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Iowa State College

1918

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CHEMICAL CHANGES DURING SILAGE FORMATION

BY RAY E. NEIDIG

AGRICULTURAL EXPERIMENT STATION
IOWA STATE COLLEGE OF AGRICULTURE AND
THE MECHANIC ARTS

CHEMICAL SECTION

AMES, IOWA
SUMMARY.

Examination of the contents of the three types of silo in use at the Iowa Agricultural Experiment Station showed the following chemical changes during the actual period of silage formation.

1. Non-reducing sugar was rapidly changed to reducing sugar, and the latter then decreased in amount but did not disappear completely.
2. The amount of volatile acids increased daily.
3. In the concrete silo, as already demonstrated for the hollow tile and wooden stave silos, the racemic lactic acid produced showed a daily increase.
4. Alcohol was formed in small amounts in each silo.
5. Carbon dioxide developed very rapidly after filling the silo.
6. Free oxygen disappeared entirely after the second or third day.
7. The maximum temperature observed in any of the three silos was 91° Fahrenheit.
8. Within the limits of this investigation, no differences were noted which might be attributed to difference in the material of which the silos were constructed.
CHEMICAL CHANGES DURING SILAGE FORMATION

By RAY E. NEIDIG

INTRODUCTORY.

Studies on the volatile aliphatic acids and the lactic acid in corn silage have been reported in previous publications. It was shown that both non-volatile and volatile acids are present in considerable amount and occur in the ratio of about four parts of the former to three of the latter. The principal volatile acids were acetic and propionic, these being present in the proportion of about ten to one. The non-volatile acid was found to be lactic acid. In the three types of silos examined very little difference was noted in the above ratios.

The results thus far reported deal principally with the composition of silage at various periods subsequent to the fermentation. The uniformity of the successive samples throughout the feeding season seems to indicate that whatever changes occur after the early period of silage fermentation are of only minor importance. Chemical changes taking place during the fermentation period were not discussed at any length in the previous reports. It is known, of course, that the freshly cut corn contains soluble sugars and very little acid, whereas the silage made from it contains considerably less sugar but an abundance of acid. It is reasonable to assume, therefore, that the acidity of silage is developed at the expense of the sugar. The mechanism of the process has not, however, been satisfactorily explained. Babcock and Russell of Wisconsin attribute the formation of silage mainly to intramolecular respiration of the green plant cells and regard bacterial activities as only of secondary importance. Russell of Rothamstead, and Hart and Willaman of Wisconsin, still maintain this view. On the other hand Esten and Mason find yeasts and lactic acid bacteria in silage in such enormous numbers that their chemical activities cannot be disregarded.

It is already known that the acidity of silage practically reaches its maximum in less than three weeks. Esten and Mason observed a still more rapid fermentation, most of the acid hav-
ing developed in the first seven days with only a slight increase thereafter. It is evident, therefore, in order to follow the chemical changes that constitute silage formation, samples must be examined at frequent intervals during the two weeks immediately following the time of filling the silo. The work described in this paper represents a series of observations made upon the contents of three distinct types of silos during the actual process of silage formation.

Investigations upon the hollow clay tile silo and the wooden silo were conducted in the fall of 1912. These silos have been described in the work already mentioned. The third, a concrete silo of monolithic type, is forty feet high and fourteen feet in diameter. Investigations on this silo were conducted during the fall of 1913.

**METHODS.**

The determinations made upon the fermenting silage include the following: reducing sugars, both before and after inversion, volatile acid, lactic acid, alcohol, and analyses of the gas from the interior of the silo. Temperatures of the silage were also taken in the silos. As in the previous work, samples were secured by means of an augur, subjected to a pressure of 350 kg. per sq. cm. and the juice thus obtained was used for the analytical determinations.

*Determinations of sugar:* To 100 grams of the juice, 15 cc. of a normal solution of neutral lead acetate was added, the volume made up to 250 cc. and the solution filtered. Two hundred cc. of the filtrate was treated with just sufficient anhydrous sodium carbonate to precipitate the lead, and the solution again filtered. Duplicate portions of 25 cc. of this filtrate were used for the sugar determinations by Allihn's method. For the determination of reducing sugars after inversion, 50 cc. of the lead free solution was mixed with 5 cc. concentrated hydrochloric acid, allowed to stand 24 hours, then neutralized and diluted to 100 cc. The reducing sugar in 25 cc. of this solution was determined as above. Cuprous oxide was weighed directly and calculated as glucose in both cases.

*Determinations of volatile acids:* Volatile acids were determined by the Duclaux method as in the method cited (Research Bulletin No. 7, Iowa Agr. Exp. Station). The Duclaux fractionation was made upon one half of the solution after removal of the barium sulfate instead of first distilling into two fractions and making separate titrations and calculations. On account of the great preponderance of acetic acid, very little is accomplished by this preliminary fractionation while the calculation is rendered still more laborious.
Determination of lactic acid: After removing the volatile acids by distilling with steam under reduced pressure, lactic acid was determined as in the previous work by extracting the residue with ether and crystallizing out the zinc salt. Observations were then taken with the polariscope to determine optical activity and the zinc salt was identified by determining the percentage of zinc present.

Determination of alcohol: 100 grams of the silage juice was neutralized with sodium hydroxide, transferred to a 200 cc. distilling flask and distilled from a glycerol bath. The volume of the distillate collected in each case was 75 cc. This was diluted to 100 cc., and 50 cc. was transferred to a 300 cc. flask and boiled for 15 hrs. under a reflux condensor with 5 g. chromic acid and 5 cc. concentrated sulfuric acid. The volatile acids resulting from the oxidation of the alcohols were distilled with steam until a distillate of 1 liter was collected. This was titrated with deci-normal barium hydroxide, using phenolphthalein as an indicator. The acid was calculated as acetic acid and recalculated to ethyl alcohol.

Gas analysis: The apparatus used for obtaining samples of gas from the interior of the silo was made as follows: A one-half inch galvanized pipe was set up at the bottom of the silo, at the center, extending to a height of about ten feet. At the upper end, a two-foot piece of one-inch pipe containing numerous small holes was attached. At the lower end another section of pipe was attached to extend along the floor and up the side of the silo to a height of about six feet where it passed through the wall, a stopcock being fitted to the outer end. The entire length of pipe was given a thin coat of shellac on the inside. The purpose of inserting the pipe vertically from the bottom of the silo was to prevent any interference with the normal settling of the silage. The above described apparatus was installed in the three silos, constructed of hollow clay tile, wooden staves, and concrete respectively, before filling.

Samples of gas, representing in each case the gas from the center of the silo at a height of about twelve feet from the bottom, were obtained by means of an aspirator. The analysis was made in an Orsatt gas apparatus. One hundred cc. of the gas was measured into the burette, then transferred to the pipette containing the potash solution (1 part KOH to 2 parts H₂O) and allowed to remain there ten minutes, the operation being repeated in order to insure complete absorption of the carbon dioxide. The gas was then transferred to the burette and the decrease in volume noted. This decrease represents carbon dioxide. The contents of the burette were now transferred to the pipette containing alkaline pyrogallol solution (1 part 25%
pyrogallol solution to 6 parts 60% KOH) and subjected to the same manipulation as before. The loss in volume represents percentage of oxygen. The residual gas consists chiefly of nitrogen.

Temperature observations: The apparatus used in obtaining the temperature of different parts of each silo at frequent intervals consisted of a portable indicator and twelve electric thermometers, each connected with 15 feet of insulated wire. Four of the thermometers were buried in each silo at the time of filling, about fifteen feet from the top. The position of the thermometers in each silo was the same; one at the center, one at the outside edge, next to the silo wall, and the remaining two on a direct line between the first two, equidistant from each other and from the center and outside thermometers. When readings were desired each thermometer was connected with the indicator and on passing an electric current through the apparatus, the temperatures were read directly in degrees Fahrenheit on the indicator scale. The temperature observations on the wooden stave and hollow clay tile silos were taken a year later than the chemical studies on these two silos, yet, for the sake of comparison, they are included in this paper.

EXPERIMENTAL.

Wooden Stave Silo.

The wooden stave silo was filled Sept. 23, 1912, with Reid's yellow dent corn. The stalks were somewhat green and the corn well dented in the ear. Water was added to the top of the silo immediately after filling. The analytical results are opposite:

Hollow Clay Tile Silo.

The hollow clay tile silo was filled September 27, 1912. The corn was Reid's yellow dent, somewhat riper in appearance and containing less moisture than that put in the wooden stave silo. No water was added to this silo. The first sample was taken on the day of filling and represents the fresh corn juice. Samples 2 and 3 were taken Sept. 28 and 29, respectively. Owing to the small amount of acidity the volatile acids were not determined by the Duclaux method in these samples but simply calculated as acetic acid. The analytical results are opposite.

Concrete Silo.

The concrete silo was filled September 15, 1913, with Reid's yellow dent corn. The corn was well dented and quite dry owing to a lack of rain previous to filling the silo. Water was added at the top of the silo on the morning of September 20. After
TABLE I.—WOODEN STAVE SILO.

<table>
<thead>
<tr>
<th>No. of sample</th>
<th>Age of sample (days)</th>
<th>% of moisture in silage</th>
<th>Acetic acid grams</th>
<th>Propanoic acid grams</th>
<th>Butyric acid grams</th>
<th>Valeric acid grams</th>
<th>Lactic acid grams</th>
<th>Sugars Calculated as Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>76.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Before inversion: 2.570</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>79.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>After inversion: 5.1060</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
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<td>0.4178</td>
<td>0.0160</td>
<td>0.0099</td>
<td>0.0044</td>
<td>0.4427</td>
<td>0.7565</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>65.1</td>
<td>0.5122</td>
<td>0.0774</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.5929</td>
<td>0.8287</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>66.0</td>
<td>0.5000</td>
<td>0.0300</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.5566</td>
<td>0.8404</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>61.7</td>
<td>0.5848</td>
<td>0.0444</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.5729</td>
<td>0.8500</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>68.5</td>
<td>0.5251</td>
<td>0.0448</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.6070</td>
<td>0.7638</td>
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<tr>
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<td>7</td>
<td>67.6</td>
<td>0.6482</td>
<td>0.0448</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.6090</td>
<td>1.0700</td>
</tr>
<tr>
<td>9</td>
<td>8</td>
<td>67.5</td>
<td>0.5976</td>
<td>0.0414</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.6300</td>
<td>0.5630</td>
</tr>
<tr>
<td>10</td>
<td>9</td>
<td>63.3</td>
<td>0.7488</td>
<td>0.0020</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.8080</td>
<td>1.2700</td>
</tr>
<tr>
<td>11</td>
<td>10</td>
<td>68.3</td>
<td>0.5312</td>
<td>0.0588</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.5720</td>
<td>0.9857</td>
</tr>
<tr>
<td>12</td>
<td>11</td>
<td>67.2</td>
<td>0.5908</td>
<td>0.0698</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.6266</td>
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<tr>
<td>13</td>
<td>12</td>
<td>65.6</td>
<td>0.5920</td>
<td>0.0612</td>
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<td>0.0000</td>
<td>0.6602</td>
<td>1.6170</td>
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<td>14</td>
<td>13</td>
<td>65.2</td>
<td>0.5925</td>
<td>0.0726</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.6653</td>
<td>1.4690</td>
</tr>
</tbody>
</table>

Volatile and non-volatile acids were not separated in the first two samples.

TABLE II.—HOLLOW CLAY TILE SILO.

<table>
<thead>
<tr>
<th>No. of sample</th>
<th>Age of sample (days)</th>
<th>% of moisture in silage</th>
<th>Acetic acid grams</th>
<th>Propanoic acid grams</th>
<th>Butyric acid grams</th>
<th>Valeric acid grams</th>
<th>Lactic acid grams</th>
<th>Sugars Calculated as Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>64.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Before inversion: 2.160</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>62.0</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>After inversion: 2.180</td>
</tr>
<tr>
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<td>2</td>
<td>65.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Before inversion: 2.160</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>64.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>After inversion: 2.180</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>57.9</td>
<td>0.1472</td>
<td>0.0922</td>
<td>0.0017</td>
<td>0.0038</td>
<td>0.4247</td>
<td>0.7565</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>58.1</td>
<td>0.3400</td>
<td>0.0322</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.3792</td>
<td>0.8640</td>
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<tr>
<td>7</td>
<td>6</td>
<td>62.1</td>
<td>0.2415</td>
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<td>0.3770</td>
<td>0.8640</td>
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<tr>
<td>8</td>
<td>7</td>
<td>58.8</td>
<td>0.4025</td>
<td>0.0219</td>
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<td>0.0000</td>
<td>0.4445</td>
<td>0.7990</td>
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<tr>
<td>9</td>
<td>8</td>
<td>60.9</td>
<td>0.4182</td>
<td>0.0486</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.4618</td>
<td>0.8464</td>
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<tr>
<td>10</td>
<td>9</td>
<td>63.4</td>
<td>0.3972</td>
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<td>0.4430</td>
<td>0.8210</td>
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<td>11</td>
<td>10</td>
<td>63.2</td>
<td>0.4742</td>
<td>0.0678</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.5239</td>
<td>1.1080</td>
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<tr>
<td>12</td>
<td>11</td>
<td>58.5</td>
<td>0.5045</td>
<td>0.0524</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.5672</td>
<td>1.3400</td>
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</tbody>
</table>

Volatile acids were not separated in the first three samples.

*Calculated as acetic acid.  **Calculated as ethyl alcohol.
thoroughly soaking the top of the silage, oats were sown which soon sprouted and formed a thick matted covering over the surface of the silage. No separation of the volatile acids by the Duclaux method was made on the samples of silage from this silo. The total volatile acids were calculated as acetic acid. The analytical results follow:

**TABLE III.—CONCRETE SILO.**

<table>
<thead>
<tr>
<th>No. of sample</th>
<th>Age of sample (days)</th>
<th>% of moisture in silage</th>
<th>Analysis of 100 Grams Silage Juice</th>
<th>Gas Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>% total volatile acid grams</td>
<td>% of CO₂</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lactic acid grams</td>
<td>% of oxygen</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>sugars calculated as glucose</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before inversion</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>After inversion</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Alcohol grams</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>50.0</td>
<td>0.0114</td>
<td>8.068</td>
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<tr>
<td>2</td>
<td>1</td>
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<td>8.942</td>
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<tr>
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<td>2</td>
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<td>61.6</td>
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<td>7.004</td>
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<td>5</td>
<td>62.0</td>
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<td>0.5680</td>
<td>5.573</td>
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<td>8</td>
<td>7</td>
<td>62.4</td>
<td>0.6960</td>
<td>5.770</td>
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<td>9</td>
<td>8</td>
<td>63.3</td>
<td>0.8660</td>
<td>5.770</td>
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<td>64.5</td>
<td>1.0115</td>
<td>5.770</td>
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<td>65.6</td>
<td>1.4068</td>
<td>5.770</td>
</tr>
</tbody>
</table>

*Calculated as acetic acid.  **Calculated as ethyl alcohol.

**TABLE IV.—TEMPERATURE READINGS—WOODEN STAVE SILO.**

Filled September 13, 1913.

<table>
<thead>
<tr>
<th>Date of reading</th>
<th>Time of reading</th>
<th>Readings of Thermometers Placed at Center</th>
<th>Two feet from Center</th>
<th>Two feet from Wall</th>
<th>at Wall</th>
<th>Temperature of atmosphere Degrees F.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept. 12</td>
<td>8:00 A. M.</td>
<td>80</td>
<td>80</td>
<td>76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sept. 14</td>
<td>7:00 A. M.</td>
<td>80</td>
<td>77</td>
<td>66</td>
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<td></td>
</tr>
<tr>
<td>Sept. 15</td>
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<td>77</td>
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<td>76</td>
<td>74</td>
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<tr>
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<td>66</td>
<td>64</td>
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<td>80</td>
<td>60</td>
<td>48</td>
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</table>
### TABLE IV.—TEMPERATURE READINGS—WOODEN STAVE SILO—Continued.

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<th>Date of reading</th>
<th>Time of reading</th>
<th>Readings of Thermometers Placed</th>
<th>Temperature of Atmosphere Degrees F.</th>
</tr>
</thead>
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<td></td>
<td>at Center</td>
<td>Two feet from Center</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Degrees Fahrenheit</td>
<td>Degrees F.</td>
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<td>Sept. 24</td>
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<td>82</td>
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<td>Sept. 26</td>
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<td>83</td>
<td>83</td>
</tr>
<tr>
<td>Sept. 27</td>
<td>2:00 P. M.</td>
<td>84</td>
<td>83</td>
</tr>
<tr>
<td>Sept. 28</td>
<td>5:15 P. M.</td>
<td>84</td>
<td>83</td>
</tr>
<tr>
<td>Sept. 29</td>
<td>5:00 P. M.</td>
<td>84</td>
<td>83</td>
</tr>
<tr>
<td>Sept. 30</td>
<td>4:45 P. M.</td>
<td>83</td>
<td>82</td>
</tr>
<tr>
<td>Oct. 1</td>
<td>5:00 P. M.</td>
<td>84</td>
<td>84</td>
</tr>
<tr>
<td>Oct. 2</td>
<td>3:30 P. M.</td>
<td>85</td>
<td>84</td>
</tr>
<tr>
<td>Oct. 3</td>
<td>2:30 P. M.</td>
<td>86</td>
<td>84</td>
</tr>
<tr>
<td>Oct. 4</td>
<td>2:40 P. M.</td>
<td>86</td>
<td>84</td>
</tr>
<tr>
<td>Oct. 5</td>
<td>11:50 A. M.</td>
<td>85</td>
<td>84</td>
</tr>
</tbody>
</table>

*Water was turned on the top of the silo at 8:30 P. M. and allowed to flow all night.

### TABLE V.—TEMPERATURE READINGS—HOLLOW TILE SILO.
Filled September 18, 1913.

<table>
<thead>
<tr>
<th>Date of reading</th>
<th>Time of reading</th>
<th>Readings of Thermometers Placed</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>at Center</td>
<td>Two feet from Center</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Degrees Fahrenheit</td>
<td>Degrees F.</td>
</tr>
<tr>
<td>Sept. 18</td>
<td>1:00 P. M.</td>
<td>77</td>
<td>78</td>
</tr>
<tr>
<td>Sept. 19</td>
<td>8:00 A. M.</td>
<td>81</td>
<td>82</td>
</tr>
<tr>
<td>Sept. 20</td>
<td>7:45 A. M.</td>
<td>83</td>
<td>85</td>
</tr>
<tr>
<td>Sept. 21</td>
<td>7:15 A. M.</td>
<td>85</td>
<td>86</td>
</tr>
<tr>
<td>Sept. 22</td>
<td>8:30 A. M.</td>
<td>86</td>
<td>87</td>
</tr>
<tr>
<td>Sept. 23</td>
<td>7:30 A. M.</td>
<td>87</td>
<td>88</td>
</tr>
<tr>
<td>Sept. 24</td>
<td>7:30 A. M.</td>
<td>88</td>
<td>89</td>
</tr>
<tr>
<td>Sept. 25</td>
<td>7:45 A. M.</td>
<td>88</td>
<td>90</td>
</tr>
<tr>
<td>Sept. 26</td>
<td>7:15 A. M.</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Sept. 27</td>
<td>2:00 P. M.</td>
<td>90</td>
<td>91</td>
</tr>
<tr>
<td>Sept. 28</td>
<td>5:15 P. M.</td>
<td>90</td>
<td>91</td>
</tr>
<tr>
<td>Sept. 29</td>
<td>5:00 P. M.</td>
<td>90</td>
<td>91</td>
</tr>
<tr>
<td>Sept. 30</td>
<td>4:45 P. M.</td>
<td>90</td>
<td>91</td>
</tr>
<tr>
<td>Oct. 1</td>
<td>5:00 P. M.</td>
<td>90</td>
<td>91</td>
</tr>
<tr>
<td>Oct. 2</td>
<td>3:30 P. M.</td>
<td>90</td>
<td>91</td>
</tr>
<tr>
<td>Oct. 3</td>
<td>2:30 P. M.</td>
<td>90</td>
<td>91</td>
</tr>
<tr>
<td>Oct. 4</td>
<td>2:40 P. M.</td>
<td>90</td>
<td>91</td>
</tr>
<tr>
<td>Oct. 5</td>
<td>11:30 A. M.</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Oct. 6</td>
<td>5:00 P. M.</td>
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<td>90</td>
</tr>
<tr>
<td>Oct. 7</td>
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<td>Oct. 8</td>
<td>4:30 P. M.</td>
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</tbody>
</table>
TABLE VI.—TEMPERATURE READINGS—CONCRETE SILO.
Filled September 16, 1913.

<table>
<thead>
<tr>
<th>Date of reading</th>
<th>Time of reading</th>
<th>Readings of Thermometers Placed</th>
<th>Two feet from Center</th>
<th>Two feet from Wall</th>
<th>at Wall</th>
</tr>
</thead>
<tbody>
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<td>70</td>
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<td>84</td>
<td>68</td>
<td>68</td>
</tr>
<tr>
<td>Sept. 19</td>
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<td>65</td>
<td>65</td>
</tr>
<tr>
<td>Sept. 20</td>
<td>7:45 A. M.</td>
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<td>60</td>
</tr>
<tr>
<td>Sept. 21</td>
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<td>87</td>
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</tr>
<tr>
<td>Sept. 22</td>
<td>8:30 A. M.</td>
<td>88</td>
<td>89</td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>Sept. 23</td>
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<td>88</td>
<td>89</td>
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<td>51</td>
</tr>
<tr>
<td>Sept. 24</td>
<td>7:30 A. M.</td>
<td>88</td>
<td>88</td>
<td>58</td>
<td>58</td>
</tr>
<tr>
<td>Sept. 25</td>
<td>7:45 A. M.</td>
<td>90</td>
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<td>59</td>
<td>59</td>
</tr>
<tr>
<td>Sept. 26</td>
<td>7:45 A. M.</td>
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<td>88</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td>Sept. 27</td>
<td>9:00 P. M.</td>
<td>90</td>
<td>87</td>
<td>65</td>
<td>60</td>
</tr>
<tr>
<td>Sept. 28</td>
<td>9:15 P. M.</td>
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<td>69</td>
<td>69</td>
</tr>
<tr>
<td>Sept. 29</td>
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<td>88</td>
<td>65</td>
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</tr>
<tr>
<td>Sept. 30</td>
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<td>62</td>
</tr>
<tr>
<td>Oct. 1</td>
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<td>64</td>
</tr>
<tr>
<td>Oct. 2</td>
<td>3:30 P. M.</td>
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<td>86</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Oct. 3</td>
<td>2:30 P. M.</td>
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<td>62</td>
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<tr>
<td>Oct. 4</td>
<td>2:40 P. M.</td>
<td>91</td>
<td>85</td>
<td>67</td>
<td>67</td>
</tr>
<tr>
<td>Oct. 5</td>
<td>11:30 A. M.</td>
<td>91</td>
<td>84</td>
<td>67</td>
<td>67</td>
</tr>
<tr>
<td>Oct. 6</td>
<td>5:00 P. M.</td>
<td>90</td>
<td>84</td>
<td>64</td>
<td>64</td>
</tr>
</tbody>
</table>

DISCUSSION.

Before discussing the results of the foregoing investigation on silage formation in the three types of silos, several factors which enter into the work and exert a decided influence upon the amounts of chemical products formed in the silo, must be reckoned with. Two of the chief factors are the maturity and moisture content of the corn. It is known that the more immature the corn the greater the amount of acid produced. Differences in maturity also show differences in the sugar and moisture content and, as acids are formed chiefly at the expense of the sugars, it is evident that any differences in the maturity of the corn would materially affect the quantities of the chemical products. Since the three silos were not filled with corn of uniform maturity and water was added to two of the silos, the results will not be compared quantitatively but the general ratio of the chemical products will be used for the basis of the discussion.
and the merits of the three types of silos discussed from this viewpoint.

A comparison of the sugar determinations shows that in each case the juice from the green plant contains about equal amounts of reducing sugar and non-reducing sugar. The latter may be considered sucrose. During the first two days after filling the silo, the most noticeable change is the inversion of the sucrose. When the sugar determinations before and after inversion coincided, the inversion was considered complete and no further determinations were deemed necessary. The rate of inversion was not the same in the three silos; the wooden silo requiring three days, the hollow clay tile silo four and the concrete silo five days. No significance, however, can be attached to these differences, inasmuch as the chemical changes vary at the same rate. The inversion of sucrose is probably due to the greater extent to the enzyme sucrase. Acids, likewise, bring about the hydrolysis of sucrose but their effect in this instance is practically negligible for the reason that organic acids are weak catalysts and at this early period of silage formation are present only in small amounts. Although it is not possible to state the exact source of the enzyme, sucrase is known to be present in the corn plant itself, and it is also a product of numerous microorganisms including bacteria, yeasts and molds.

From the time of filling the silo, the total sugar decreases in amount until a minimum is reached in about three weeks. In the case of the hollow tile silo this minimum was reached in twelve days. In no instance does the reducing sugar disappear completely. Even after a much longer period the presence of reducing sugars can be demonstrated. A sample of silage juice, 120 days old, from the concrete silo was found to contain 3.1 grams reducing sugar per 100 grams juice. In order to identify the sugar a portion of the juice was clarified with neutral lead acetate in the usual manner, concentrated on the water bath, and then treated with phenylhydrazine hydrochloride and sodium acetate in a flask immersed in boiling water. After a few minutes yellow crystals separated from the hot solution. These were filtered out and washed with cold water, then recrystallized five times from 50% alcohol. The substance showed a melting point of 204°-206°. Phenylglucosazone melts at 205°. From the melting point, the insolubility in hot water, and the crystalline form of the osazone, it is evident that the sugar was either glucose or fructose or a mixture of the two. Examination of the clear sugar solution in a polariscope showed dextro rotation, indicating, therefore, that the sugar consisted of glucose either in the pure state or greatly in excess of the fructose.
The results on the acidity of silage show that in each case there is a gradual rise in volatile acids. In the two silos, wooden stave and hollow clay tile, the individual volatile acids were determined and approximately the same proportions of propionic to acetic acid were found as noted in our previous publications on this subject. Butyric and valeric acid were found to occur in traces only in one sample from the wooden silo. The three silos show differences in the rate of formation and the maximum yield of volatile acids but this cannot be attributed to the different types of silos. The ratio of volatile acids to lactic acid is approximately a constant for the three silos. Lactic acid was found to increase in the concrete silo in similar manner to that of the wooden and hollow clay tile silos. The samples of zinc lactate examined in a polariscope proved to be the racemic or inactive variety. While the maximum yield of lactic acid and volatile acids varied for the three silos, yet no significance can be attached to these differences since factors which would influence the chemical changes in silage were noticed at the time of filling the silos, such as the moisture content and the maturity of the corn.

The alcohols found in silage were calculated as ethyl alcohol, although it is known that other alcohols are produced in small amounts during silage formation. The tables giving the results for the hollow clay tile and concrete silos show a gradual increase in the amount of alcohol, while the results for the wooden stave silo indicate that the amount of alcohol found on the third day after filling the silo is almost equal to the maximum amount observed during the period of investigation for this silo. It is further noted that the results on this silo are quite irregular and do not follow any gradual increase as in the case of the hollow clay tile or concrete silos. The alcohol found probably owes its origin, in the greater part, to yeasts, since these are known to be present in enormous quantities at the beginning of silage formation. It is difficult to state with the present evidence whether or not alcohol occurs as a transitory product. It is quite probable, however, that a small part of the acetic acid which is found in silage is the result of oxidization of some of the alcohol. The mechanism of this reaction cannot be definitely explained as yet, since free oxygen is absent from the interior of the silo except in traces. It is possible, however, that microorganisms are an aid in this oxidization.

The gas analyses on the three silos show practically the same results: carbon dioxide is formed very rapidly during the first few days, and after reaching a maximum, which differs slightly for each silo, gradually decreases until a minimum of approxi-

\[\text{Loc. cit.}\]
mately 20% of the entire gas is reached. Oxygen, on the other hand, disappears entirely during the first few days. The residual gas consists mainly of nitrogen, a considerable amount of which must gain access to the silo from the top. As air diffuses downward the nitrogen replaces the carbon dioxide and accumulates, while the atmospheric oxygen is used by the aerobic bacteria and fungi found at the surface of every silo. This, it appears, satisfactorily explains the presence of two or three feet of spoiled silage always found at the top of silos.

The temperature observations on the three silos were taken every eight hours during the early period of silage formation. Since no appreciable differences were observed during these short periods, only the temperatures taken twenty-four hours apart are given in the foregoing tables. The question of the temperatures attained in the silo during the early period of silage formation is one which is very much in dispute and it is hoped that the results obtained will greatly aid in clearing some of the erroneous conceptions commonly held. As the silage in each of the three silos was pronounced to be in excellent condition by experts, it is only reasonable to suppose that the observed temperatures are the actual temperatures prevailing during the formation of good normal silage.

It is well known that higher temperatures are reached at the top of the silos where oxygen gains entrance and partially spoiled silage results. Similarly, if oxygen gained access to the silage, due to insufficient settling of the silage or to faulty construction of the silo, higher temperatures would be noted. In the author's opinion, a great number of the conflicting statements found in the literature on the temperatures observed in silos can be directly attributed to the factors just mentioned.

The results on the three silos during the period of examination indicate that no importance can be attached to the superiority of any one type of silo over others in regard to the conductivity of heat through the silo walls. It was intended to continue the temperature observations throughout the winter, with a view of ascertaining the relative conductivity of the silo walls, but since the silage was fed below the depth of the thermometers before cold weather, this point could not be determined and is, therefore, reserved until a future date. However, it is quite probable that the three silos will show similar properties.

The chemical changes occurring during the early period of silage fermentation are very similar for the three silos. The only differences that were noted are in the rate of formation and the quantity of the chemical constituents produced. When the fact is taken into consideration that the corn was in a different
state of maturity when put into the different silos, it is not surprising that such results are obtained and they can hardly be attributed to differences in the types of silo.

A complete study of the bacterial flora of silage is necessary, in the author's opinion, before definite statements can be made upon the causes of silage formation. It is hoped that these results on the chemical changes in silage may be an aid in such an investigation.

CONCLUSIONS.

The results show that no differences were noted in the chemical changes of the silage in the three silos which could be attributed to the effect of different types of building materials upon the process of silage formation. It is readily seen that approximately the same results are obtained in the temperature observations, gas analyses and determinations of alcohol, sugars and volatile and non-volatile acids of the silage from the three silos. The only differences noted are differences in quantity; the ratio of the chemical substances, however, is very nearly the same for each silo.

The question now arises as to the comparative value of the silage. Is silage of high acidity of more value than that of low acidity? In the author's opinion silage must have a sufficient amount of acidity to insure its keeping but beyond this point additional acid is neither essential or beneficial. A large amount of acid is known to be harmful but the quantity which gives the maximum food value has not been determined.

REMARKS CONCERNING THE VALUE OF DIFFERENT TYPES OF SILOS.

Since the three silos produced excellent silage a short discussion of the relative merits of the different types of silo will be included in this work. In the past the main argument used by wooden silo manufacturers was the superiority of wood over stone, brick, hollow tile, or concrete as a material for the silo walls. They based their conclusions upon the fact that wood is more a non-conductor of heat than the other materials. The temperature results on the three types of silos show that little stress can be laid on this point as all three silos showed relatively the same temperatures. The results also show that a high temperature is not an essential factor in the production of good silage since the maximum temperature noted in the three silos was 91° Fahrenheit. Moreover, the period of silage formation
is of short duration, about three weeks at the most, and it occurs at a time of the year when the weather is not exceedingly cold, hence for this reason alone, conductivity of the silo wall would not play an important part in silage formation. As for silage freezing in severe cold weather in the silos, the writer has failed to notice an advantage in using any one particular material for the silo walls, as the three silos, constructed of wooden staves, hollow tile, and concrete, respectively, froze approximately the same amount in each instance. Much stress has also been laid upon the alleged deleterious effect of the acids upon the stone, hollow tile and concrete walls which results in forming a portion of spoiled silage next to the walls. The writer believes that such instances are due either to the walls not being air tight, thus allowing air to gain access to the silage, or to rough surface walls, which prevent the thorough settling of the silage near the walls. Examination of the concrete and hollow tile silos, which have smooth walls and contained silage which was properly packed, failed to show any spoiled silage. The chief factors necessary for the production of good silage are, therefore, smooth air-tight walls, corn in the right state of maturity, the proper amount of moisture, and carefulness in filling. These four factors properly carried out will insure a good quality of silage with a minimum loss due to spoiled silage. It, therefore, seems that a great amount of the literature which has been scattered broadcast mainly for advertising purposes, is unreliable and the author suggests that the silo buyer base his choice from the various types of silos upon the economic standpoint which means considering the original cost, the cost of maintenance, and the approximate life of the silo.

ACKNOWLEDGEMENTS.

The writer wishes to thank Professor J. B. Davidson for his co-operation in securing the temperature observations, also, Dr. A. W. Dox for the interest taken in this investigation.
APPENDIX—DUCLAUX TABLES—Wooden Stave Silo.

SAMPLE 3. FRACTIONATION.*

<table>
<thead>
<tr>
<th></th>
<th>10 cc</th>
<th>20 cc</th>
<th>30 cc</th>
<th>40 cc</th>
<th>50 cc</th>
<th>60 cc</th>
<th>70 cc</th>
<th>80 cc</th>
<th>90 cc</th>
<th>100 cc</th>
<th>Acid</th>
<th>Molecular proportions</th>
<th>cc Decinormal in total distillate</th>
<th>Grams in distillate and residue</th>
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<tbody>
<tr>
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<tr>
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<td>Total</td>
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</tbody>
</table>

One-half of the original solution was used in this as well as in the succeeding samples.

SAMPLE 4. FRACTIONATION.

<table>
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<tr>
<th></th>
<th>10 cc</th>
<th>20 cc</th>
<th>30 cc</th>
<th>40 cc</th>
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<th>60 cc</th>
<th>70 cc</th>
<th>80 cc</th>
<th>90 cc</th>
<th>100 cc</th>
<th>Acid</th>
<th>Molecular proportions</th>
<th>cc Decinormal in total distillate</th>
<th>Grams in distillate and residue</th>
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<td>4.55</td>
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SAMPLE 5. FRACTIONATION.

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Propionic 8  2.851  0.9444  
Total 38.499  0.5792  

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Propionic 8  3.2  0.9446  
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Propionic 4  2.881  0.9448  
Total 46.100  0.9630  

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<td>Total</td>
<td>35.199</td>
<td>0.6658</td>
<td></td>
</tr>
</tbody>
</table>
Hollow Clay Tile Silo.

The hollow clay tile silo was filled September 27, 1912. The corn was somewhat riper in appearance and contained less moisture than that put in the wooden stave silo. The first sample was taken September 27, 1912, and represents the fresh corn juice. Samples two and three were taken September 28th and 29th respectively. On account of the small amount of acid the volatile acid was not determined by the Ducaux method in these three samples but instead the volatile acidity was calculated as acetic acid.

Sample four was taken September 30, 1912.

**SAMPLE 4. FRACTIONATION.**

<table>
<thead>
<tr>
<th>Acidity</th>
<th>Molecular proportions</th>
<th>cc Decinormal in total distillate</th>
<th>Grams in distillate and residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic</td>
<td>100</td>
<td>19.829</td>
<td>0.1472</td>
</tr>
<tr>
<td>Propionic</td>
<td>6</td>
<td>1.177</td>
<td>0.0092</td>
</tr>
<tr>
<td>Butyric</td>
<td>1</td>
<td>0.166</td>
<td>0.0025</td>
</tr>
<tr>
<td>Valeric</td>
<td>1</td>
<td>0.166</td>
<td>0.0025</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>21.198</td>
<td>0.1601</td>
</tr>
</tbody>
</table>

Only one portion. Was not diluted one-half.

**SAMPLE 5. FRACTIONATION.**

<table>
<thead>
<tr>
<th>Acidity</th>
<th>Molecular proportions</th>
<th>cc Decinormal in total distillate</th>
<th>Grams in distillate and residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic</td>
<td>100</td>
<td>36.00</td>
<td>0.2700</td>
</tr>
<tr>
<td>Propionic</td>
<td>10</td>
<td>3.6</td>
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</tr>
<tr>
<td>Total</td>
<td></td>
<td>39.6</td>
<td>0.2981</td>
</tr>
</tbody>
</table>

**SAMPLE 6. FRACTIONATION.**

<table>
<thead>
<tr>
<th>Acidity</th>
<th>Molecular proportions</th>
<th>cc Decinormal in total distillate</th>
<th>Grams in distillate and residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic</td>
<td>9</td>
<td>22.65</td>
<td>6.310</td>
</tr>
<tr>
<td>Propionic</td>
<td>1</td>
<td>2.62</td>
<td>0.0892</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>25.27</td>
<td>6.3992</td>
</tr>
</tbody>
</table>

*Diluted one-half. The remaining samples of the hollow tile silo are also diluted in the same manner.*
### SAMPLE 7. FRACTIONATION.

<table>
<thead>
<tr>
<th></th>
<th>10 cc</th>
<th>20 cc</th>
<th>30 cc</th>
<th>40 cc</th>
<th>50 cc</th>
<th>60 cc</th>
<th>70 cc</th>
<th>80 cc</th>
<th>90 cc</th>
<th>100 cc</th>
<th>Acid</th>
<th>Molecular proportions</th>
<th>ee-Decinormal in total distillate</th>
<th>Grams in distillate and residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.0</td>
<td>2.0</td>
<td>2.1</td>
<td>2.2</td>
<td>2.3</td>
<td>2.4</td>
<td>2.5</td>
<td>2.7</td>
<td>3.1</td>
<td>3.7</td>
<td>Acetic</td>
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<td>0.03416</td>
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<td>4.0</td>
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<td>8.3</td>
<td>10.6</td>
<td>13.0</td>
<td>15.5</td>
<td>18.25</td>
<td>21.35</td>
<td>25.05</td>
<td>Propionic</td>
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<td>2.277</td>
<td>0.0554</td>
</tr>
<tr>
<td>C</td>
<td>7.98</td>
<td>15.96</td>
<td>24.35</td>
<td>33.13</td>
<td>42.32</td>
<td>51.9</td>
<td>61.88</td>
<td>72.86</td>
<td>85.28</td>
<td>100.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>7.82</td>
<td>16.</td>
<td>24.48</td>
<td>33.29</td>
<td>42.51</td>
<td>51.97</td>
<td>62.00</td>
<td>73.09</td>
<td>85.18</td>
<td>100.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Total**

|     |       |       |       |       |       |       |       |       |       | 25.050 |             | 0.3770                  |                          |                          |

### SAMPLE 8. FRACTIONATION.

(Only 75 grams sample taken)

<table>
<thead>
<tr>
<th></th>
<th>10 cc</th>
<th>20 cc</th>
<th>30 cc</th>
<th>40 cc</th>
<th>50 cc</th>
<th>60 cc</th>
<th>70 cc</th>
<th>80 cc</th>
<th>90 cc</th>
<th>100 cc</th>
<th>Acid</th>
<th>Molecular proportions</th>
<th>ee-Decinormal in total distillate</th>
<th>Grams in distillate and residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.7</td>
<td>1.8</td>
<td>1.9</td>
<td>2.0</td>
<td>2.0</td>
<td>2.1</td>
<td>2.25</td>
<td>2.45</td>
<td>2.70</td>
<td>3.25</td>
<td>Acetic</td>
<td>100</td>
<td>29.136</td>
<td>0.502</td>
</tr>
<tr>
<td>B</td>
<td>1.7</td>
<td>3.5</td>
<td>5.4</td>
<td>7.4</td>
<td>9.4</td>
<td>11.5</td>
<td>13.75</td>
<td>16.2</td>
<td>18.9</td>
<td>22.15</td>
<td>Propionic</td>
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<td>2.0186</td>
<td>0.0514</td>
</tr>
<tr>
<td>C</td>
<td>7.67</td>
<td>15.8</td>
<td>24.38</td>
<td>33.41</td>
<td>42.44</td>
<td>51.92</td>
<td>62.08</td>
<td>73.14</td>
<td>85.33</td>
<td>100.</td>
<td></td>
<td>(100 gram sample)</td>
<td>0.4926</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>7.82</td>
<td>16.</td>
<td>24.48</td>
<td>33.29</td>
<td>42.31</td>
<td>51.97</td>
<td>62.01</td>
<td>73.09</td>
<td>85.18</td>
<td>100.</td>
<td></td>
<td>(100 gram sample)</td>
<td>0.0419</td>
<td></td>
</tr>
</tbody>
</table>

**Total**

|       | 0.4445 |

### SAMPLE 9. FRACTIONATION.

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<thead>
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<th></th>
<th>10 cc</th>
<th>20 cc</th>
<th>30 cc</th>
<th>40 cc</th>
<th>50 cc</th>
<th>60 cc</th>
<th>70 cc</th>
<th>80 cc</th>
<th>90 cc</th>
<th>100 cc</th>
<th>Acid</th>
<th>Molecular proportions</th>
<th>ee-Decinormal in total distillate</th>
<th>Grams in distillate and residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.4</td>
<td>2.5</td>
<td>2.6</td>
<td>2.68</td>
<td>2.8</td>
<td>2.95</td>
<td>3.15</td>
<td>3.4</td>
<td>3.75</td>
<td>4.45</td>
<td>Acetic</td>
<td>100</td>
<td>27.89</td>
<td>0.4182</td>
</tr>
<tr>
<td>B</td>
<td>2.4</td>
<td>4.9</td>
<td>7.5</td>
<td>10.18</td>
<td>12.98</td>
<td>15.98</td>
<td>19.08</td>
<td>22.48</td>
<td>26.23</td>
<td>30.63</td>
<td>Propionic</td>
<td>10</td>
<td>2.789</td>
<td>0.0436</td>
</tr>
<tr>
<td>C</td>
<td>7.82</td>
<td>15.97</td>
<td>24.44</td>
<td>33.11</td>
<td>42.31</td>
<td>51.92</td>
<td>62.18</td>
<td>72.27</td>
<td>85.49</td>
<td>100.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>7.82</td>
<td>16.</td>
<td>24.48</td>
<td>33.29</td>
<td>42.31</td>
<td>51.97</td>
<td>62.01</td>
<td>73.09</td>
<td>85.18</td>
<td>100.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Total**

|     | 30.679 | 0.4618 |

---

**SAMPLE 10. FRACTIONATION.**

(Only 75 grams sample taken)

<table>
<thead>
<tr>
<th></th>
<th>10 cc</th>
<th>20 cc</th>
<th>30 cc</th>
<th>40 cc</th>
<th>50 cc</th>
<th>60 cc</th>
<th>70 cc</th>
<th>80 cc</th>
<th>90 cc</th>
<th>100 cc</th>
<th>Acid</th>
<th>Molecular proportions</th>
<th>ee-Decinormal in total distillate</th>
<th>Grams in distillate and residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.4</td>
<td>2.5</td>
<td>2.6</td>
<td>2.68</td>
<td>2.8</td>
<td>2.95</td>
<td>3.15</td>
<td>3.4</td>
<td>3.75</td>
<td>4.45</td>
<td>Acetic</td>
<td>100</td>
<td>27.89</td>
<td>0.4182</td>
</tr>
<tr>
<td>B</td>
<td>2.4</td>
<td>4.9</td>
<td>7.5</td>
<td>10.18</td>
<td>12.98</td>
<td>15.98</td>
<td>19.08</td>
<td>22.48</td>
<td>26.23</td>
<td>30.63</td>
<td>Propionic</td>
<td>10</td>
<td>2.789</td>
<td>0.0436</td>
</tr>
<tr>
<td>C</td>
<td>7.82</td>
<td>15.97</td>
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<td>33.11</td>
<td>42.31</td>
<td>51.92</td>
<td>62.18</td>
<td>72.27</td>
<td>85.49</td>
<td>100.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>7.82</td>
<td>16.</td>
<td>24.48</td>
<td>33.29</td>
<td>42.31</td>
<td>51.97</td>
<td>62.01</td>
<td>73.09</td>
<td>85.18</td>
<td>100.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Total**

|     | 30.679 | 0.4618 |
### SAMPLE 10. FRACTIONATION.

<table>
<thead>
<tr>
<th></th>
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<th>20 cc</th>
<th>30 cc</th>
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<th>50 cc</th>
<th>60 cc</th>
<th>70 cc</th>
<th>80 cc</th>
<th>90 cc</th>
<th>100 cc</th>
</tr>
</thead>
<tbody>
<tr>
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<td>3.6</td>
<td>4.2</td>
</tr>
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<td>4.7</td>
<td>7.2</td>
<td>9.8</td>
<td>12.5</td>
<td>15.3</td>
<td>18.2</td>
<td>21.6</td>
<td>25.2</td>
<td>29.4</td>
</tr>
<tr>
<td>C</td>
<td>7.82</td>
<td>15.98</td>
<td>24.49</td>
<td>33.33</td>
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<td>62.16</td>
<td>73.21</td>
<td>85.26</td>
<td>100.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Acid</th>
<th>Molecular proportions</th>
<th>ee Decinormal in total distillate</th>
<th>Grams in distillate and residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic</td>
<td>9</td>
<td>26.46</td>
<td>0.3772</td>
</tr>
<tr>
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Total

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<th>ee Decinormal in total distillate</th>
<th>Grams in distillate and residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic</td>
<td>85</td>
<td>31.629</td>
<td>0.4742</td>
</tr>
<tr>
<td>Propionic</td>
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<td>3.721</td>
<td>0.0578</td>
</tr>
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</table>

Total

<table>
<thead>
<tr>
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<th>Molecular proportions</th>
<th>ee Decinormal in total distillate</th>
<th>Grams in distillate and residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic</td>
<td>100</td>
<td>33.654</td>
<td>0.5048</td>
</tr>
<tr>
<td>Propionic</td>
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<td>3.365</td>
<td>0.0624</td>
</tr>
</tbody>
</table>

Total

### SAMPLE 11. FRACTIONATION.

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<th>10 cc</th>
<th>20 cc</th>
<th>30 cc</th>
<th>40 cc</th>
<th>50 cc</th>
<th>60 cc</th>
<th>70 cc</th>
<th>80 cc</th>
<th>90 cc</th>
<th>100 cc</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
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<td>2.9</td>
<td>3.0</td>
<td>3.1</td>
<td>3.25</td>
<td>3.4</td>
<td>3.65</td>
<td>3.9</td>
<td>4.3</td>
<td>5.05</td>
</tr>
<tr>
<td>B</td>
<td>2.8</td>
<td>5.7</td>
<td>8.7</td>
<td>11.8</td>
<td>15.05</td>
<td>18.45</td>
<td>22.1</td>
<td>26.0</td>
<td>30.3</td>
<td>35.35</td>
</tr>
<tr>
<td>C</td>
<td>7.92</td>
<td>16.12</td>
<td>24.61</td>
<td>33.38</td>
<td>42.57</td>
<td>52.19</td>
<td>62.52</td>
<td>73.55</td>
<td>85.71</td>
<td>100.</td>
</tr>
<tr>
<td>D</td>
<td>7.89</td>
<td>16.12</td>
<td>24.65</td>
<td>33.49</td>
<td>42.57</td>
<td>52.20</td>
<td>62.24</td>
<td>73.27</td>
<td>85.31</td>
<td>100.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Acid</th>
<th>Molecular proportions</th>
<th>ee Decinormal in total distillate</th>
<th>Grams in distillate and residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic</td>
<td>85</td>
<td>31.629</td>
<td>0.4742</td>
</tr>
<tr>
<td>Propionic</td>
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<td>3.721</td>
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</tr>
</tbody>
</table>

Total

<table>
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<tr>
<th>Acid</th>
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<th>ee Decinormal in total distillate</th>
<th>Grams in distillate and residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic</td>
<td>100</td>
<td>33.654</td>
<td>0.5048</td>
</tr>
<tr>
<td>Propionic</td>
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<td>3.365</td>
<td>0.0624</td>
</tr>
</tbody>
</table>

Total

### SAMPLE 12. FRACTIONATION.

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<th>10 cc</th>
<th>20 cc</th>
<th>30 cc</th>
<th>40 cc</th>
<th>50 cc</th>
<th>60 cc</th>
<th>70 cc</th>
<th>80 cc</th>
<th>90 cc</th>
<th>100 cc</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.9</td>
<td>2.97</td>
<td>3.11</td>
<td>3.25</td>
<td>3.40</td>
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<td>3.8</td>
<td>4.1</td>
<td>4.55</td>
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<td>5.87</td>
<td>8.97</td>
<td>12.22</td>
<td>15.62</td>
<td>19.17</td>
<td>22.97</td>
<td>27.07</td>
<td>31.62</td>
<td>37.02</td>
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<tr>
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<td>24.23</td>
<td>33.01</td>
<td>42.19</td>
<td>51.78</td>
<td>62.05</td>
<td>73.12</td>
<td>85.41</td>
<td>100.</td>
</tr>
<tr>
<td>D</td>
<td>7.82</td>
<td>16.00</td>
<td>24.48</td>
<td>33.29</td>
<td>42.31</td>
<td>51.97</td>
<td>62.01</td>
<td>73.09</td>
<td>85.18</td>
<td>100.</td>
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</table>

<table>
<thead>
<tr>
<th>Acid</th>
<th>Molecular proportions</th>
<th>ee Decinormal in total distillate</th>
<th>Grams in distillate and residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic</td>
<td>100</td>
<td>33.654</td>
<td>0.5048</td>
</tr>
<tr>
<td>Propionic</td>
<td>10</td>
<td>3.365</td>
<td>0.0624</td>
</tr>
</tbody>
</table>

Total

<table>
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<th>Molecular proportions</th>
<th>ee Decinormal in total distillate</th>
<th>Grams in distillate and residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic</td>
<td>100</td>
<td>33.654</td>
<td>0.5048</td>
</tr>
<tr>
<td>Propionic</td>
<td>10</td>
<td>3.365</td>
<td>0.0624</td>
</tr>
</tbody>
</table>

Total

<table>
<thead>
<tr>
<th>Acid</th>
<th>Molecular proportions</th>
<th>ee Decinormal in total distillate</th>
<th>Grams in distillate and residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic</td>
<td>100</td>
<td>33.654</td>
<td>0.5048</td>
</tr>
<tr>
<td>Propionic</td>
<td>10</td>
<td>3.365</td>
<td>0.0624</td>
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