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INTRODUCTION

In an earlier article the writers (7) have discussed the inhibiting effect of carbon dioxide on the growth of fruit-rotting fungi and its retarding effect on the ripening processes of fruits and vegetables in storage. The present paper supplements the previous one by presenting the results of chemical analyses of fruits and vegetables treated with carbon dioxide.

WORK OF OTHER INVESTIGATORS

Many investigators have studied the chemical changes that take place in fruits and vegetables after harvest and during storage. Boswell (5) has shown that in the ripening processes of pea seeds there occurs a rapid decrease in the percentage of sugar and soluble nitrogenous substances and an increase in starch, total acid-hydrolyzable substances, and insoluble nitrogen compounds (largely proteins). He adds that these changes are so rapid that a slight delay in harvesting results in markedly low quality, and that high sugar and low starch content in young peas is essential for high quality.

Jones and Bisson (8a) found that at 0° C. no important changes occur in the carbohydrate content of freshly harvested peas. At temperatures above 0° there is a loss in percentage of the more mobile carbohydrates and an increase in percentage of the more stable carbohydrates. These changes appeared to occur most rapidly at 25°, and more rapidly in shelled than in unshelled peas.

Kertesz (8b) reported that changes in the chemical composition of green peas begin immediately after shelling. Important among these changes are loss of sucrose and increase in alcohol-insoluble residue.

A number of investigators have studied the rapid depletion of sugar in green sweet corn after its removal from the stalk. That sweet corn loses a large percentage of its sugar content after removal, especially at summer temperatures, has been demonstrated by Straughn (11), Straughn and Church (12), Stevens and Higgins (10), Appleman and Arthur (1), and others. Appleman and Arthur found that the depletion of sugar does not proceed at a uniform rate but is more rapid at first and slows down as it approaches equilibrium, which is reached at the point where about 62 per cent of the total sugar and about 70 per cent of the sucrose have been lost. They state that in general
the rate of loss is doubled for every 10 degrees' increase in temperature between 10° and 30° C. until it reaches 50 per cent of the initial total sugar and 60 per cent of sucrose.

Sweet cherries have been studied by Hartman and Bullis (8), who report that in ripening there is an increase in the sugar and solid content of the fruit, a decrease in acidity and astringency, and an increase in specific gravity of both the fruit and fruit juice. Except for loss of weight and volume, there were no striking chemical or physiological changes in the fruit immediately after removal from the tree.

Bigelow and Gore (4) conducted studies on the after-harvest changes in "market-ripe" peaches at three different storage temperatures. They divided the actual daily loss in grams of the substance per peach by the solid matter in the flesh at the beginning of storage. Averaging the results of several varieties, they found a loss of "marc," acids, and sucrose at each temperature and in general an increase of reducing sugars.

Appleman and Conrad (2), in studies of the pectic constituents of peaches, showed that the conversion of protopectin to pectin could be greatly retarded at low temperatures.

Kidd and West (9) reported that an atmosphere of 5 to 15 per cent O₂ combined with 10 to 15 per cent CO₂ about doubles the storage life of apples so long as the temperature is above that associated with low-temperature injury, and that the rate of loss of sugars was about halved under optimum gas-storage conditions as compared with storage in air at the same temperature.

Recently Thornton (13) reported on the tolerance of fruits, vegetables, and flowers to CO₂ and discussed the use of the gas for retarding the ripening of bananas and for removing the astringency of pears.

EXPERIMENTAL PROCEDURE

MATERIAL AND TREATMENT

In the present work the fruits and vegetables were treated with various percentages of CO₂ for certain periods of time. Comparable material was placed at each of the temperatures 0°, 5°, 10°, 15°, and 20° C. In some of the experiments a temperature range of 5°, 10°, 15°, 20°, and 25° was substituted. Constant temperatures were maintained by means of the chambers employed by Brooks and Cooley (6). There were two lots at each temperature, one receiving the CO₂ treatment and the other not treated and therefore considered as a control. The treated lots were placed in 9-liter glass jars, the ground-glass covers of which had been replaced by 2-hole metal covers sealed with plasticine. The controls were held in glass moist chambers or similar 9-liter glass jars without stoppers.

A constant percentage of carbon dioxide was maintained in the test chambers by means of a continuous renewal of the storage air. The desired concentration of CO₂ was obtained by mixing ordinary laboratory air with CO₂. By means of water displacement the gaseous mixture was driven through copper tubing into the experimental jars in the constant-temperature chambers. The gas was passed through coils of copper tubing within the chambers before entering the jars in order to bring it to the same temperature as the fruit in the jars. By means of a pinch clamp the rate of flow of the gas was so regulated as to permit about 90 gallons of gas to pass through in a period of 24
hours. At the beginning of each experiment the gas was first bubbled through very rapidly until the atmosphere surrounding the fruit had attained the desired concentration of CO₂. The exact concentration of CO₂ employed in the experiment was determined by means of an Orsat gas apparatus, the percentage usually falling a little below the theoretical.

Peas, sweet corn, peaches, and cherries were used for the experiments. Alaska, Gradus, and Nott Excelsior peas were obtained from the Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C. Sutton Perfection peas, grown at Mountain Lake Park, Md., were brought to Washington under "top icing." A recording thermometer in the pack indicated that the temperature was about 0° C. while the peas were in transit. Cherries and peaches were procured from the open market, the peaches being in what was apparently the "market ripe" stage of maturity. The sweet corn was obtained either from the Arlington farm or from Falls Church, Va., and was harvested early in the morning and quickly brought to the laboratory.

The amount of material employed for each lot was 250 g of peas in pod, 100 g of cherries, 10 to 15 peaches, and 4 half ears of corn in the "milk" stage. The ears of corn were cut in half transversely, and one half ear with husk was placed in the CO₂ lot and the other half in the control. Thus each lot contained the halves of four different ears. Alternate ears were reversed in order that each lot might receive first the tip half and then the butt half.

When the treatment extended over a period of from three to six days samples were removed every 24 or 48 hours. In these instances over a kilogram of peas and 10 ears of corn were employed for each lot.

Sampling

At the end of the CO₂ treatment the lots were removed, tested for flavor, and preserved for subsequent moisture and carbohydrate determinations.

For moisture samples, 3 to 5 g of macerated peas or corn and 5 to 10 g of peaches or cherries were placed in a weighing bottle, weighed, covered with 10 c c of absolute alcohol, and dried to constant weight at 70° to 80° C in a vacuum equal to 25 inches of mercury.

The material for the carbohydrate analyses was preserved in alcohol. The peas were hulled and sampled whole, the cherries were pitted and sliced, the peaches were peeled, pitted, and ground in a food grinder, and two to four rows of sweet-corn kernels were completely removed from each ear by means of a sharp scalpel. The fresh material was dropped into a counterpoised Kohlrausch flask containing 0.25 g of CaCO₃, weighed on a torsion balance, and covered with sufficient hot 95 per cent alcohol to make the final mixture 80 per cent. The samples were boiled on a water bath for from three to five minutes. Duplicate samples contained 20 g of peas, 40 g of cherries, 20 or 40 g of peaches, and 16 or 20 g of corn.

Chemical Analyses

Sugar was extracted from the sweet-corn samples either by the method of Appleman and Arthur (1) or in accordance with the recommendations of the Association of Official Agricultural Chemists (3). When the extract was cooled to 20° C. the volume was made up to 200 c c, filtered, and 150 c c was withdrawn for analyses.
With peaches and cherries the supernatant alcohol was decanted off and the sample given three 30-minute refluxings with 80 per cent alcohol. Peas were extracted in the same manner, except that they were ground in a mortar before refluxing. The alcoholic extract of peas, cherries, and peaches was made up to a volume of 500 c.c.

The alcoholic aliquots from all samples were evaporated down on a water bath, the alcohol being replaced with distilled water. When cooled again the aqueous solution was transferred to 200 c.c flasks, clarified with neutral lead acetate, and the excess lead removed by means of sodium carbonate or potassium oxalate. The volume was made up to 200 c.c, and reducing sugars were determined by the Munson and Walker method. Aliquots of 50 c.c were reserved for total sugars. In some experiments the total sugar was determined as reducing sugar after inversion with hydrochloric acid and neutralization with sodium carbonate. In other experiments the inversion was made by a commercial preparation of invertase and reported as sucrose. In either case the results were computed as sucrose. Total sugar reported for peaches and sweet corn represents the sum of reducing sugar and sucrose. In peas only traces of reducing sugar were found, and in cherries only traces of sucrose.

The sugar-free residues were retained for determination of acid-hydrolyzable polysaccharides. (Completeness of sugar extraction was determined by the alpha-naphthol test.) The dried sample was placed in a Kjeldahl flask containing 200 c.c of distilled water and 20 c.c of dilute hydrochloric acid (specific gravity 1.125) and hydrolyzed for three hours in a water bath. The solution was neutralized with sodium carbonate, and reducing substances were determined by the Munson and Walker method. The results were computed as starch and reported as acid-hydrolyzable polysaccharides.

RESULTS

PEAS

The effect of the treatment with CO₂ on the carbohydrate content of Alaska and Sutton Perfection peas is shown in Table 1.

Table 1.—Total sugar and acid-hydrolyzable polysaccharides in peas treated with CO₂

[Expressed as percentage of fresh weight]

<table>
<thead>
<tr>
<th>Variety</th>
<th>Treatment</th>
<th>Total sugar</th>
<th>Acid-hydrolyzable polysaccharides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CO₂</td>
<td>Temperature (°C.) and time</td>
<td>In treated product</td>
</tr>
<tr>
<td></td>
<td>Per cent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alaska</td>
<td>44</td>
<td>25°, 1 day.</td>
<td>4.6</td>
</tr>
<tr>
<td>Sutton Perfection</td>
<td>42</td>
<td>25°, 1 day.</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15°, 1 day.</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10°, 2 days.</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5°, 2 days.</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20°, 1 day.</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15°, 1 day.</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10°, 1 day.</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5°, 2 days.</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0°, 2 days.</td>
<td>6.4</td>
</tr>
</tbody>
</table>
With both varieties the CO₂ treatment resulted in the retention of a higher percentage of total sugar and a lower percentage of polysaccharides at all but the lower temperatures. The difference between the treated and control lots is much more pronounced with the Alaska peas than with the Sutton Perfection. A possible explanation of this may be found in the fact that in 1930 this section of the country experienced the worst drought on record, the effects of which were not so evident when the Alaska peas were harvested as later when the Sutton Perfection peas were ripe. In the case of the Alaska peas it will be observed that the treated lots at 10°, 15°, and 20° C. retained 35, 51, and 43 per cent more total sugar, respectively, than did the controls at these temperatures. At 25° there was 76 per cent more sugar in the treated lots than in the controls.

With the Sutton Perfection peas the treated lots at 10° and 15° each contained 5 per cent more sugar than the controls, and at 20° there was 18 per cent more sugar in the treated lots than in the controls.

The results for the Gradus and Nott Excelsior peas appear in Figures 1 and 2, respectively. Both treated and control lots at 5°, 15°, and 25° were compared with a control at 0° C.

The excess of sucrose in the treated lots over the controls is most pronounced at 25° and 15° C. and is more marked as the time element increases. At the end of two days the treated Gradus peas at 15° contained over 10 per cent more sugar than did the controls at the end of the same period. During this time Nott Excelsior peas at 15° contained over 20 per cent more sugar than the controls. The contrast in the case of the Gradus peas was much more pronounced for the 3-day treatment. At the end of three days the flavor of the Nott Excelsior peas at 15° and 25° was objectionable. The low temperature of 5° appeared as effective as the CO₂ treatment in retaining the sucrose in the peas.

**SWEET CORN**

The results of the CO₂ treatment upon the sugar content of Stowell Evergreen sweet corn will be found in Table 2.
TABLE 2.—Total sugar in Stowell Evergreen sweet corn treated with CO₂

[Expressed as percentage of fresh weight]

<table>
<thead>
<tr>
<th>Temperature (° C.) and time of treatment</th>
<th>35 per cent CO₂</th>
<th>45 per cent CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated</td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>20°, 1 day</td>
<td>3.7</td>
<td>3.1</td>
</tr>
<tr>
<td>15°, 1 day</td>
<td>3.3</td>
<td>3.2</td>
</tr>
<tr>
<td>10°, 1 day</td>
<td>3.8</td>
<td>3.4</td>
</tr>
<tr>
<td>5°, 2 days</td>
<td>4.5</td>
<td>4.2</td>
</tr>
<tr>
<td>0°, 2 days</td>
<td>4.8</td>
<td>5.2</td>
</tr>
</tbody>
</table>

It will be noted that with 45 per cent CO₂ the treated lots at all temperatures retained a higher percentage of total sugar than did the controls, the contrast being greater at the higher temperatures. The sweet corn treated at 20° C. contained about 30 per cent more sugar than the untreated lot at the same temperature, whereas at 15° and 10° the treated lots contained over 10 per cent more sugar than the untreated lots. The effect of 35 per cent CO₂ on sweet corn was similar to, though not so great as, that of 45 per cent CO₂.

The results for the Early Evergreen and Golden Bantam sweet corn appear in Figures 3 and 4, respectively.

In contrast to the results for peas, the effect of CO₂ on the sugar content of sweet corn was less pronounced at 25° C. than at the lower temperatures. This is especially true for Golden Bantam corn. The general effect of the CO₂ on both varieties was to retard the sugar loss during the storage period. In the results for Early Evergreen sweet corn in Figure 3 it is interesting to note the grouping of the curves for the second day. Apparently the CO₂ treatment was equivalent to a 10-degree drop in temperature for the 25° and 15° lots and a 5-degree drop for the 5° lot.

In the Golden Bantam variety the CO₂ was ineffective in retarding the loss of sugar at 25° C. At 15°, however, there was over 20 per cent more total sugar in the treated than in the untreated lots for the first and second days. On the third day the sugar in the treated lot had dropped to the level of the control. At 5° the treated lots contained over 20 per cent more total sugar than the controls for two, four, and six days, but the corn had begun to lose its palatability on the sixth day.
PEACHES AND CHERRIES

In Table 3 will be found the results of CO₂ treatment of sour cherries, sweet cherries (Bing variety), and Belle peaches. The sour cherries received 45 per cent CO₂ for 2 and 4 days; the sweet cherries 44 per cent CO₂ for 2 and 3 days; and the peaches 35, 43, 44, and 47 per cent CO₂ for 1 and 2 days. The variation in the results with these different percentages of CO₂ on the peaches was very slight, and they have been averaged for the reader's convenience.
TABLE 3.—Reducing sugar, total sugar, and acid-hydrolyzable polysaccharides in cherries and peaches treated with CO₂

[Expressed as percentage of fresh weight]

<table>
<thead>
<tr>
<th>Fruit</th>
<th>Treatment</th>
<th>Reducing sugar</th>
<th>Total sugar</th>
<th>Acid-hydrolyzable polysaccharides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CO₂</td>
<td>In treated product</td>
<td>In controls</td>
<td>In treated product</td>
</tr>
<tr>
<td></td>
<td>Temperature (° C.) and time</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per cent</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sour cherries</td>
<td>45</td>
<td>20°, 2 days</td>
<td>7.5 6.9</td>
<td>0.62 1.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15°, 2 days</td>
<td>7.5 7.3</td>
<td>0.53 0.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10°, 2 days</td>
<td>6.5 7.2</td>
<td>0.63 0.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5°, 4 days</td>
<td>6.5 7.8</td>
<td>0.54 0.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2°, 4 days</td>
<td>7.2 7.2</td>
<td>0.55 0.59</td>
</tr>
<tr>
<td>Sweet cherries (Bing variety)</td>
<td>44</td>
<td>25°, 2 days</td>
<td>12.0 13.2</td>
<td>0.68 0.88</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15°, 2 days</td>
<td>13.6 12.2</td>
<td>0.78 0.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10°, 2 days</td>
<td>13.0 12.4</td>
<td>0.82 0.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10°, 3 days</td>
<td>11.1 12.2</td>
<td>0.73 0.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10°, 3 days</td>
<td>11.5 11.8</td>
<td>0.75 0.68</td>
</tr>
<tr>
<td>Peaches (Belle)</td>
<td>35–47</td>
<td>20°, 1 day</td>
<td>2.2 2.9</td>
<td>6.7 7.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15°, 1 day</td>
<td>2.2 2.3</td>
<td>6.3 6.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10°, 2 days</td>
<td>2.0 2.4</td>
<td>5.8 7.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20°, 3 days</td>
<td>2.2 2.2</td>
<td>7.3 6.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0°, 2 days</td>
<td>2.2 2.0</td>
<td>7.1 7.0</td>
</tr>
</tbody>
</table>

* Average of four experiments in which the percentage of gas was 35, 43, 44, and 47, respectively.

Apparently the CO₂ produced no effect upon the carbohydrate content of the peaches and cherries. The percentage of sugar and acid-hydrolyzable polysaccharides remained practically constant for both lots at all temperatures with the sour cherries, sweet cherries, and peaches.

**DISCUSSION**

The fact that there were no significant effects on carbohydrate transformation in the cherries and peaches as a result of the CO₂ treatment may help to answer a question frequently asked concerning the effect of CO₂ on fresh fruits. In a previous paper (7) reference was made to the deleterious effect of too great a concentration of CO₂ on the flavor of certain fruits such as peaches, strawberries, and raspberries. The question naturally arises as to whether the actual food value is also affected. From the evidence presented, the carbohydrate content of peaches and cherries appears to undergo no significant change from the CO₂ treatment given them. It should be recalled that the after-harvest behavior of peaches and cherries is very different from that of seeds like peas and corn. In other words, fruits are not converting their sugar to starch after harvesting, as is the case with peas and corn, and the amount of sugar consumed in respiration does not seem to be influenced by the CO₂ treatment. With the fresh fruits the CO₂ serves its purpose by keeping them firmer for a longer time than would be the case with storage in air.

By far the most pronounced effect of CO₂ on carbohydrate content was noted with peas and corn. It is common knowledge that these vegetables rapidly lose their palatability after being harvested, especially if held at summer temperatures, and this loss of flavor is largely the result of the conversion of sugar to polysaccharides.

The results of this work indicate that the conversion of sugar to starch in peas and corn can be considerably retarded by treatment
with CO₂ of the proper concentration. The greatest effect on peas was noticed at temperatures of 15°, 20°, and 25° C. At 5° and 0° the effect was not marked. Treated Sutton Perfection peas at 20° were 18 per cent higher, and Alaska peas at 25° were 76 per cent higher in total sugar than were the controls. The results for the Nott Excel- sior and Gradus peas were similar though not so pronounced.

With all of the sweet corn except Golden Bantam the proper concentration of CO₂ retarded the sugar loss at all temperatures employed. In contrast to the results with peas, the effect of CO₂ on sweet corn was even more pronounced at the lower temperatures. In the experiments on Early Evergreen and Golden Bantam the carbohydrate changes could be easily followed by means of the consecutive sampling of the same lot.

At the time of sampling, the fruits and vegetables were tested for flavor. In all the peaches treated with CO₂ at 15° and 20° C. there was noticed a distinctly overripe flavor; those at 10° were generally questionable or on the border line, whereas at 5° and 0° no effect was noticed. Cherries were generally unaffected by the CO₂ treatment.

Data on the effect of CO₂ on the flavor of peas and corn were obtained by the continual exposure to the gas indicated in Figures 1-4. Forty-seven per cent CO₂ for 2 days did not affect the flavor of Nott Excel- sior peas held at 5°, 15°, and 25° C. By the end of 3 days the 15° and 25° lots were objectionable in flavor, but those at 5° were still satisfactory. The Gradus peas at 25° endured 44 per cent CO₂ for 2 days and those at 15° for 3 days without any noticeable effect on flavor. The flavor of the 25° lot was objectionable at the end of 3 days and that of the 15° lot by 4 days of treatment. The 5° lot was satisfactory in regard to flavor by the end of 4 days.

The treatment of the Alaska and Sutton Perfection peas was not continued long enough to obtain data on the effect on flavor.

The Early Evergreen sweet corn reacted similarly to peas under the CO₂ treatment, but the Golden Bantam variety proved a little more sensitive. Both varieties endured the CO₂ treatment (43 to 49 per cent) at 5° C. for 5 days without impairment of flavor. The flavor of the Early Evergreen corn at 5° was questionable at the end of 6 days. The Golden Bantam corn could not be sampled after the fifth day because of insufficient amount of material offered by the normally small ears. The 25° lots of Early Evergreen corn withstood the CO₂ treatment for 2 days and the 15° lot for 3 days without loss of palatability. With the Golden Bantam the limit of treatment was 1 day at 25° and 2 days at 15°.

The treatment of the Stowell Evergreen sweet corn was not continued long enough to obtain data on effect on flavor.

**SUMMARY**

Peaches, cherries, sweet corn, and garden peas were treated with carbon dioxide gas at different temperatures for periods of one to six days and subsequently analyzed for sugars and acid-hydrolyzable polysaccharides.

The temperatures employed were 0°, 5°, 10°, 15°, 20°, and 25° C. Treatment at the higher temperatures was usually for one to three days, and that at the lower temperatures was usually from two to six days.
No significant difference in percentage of reducing sugar, total sugar, or acid-hydrolyzable polysaccharides was observed when sour cherries, sweet cherries, and Belle peaches were treated with CO₂ in concentrations of from 35 to 47 per cent.

Similar concentrations of CO₂ retarded the rate of sugar loss in peas and sweet corn.

Treatment of peaches with 35 to 47 per cent CO₂ at the higher temperatures (15° and 20° C.) produced a characteristic overripe flavor. In similar experiments the flavor of cherries was not affected by the CO₂ treatment. Exposure to 43 to 47 per cent CO₂ for two days did not affect the flavor of Gradus and Nott Excelsior peas or Early Evergreen sweet corn when held at 25° and 15°. One day at 25° and two days at 15° were the limit of tolerance to CO₂ for Golden Bantam sweet corn. At 5° the flavor of both varieties of peas was normal for three days and that of both varieties of sweet corn for four days. Increasing the time of exposure in any of the above cases was usually detrimental to flavor.

Within the limit of tolerance the treated sweet corn seemed sweeter than the controls when sampled.

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(12) and Church, C. G.

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