CHANGES IN THE PECTIC CONSTITUENTS OF APPLES IN RELATION TO SOFTENING

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INTRODUCTION

One of the principal changes that takes place in apples as they ripen on the tree and in storage is the softening of the flesh of the fruit. In recent years many studies have been made on the ripening and storage of apples (10), and in these the softening of the fruit has been given major consideration. In general, it has been found that there is a gradual, though somewhat irregular, softening of the fruit on the tree as it becomes mature. This softening continues in storage and is the outstanding change that takes place during the process of ripening, the fruit changing from the hard unripe condition to the relatively soft, eating-ripe condition, and finally to the very soft, mealy, overripe condition. The rate at which softening takes place in storage depends primarily upon the temperature at which the apples are kept. For a number of varieties the rate of softening at 40° F. was found to be slightly more than double that at 32°, whereas that at 50° was slightly less than double that at 40°, and that at 60° was nearly double the rate at 50°.

In these studies (10) a fruit pressure tester (9) was used to measure the firmness of the apples. This tester records the pressure in pounds necessary to force a plunger seven-sixteenths of an inch in diameter into the fruit a distance of five-sixteenths of an inch. In penetrating the fruit the tester crushes some of the cells and separates and pushes aside others. The firmness of the apples as measured by the pressure tester is probably due principally to three factors—the size of the cells and intercellular spaces, the thickness of the cell walls, and the ease with which the cells separate. The last two factors are of particular interest in relation to the studies herein reported, as the thickness of the cell walls depends on the thickness of the cellulose or substance of which they are composed and upon the thickness of the layer of cementing substance or pectic material of the middle lamella, while the ease with which the cells separate depends upon the amount of pectic material cementing them together. All three factors might play a part in the softening of the fruit on the tree. In storage, however, there would be no further increase in the size of the cells and intercellular spaces, and softening should be due to changes in the thickness of the cell wall and the ease with which the cells separate.

The studies reported in this paper were undertaken to obtain a better understanding of the underlying chemical changes that take place in apples during the softening process.

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2 Acknowledgment is made to J. R. Magness, of the Office of Horticultural Crops and Diseases, Bureau of Plant Industry, for helpful suggestions and criticisms during the progress of this work.
3 Reference is made by number (italic) to "Literature cited," p. 746.
REVIEW OF LITERATURE

In the recent literature dealing with the pectic material in fruit, particularly in apples, the following pectic substances have been recognized: Pectin, which is water soluble and is derived from protopectin probably by enzyme action; protopectin or pectose, which is water insoluble; and the pectic substance of the middle lamella, which is also water insoluble. The total pectic substances really include all three of these, but in this paper only the pectin and protopectin are included as the pectic substance of the middle lamella was not determined.

Several investigators have found softening in fruit to be associated with changes in the pectic material in the cell walls and middle lamella. Carré (5, 4) made extensive studies of the pectic materials of apples in storage. She associated ripening in storage with an increase in pectin and a corresponding decrease in protopectin (pectose), the total pectic materials remaining constant. In the latter stages of storage in which the fruit becomes mealy and breaks down she observed a breaking down of what she termed the “pectic substance of the middle lamella.” She found that during this period there is a temporary increase in protopectin, which again decreases. These changes were observed to take place more rapidly at higher temperatures. Similar results were observed by Carré and Horne (6) when microchemical methods were used. With peaches, Appleman and Conrad (1) found that there was a decrease in protopectin and a corresponding increase in pectin with ripening in storage, the rate of these changes increasing with the temperature. When the fruit ripened on the tree very little change was noted in the pectic materials. Emmett (8), working with pears, obtained similar results. At 1° C. the development of pectin was greatly retarded, as was also the softening of the fruit. Appleman and Conrad (2) found an increase in pectin and a decrease in protopectin with increased maturity of tomatoes while on the vine. In canned tomatoes a close relationship existed between the amount of disintegration and the ratio of pectin to protopectin.

From the investigations discussed above it would seem that the cell walls are thickened and cemented together by an insoluble pectic material, usually protopectin. As softening takes place the protopectin is changed to a soluble form, thus permitting the cells to separate readily and decreasing the thickness of their walls. These changes are accelerated by an increase in temperature.

In the investigations previously cited no measurements were made of the firmness of the fruit, except with tomatoes (2), under the various conditions of the experiments. It was the purpose of the present investigation to determine to what extent the changes in the pectic materials of apples are responsible for the softening of the fruit as measured by the pressure tester.

METHODS

The determination of the pectic materials (pectin, total pectic material, and protopectic) was carried out according to the method described by Carré and Haynes (4, 5) with some modifications.

A composite sample of at least 20 apples was used for each determination. The firmness of the fruit was first determined by means
Changes in Pectic Constituents of Apples

The soluble pectin was determined in two ways, as follows:

1. Samples of 100 gm. of pulp were frozen overnight. After thawing, the juice was pressed out through muslin. The pulp was then repeatedly washed with cold water until no appreciable amount of pectin remained. Preliminary investigations had shown that seven washings with 150 to 200 milliliters of water were sufficient to accomplish this. The juice and the first washing were combined, as were also each additional two washings. They were then brought to a boil, cooled, and made to a 500-ml. volume. After filtering, aliquots of sufficient size to yield if possible 0.02 to 0.03 gm. of calcium pectate were transferred to beakers, diluted to 300 ml., made alkaline with 100 ml. of 0.1 normal sodium hydroxide, and allowed to stand overnight. They were then acidified with 50 ml. of normal acetic acid, and the calcium pectate was precipitated by the addition of 50 ml. of molar calcium chloride. The precipitate was then brought to a boil, filtered, and washed with hot water until free from chlorides. The precipitate was then washed back into the beaker, boiled, filtered again, and washed. It was then washed into a small tared beaker, dried, and weighed.

2. Portions of the pulp were frozen overnight. After thawing, the juice was pressed out through muslin. Definite volumes, 100 ml. of the juice were brought to a boil, cooled, and made to a 500-ml. volume. After filtering, aliquots of sufficient size to yield 0.02 to 0.03 gm. of calcium pectate were used, and from this point the determinations were carried on as in the first method.

In Tables 1 and 2 the two methods are compared. The soluble pectin in the pulp was obtained by the first method, while that in the juice was obtained by the second. As the results by both methods were practically the same, the second method, which was much shorter, was used in the later determinations.

The determination of the total pectic substances was carried on as follows: The juice from 100-gm. samples of pulp was pressed out through muslin, and the pulp was washed with cold water to remove the organic acids and most of the soluble pectin. The juice and the wash water were combined, brought to a boil, cooled, and made to volume. The residue was then transferred to a flask, covered with 1/75 normal hydrochloric acid, and boiled for an hour under a reflux condenser. The solution was then pressed out through muslin and made to a 500-ml. volume. Extraction of the residue with 1/75 normal hydrochloric acid was repeated twice. After filtering, aliquots of each extraction of sufficient size to yield, if possible, 0.02 to 0.03 gm. of calcium pectate were used, and the determinations were carried on from this point as for soluble pectin.

Protopectin was obtained by subtracting the soluble pectin from the total pectic substance.

EXPERIMENTAL DATA

SOFTENING ON THE TREE IN RELATION TOPECTIC CHANGES

The data on the relation of softening of fruit while on the tree to the changes in the pectic constituents are presented in Table 1 and Figure 1. The amount of soluble pectin in apples as they ripen...
on the tree is very small and remains practically constant. As the fruit softens there is a gradual decrease in the percentage of protopectin and a corresponding decrease in total pectin in the Ben Davis apples. In the Jonathan, however, the pectic changes during the first period are negligible even though there is a softening of 6.5 pounds. During the second period the changes correspond to those in the Ben Davis apples. Although the percentage of protopectin decreases, it is probable that the actual amount does not decrease but seems to be less because of the increased size of the fruit. Softening on the tree appears to be associated in part with the apparent decrease in the protopectin content. Other factors, such as increase in the size of the cells and intercellular spaces, may be the principal ones responsible for softening in the rapidly growing fruit.

![Graph](attachment:image.png)

**Figure 1.**—Pectic changes during softening of apples on the tree. Solid line represents Ben Davis in 1926; broken line, Jonathan in 1927

**Table 1.**—Softening of apples on the tree in relation to changes in pectic constituents

| Variety of apple and date of test | Pressure test | Soluble pectin | | Total pectic substances |
|----------------------------------|--------------|----------------|-----------------------------|
|                                  | Pounds       | In juice       | In pulp | Per cent | Per cent | Per cent | Per cent |
| Ben Davis:                       |              |                |         |          |          |          |          |
| Sept. 1, 1926                    | 23.6         | .038           | .048   | .851     | .899     |
| Sept. 17, 1926                   | 21.7         | .052           | .053   | .765     | .818     |
| Oct. 14, 1926                    | 18.4         | .051           | .047   | .668     | .715     |
| Jonathan:                        |              |                |         |          |          |          |          |
| July 22, 1927                    | 21.1         | .033           |        | .907     | .840     |
| Aug. 22, 1927                    | 14.6         | .037           |        | .796     | .833     |
| Sept. 14, 1927                   | 11.9         | .034           |        | .673     | .707     |
SOFTENING IN STORAGE AT 32° F. IN RELATION TO PECTIC CHANGES

Ben Davis, Winesap, and two pickings of Jonathan were used to determine the relation of softening in storage at 32° F. to pectic changes. As softening took place there was a gradual increase in total pectic materials (Table 2 and fig. 2) in Ben Davis and Winesap apples, whereas in Jonathan the total pectic materials remained fairly constant but showed a slight decrease. There was a gradual decrease in protopectin with softening in all varieties except for the last determination on Ben Davis and Winesap apples, when there was an increase. These increases may have been due in part to loss of water by the fruit, which showed considerable shriveling. It is more probable, however, that they were due to the formation of protopectin from the so-called pectic substance of the middle lamella described by Carré, as the increases in both protopectin and total pectic substances were greater than would be expected, from loss of water, especially as there was no corresponding increase in pectin.

Fruit stored at 32° F. showed a regular increase in soluble pectin, due principally to the hydrolysis of protopectin. In contrast, fruit allowed to ripen on the tree showed no change in soluble pectin during the softening process.

There was no relation between the firmness of the three varieties and their pectic constituents. Jonathan, which was much softer when picked than Winesap and Ben Davis, had a higher protopectin and total pectin content.

The total pectic material and soluble pectin were at all times lower in the later picking of Jonathan than in the earlier picking, while the protopectin was nearly equal in the two pickings at the various stages of softening.
TABLE 2.—Softening of apples in storage in relation to changes in pectic constituents

[Pectic substances expressed as calcium pectate in percentage of fresh weight]

<table>
<thead>
<tr>
<th>Variety of apple and date of test</th>
<th>Temperature</th>
<th>Pressure test</th>
<th>Soluble pectin</th>
<th>Protopectin</th>
<th>Total pectic substances</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>32°F</td>
<td>Pounds</td>
<td>Per cent</td>
<td>Per cent</td>
<td>Per cent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>In juice</td>
<td>In pulp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ben Davis:</td>
<td>12.9</td>
<td>0.138</td>
<td>0.047</td>
<td>0.658</td>
<td>0.715</td>
</tr>
<tr>
<td>Jan. 31, 1927</td>
<td>9.8</td>
<td>0.211</td>
<td>0.214</td>
<td>0.587</td>
<td>0.801</td>
</tr>
<tr>
<td>Apr. 6, 1927</td>
<td>10.9</td>
<td>0.260</td>
<td>0.262</td>
<td>0.552</td>
<td>0.814</td>
</tr>
<tr>
<td>July 18, 1927</td>
<td>32</td>
<td>0.221</td>
<td>0.228</td>
<td>0.656</td>
<td>0.884</td>
</tr>
<tr>
<td>Winesaps:</td>
<td>18.0</td>
<td>0.036</td>
<td>0.060</td>
<td>0.504</td>
<td>0.564</td>
</tr>
<tr>
<td>Feb. 14, 1927</td>
<td>14.6</td>
<td>0.154</td>
<td>0.175</td>
<td>0.436</td>
<td>0.611</td>
</tr>
<tr>
<td>Apr. 22, 1927</td>
<td>12.5</td>
<td>0.262</td>
<td>0.251</td>
<td>0.371</td>
<td>0.602</td>
</tr>
<tr>
<td>July 22, 1927</td>
<td>11.1</td>
<td>0.263</td>
<td>0.275</td>
<td>0.425</td>
<td>0.700</td>
</tr>
<tr>
<td>Jonathan I:</td>
<td>14.6</td>
<td>0.037</td>
<td></td>
<td>0.796</td>
<td>0.833</td>
</tr>
<tr>
<td>Aug. 22, 1927</td>
<td>13.6</td>
<td>0.032</td>
<td></td>
<td>0.760</td>
<td>0.801</td>
</tr>
<tr>
<td>Oct. 5, 1927</td>
<td>11.0</td>
<td>0.166</td>
<td></td>
<td>0.597</td>
<td>0.745</td>
</tr>
<tr>
<td>Nov. 14, 1927</td>
<td>8.7</td>
<td>0.223</td>
<td></td>
<td>0.591</td>
<td>0.734</td>
</tr>
<tr>
<td>Dec. 21, 1927</td>
<td>7.6</td>
<td>0.225</td>
<td></td>
<td>0.598</td>
<td>0.733</td>
</tr>
<tr>
<td>Jonathan II:</td>
<td>11.9</td>
<td>0.034</td>
<td></td>
<td>0.673</td>
<td>0.707</td>
</tr>
<tr>
<td>Sept. 14, 1927</td>
<td>12.0</td>
<td>0.018</td>
<td></td>
<td>0.687</td>
<td>0.705</td>
</tr>
<tr>
<td>Oct. 14, 1927</td>
<td>11.0</td>
<td>0.063</td>
<td></td>
<td>0.618</td>
<td>0.681</td>
</tr>
<tr>
<td>Nov. 16, 1927</td>
<td>9.3</td>
<td>0.107</td>
<td></td>
<td>0.574</td>
<td>0.681</td>
</tr>
<tr>
<td>Dec. 21, 1927</td>
<td>8.4</td>
<td>0.130</td>
<td></td>
<td>0.560</td>
<td>0.690</td>
</tr>
<tr>
<td>Mar. 3, 1928</td>
<td>7.7</td>
<td>0.138</td>
<td></td>
<td>0.542</td>
<td>0.610</td>
</tr>
<tr>
<td>Jonathan III:</td>
<td>14.6</td>
<td>0.037</td>
<td></td>
<td>0.796</td>
<td>0.833</td>
</tr>
<tr>
<td>Aug. 22, 1927</td>
<td>13.5</td>
<td>0.035</td>
<td></td>
<td>0.705</td>
<td>0.760</td>
</tr>
<tr>
<td>Sept. 15, 1927</td>
<td>9.7</td>
<td>0.159</td>
<td></td>
<td>0.618</td>
<td>0.760</td>
</tr>
<tr>
<td>Oct. 5, 1927</td>
<td>8.5</td>
<td>0.229</td>
<td></td>
<td>0.447</td>
<td>0.676</td>
</tr>
<tr>
<td>Oct. 24, 1927</td>
<td>7.7</td>
<td>0.202</td>
<td></td>
<td>0.435</td>
<td>0.687</td>
</tr>
<tr>
<td>Dec. 17, 1927</td>
<td>14.6</td>
<td>0.037</td>
<td></td>
<td>0.796</td>
<td>0.833</td>
</tr>
<tr>
<td>Sept. 8, 1927</td>
<td>13.2</td>
<td>0.048</td>
<td></td>
<td>0.737</td>
<td>0.785</td>
</tr>
<tr>
<td>Sept. 26, 1927</td>
<td>8.7</td>
<td>0.237</td>
<td></td>
<td>0.454</td>
<td>0.691</td>
</tr>
<tr>
<td>Oct. 26, 1927</td>
<td>7.2</td>
<td>0.278</td>
<td></td>
<td>0.384</td>
<td>0.662</td>
</tr>
<tr>
<td>Jonathan IV:</td>
<td>14.6</td>
<td>0.037</td>
<td></td>
<td>0.796</td>
<td>0.833</td>
</tr>
<tr>
<td>Aug. 22, 1927</td>
<td>14.8</td>
<td>0.018</td>
<td></td>
<td>0.730</td>
<td>0.745</td>
</tr>
<tr>
<td>Aug. 14, 1927</td>
<td>9.3</td>
<td>0.185</td>
<td></td>
<td>0.549</td>
<td>0.734</td>
</tr>
<tr>
<td>Aug. 22, 1927</td>
<td>7.5</td>
<td>0.270</td>
<td></td>
<td>0.408</td>
<td>0.678</td>
</tr>
</tbody>
</table>

* Date picked and placed in storage.

SOFTENING IN STORAGE AT VARIOUS TEMPERATURES IN RELATION TO PECTIC CHANGES

Fruit from the second picking of Jonathan was placed in storage at 32°, 40°, 50°, and 60° F. Determinations were made at intervals, and the data are presented in Table 2 and Figure 3.

There was some irregularity in the percentage of total pectic substances and protopectin at the various temperatures. In general, there was a tendency for the amount of total pectic substances to decrease slightly with softening. There was a decrease in protopectin with softening, as shown in Figure 3, from more than 0.8 per cent at the start with a pressure test of 14.6 pounds to slightly less than 0.45 per cent at 40°, 50°, and 60°, and 0.53 per cent at 32° F., when the fruit tested 8 pounds. A very consistent increase in soluble pectin occurred at all temperatures, with softening from 0.04 per cent at the start to about 0.25 per cent when the fruit tested 8 pounds.

That the rate of softening is proportional to the rate at which the protopectin is converted into pectin is shown in Table 3. In storage at 32° F., 100 days were required for the fruit to soften from a pressure test of 14.6 pounds, the hard-ripe condition at picking time, to 10
pounds, which represents a full-ripe condition. At the same time the protopectin decreased from 0.796 to 0.585 per cent and the pectin increased from 0.037 to 0.195 per cent. Using the conditions found at 32° storage as standards for comparison with those at the other temperatures, it was found that at 40° it required 42 days to reach the same condition of softness, and 44 and 51 days, respectively, for the corresponding changes in the protopectin and pectin to take place. At 50° it required 29 days for the pressure test to become 10 pounds, and 27 and 31 days, respectively, to reach 0.585 per cent protopectin and 0.195 per cent pectin, while at 60° the corresponding changes took place in 16, 15, and 19 days, respectively.

Table 3.—Comparison of rate of softening of apples in storage with rate of change in percentage of protopectin and pectin at different temperatures

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Days required to reach—</th>
</tr>
</thead>
<tbody>
<tr>
<td>°F.</td>
<td>°C.</td>
</tr>
<tr>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>40</td>
<td>4.4</td>
</tr>
<tr>
<td>50</td>
<td>10.0</td>
</tr>
<tr>
<td>60</td>
<td>15.5</td>
</tr>
</tbody>
</table>

At the time the first determination was made on the fruit stored at 60° F. there was a slight increase in firmness which was associated with a slight decrease in pectin and a considerable decrease in protopectin and total pectic substances. With this exception, the changes in the pectic constituents at the various temperatures were proportional to the rate of softening at each temperature.

SUMMARY AND CONCLUSIONS

The relation between the softening of the fruit on the tree and in storage, as measured by a mechanical pressure tester, and changes in the pectic constituents of the fruit was studied, and from the data obtained the following conclusions seem justified:
Softening on the tree as apples approach maturity is to some extent associated with a decrease in the percentage of protopectin and a corresponding decrease in total pectic substances, whereas the pectin, which is present in very small amounts, remain constant. Softening on the tree can not be entirely accounted for by changes in the pectic constituents.

The relative firmness of different varieties of apple likewise can not be accounted for by differences in their pectic constituents.

Softening in storage is apparently due to the conversion of insoluble pectic substances, principally protopectin, into soluble form. The rate of conversion at different temperatures is proportional to the rate of softening.

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