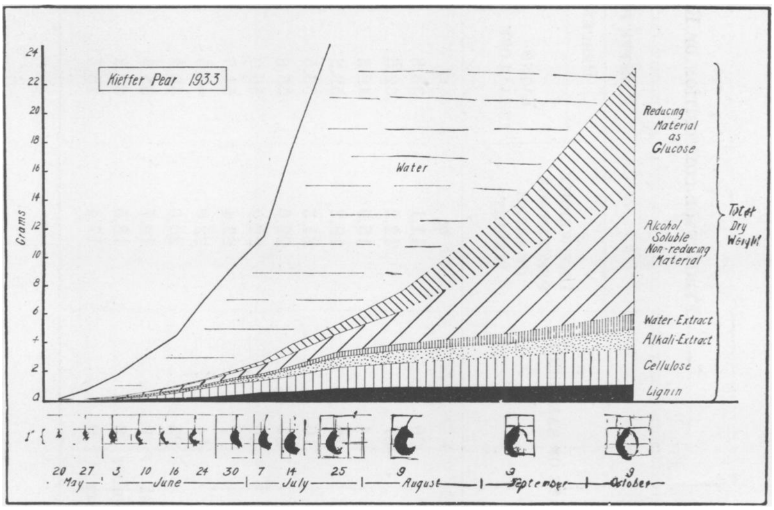


FIG. 5. Seasonal course of actual weight of constituents. Kieffer pear fruit, 1933.

TABLE V

PERCENTAGE COMPOSITION OF KIEFFER PEAR AND WAGENER APPLE, 1933, DRY WEIGHT BASIS

DATE OF SAMPLING	KIEFFER PEAR				WAGENER APPLE			
	DRY WEIGHT PERCENTAGE OF FRESH WEIGHT	PERCENTAGE OF DRY WEIGHT			DRY WEIGHT PERCENTAGE OF FRESH WEIGHT	PERCENTAGE OF DRY WEIGHT		
		LIGNO-CELLULOSE	LIGNIN	REDUCING MATERIAL AS DEXTROSE		LIGNO-CELLULOSE	LIGNIN	REDUCING MATERIAL AS DEXTROSE
	%	%	%	%	%	%	%	%
May 20	11.1	33.8	11.7	1.0	......	......	......	......
" 27	12.2	32.6	13.4	4.5	13.5	30.5	12.5	2.8
June 4	15.9	42.3	15.8	5.2	12.7	24.3	11.8	7.2
" 10	19.2	52.2	18.7	4.7	12.7	23.3	8.2	9.1
" 16	21.3	53.5	17.8	5.3	13.9	20.2	6.5	14.8
" 24	22.0	55.6	18.4	5.3	14.4	19.2	4.9	18.4
" 30	22.0	56.0	18.9	5.3	13.9	20.6	4.7	21.7
July 7	23.4	51.5	17.4	7.9	14.2	20.0	4.8	23.1
" 14	22.0	45.5	16.2	9.6	14.0	17.9	4.0	26.3
" 25	20.0	40.2	14.2	11.2	14.5	18.8	3.3	29.0
August 9	18.7	34.3	11.0	18.2	14.2	15.2	2.4	31.7
September 9	18.5	22.3	6.9	31.3	15.1	14.5	1.9	36.6
October 9	17.4	15.5	4.3	37.2	13.5	8.9	1.1	39.1

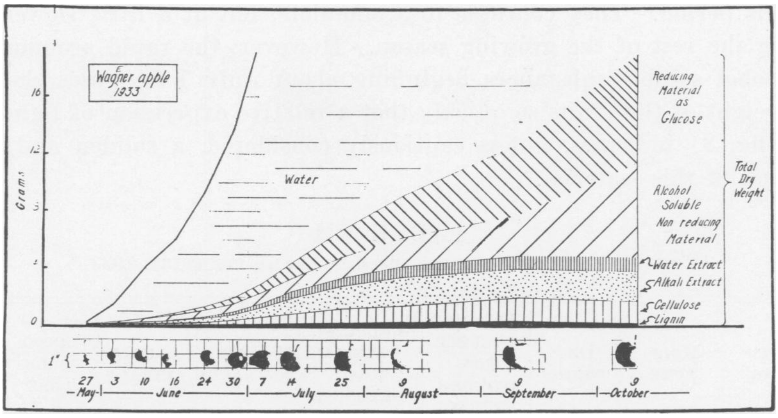


FIG. 6. Seasonal course of actual weight of constituents. Wagener apple fruit, 1933.

pear fruits decreases, but each one actually increases throughout the growing season. Figure 5 shows clearly that the apparent increase and decrease in lignocellulose, when expressed on a dry weight basis, is due only slightly to changes in the rate of accumulation of lignin and cellulose, and principally to changes of the total dry weight. The chief variable of the dry

TABLE VI

CONSTITUENTS OF KIEFFER PEAR IN GRAMS PER FRUIT, 1933

DATE OF SAMPLING		MOISTURE	DRY WEIGHT	ALCOHOL SOLUBLE NON-REDUCING EXTRACT	REDUCING MATERIAL AS DEXTROSE	WATER EXTRACT	ALKALI EXTRACT	LIGNO-CELLULOSE	LIGNIN
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
May	20	0.08	0.01	0.003	0.0001	0.0019	0.0012	0.0011	0.0013
"	27	0.71	0.10	0.040	0.0040	0.0103	0.0121	0.0189	0.0132
June	4	1.52	0.28	0.095	0.0149	0.0166	0.0425	0.0306	0.0905
"	10	2.64	0.62	0.162	0.0293	0.0321	0.0839	0.2101	0.1169
"	16	3.53	0.95	0.232	0.0508	0.0454	0.1202	0.3405	0.1700
"	24	5.13	1.44	0.325	0.0761	0.0645	0.1839	0.5387	0.2662
"	30	7.37	2.07	0.451	0.1093	0.1015	0.2457	0.7721	0.3925
July	7	8.85	2.70	0.608	0.2131	0.1103	0.3438	0.7769	0.6160
"	14	13.83	3.90	0.927	0.3727	0.1724	0.4375	1.1419	0.6336
"	25	20.92	5.23	1.369	0.6465	0.2799	0.6995	1.499	0.8202
August	9	33.34	7.66	2.320	1.3958	0.3596	0.9312	1.7843	0.8474
September	9	60.45	13.71	5.433	4.3308	0.6299	0.9611	2.1299	0.9608
October	9	109.86	23.14	9.773	8.6350	1.0775	1.3871	2.6135	0.9943

weight is the alcohol soluble substances, especially reducing sugars. Lignin and cellulose accumulate faster during May and June than the other dry weight constituents, making a great relative increase of these substances for this period. They continue to accumulate, but at a little slower rate, during the rest of the growing season. However, the rapid accumulation of alcohol soluble substances beginning about July 1 increases the total dry weight of the fruits so quickly that a relative expression of lignocellulose (fig. 3) indicates, unless cautiously considered, a sudden and rapid decrease of this material.

TABLE VII

CONSTITUENTS OF WAGENER APPLE IN GRAMS PER FRUIT, 1933

DATE OF SAMPLING	MOISTURE	DRY WEIGHT	ALCOHOL SOLUBLE NON-REDUCING EXTRACT	REDUCING MATERIAL AS DEXTROSE	WATER EXTRACT	ALKALI EXTRACT	LIGNO-CELLULOSE	LIGNIN
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
May 27	0.26	0.04	0.0160	0.0011	0.0049	0.0065	0.0128	0.0052
June 4	1.64	0.20	0.0881	0.0145	0.0139	0.0346	0.0489	0.0237
" 10	4.13	0.53	0.2247	0.0483	0.0427	0.1063	0.1245	0.0438
" 16	5.98	0.84	0.2985	0.1263	0.0645	0.2018	0.1728	0.0557
" 24	9.19	1.33	0.3740	0.2465	0.1257	0.3093	0.2560	0.0656
" 30	13.61	1.89	0.4775	0.4135	0.2003	0.4573	0.3917	0.0903
July 7	19.53	2.97	0.6710	0.6871	0.3213	0.7377	0.5973	0.1431
" 14	30.40	4.43	0.9898	1.1687	0.4848	1.1137	0.7940	0.1789
" 25	42.36	6.69	1.5445	1.9462	0.5712	1.6000	1.2585	0.2184
August 9	58.50	9.10	2.1508	2.9048	0.7550	2.0561	1.3859	0.2233
September 9	81.33	13.77	4.7782	5.0919	0.9004	2.0052	2.0147	0.2696
October 9	126.24	18.96	8.6483	7.6017	1.0667	2.2098	1.7275	0.2176

During May and June the increase in size of the fruit is due largely to the formation of new cells and at this time cell wall material accounts for most of the dry weight of the fruit. After the last of June, increase in fruit size is due to expansion of the already formed cells and to enlargement of the intercellular spaces (TETLEY, **36**). This behavior would occasion a progressive decrease in the proportion of cell wall to cell contents, thereby showing a less rapid increase in cell wall materials (lignocellulose) during the remainder of the growing season. Alcohol soluble materials are present chiefly in the vacuoles of the cells and, as would be expected, the larger the cells the greater the proportion of cell inclusions to cell wall constituents. Thereafter, it would seem that the great increase in dry weight is due to cell inclusions and should be considered separately from the cell wall constituents.

Hardness.—Data on hardness of fruits in terms of pounds pressure as determined by the standard pressure tester are presented in figure 8 and table IX.

TABLE VIII

WEIGHT AND VOLUME OF KIEFFER PEAR AND WAGENER APPLE FRUITS, 1933

Date of sampling		Kieffer pear			Wagener apple		
		Av. weight	Av. volume*	Sp. gr.	Av. weight	Av. volume*	Sp. gr.
		gm.	*cc.*		*gm.*	*cc.*	
May	20	0.09	0.09	1.00	....	....	....
"	27	0.75	0.73	1.02	0.30	0.30	1.00
June	3	1.92	1.84	1.04	1.84	1.89	.97
"	10	3.51	3.22	1.09	4.66	4.91	.94
"	16	4.82	4.45	1.08	6.82	7.63	.89
"	24	7.08	6.62	1.07	10.52	11.53	.91
"	30	10.16	9.27	1.09	15.50	17.72	.87
July	7	12.34	11.53	1.07	22.50	25.76	.87
"	14	18.70	17.76	1.05	34.83	40.00	.87
"	25	27.60	26.15	1.05	49.05	55.70	.88
August	9	42.80	40.60	1.05	67.80	77.41	.87
September	9	77.50	75.00	1.03	95.10	111.42	.85
October	9	138.20	125.00	1.10	145.20	164.00	.88

* Volume determined by displacement.

TABLE IX

HARDNESS OF KIEFFER PEAR AND WAGENER APPLE FRUITS, 1933

Date of sampling		Kieffer pear				Wagener apple			
		Mean	S.D.*	P.E.†	Corrected for Gov't. pressure tester‡	Mean	S.D.*	P.E.†	Corrected for Gov't. pressure tester‡
		lb.			*lb.*	*lb.*			*lb.*
June	10	20.52	1.58	1.06	27.6	20.04	1.75	1.18	27.5
"	16	22.90	1.77	1.19	30.9	19.52	1.06	0.71	26.3
"	24	24.11	0.38	0.25	32.5	20.65	1.78	1.20	27.8
"	30	23.44	1.67	1.12	31.5	22.31	2.14	1.44	30.1
July	7	25.44	1.60	1.07	34.2	20.71	1.42	0.95	28.0
"	14	24.27	1.21	0.82	32.6	20.08	0.87	0.58	27.0
"	25	22.40	0.35	0.23	30.2	18.38	2.43	1.63	24.8
August	9	20.79	1.30	0.87	28.0	16.63	0.80	0.53	22.4
September	9	17.29	1.10	0.74	23.3	12.00	1.55	1.04	16.2
October	9	12.84	0.40	0.26	17.2	10.80	0.79	0.53	14.5

* Standard deviation = $\sqrt{\frac{\text{Summation } X^2}{n} - M^2}$.

† Probable error = S.D. × .6745.

‡ Times factor 1.35 to compare with 5/16 regulation size plunger.

Storage studies

For storage studies, Kieffer fruits were picked at three dates: the first lot on September 29, before the normal picking date for this variety; the second on October 13, about the regular picking time; and the last lot on October 27, later than they are usually harvested. The fruits were picked into baskets and placed immediately in cold storage at 33° F. Samples for chemical analyses were taken monthly during storage. Notes on the condition of the fruits were made at time of sampling.

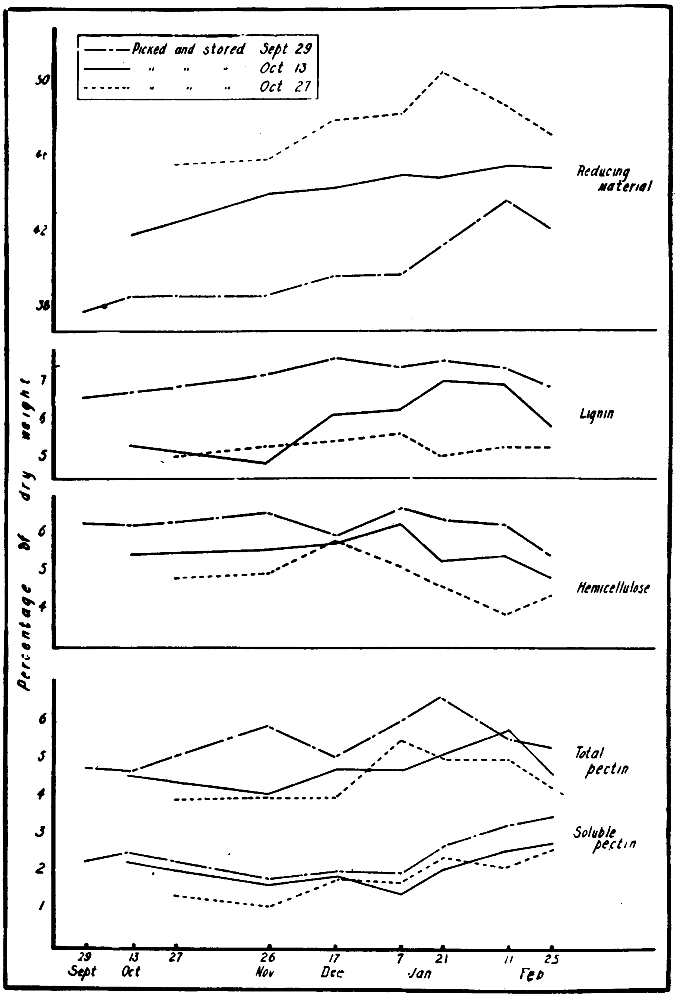


FIG. 7. Percentage change in constituents of Kieffer pear fruits in storage at 33° F., 1931–32.

EMMETT (**13**) in an investigation of changes in pear fruits found that "loss of weight in storage is due chiefly to transpiration." In this study in 1931–1932 the pear fruits showed a loss of 1.5 per cent. of their fresh weight per month. The dry weight concentration of the fruit actually decreased with a corresponding increase in precentage of moisture. In spite of this increase in percentage of moisture the major loss in weight of the fruit is moisture. Thus, paradoxically, the moisture decreases in amount while it is increasing in percentage.

Of the three picking dates represented, October 13 proved to be the best for storage. Determinations of reducing materials, soluble pectin, total pectins, hemicelluloses, and lignin made on these fruits during storage are presented in table VII and figure 7. On the dry weight basis these data show increase in all constituents except hemicellulose during storage. WIDDOWSON found hemicelluloses to decrease in apples in storage. The later-picked fruits had a higher concentration of sugars and lower concentration of lignin, pectins, and hemicelluloses. The intermediate picking was intermediate in all these respects. It may be significant that sugars show a sharp rise followed by a sharp decrease in the early and late pickings. The last picking indicates this break first and this is the inverse order of their keeping quality in storage. This also seems to be the case with total pectins which disappear as pears become overripe and start to break down. EMMETT found this to be the case in Bartlett pears. In general, chemical changes during the ripening and breakdown in storage

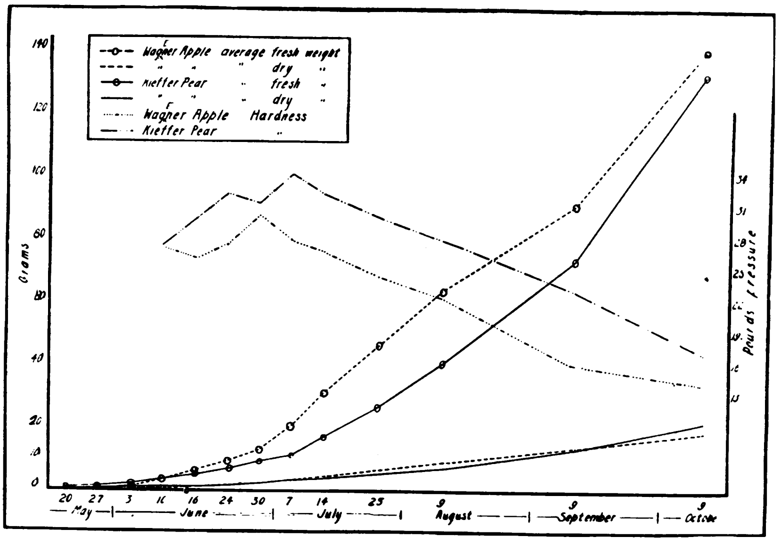


FIG. 8. Dry weight, fresh weight, and hardness of Kieffer pear and Wagener apple fruit, 1933.

are similar in pears and apples. The hemicelluloses seem to be the original source of respirable material.

The Kieffer pear fruits picked on October 13 with a pressure of 14.8 pounds skinned or 18.2 pounds unskinned, kept much better in storage than the later or earlier picked fruits. The reducing material content at that time was 42 per cent. of the fruits' dry weight, although as MAGNESS (**19**) states, "differences in chemical composition due to variations in growing conditions are so great in relation to those due to stage of maturity that any picking test based on chemical composition would prove unsatisfactory."

Discussion and conclusions

Assuming that the course of events in the process of lignification occurs as diagrammed, the constituents in order of their complexity would follow the scheme outlined by ONSLOW (**26**). First, some of the sugar becomes oxidized to polyuronic acids, such as galacturonic and glucuronic, which may combine with condensation products of the sugars, such as arabinose and galactose, to form pectic substances. These pectic substances, then, by decarboxylation form five-carbon sugars, such as arabinose, xylose, and some hexoses and uronic acids which together make up the hemicelluloses.

The hemicelluloses may go to lignin, a substance having an uncertain empirical formula. NORRIS and SCHRYVER (**24**) were able to produce some hemicellulose-like material by treating a pectin preparation. CANDLIN and SCHRYVER (**5**), also by treating pectin with alkalis, secured hemicellulose, similar to that isolated from wood in all respects, and some unidentified residues which they state might possibly form combinations with cellulose to produce lignocellulose.

The cellulose seems to be composed of pure glucose and probably is formed directly by condensation of glucose (**27**, p. 67). Lignocellulose has a composition of about 60 per cent. cellulose and 40 per cent. lignin (**7, 27**). Two general theories as to the formation of lignin exist (**16**, p. 49); first, that cellulose of the cell wall is converted directly to lignin or lignocellulose; and second, that materials other than cellulose are lignin precursors.

ONSLOW (**26**, p. 69) supports the first view: "as the cells in plants grow older the walls usually become lignified; that is, part of the cellulose becomes converted to lignocellulose." KÖNIG and RUMP (**18**) also suggest the conversion of cellulose to lignin.

The changes in the relative amounts of the constituents, as indicated in figures 1, 2, 3, and 4, strengthen the hypothesis presented in the lignification diagram. We may consider that the sugars, which the leaves are supplying to the fruits, are being converted to pectins and then to hemicelluloses and finally to lignocellulose. The data show a relative decrease in sugars during lignification in Kieffer fruit. In the Bartlett fruit with less lignification, the accumulation of sugars is retarded less; and in the apple, with no lignification, no checking of the concentration of sugars occurs. We should expect the pectins, being intermediate products, to be more uniform; and hemicelluloses, because of their greater complexity, to vary more with the end product. This is borne out by their relative concentrations (fig. 2). Associated with the decrease in lignocellulose is an intermediate decrease in hemicelluloses and a sharp increase in sugars. From the graphs showing changes as percentage of the dry weight it is easy to imagine the lignocellulose being broken down to hemicelluloses and then to sugars.

MAGNESS (**19**), referring to his work with Bartlett pears, concludes that "as fruits ripen on the tree, much material other than starch is converted into sugars." MURNEEK (**22**) suggests that in the apple, hemicelluloses are a source of sugar for the maturing fruit. CRIST and BATJER (**7**) suggest a destruction of lignocellulose, and from histological studies, find the clusters of "grit cells" apparently becoming smaller as there is more unlignified tissue between the clusters. These findings seem to support the possibility of a breaking down of the more complex materials to simpler ones in Kieffer pear fruits during the latter part of the growing season.

If the same data are plotted (fig. 5) as absolute amounts per fruit during the growing season, it becomes difficult to imagine any of the constituents breaking down. The data show clearly an increase in every fraction. Hemicellulose changes are almost identical with lignin changes (table III, fig. 2) and would, if presented as absolute amounts per fruit, show the same increase during the growing season. With these particular data, the possibility of hemicellulose supplying sugar to the maturing Kieffer pear fruit is not supported. The case may be quite different in the apple, however, as an inspection of figure 6 reveals a slight decrease during the latter part of the growing season in total amounts of lignin and cellulose. As the absolute amount of lignocellulose in the pear fruit does not decrease, but actually increases, the destruction of lignocellulose (**7**) could be accounted for by its being formed in new parts of the fruit, as nearer the periphery, faster than it is destroyed in the more concentrated areas. The writer doubts that actual destruction of lignocellulose occurs. An apparent decrease in size of the "grit cell" clusters may be due to the clusters being pushed farther apart as the fruit increases in size, due to increase in size of individual cells in the latter part of the growing season, thus distributing the stone cells over a greater area.

It therefore becomes apparent that there is no basis for the support of the supposition that lignocellulose is being converted over to sugars or to any other material. However, it does seem quite probable that the building up of lignocellulose is through these intermediate materials.

It is evident, therefore, that the amount of grit cells of pears depends principally on the extent of grit cell formation during early stages of the formation of the fruit and apparently is not reduced by changes taking place in the grit aggregation during the latter part of the growing season or during ripening. This in turn means that the process is not likely to be materially influenced by cultural or handling practices and bears out the suggestion of CRIST and BATJER that only through his choice of varieties does the pear grower have any considerable control over this more or less objectionable characteristic of pear fruits. A distinction between absolute amount and apparent grittiness is evident when the fruit becomes soft, as when allowed to ripen on the tree, permitting the grits to separate easily from the pulp and making them more noticeable. A fruit ripening on the tree is increasing more rapidly in substances other than grit cells, although it is actually increasing slightly in absolute amounts of grit cells. In apparent grittiness it is increasing greatly. This accounts for the popular impression that "grittiness" increases when pear fruits are allowed to ripen on the tree.

Summary

1. Existing data (and the first two years' results of this study) showed such a tremendous decrease of the percentage of lignocellulose (stone cells) accompanied by an equally great increase of reducing materials, in maturing Kieffer pear fruits, that it suggested that part of the lignocellulose had been converted to reducing substances. Investigations of these changes in the Bartlett pear, a fruit in which lignocellulose occurs in smaller quantities, showed that as a percentage of the dry weight the lignocellulose began to decrease, with a corresponding increase in reducing substances, about 10 days earlier than in the Kieffer. Similar studies of the Wagener apple, a fruit which contains very little lignocellulose, indicated decreases of lignocellulose and accumulation of reducing substances occurring in the first samples taken soon after petal fall. These results strengthened the supposition that lignocellulose may be converted to reducing materials.

2. During the growing season of 1933, changes in absolute amounts of these materials in the Kieffer pear and Wagener apple were determined. These determinations show that there was no actual decrease in lignocellulose, but because of the great increase of alcohol soluble materials, the *percentage* of lignocellulose decreased rapidly. The findings in 1933 indicate that lignified tissue does not break down to form less complex materials in these fruits during growth.

3. Calculated changes in composition may be misleading when presented as percentages. Total "grit" in pear is not reduced during ripening, but "grittiness" is masked by the increased amounts of other constituents of the fruit.

4. There is evidence, however, that the sugars are built up, through the compounds studied, to lignified tissue.

5. In storage, hemicelluloses decreased more than any other constituents; this suggests that these materials may be the source of respirable substances for the fruit after its removal from the tree.

6. The modified SHAFFER-HARTMANN titration method, employed for determining amounts of reducing material during the first two years' study, was found to give higher values on young pear and young apple fruit extracts than the amounts of copper reduced warrants. The high proportion of skin to flesh in the samples taken when the fruits were small is so closely associated with these unwarranted high values that adsorption of the iodine reagent by some material in the skin is indicated.

7. The suggestion of CRIST and BATJER that the grower has little control, other than variety selection, over the "grittiness" of his pear fruits, is supported.

The writer wishes to acknowledge the assistance and encouragement of Dr. J. W. CRIST in the inception and execution of this study; the constructive criticism and suggestions of Professor V. R. GARDNER in preparation of the manuscript; and the helpful suggestions of Professor C. D. BALL on chemical technique.

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