# THE COURSE OF STONE CELL FORMATION IN THE PEAR FRUITS

·By

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#### 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 of the constituents was consistent in the several samples tested, and made possible 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 same 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 two varieties of pears studied, can be seen in Figure 21 of Crist and Batjer's report (7). For example, the Kieffer fruits on May 18 had an alcohol extractable content of 55 percent of the dry weight, which dropped to nearly 25 percent the latter part of June, and then increased steadily, reaching a concentration of 55 percent again by September 2. The alcohol extract is

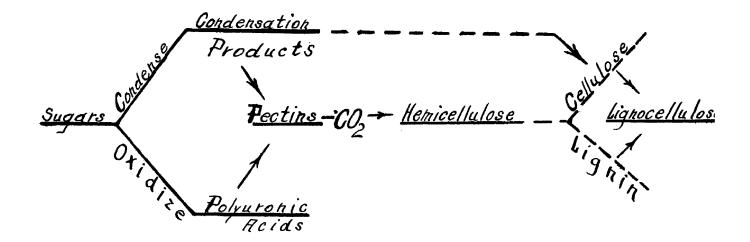
composed largely of sugars. The lignocellulose started off with a concentration of about 25 percent, reached a maximum the last of June of over 55 percent and then dropped off to 25 percent by September.

This same inter-relation of these materials was found in the Bartlett variety of pears, which in contrast to 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 reversion 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 tissue 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. A great deal of investigation has been done, chiefly on woody
material, to get a clue to this course of events (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, - galocturonic, 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 on its 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 over at 65° C. For carbohydrate and pectin analyses similar portions of the fruits, as were used for lignocellulose, were taken. These were finely ground in a meat grinder and thoroughly mixed. Small amounts were placed in weigh 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 percent alcohol of sufficient volume to give a final concentration, including the moisture of the sample, of 80 percent alcohol. Boiling was continued for 10 minutes after which the jars were sealed and stored for analyses. 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. Archbald (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 future samples.

<u>Analysis</u> - Benzene extractions, alcohol extractions, water extractions, and alkali extractions were made and the cellulose and lignin determinations secured as described by Crist and Batjer (7).

Total sugars, sucrose, dextrins, starch and hemicelluloses 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 Carre and Haynes (6) and employed by Appleman and Conrad (1).

During the growing season 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 phlorhizin, causing an error in the determination of the reduced 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 (30) in which titration was carried out in the presence of the plant extract and second, by the Volumetric Thiosulfate 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. Titration results are presented in Tables I and II for apple and pear.

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 colored and contain larger proportions of skin to flesh of the fruit.

The above 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 less. The greatest loss was in 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 data that this substance is associated with the skin or pigments in both apple and pear fruits.

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

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 render it possible to get readings on the early hard fruits. The pressures thus obtained may be converted, approximately, over to 5/16 plunger values, by a factor 1.42 when skin is removed and 1.35 when skin is not removed. These factors were arrived at 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 pear, Bartlett pear and Wagener apple fruits from vigorous, good producing trees.

Changes in the amounts 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.

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 (Figures 1 and 2), consisting of a very rapid accumulation during the first four weeks after fruit setting followed by a less rapid falling off, 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 a few days before the peak of concentration of lignocellulose is reached. The sugar curves then indicate a piling up of sugars coincidental with the decrease in lignocellulose. The difference between total and reducing sugars is reported as sucrose and persists in relative 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.

A 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 (7). As would be expected after considering their findings, the percentage of lignified tissue does not increase in the "grit cell"-free Wagener apple fruits (Figure 1). Although lignocellulose starts at a concentration equal to that in the pear, it decreases continuously throughout the season, except for a short period in July. Accumulation of sugars is not delayed as in the pear fruits, but proceeds at a uniform rate from the very start.

Hemicelluloses in the Kieffer pear (Figure 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 hemicelluloses 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, as found by Widdowson (37) and Tetley (35), have the same character and value 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 lower peak earlier in the season and fall off to a lower level than those for the Kieffer (Figure 1). Although total sugars show a decrease for the first two weeks after fruit set, it is not as pronounced as in the Kieffer and accumulation of sugars starts about 10 days earlier (Figure 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 for Kieffer.

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

There is a rapid accumulation of sugars, showing a steady increase in sucrose, and a gradual decrease in lignocellulose from the very earliest sampling in the Wagener apple. As in the pear fruits, protopectin goes over to soluble pectin early in the season. The increase of both total and soluble pectins found in the apple 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 (7) show lignin to account consistently for about one-third of the lignocellulose. These data co-incide with their findings and, as would be expected, lignin changes are qualitatively the same as lignocellulose (Figure 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 do the lignin and maintain a difference of about 4 percent of the dry weight. It may be significant that this over-rapid decrease in lignin, and slowing of the hemicellulose decline, coincide nicely with the high concentration of starch.

Starch remains insignificant, (less than 1 percent) until early July when it begins to accumulate rapidly, reaching a concentration of 5 percent. 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  $\frac{1}{2}$  to  $1\frac{1}{2}$  percent 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, Beurre Hardy, Angoulene and Comice. If this is true in the Kieffer pear, sampling which includes the whole fruit would be more representative of the sugar content. It was also desirable to express constituents as an absolute quantity per fruit. Accordingly the samples in 1933, of Kieffer pear fruits and Wagener apple fruits from the college orchard, were made up from whole fruits from which the loculi of the carpels with their contents were removed. The average weight and volume of the fruits was determined at each sampling.

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

An 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 pear fruits decrease, 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, but principally to changes of the total dry weight. The chief variable of the dry weight is the alcohol soluble substances,

especially reducing sugars. Lignin and cellulose accumulate faster during May and June than the other dry weight constituents, thus showing 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 piling up of alcohol soluble substances beginning about July first increases the total dry weight of the fruits so quickly that a relative expression of lignocellulose (Figure 3) indicates, unless cautiously considered, a sudden and rapid decrease of this material.

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. Therefore, 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 - Hardness of fruits in terms of pounds pressure as determined by the Government Standard Pressure Tester are presented in Figure 8 and Table 9.

#### STORAGE STUDIES

For storage studies, fruits were picked at three dates; the first lot on September 29, before the normal picking date for this variety, the second lot 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 in cold storage immediately 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.

Emmett (13) in an investigation of changes in pear fruits found that "loss of weight in storage is due chiefly to transpiration". If this is the case in our material, then we would expect a loss of water and increased concentration of dry matter, which would, if used as a basis of calculations, show no relative increase of materials. Under these conditions, if calculations are made on a fresh weight basis, they would show an increase in constituents. In our case, with three different pear populations in storage from October to February an increase in concentration of all constituents is shown except for hemicellulose, whether on fresh or dry weight basis. Undoubtedly the conditions of storage determine the major loss in weight. In this study in 1931-1932 the pear fruits showed a loss of  $1\frac{1}{2}$  percent of their fresh weight per month. The dry weight concentration of the fruit actually decreased which indicated the major loss in weight was due to respiration and not to transpiration.

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, were made on these fruits during storage and are presented in Table VII and Figure 7. On a percentage of the dry weight basis these data show an increase in all constituents except hemicellulose during storage. Widdowson (37) 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 might be significant that sugars show a sharp rise followed by a sharp decrease in the early and late pickings. The last picking indicate 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 show a disappearance of pectins as pears get over-ripe and start breaking down. Emmett (13) found this to be the case in Bartlett pears. In general, chemical changes during ripening and breakdown in storage 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. It may be of interest to note that the reducing material content at that time was 42 percent 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 the course of events in the process of lignification to occur as diagrammed, the constituents in order of their complexity would follow the scheme outlined by Onslow (26). First, some of the sugar became 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 in all respects to that isolated from wood, 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 percent cellulose and 40 percent lignin (7) (27). Two general theories as to the formation of lignin exist (16, p.49); one, that cellulose of the cell wall is converted directly to lignin or lignocellulose, and two, 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". Konig 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 imagine the sugars, which the leaves are supplying to the fruits, are being converted to pectins and then to hemicelluloses and finally lignocellulose. The data show a relative decrease in sugars during lignification in Kieffer fruit. In the Bartlett fruit with a less amount of lignification, the accumulation of sugars is retarded to a less extent, and in the apple, with no lignification, no checking of the concentration of sugars occurs. We would expect the pectins, being intermediate products, to be more uniform and hemicalluloses, because of their greater complexity, to vary more with the end product. This is borne out by their relative concentrations (Figure 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 the Kieffer pear fruits during the latter part of the growing season.

If the same data are plotted (Figure 5) as absolute amounts of constituents per fruit for different dates 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, Figure 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 lignoecllulose 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 author is doubtful 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.

If now we consider Figure 5, it 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 "grittiness" 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 (7) 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.

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#### SUMMARY

Existing data (and the first two year's 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.

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 <u>percentage</u> of lignocellulose decreased rapidly. The findings in 1933 indicates that lignified tissue does not break down to form less complex materials, in these fruits during growth.

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.

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

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

The modified Shaffer-Hartmann titration method, employed for determining amounts of reducing material during the first two year's 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.

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.

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TABLE I.

AVERAGE c.c. OF O.1 N., SODIUM THIOSULPHATE SOLUTION REQUIRED TO TITRATE REDUCTION OF FEHLING SOLUTION SECURED WITH 50 c.c. OF THE ALCOHOL SOLUBLE EXTRACT OF APPLE FRUITS.

Date of Sam-			UNCLEA	RED SOI	LUTIONS				RED SO	LUTIONS of Neut	ral
pling*	Titration in presence of plant extract by Shaffer-Hartmann Hethod.	Reduced Copper separated from plant extract and titrated by Volumetric Hand Thiosulfate Method.	Difference between the two Methods.	act	Reduced Copper separated from plant extract and titrated by Volumetric by Thiosulfate Method.	Shaffer-Hartmann Titration of Filtrate #from Volumetric-Thiosulfate 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-Thiosulfate Method.	Shaffer-Hartmann Titration of Filtrate from Volumetric-Thiosulfate Method.	Copper Titration plus Filtrate Titration.
May 27 June 4 10 16 24 30 July 7 14 25 Aug. 9 Sept. 9 Oct. 9	9.5 10.7 11.8 14.5 13.8	2.4 3.0 5.7 6.7 7.7 8.1 9.5 9.7 11.8 14.3 13.5	2.3 1.5 1.2 1.2 .5 .3  1.0	3.4 4.8 4.8 6.9 8.0 8.3 8.7 9.9 11.2 11.7 14.6 13.8	1.0 2.4 3.0 5.3 6.8 7.7 8.3 9.4 10.7 11.4 14.0 13.4	2.4 2.4 1.7 1.6 1.0 .7 .6 .5 .6 .4 .4	3.4 4.8 4.7 6.9 7.8 8.4 8.9 9.9 11.3 11.8 14.4 13.7	1.9 3.2 3.5 5.7 6.0 7.6 8.4 9.6 11.5 11.0 13.9	.3 2.0 2.6 5.1 6.2 7.4 8.0 9.2 11.3 10.7 13.6 13.1	1.5 1.0 .7 .8 .4 .5 .4 .2 .4	1.8 3.0 3.5 5.9 6.6 7.9 8.4 9.6 11.5 11.1 13.8 13.3

AVERAGE c.c. OF O.1 N SODIUM THIOSULFATE SOLUTION REQUIRED TO
TITRATE REDUCTIONS OF FEHLINGS SOLUTION SECURED
WITH 50 c.c. OF THE ALCOHOL SOLUBLE EXTRACT OF PEAR FRUITS.

Date o		presence of plant extract by Shaffer-Hartmann Method.	separated from plant extract and titrated by Volumetric-Thiosulfate Method.	Volumetric- Thiosulfate Method titrated by Shaffer- Hartmann Method.	tion plus filtrate titration
		7.0	~	2	0
May	20	1.6	•3	.6	.9
_	27	3.2	1.5	1.5	3.0
June	4	3.7	2.2	.9	3.1
	10	3.1	2.3	•9	3.2
	16	4.1	2.9	•6	<b>3</b> •5
	24	3.8	3.0	•4	3.4
	30	3.7	3.0	.2	3.2
July	7	5.2	4.6	.2	4.8
	14	6.3	5 <b>.4</b>	•3	5.7
	25	7.3	6.3	•3	6.6
Aug.	9	9.6	8.7	•1	8.8
Sept.	9	15.8	14.7	.1	14.8
Oct.	9	16.7	16.3		16.3
Nov.	4	19.0	18.6	•2	18.8

\*80 grams fresh weight per sample

TABLE III PERCENTAGE COMPOSITION OF KIEFFER PEAR FRUITS - 1931 - DRY WT. BASIS.

Date	of		Dry Wt.	Dry Wt. of	Reducing			Hemi-				Total
Sampling	ing	Moisture	of Fruit	Alc. Ext.	Sugars	Dextrins	Starch	Cellu- lose	Residue*	Lignin	Pectin	Pectin
June	63	84.87	15.13	27*67	10.63	49.	•76	11.14	19.07		.75	7.30
	വ	84.27	15.73	42.82	8.46	.61	•54	14.91	36.20		66.	5.61
	σ	83.54	16.46	43.25	8.62	.54	.77	15.01	39.10		•75	5.52
	7	82.43	17.57	42.19	8.12	-64	.67	15.93	39.79		.87	5.42
	14	81.23	18.77	34.62	6.92	.62	•74	17.82	46.22		86.	4.08
	17	78.92	21.08	34.83	7.44	980	99•	17.64	48.15		-74	4.81
	88	78.26	21.74	32.58	6.20	.42	.63	19.59	41.74	19.20	1.07	3.95
	83	77.63	22.37	29.96	6.25	.83	.75	19.63	40.22	20.70	1.10	3,85
	36	77.58	22.42	30.50	6.19	.71	99•	19.61	41.62	20.80	1.10	3,68
	23	77.53	22.47	31.64	6.36	1.00	.85	18,82	42.87	20.30	•85	4.75
July	જ	76.83	23.17	29.77	6.38	1.07	•75	19.23	42.90	21.28	.71	3,68
	വ	75,85	24.15	33,28	7.94	1.80	.87	17,75	42.98	19.80	17.	4.30
	ထ	76.34	23.66	34.79	8.45	. 95	1.62	17.50	40.01	18,36	1.19	3,89
	77	76.55	23.45	36.67	9,56	1.07	20.2	16.30	38.69	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	.65	3.79
	22	77.00	23.00	44.69	12.60	1.60	5.40	14.60	28.97	12.40	1.61	4.34
Aug.	4	78.00	22.00	49.42	14.72	1.75	6.10	13.97	26.66	11.05	2,68	4.62
	ឧ	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	90.7	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	8.6	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
Sept.		80.91	19,09	71.83	31.40	1.32	4.12	7.65	14.27	6.86	19.1	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
	88	82.58	17.42	76.23	35.87	66•	2.10	6.53	11.97	6.05	1.60	4.58
Oct.	9	82.65	17,35	80.03	39.59	.93	1.14	5.94	10.79	5.44	1.47	4.41
	13	82.18	17.82	80.61	40.17	69•	.39	5.79	10.65	5.27	1.42	3.73
	20	82.26	17.74	84.72	43.29	99•	trace	5,38	9.72	4.67	1.26	3.67
	27	82.16	17.84	86.49	46.07	19.	trace	5.03	*	4.34	1.76	3.91
Nov.	ы		17.38	2	44.76	.87	.13	4.85	•	3.96	1.62	3.85
	20	84.00	17.00	98.85	49.87	ಜ್	. 25	5.37	10.13	4.22	1.35	3.88
*	*Fraction	οŢ	sample remaining	ning after ca	rbohydrate	extraction	n.					

PERCENTAGE COMPOSITION OF KIEFFER PEAR FRUITS IN STORAGE AT 53° F. - DRY WT. BASIS TABLE IV

Date of Sempling	ng ng	Moisture	Dry Wt. of Fruit	Dry Wt. of Alc. Ext.	Reducing Sugara	Dextrine	Starch	Hemi- Cellu- lose	Residue*	Lignin	Pectin	Total Pectin
- HAR	HARVESTED	AND	PLACED IN ST	STORAGE SEPTEM	EMBER 29, 1931	31						
Sept.	58	82,30	17.70		37.92	66*	49*	6.39	14.07	6.67	2.32	4.76
	13	82.42	17.58		38.81	98•	.31	6.34	14,99	6.84	2.50	4.64
Nov.	86	82,70	17.30		38.84	.73	•45	6.76	15.77	7.29	1.79	5.82
Dec.	17	83.31	16.69		39.98	68.	-34	6.04	16.19	•	2.07	5.05
Jan.	~	83,13	16.87		40.02	1.30	35	6.82	15,52	7.51	1.98	5.88
ŧ	23	84.37	15.63		41.59	1.36	.31	6.50	16,89	7.72	2.73	6.77
Feb.	11	84.90	15.10		43.97	1.08	.42	6.42	•	7.53	3.19	5.56
¥	25	84.30	15.70		42.47	1.24	•98	5.57	15.75	7.16	3.48	5.32
HA	HARVESTED	AND	PLACED IN ST	STORAGE OCTOBER	ER 13, 193.	1						
Oct.	13	82.44	17.56	79.72	42.14	64.	.22	5.55	12.41	5.33	2.22	4.55
Nov.	28	82.54	17,46	83.61	44.26	.75	28	5.77	18.91	4.93	1.65	4.17
Dec.	17	83,00	17.00	85.29	44.70	•78	.24	5.84	18.91	6.25	1,81	4.70
	2	83,10	16.90	85.02	45.20	!	.32	5.39	13.64	6.44	1.46	4.73
E	21	84.21	15,79	85.54	45.22	1.16	•39	6.48	15.52	7.16	8.00	1
Feb.	I	83.60	16.40	83.92	45.85	1.16	*84	5.60	14.31	7.12	2.53	5.70
Feb.	25	83.57	16.43	84.50	45.74	06.	•35	5.49	13.64	60.9	2.73	4.54
HA	HARVESTED	AND	PLACED IN ST	STORAGE OCTOBER	ER 27, 1931							
Oct.	22	82.40	17.60		45.79	89•	<b>9T°</b>	4.90	11.87	5.13	1,30	3.88
Nov.	26	82.74	17.26		46.16	.63	-28	5.04	11.30	5.36	1001	
	17	83.44	16.56		48.26	62	.28	5.89	12,43	5.51	1.87	4.00
ត់	~	83.72	16.28		48.69	1.11	94.	5.40	12,20	5.77		5.52
	21	84.16	15,84		50.75	06.	.22	4.74	12,11	5.19	2.27	5.15
ۇ.	ı	•	15.78		49.10	8.	<b>.</b>	4.08	12,53	5.47		5.06
<b>=</b>	22	83.78	16.22		47.59	<b>1</b> 8•	•63	4.53	12,75	5.48	2.51	4.26
F	*Fraction	of	Sample remaining	after	carbohydrate	extraction	•uo					

TABLE V PERCENTAGE COMPOSITION OF KIEFER PEAR AND WAGENER APPLE - 1933 - DRY WT. BASIS.

Date of		KIEFER PEAR				M	WAGENER APPLE	PLE
Sampling	Dry Weight	Percentage	e of Dry	y Weight	Dry Weight	Percen	Percentage of I	Dry Weight
	Percentage of	Ligno-	Lignin	Lignin Reducing	Percentage of	Ligno-	Lignin	Reducing
	Fresh Weight	Cellulose		Waterial	Fresh Weight	Cellulose		Meterial as
				Dextrose				Dextrose
	r	9	!					
May 60	T•TT	χ. 20.00 10	11.7	7.0				
	12.2	32.6	13.4	4.5	13.5	30.5	ני	80
June 4	15.9	42.3	15.8	ໝ	12.7	24.3	2 0	2,2
업	19.2	52.2	18.7	4.7	12.7	23.23	) o	· 6
16	21.3	53.5	17.8	5.3	13.9	808	์ วัก	14.8
24	22.0	55.6	18.4	5.3	14.4	19.2	9 4	18.4
	22.0	56.0	18.9	5.3	13.9	20.6	•	21.7
July 7	23.4	51.5	17.4	7.9	14.2	0.02	. α	23.1
14	0.88	45.5	16.2	9.6	14.0	17.9	) C	26.3
22	20.0	40.2	14.2	11.2	14.5	18.8	) b(	0 68
Aug. 9	18.7	34.3	11.0	18.2	14.2	15.2	9 0	31.7
	18.5	22.33	6.9	31.3	15.1	14.5	# o	36.6
0ct. 9	17.4	15.5	4.3	37.2	13.5	ο Θ	,	30.1
						•	4	1

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
May 20	.08	10.	.003	.0001	.0019	2100*	1100.	.0013
	1.52	88	0.00	.0149	0166	0426	0306	20T0.
	2.64	89.	.162	.0293	.0321	.0839	2101	1169
	3.53	.95	83.22	•0508	.0454	.1202	.3405	.1700
	5.13	1.44	325	.0761	.0645	.1839	.5387	.2662
	7.37	2.07	127.	.1093	1015	.2457	.7721	.3925
	GB*85	2.70	809	.2131	.1103	.3438	.7769	.6160
	13.83	3.90	.927	.3727	.1724	.4375	1.1419	.6336
	20.92	5.23	1,369	. 6465	.2799	.6995	1,499	8202
	33.34	7.66	2,320	1,3958	.3596	.9312	1,7843	.8474
	60.45	13.71	5.433	4.3308	•6889	.9611	2,1299	9096
0ct. 9	109,86	23.14	9.773	8.6350	1.0775	1.3871	2,6135	.9943

TABLE VII CONSTITUENTS OF WAGENER APPLE IN GRAMS PER FRUIT - 1933

Date of Sempling	g Moisture	Dry Weight	Alcohol Soluble non-reducing Estract	Reducing Material as Dextrose	Water Extract	Alkali Extract	Ligno- Cellulose	Lignin
l	├	40.	•0160	.0011	.0049	9000	.0128	.0052
		02.	•0881	.0145	.0139	.0346	.0489	.0237
		53	. 2247	.0483	.0427	.1063	.1245	.0438
		<b>8</b>	. 2985	.1263	.0645	.2018	.1728	.0557
€ 1	24 9.19	1.33	.3740	,2465	.1257	.3093	.2560	•0656
		1.89	.4775	.4135	.2003	.4573	.3917	.0903
		2.97	.6710	.6871	.3213	.7377	.5973	.1431
		4.43	8686	1,1687	•4848	1,1137	.7940	.1789
	—	69.9	1.5445	1.9462	.5712	1,6000	1.2585	.2184
		9.10	2,1508	2,9048	.7550	2,0561	1,3859	. 2233
	9 81.33	13.77	4.7782	5.0919	₹006	2,0051	2.0147	.2696
	9 126.24	18.96	8.6483	7.6017	1,0667	2,2098	1,7275	.2196

TABLE VIII WEIGHT AND VOLUME OF KIEFFER PEAR AND WAGENER APPLE FRUITS - 1933

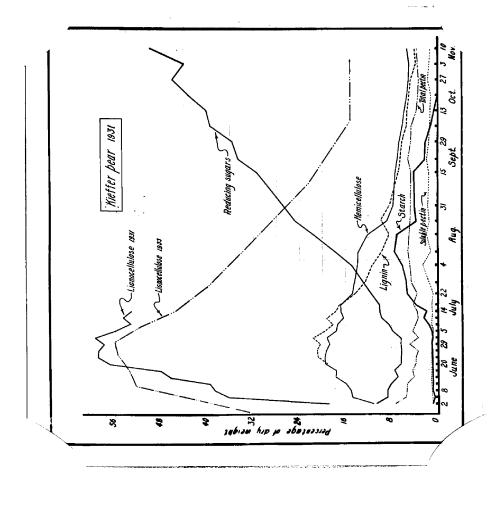
		תוש דויסיקא	A OTTOME OF A	Trad radia	AND VOLUME OF ALEFFER FEAR AND WARFING AFFILE FROILS	COST CTIONS W	
Date of	of	Ĭ.Ž.	Kieffer Pear		M	Wagener Apple	
Sampling	ling	Ave. Weight Grams	Ave. Volume C. Cm.*	Sp. Gr.	Ave. Weight Grems	Ave. Volume C. Cm.	Spr. Gr.
May	20	60°	60*	00°⊺			
	27	.75	.73	1.02	•30	30	1.00
June	ເນ	1.92	1,84	1.04	1,84	1.89	.97
•	97	3,51	3,22	1.09	4.66	4.91	• 94
	16	4.82	4,45	1.08	6,82	7.63	68°
	₹	7.08	6,62	1.07	10,52	11.53	.91
	30	10.16	9.27	1.09	15,50	17.72	.87
July	2	12.34	11,53	1.07	22,50	25.76	.87
	14	18.70	17.76	1,05	34.83	40.00	.87
	22	27.60	26.15	1.05	49.05	55.70	88•
Aug.	o,	42.80	40.60	1,05	67.80	77.41	.87
Sept.	O.	77.50	75.00	1.03	95.10	111.42	•85
Oct.	O)	138.20	125,00	1.10	145.20	164.00	<b>.</b> 88

\*Yolume determined by displacement.

TABLE IX
HARDNESS OF KIEFFER FEAR AND WAGENER APPLE FRUITS - 1933.

Date Sampl			Kieffer	r Pear			Wagen	er Apple	
		Mean	S.D.#	P.E.**	Corrected for Gov't. Pressure Tester*	Mean	S.D.#	P.E.**	Corrected for Gov't Pressure Tester*
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	.71	26.3
	24	24.11	•38	.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	•95	28.0
•	14	24.27	1.21	.82	32.6	20.08	<b>.</b> 87	•58	27.0
	25	22.40	.35	.23	30.2	18.38	2.43	1.63	24.8
Aug.	9	20.79	1.30	.87	28.0	16.63	-80	•53	22.4
Sept.	9	17.29	1.10	.74	23.3	12.00	1.55	1.04	16.2
Oct.	9	12.84	.40	.26	17.2	10.80	.79	•53	14.5

<sup>\*</sup> Times factor 1.35 to compare with 5/16 regulation size plunger.



----- Bortleit pear ----- Hagher apple

. 20.

\$

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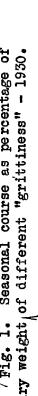
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Percentage

/Fig. 1. Seasonal course as percentage of dry weight of different "grittiness" - 1930.



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NAV E

Fig. 2. Seasonal course as percentage of dry weight of several constituents of Kieffer pear fruit - 1931.

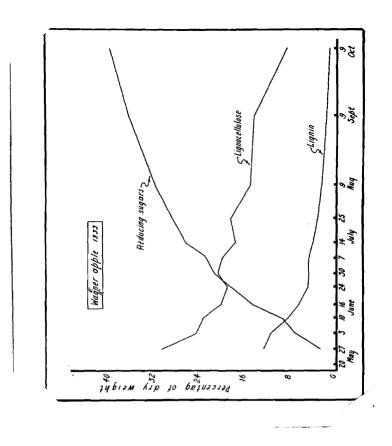


Fig. 4. Seasonal course as percentage of dry weight. Wagener apple fruit - 1933.

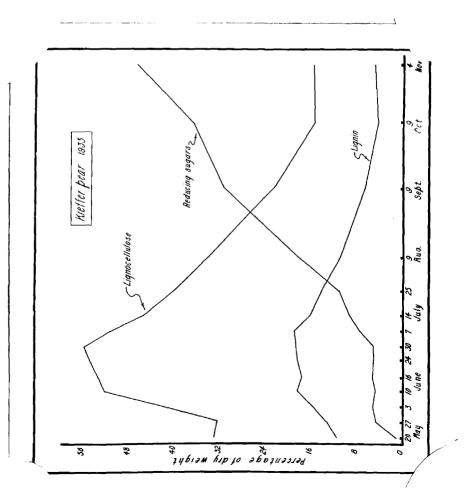


Fig. 3. Seasonal course as percentage of dry weight. Kieffer pear fruits - 1933.

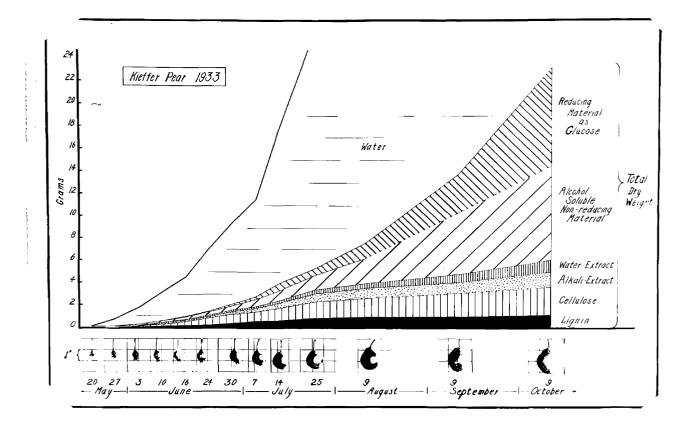


Fig. 5. Seasonal course of actual weight of constituents. Kieffer pear fruit - 1933.

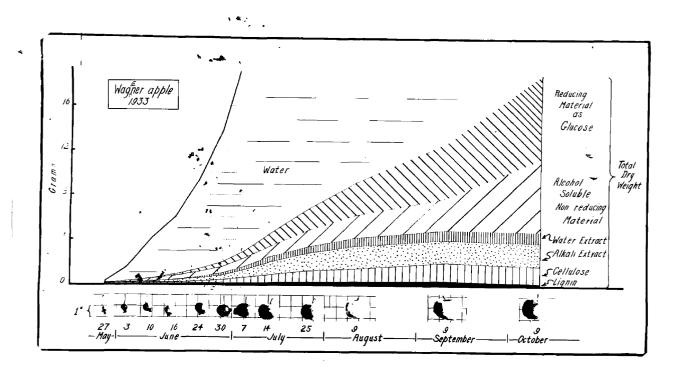


Fig. 6. Seasonal course of actual weight of constituents. Wagener apple fruit - 1933.

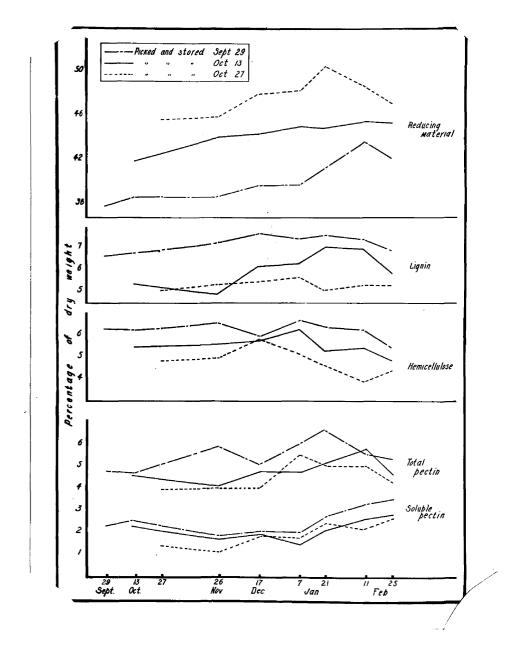


Fig. 7. Percentage change in constituents of Kieffer pear fruits in storage at 33° F. - 1931-32.

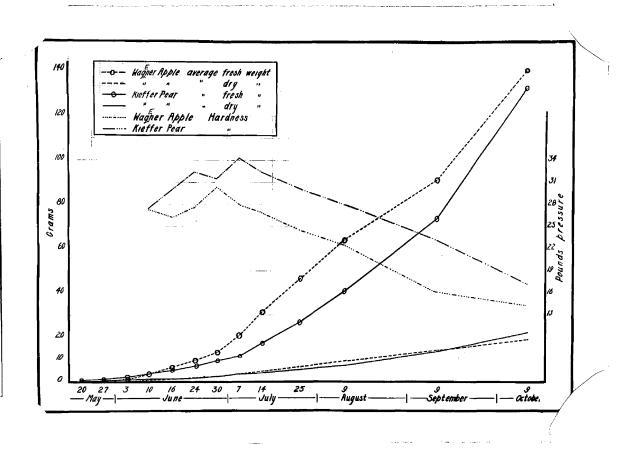


Fig. 8. Dry weight, fresh weight, and hardness of Kieffer pear and Wagener apple fruits - 1933.