Introductory

Sugar Manufacture

And

Analysis

By

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A thesis presented to the department of Chemistry in the American University of Beirut in partial fulfilment of the requirements for the degree of Master of Arts in Chemistry.

June, 1944.
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INTRODUCTION

The sugar industry ought to be one of the important industries in the Arab countries of the Near East, Iraq, Syria, and Lebanon; for the climate is suitable for the cultivation of sugar cane or sugar-beet; Yet, hundreds of thousands of pounds worth of sugars and saccharine materials are imported annually into these countries.

The following table '1' gives the quantities and value of the imported sugars into Iraq alone during the years: 1936 - 1942

<table>
<thead>
<tr>
<th>Material Imported</th>
<th>1936</th>
<th></th>
<th>1937</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Quantity</td>
<td>Value</td>
<td>Quantity</td>
<td>Value</td>
</tr>
<tr>
<td></td>
<td>in tons</td>
<td>in I. Dinars</td>
<td>in tons</td>
<td>in I. Dinars</td>
</tr>
<tr>
<td>1. Sucrose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. In loose cores, or sugar candy blocks</td>
<td>22,025</td>
<td>251,829</td>
<td>23,931</td>
<td>292,846</td>
</tr>
<tr>
<td>b. Granulated or powder</td>
<td>14,885</td>
<td>121,293</td>
<td>17,828</td>
<td>146,203</td>
</tr>
<tr>
<td>c. Unrefined</td>
<td>1</td>
<td>10</td>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td>2. Other Sugars (Glucose, maltose, Lactose etc.)</td>
<td>85</td>
<td>855</td>
<td>55</td>
<td>985</td>
</tr>
<tr>
<td>3. Molasses</td>
<td>1</td>
<td>11</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4. Confectionery</td>
<td>461</td>
<td>18,449</td>
<td>723</td>
<td>30,075</td>
</tr>
<tr>
<td>5. Other Saccharine Materials</td>
<td>13</td>
<td>605</td>
<td>22</td>
<td>1,138</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>393,052</strong></td>
<td><strong>451,466</strong></td>
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</table>

'1' Foreign Trade Statistics
Department of Customs and Excise, Ministry of Finance, Government of Iraq.
<table>
<thead>
<tr>
<th>Imported Material</th>
<th>1938 Quantity</th>
<th>Value in I. Dinars</th>
<th>1939 Quantity</th>
<th>Value in I. Dinars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>in Tons</td>
<td></td>
<td>in Tons</td>
<td></td>
</tr>
<tr>
<td><strong>1. Sucrose</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. In leaves, cones or blocks and candy</td>
<td>24,642</td>
<td>334,069</td>
<td>23,770</td>
<td>341,554</td>
</tr>
<tr>
<td>b. Granulated or powdered</td>
<td>19,204</td>
<td>170,796</td>
<td>21,400</td>
<td>212,219</td>
</tr>
<tr>
<td>c. Unrefined</td>
<td>6</td>
<td>94</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td><strong>2. Other Sugars</strong> (Glucose, Maltose, Lactose, etc.)</td>
<td>15</td>
<td>288</td>
<td>18</td>
<td>395</td>
</tr>
<tr>
<td><strong>3. Molasses</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Confectionary</td>
<td>660</td>
<td>16,685</td>
<td>477</td>
<td>18,708</td>
</tr>
<tr>
<td><strong>5. Other Saccharine Materials</strong></td>
<td>23</td>
<td>1,162</td>
<td>32</td>
<td>1,922</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>533,074</td>
<td>564,810</td>
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<table>
<thead>
<tr>
<th>Imported Material</th>
<th>1940 Quantity</th>
<th>Value in I. Dinars</th>
<th>1941 Quantity</th>
<th>Value in I. Dinars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>in Tons</td>
<td></td>
<td>in Tons</td>
<td></td>
</tr>
<tr>
<td><strong>1. Sucrose</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. In leaves, cones or blocks and candy</td>
<td>17,116</td>
<td>366,275</td>
<td>7,462</td>
<td>193,357</td>
</tr>
<tr>
<td>b. Granulated or powdered</td>
<td>23,855</td>
<td>383,433</td>
<td>32,054</td>
<td>632,147</td>
</tr>
<tr>
<td>c. Unrefined</td>
<td>1</td>
<td>20</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td><strong>2. Other Sugars:</strong> (Glucose, Maltose, Lactose etc.)</td>
<td>23</td>
<td>561</td>
<td>29</td>
<td>772</td>
</tr>
<tr>
<td><strong>3. Molasses</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Confectionary</td>
<td>203</td>
<td>13,313</td>
<td>66</td>
<td>6,826</td>
</tr>
<tr>
<td><strong>5. Other Saccharine materials</strong></td>
<td>10</td>
<td>803</td>
<td>13</td>
<td>1,452</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>767,405</td>
<td>634,559</td>
<td></td>
</tr>
<tr>
<td>Imported Material</td>
<td>1942 Quantity Value</td>
<td>1942 Value in I. Dinars</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>---------------------</td>
<td>------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>in Tons</td>
<td>in I. Dinars</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Sucrose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. In leaves, canes or blocks and candy</td>
<td>1,039</td>
<td>41,992</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Granulated and powdered</td>
<td>15,904</td>
<td>385,743</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. Unrefined</td>
<td>109</td>
<td>4,049</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Other Sugars (Glucose, Maltose, Lactose, etc.)</td>
<td>9</td>
<td>354</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Molasses</td>
<td>-</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Confectionary</td>
<td>77</td>
<td>12,240</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Other Saccharine materials</td>
<td>16</td>
<td>5,057</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>449,437</strong></td>
<td><strong>449,437</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

These mentioned figures illustrate the necessity of establishing sugar factories better than any other way.

The Iraq government started the research to ascertain the suitability of Iraq climate and soil for the cultivation of sugar-beet or sugar-cane. The results of the experiments showed that sugar-beet grows successfully in the northern part of Iraq.

When Dr. N.D. Constan suggested to the Iraqi Government that we take up the study of sugar manufacture for my Master of Arts degree, my Government welcomed the idea and I was asked to study the methods of sugar manufacture as far as available in this University,
The practical part of my work consisted of sugar analysis and visits to sugar factories. Many different samples of saccharine materials were analyzed such as row sugar, sugar-cane, molasses, jams, etc., the results of the analysis are not included in this theses as they mean little or nothing, to the reader, or, in view of any originality.

I worked for two weeks in Tripoli Sugar Factory studying the manufacture of cane sugar.

Commercial glucose was studied in the Cipa Glucose Plant, and experiments in the laboratory were made to that end.

Parts I, II of this thesis present a condensed review of the methods of sugar manufacture and refining and the manufacture of commercial glucose, stressing on the theory of the process and explaining it whenever possible.

The description of the different types of machinery was avoided except for the very general type which was the basis for any further development.

Part IV is devoted to the general methods of sugar analysis. The first chapter, the qualitative analysis, summarises the most important chemical reactions of sugars in general, besides describing the important procedures for the qualitative identification of sugars. The methods on the quantitative analysis of sugars described in the second chapter of part IV are only those which I used in my work.
I wish to express my thanks and gratitude to all the personnel of the chemistry department and especially to Dr. N.D. Constan for suggesting the subject and for his invaluable helps and suggestions for carrying out my work.
CHAPTER 1.

INTRODUCTION

The term sugar was originally employed and intended to classify all substances having a sweet taste, and thus came to be used indiscriminately for cane sugar, fruit sugar, lead acetate (so-called sugar of lead) and other compounds possessing this property.

At present, in a general sense, it is reserved almost exclusively to denote crystallized sugar from the tropical cane or the sugar beet, which is sucrose, or cane sugar. The commercial sugar whether derived from the sugar cane, sugar beet, the maple tree, the palm or some other sources, is almost pure sucrose.

History of sugar:— Etymologically, sugar would seem to be of Indian origin, the earliest from of the word seem to be "Garkara" in Sanskrit and "Sakkara" in Prakrit hence it may be traced through all the Aryan and Semitic languages, as "Shakar" in Persician, "Sukkar" in Arabic, "Suicar" in Assyrian and Phenician, "Saccharum" in Latin, "Azucar" in Spanish and Portuguese, "Zuccher" in Italian, "Sucre" in French, "Sucker" in German etc.

The cultivation of the genuine sugar cane "Saccharum officinarum" appears to have been common in India and China in very remote times, but there is no documentary evidence on this point earlier than Herodotus. Frequent mention of the "Sweet cane" occurs in the Scriptures but the plant referred to is doubtful. An Indian reed yielding honey is alluded to by Strobe and a similar statement concerning an Egyptian reed is made by Thiophrastus; while Dioscorides actually gives the name "Saccharum" to a kind
of honey obtained from reeds in Arabian Felix and India; Both he and Pliny accurately describe the product as being white and brittle and of salt like consistency.

Later it seems to have been generally termed "Indian Salt" among the Greeks and the Romans, by whom it was obtained in small quantities at great expense from India, and used medicinally.

The introduction of sugar cane in the Mediterranean basin must have occurred as an early date; for it was found growing at Assouan, on the Nile in upper Egypt, in 714 A.D. and was carried into Spain by the Moors in 766 while Sicily engaged in the culture about 1060 to 1090. During the religious wars of the middle ages, the "Sweet honeyed reed" called "Zuera" which abounded in the meadows about Tripoli, were consumed by the Crusaders; and it is evident that sugar making in that neighbourhood was conducted in a wholesale and systematic manner. From Cyprus and Madeira, the industry extended, in 1500 to 1600, to most of the West Indies where it was carried on by Spanish and British colonists; but there is strong evidence in favor of the supposition that several kinds of sugar cane are indigenous to both the West Indies and to almost the whole continent of South America.

Establishment of Refineries:— From the extensive growth of sugar in the western tropics there resulted large importation of the raw article into Europe. The introduction of tea and coffee about the same time created a general and wide demand for what had hitherto been regarded as a medicine rather than a nutritive article of diet. Sugar refining appears to have been copied from
the Arabs by the Venetians. Refineries were established in England and Germany in the 16th century, and in Holland soon after, and utilized the imported raw sugar.

Sugar from Beets:— Up to this time sugar derived from cane was the only kind known in commerce. But in 1747, A.S. Margroff, Director of the physical class of the Academy of Science at Berlin, demonstrated the existence of about 6% of sugar in beet root and recommended the cultivation thereof; and in 1795 Franz Carl Arehard, Director of the physical Class at the Academy of Science at Berlin, succeeded in manufacturing beet sugar on his farm in Silesia, Germany, and presented loaves of refined sugar to Frederick William the III of Prussia in 1799, and established the first beet sugar factory at Steinan, Germany. About 10 years later Napoleon used extraordinary efforts to porter the production of native-grown sugar, and grapes, plums, maize, sorghum, carrots, etc. were also experimented on. The results obtained did not excell those from beets, and the first French factory for making beet-sugar was established at Illie, in 1810 by Crespel-Delisse. Today Europe alone produces annually nearly 7 million tons of beet-sugar.

Chief Sources of Sugar:— From the preceding statements, it may be seen that the world's sugar supply is obtained almost exclusively from two plants, the sugar-cane and the sugar-beet. Only a comparatively small quantity of palm sugar is produced, and the small quantity of maple sugar made in America and Canada is consumed in the raw state.
The Sugar Cane: "Saccharum Officinarum" is a very large grass; its stalk is round and pointed. Normal cane are never hollow. The exterior color varies with the variety. The stalk, of the variety usually grown for sugar production are of a greenish yellow, or purple, or purplish red color, or have stripes of red or purple and green. In certain varieties, the green is so light that the canes are after spoken of as being white. The light to which the cane grows varies greatly with the soil and climatic conditions and range from 2 to 6.5 meters, seldom attaining the latter height. The cane grown in this country, Lebanon and Syria, never reaches full maturity, it attains a height of about 2 – 3 meters. The Cuban cane under favorable conditions reaches a height of from 3.5 to 4 meters. The leaves of the sugar cane are ribbed, and in different parts of the plant their length varies from few centimeters to 2 meters. Sugar cane is a tropical plant though it grows successfully in subtropical regions. Outside the tropics it rarely reaches full maturity and produces seed.

Composition of Cane and Cane Juice: The composition of the cane is extremely variable and is influenced by the variety, soil, the climate, and the age of the plant. According to Dr. C.H. Browne Jr. of the Louisiana Sugar experiment Station, the composition of the purple variety of Louisiana cane, as indicated by the results of many analyses at the time of harvesting, in November-December, is as follows:
<table>
<thead>
<tr>
<th>Component</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>74.5</td>
</tr>
<tr>
<td>Ash</td>
<td>0.5</td>
</tr>
<tr>
<td>Fiber</td>
<td>10.0</td>
</tr>
<tr>
<td>Nitrogenous bodies</td>
<td>0.4</td>
</tr>
<tr>
<td>Sugars (like sucrose, glucose, and fructose)</td>
<td>14.0</td>
</tr>
<tr>
<td>Fat, wax, pectin (Gum) free acids, combined acids</td>
<td>0.8</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
</tr>
</tbody>
</table>

The reaction of cane juice with litmus is always acidic. The preceding analysis refers only to that part of the cane harvested for sugar making. The unripe top joints of the stalk are always lifted in the field.

The average sucrose content in the juice of extracted in the sugar factory in Tripoli was 16 - 17.5%, and reducing sugars were 0.5%.

Coefficients used in Sugar Manufacture

For convenience and brevity, several expressions, or coefficients, are used by sugar makers and chemists in describing the condition of the juice and products at various stages of the manufacture. These coefficients may be defined as follows:

Coefficient of Quotient of Purity:- The coefficient or quotient of purity shows the relation between the sucrose and the total solids contained in the material; it is the percentages of the sucrose in the total solid material in the substance. In calculating the "apparent coefficient", that is the coefficient ordinarily employed, the total solids are determined by a Brix hydrometer and the sucrose by the direct polarization. In the actually "true coefficient of purity" the total solids are determined by actually drying the material.
The **true** coefficient is used in comparative laboratory tests and in special researches but not usually in conducting the manufacture.

The apparent coefficient is meant when the word "true" is not prefixed, and usually only the word "purity" is used to designate this coefficient. Purities are commonly expressed with the degree mark instead of the percentage sign, or both signs are omitted.

The purity coefficient gives approximate indication to the amount of crystallizable sucrose, as every part of solid non sucrose prevent the crystallization of one and a half part of sucrose, approximately. So in a juice of purity 90, which is considered very high, there are 10 parts of solid non sucrose which binders the crystallization of 15 parts of sucrose having the crystallizable sucrose 75 or 75/90 = 83% of the sucrose present. In a juice of purity 80, which is considered very low, the crystallizable sucrose is $\frac{30- (20 \times 1.5)}{80} = 63\%$ of the sucrose present.

The purity of the juice in Tripoli Sugar Factory ranged between 85 - 87, that is, this crystallizable sucrose ranged form 74 - 78% of sucrose present.

2. **Glucose Coefficient or Glucose Ratio**:

The glucose coefficient is the ratio of the glucose (reducing sugars are termed glucose by sugar manufacturers) to the sucrose multiplied by 100. An increase in this coefficient from a stage of manufacture to another is usually indicative of inversion of sucrose. This is not necessarily the case however, since the
sucrose may not be removed in like proportions in the process of manufacture and the mechanical loss may not balance each other.

5. **Solute Coefficient:**

The solute coefficient is the ratio of the percentage of ash to that of sucrose. It is little used in the cane sugar industry.
MANUFACTURE OF CANE SUGAR

The various stages of the manufacture of cane sugar may be briefly outlined as follows:-

1. The extraction.
   a. Milling process.
   b. Diffusion process.

2. The purification of the juice. That is defecation and clarification.

3. The concentration of the juice to a syrup.

4. The crystallization of the sugar
   a. Crystallization in the vacuum pan.
   b. Crystallization at rest.
   c. Supplement crystallization with motion.

5. Curing of the Sugar:
   a. Centrifuging and washing
   b. Drying or granulation of the sugar
   c. Formation of cubes, loaves, etc.
EXTRACTION OF THE JUICE.

Milling process:-

The oldest and the most employed method of extracting the juice from the sugar cane is by means of mills.

The most common forms of mill is the three roller type or combination of three-rollers mill. Two of the rollers are on the same level and turn in the same direction. The first is called "feed-roller" and the second "bagasse roller". The third roller is placed above the other two and turns in the opposite direction. It is called "top-roller". As a rule the rollers of the first mill are placed in such a way that the cane can enter easily and is but slightly squeezed, but the space between the top and the bagasse roller is so small that only the mill squeezed cane can pass, and it is here that the actual crushing takes place. The distance between the top roller and the feed-roller and between the former and the bagasse-roller of the second mill is smaller than those in the first, and still smaller the third one in order to obtain maximum extraction.

The extracted juice falls from the rollers on to the head plate of the mill which has the form of a collecting trough with perforated bottom, which keeps back the coarser particles of cane and allows the juice to pass.

In the extraction of the juice in "Tripoli Sugar factory" two-and three roller mills are used. The whole cane are introduced into the first mill the roller of which are provided with V shaped teeth, which are placed so close together that the cane passing through them are slightly squeezed. The emerging cane is crushed
soft and goes to the second mill, the rollers of which are quite close together with small teeth about 9 to the square inch where the cane are crushed further and the bagasse emerges in the form of small chips.

The sugar content of the bagasse was about 5% and sometimes went up to 6.5%.

To extract this sugar left, the bagasse is macerated with water and recrushed. This procedure is not adopted in Tripoli Sugar Factory.

Usually in three mills extraction the bagasse is macerated twice. The first time in the second mill and the juice from the first and second mills go to the defacator. The second maceration is in the third mill and the juice obtained is used for maceration, instead of water, in the second mill.

Maceration can be carried with hot water insted of cold water, but experiments showed that there is no difference between the two methods in the extracting power.

The bagasse is used as a fuel and seldom as cattle food, and it usually have the following average composition:

<table>
<thead>
<tr>
<th></th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber</td>
<td>46.5</td>
</tr>
<tr>
<td>Water</td>
<td>47.5</td>
</tr>
<tr>
<td>Sucrose</td>
<td>4.5</td>
</tr>
</tbody>
</table>

Of course, higher or lower values may occur, depending on the quality of the crushing and the hardness of the cane fibers.

**Diffusion Process**

As the diffusion process is used exclusively in beet-sugar manufacture, and only to a very limited extent in cane
sugar factories, it will be described fully in the section on
beet-sugar industry. A brief outline of the process as applied to
cane, however, will be given here.

The cane is reduced to fine shreds or match-like fragments and
is then introduced into a series of cylindrical vessels. Hot
water at 85°C is introduced into the first vessel which contains
the exhausted cane and then to the second, then the third until
it reaches the last one which contains the fresh cane. The juice
and cane move on the counter-current principles, the juice becoming
more and more concentrated by passing from one vessel to the other
and the cane becomes more and more exhausted. When the cane in the
first vessel is completely exhausted it is refilled with fresh
cane and attached at the end so the second vessel becomes the
first and so on.

Owing to the scarcity of water and the high price of fuel,
on most plantations and to the difficulty of disposing of the
residue or exhausted chips. This process is little used in cane
factories. From the point of view of the extraction of sugar,
however, it is an ideal process. For example, the loss in sucrose
(unextracted) by the best of mills is never less than 5% and
usually about 10% while by diffusion the loss in sucrose is 0.97%.

Another advantage is the absence of gums and pectins and
waxes expressed with the juice due to the use of the heavy press-
use in milling, yet the big amounts of water used, the inability
to use the bagasse as fuel, due to the high water content, without
drying, the fixed capacity of the extracting vessels which does
not permit variations to meet the necessities which might be
encountered during the other stages of manufacture, renders the
diffusion process impractical in cane-sugar industry.
THE PURIFICATION OF THE JUICE.

The turbid, viscous juice from the mills is not fit to be worked up into sugar without clarification, and the first step is to submit the juice to such treatment as will remove the viscous and gummy constituent. As sucrose is very liable to decomposition by micro-organisms, which results in sourness, inversion and fermentation, and as the cane juice is very suitable medium for their development, it is necessary to boil the juice immediately; whereby the albuminoids are coagulated and the further development of the micro-organisms checked. It is also necessary to remove all impurities, both dissolved and suspended, so far as it is practical without spending too much on clarifying agents, which may cost more than the results are worth.

The purpose of clarification is, therefore, to free the juice as quickly as possible from all constituents except sucrose, without delay, since it is only in concentrated solution that sugar is unaffected by ferments and micro-organisms.

Besides heat, which coagulates the albuminoids and kills the germs, the agent which has been used for clarification from the earliest times is lime. This precipitates gummy and albuminoid matter which forms a muddy sediment, and a top layer of scum, between which the bulk of the juice is clear and limpid.

The method of clarification may be divided into three kinds:

1. Defecation with the right amount of lime so there is no excess lime left.

2. The juice is heavily limed and the excess lime is removed by:
   a. Carbon dioxide.
b. Sulfer dioxide.

3. The juice is treated with sulfer dioxide and then lime is added to neutrality.

1. The quantity of lime used on the defecation process is just sufficient to neutralize the free acids of the juice, preventing them from afterwards causing inversion; further, the lime combines with the albumin, which had previously been suspended in the juice in a gelatinous form, but is now coagulated both by the action of lime and heat. The acid calcium phosphates are precipitated as flocculant tribasic phosphate, as well as a part of the pectin. All these flocculant precipitates envelop the suspended particles of bagasse, wax, clay etc, and partly rise to the surface and partly sink as soon as the juice begins to boil.

The amount of lime necessary for tempering and ascertained in many ways. Some use as much lime as necessary for the juice to give a faint alkaline reaction to litmus paper. Others ascertain in the laboratory how many cubic centimeters of milk of lime are needed to clarify one liter of the juice and use that proportion in the factory. The best method is to add milk of lime in the approximate proportion, filter some of the juice after boiling and add few drops of a solution of saccharate of lime more.

If a precipitate is formed then too little lime was added so the lime is increased in the next batch. If no precipitate is formed then either too much lime was added or just the right amount to make sure of this point the quantity of lime is decreased in the

1 The solution is made by shaking up 80% solution of sucrose with lime and filtering the solution.
following batches gradually until a precipitate begins to form on the addition of saccharate of lime solution then the lime is increased slightly and that proportion adopted as the correct one.

The milk of lime used is a 15 $\text{B}^\circ$ suspension or 150 grm. calcium oxide per liter.

Owing to the variable composition of the raw juice the defecated juice should be constantly tested in the laboratory for the amount of lime added and changing it whenever necessary.

After the addition of lime, in the liming tanks, the limed juice is pumped to the boiling tanks where the juice is heated just to boiling by a closed steam pipes in the bottom of the tank. When the limed juice starts to boil, the steam is shut off and the juice transferred to the settling tanks, usually situated at the top of the factory so the clear juice can be transferred to the storage tanks and then to the evaporators by the action of gravity.

In the settling tanks the heavy sediment sinks to the bottom and the light sediments floats. It is left for some time, 1/2 hrs. depending on the quality of the juice, and then is drawn to the storage tanks through a pipe in the bottom slightly elevated so no sediment may be drawn with the clear juice. The sediment and scum is then sent to the filter press where the clear filtrate is sent to the storage tanks.
2. Juice treated by the defocation process still contains so many gummy constituents that it is very difficult to filter, and is therefore never quite clear. The manufacture of white granulated sugar demands more care and necessitates filtration of the clarified juice as well as of the syrup, and consequently a more thorough elimination of impurities than the simple defocation process permits of. Such purification is brought about by adding a large excess of lime which precipitates albumin and gums and decomposes reducing sugars. When this action of lime taken place at the temperature of boiling juice the lime salts are dark colored, viscous, and very troublesome, but if the lime acts upon glucose at a temperature below 55 °C, the lime salts are partly insoluble and easily removed, the remainder being colorless and very clear. As soon as the lime has finished its purifying action, the excess is removed by carbon dioxide or sulfur dioxide, which combines with the lime to form the insoluble calcium carbonate or calcium sulfite.

a. The saturation with carbon dioxide may be affected in one or in two operations and according as this is done, the process may be distinguished by the name of single or double carbonation, both of which have their advantages as we shall see.

**Description of the carbonation process:**

The raw juice, from the mill, is mixed with as much lime cream as is thought necessary in large tanks provided with stirring apparatus, and then passed through juice heater where it is warmed to 40 - 50 °C. From the heater, it enters the carbonation tanks which are only half filled with the juice so as to allow for the
violent frothing which occurs during saturation with the gas. Some times these tanks are open, but in most cases they are covered with an iron plate, provided with a lid permitting samples to be taken from time to time, and allowing entrance to the tank for cleaning it. A chimney carries off the escaping gases. A coil, into which steam can be admitted, serves for heating the juice, and a perforated iron coil or cross for introducing the carbon dioxide gas.

As soon as the tank is half full with the heated juice, carbon dioxide is turned on care being taken that the temperature shall not exceed 55 °C.

At first the carbon dioxide is completely absorbed but the juice soon becomes thick and start to froth violently. Some oil sprayed on the surface helps to minimize frothing. When the juice becomes gelatinous the absorption of the gas becomes very slow until it becomes liquid again and the absorption becomes more complete until a time comes when a sample taken, the precipitate settle quickly.

Up to this point the procedure for single or double carbonation is similar, but this is no longer so.

**Single Carbonation:**

In the single carbonation the carbon dioxide is admitted until the juice gives a faint pink reaction to phenolphthalein then supply of the gas is slowly shut off, the juice being just neutral. It is then heated by the steam coil to 90 °C to decompose any calcium bicarbonate formed, and filtered or decanted,
after settling.

As a rule, 1% of lime to the weight of the juice is used or 70 liters of lime cream at 15 B° on 1000 liters of juice.

Double Carbonation:

In the double carbonation process the supply of carbon dioxide is stopped as soon as the juice settles rapidly; it then possesses an alkalinity of 0.05% CaO.

The juice is now filtered without heating. The filtered alkaline juice is pumped again to the saturation tank which can be filled to the top now as there is no more frothing. A slight amount of lime is added and saturation repeated up to neutrality; then the juice is boiled to decompose any calcium bicarbonate which may have formed and filtered or decanted after settling.

In the double carbonation process more lime is used usually 1.5% to the weight of the juice; or 100 liters cream of lime of 15 B° to 1000 liters of juice.

Third Saturation with Sulfur Dioxide:

In many cases it is impossible to obtain quite neutral juices by saturation with carbon dioxide as either the lime or the juice may contain some alkali metals, which become carbonates and thus keep the juice alkaline after saturation. In order to prevent this, and to obtain still greater decoloration, a third saturation, this time with sulfur dioxide, is sometimes used in factories working with double carbonation process. To this end the saturated juice is pumped again into the saturation tanks, and sulfur dioxide is admitted to neutrality to phenolphthalein, and remains so after being boiled, after which is it filtered.
The effect of lime on the sucrose of the juice in the carbonation process is negligible as the temperature is kept low and the heating period is short. But its effect on the reducing sugars is twofold, destructive and transforming.

Alkalies decompose reducing sugars with organic acid and their complex substances. But as the temperature is not high enough in the carbonation process, the reducing sugars merely undergo an intramolecular rearrangement or break up into few pieces so the principal products are lactic acid and saccharic acid, the calcium salts of which are insoluble in the neutral medium and are precipitated.

The excess of lime used for defecation may be removed as the insoluble calcium sulfite by using sulfur dioxide instead of carbon dioxide.

In this process excess of lime is added and the juice is not heated but is saturated with sulfur dioxide. As calcium sulfite is more soluble than calcium carbonate and as its solution is alkaline and not neutral therefore the saturation is done in the cold and the sulfur dioxide gas is shut when the juice gives a very faint red color with phenolphthalein.

After filtration or decantation the juice is boiled to coagulate the remainder of the albuminoids.

This method is quite simple and unexpensive and is superior to the direct defecation process as the juice obtained is clearer and easier to filter, yet it is very inferior to the carbonation process regarding the quality of the sugar produced and ease of operation.
As the calcium sulfite is more granular and easier to filter than calcium carbonate, some manufacturers use sulfur dioxide along with carbon dioxide in the carbonation process. Both gases being but simultaneously as soon as the juice settles rapidly so it is still alkaline, so that no danger can exist of the carbonate being converted into the soluble bisulfite.

3. In Tripoli Sugar Factory still another process is used for defecation and clarification of the juice.

The raw juice is pumped directly after screening from the mills to the top of a tower about 5 meters high, and as it trickles down it meets an ascending current of sulfur dioxide (generated by burning sulfur). It reaches the bottom nearly saturated with sulfur dioxide and there it is mixed with lime cream in such proportion that the liming juice after boiling and settling has a pH of 6.8 - 7.2.

The limed juice is then pumped to tanks heated by steam coils where it is heated first to boiling and is left to settle. It is then conveyed to the storage tanks then to the evaporators.

The owner of the factory claims that this process gives better quality of sugar than other processes.

**ADVANTAGES AND DEFECTS OF DIFFERENT PROCESSES OF CLARIFICATION.**

The advantages of carbonation over defecation are that the juice is better purified and settled from gummy constituents, it is easier to filter, and yields a more fluid molasses. Yet when we lime the juice cold, and before heating remove the
excess of lime with sulfur dioxide, a great improvement in this
direction results. Another considerable advantage is that the
clarification is affected in a single operation; for immediately
after saturation, the liquid is divided into a clear juice early
to filter, and a precipitate which settles rapidly; whereas,
in the case of defecation with or without sulfur dioxide the
precipitate settles slowly and filters badly, whilst the clari-
fied juice is gummy and opalescent.

Independent of the kind of cane crushed, we can employ alike
quantity of lime for a long time at a stretch, because if an
excess of lime has been used the carbon dioxide neutralizes it
afterwards, while the quantity of lime required in defecation
is very variable if canes from two different fields are crushed
together, and therefore one is not sure of using it in proper
quantity, which is very important point in this operation.

Owing to the carbonated juice being fuser, brighter, and
more easily worked, the quality of the sugar is better than from
similar juice clarified by the defecation process.

Against these advantages must be set certain drawback, the
chief of which is the greater expense, because more lime is req-
quired; the cost of the lime-kiln and fuel, together with that
of carbon dioxide pump and other plant, which increases the cost
price of the sugar.

If we do not expect more from the carbonation process than
a more perfect subsidation and clarification of impure juices and
do not want to make white sugar, the simple carbonation process
is amply sufficient. But even then it is too expensive because
we can obtain this by having the cold juice heavily and saturating it still cold with sulfur dioxide, which is very convenient and inexpensive process.

If on the other hand we want to make sugar for direct consumption then every thing apart to cause coloration should be avoided, and we must have recourse to the double carbonation process. This requires much lime and carbon dioxide, and thus causes considerable expenses but this is more than compensated for by the higher price such sugar fetches when compared with raw sugar.

In short, we are justified in saying that when making refining crystals or yellow sugar simple defecation and sulfitation are quite sufficient, but that the manufacture of white sugar requires double carbonation, so that in the present stage of cane sugar manufacture there is no longer any scope for single carbonation.

Before concluding this part we should mention something about the qualities of the lime used.

It should be the purest obtainable. The following is the maximum permissible impurities:-

<table>
<thead>
<tr>
<th>Impurity</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron oxide and aluminium oxide</td>
<td>2</td>
</tr>
<tr>
<td>Sulfer trioxide</td>
<td>0.2</td>
</tr>
<tr>
<td>Magnesium oxide</td>
<td>2</td>
</tr>
<tr>
<td>Silica</td>
<td>2</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>2</td>
</tr>
<tr>
<td>Moisture</td>
<td>2</td>
</tr>
</tbody>
</table>

Water and carbon dioxide through inoffensive, decrease the affect of a given quantity of lime, the same thing applies for insoluble impurities as sand and clay. So the other has fixed the limits of these impurities as given above. Iron oxide and
aluminium give rise to the formation of scale in the evaporators.

Magnesia is a very troublesome impurity in lime, when present in appreciable quantity. It discolors the juice and retards subsidation, so that the decanted juice always contains much suspended matter, which forms scales in the evaporating vessels.

Sulfates also scale the evaporators and should not occur in any appreciable quantities in the lime used for clarification. Alkalies, on the other hand, are harmless and occur usually, in small proportions in lime stone, and may be totally absent.

'1' Cane Sugar and its manufacture by H.C. Drinson Geerlgs P.160
CONCENTRATION OF THE JUICE AND CRYSTALLIZATION

Up to the stage now reached all the operations, the juice undergone, have had for their object the removal of the non-saccharine matter and the clarification of the juice. This being accomplished, the sucrose now exists in a solution containing about 15% of dry substances including those impurities which clarification failed to remove. The purpose of the next operation is, therefore, to convert the sucrose into a solid in order that it may then be separated from the impurities still remaining in solution in the mother liquor or molasses. This is affected by evaporating water from the juice until sugar saturation where the sucrose will crystallize out and can be separated from the molasses thus obtained in a pure state.

This removal of water by evaporation must be made at once and without delay, as in a dilute solution the sucrose is very liable to decomposition by micro-organisms, which cause inversion and fermentation, alcoholic and acetic, and therefore give rise to considerable loss. It is therefore necessary to deprive the clarified juice of the great bulk of its water as rapidly as possible, so as to bring it to a condition in which it is no longer liable to such decomposition, i.e. a crystalline mass containing 8 - 10 percent of moisture.

Since sucrose is liable to decomposition at high temperature it is desirable to keep the temperature as low as possible during evaporation and at the same time to reduce the time during which it remains at that temperature. This is affected by boiling the juice under reduced pressure and by means of which its boiling
point fails. Powerful pumps are used to draw off the vapors and maintain a vacuum.

The concentration of the juice is ordinarily affected in two stages, viz., evaporation, which concentrated the juice to syrup containing about 50% of solid matter, and boiling which concentrated the syrup still further and transfers it into a crystalline mass, containing only from 3 - 10% of moisture.

The sole object of evaporation is to concentrate the juice as soon as possible, with a minimum waste of steam and at a low temperature.

In boiling (during which the sucrose first assumes a crystalline form), additional care is necessary in order to obtain the maximum yield of crystallized sucrose in a pure state as possible and in such a condition as to allow it to be separated from the mother liquor with the minimum loss and trouble.

**EVAPORATION:**

The Brix degree of the thèns juice is about 25 and it must be concentrated to 55 - 60 Brx. before it can be handled easily in the crystallizer because:

1. The juice contains too large quantity of water and requires too long a time to be evaporated in the crystallizer or vacuum pan.

2. The juice contains a considerable quantity of substances which separate from the solution when boiled down to a syrupy condition and should be removed by filtration before concentration or otherwise they increase the ash content of white granulated sugar.
3. Concentration usually increases the alkalinity of the juice which should be corrected to the proper degree, since too high alkalinity renders the crystallization of sucrose difficult.

It is for these reasons that the juice is first concentration to a syrup, corrected for alkalinity, and filtered before it is sent up to the vacuum pan for crystallization.

**Construction of Evaporators:**

There are several forms of evaporators: one body (single effect) two-body (double effects), three-body (triple effects), four-body (quadruple effects), five-body (quintuple effects). These evaporators consisting of more than one body are called multiple effects.

The most common used kind is the vertical quadruple effect which it will be described briefly.

The evaporator consists of four bodies, each body consists of two sections of large cast-iron cylinders. The lower section is the heating chamber, and the upper section is the vapor chamber. The heating chamber is pierced through by numerous brass tubes which serve as heating surface as well as for the passage of the juice.

The heating chambers are filled to about three quarters full of juice and the steam turned on.

The first body is heated by the exhaust steam of the factory and is regulated such that the juice in it boils about 104 - 105 °C, and at a pressure of 2.5 lbs. per sq. inch.

The vapor developed from the juice in the first chamber passes over to the heating chamber of the second body where it heats the juice there at it boils at atmospheric pressure and at about 100 - 101 °C.
The vapors developed from the second body serve in heating the third body which should boil at 85 °C, and a vacuum of about 6 inches of mercury.

The fourth body is heated by the vapors developed from the third body: The boiling temperature 60 °C, and 23 inches of vacuum.

The low pressure in the third body is maintained by a condenser while in the fourth body it is produced by a strong air-pump and a condenser.

The juice enters the first body and moves to the second then the third and to the fourth increasing in concentration all the time until it reaches the proper concentration and then is pumped to the storage tanks.

The advantage of the multiple effect evaporators may be explained from the fact that '1'

1 Kg. of steam evaporates 0.96 Kg. of water in a single effect
and 1.90 " " double "
" 2.85 " " triple "
" 3.79 " " quadruple "
" 4.72 " " quintuple "

The advantage of a multiple effect may also be seen from a comparison of the coal consumed in evaporating the juice obtained from 10,000 metr. of beet '1'

<table>
<thead>
<tr>
<th>Effect</th>
<th>Coal Metr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>1989.7</td>
</tr>
<tr>
<td>Double</td>
<td>975.0</td>
</tr>
<tr>
<td>Triple</td>
<td>650.0</td>
</tr>
<tr>
<td>Quadruple</td>
<td>488.8</td>
</tr>
<tr>
<td>Quintuple</td>
<td>392.5</td>
</tr>
</tbody>
</table>

The syrups is left to settle, the pH corrected to 7 by some phosphoric acid, filtered and is sent to the vacuum pans.

'1' Beet-sugar making and its chemical control. Mikaide P.225.
During the boiling process the concentration of the clarified syrup is continued up to the point where there is not sufficient water for the sugar to remain dissolved, and it becomes partially crystallized.

The rapidity with which sugar crystallizes out from a solution depends, other circumstances remaining the same, on the purity of the solution, i.e. on whether much or little foreign substances are present along with the sugar. When the sugar solution is fairly pure, crystallization takes place as soon as the concentration has become too great for all the sugar to remain in solution. When an impure juice, such as molasses, is evaporated, crystallization takes place only very slowly, and no crystals are visible even when the concentration is much greater than corresponds with the solubility of sucrose.

The hot concentrated liquid is allowed to cool, and after some time sucrose crystallizes out, partly because it is less soluble in cold water than in hot, but chiefly because the super-saturated state slowly gives place to the normal state of solubility.

Boiling may therefore be performed in two ways, one of which is followed when boiling syrup, and the second when boiling molasses.

The syrup obtained by the evaporation of the juice usually has a density of 55 - 60 Brix. at 17.5 C. and contains about 40 - 45% water. If only the matter of the further concentration of the syrup were involved, it could be readily and economically accomplished in a multiple effect, but the crystallization of the
sugar must also be considered. In modern factories, the sugar is crystallized, or grained, in a single effect vacuum evaporator, termed a vacuum pan.

**Vacuum Pan.**

A vacuum pan consists of cylindrical cast-iron vessel, lined from the inside with copper as its is less reactive. The vessel has a conical bottom and a domed shape top. It is heated by steam coil, and has a large opening at the bottom for discharging the contents. An air-pump and condenser are used to produce the low pressure 25 - 26 inches of vacuum.

**TERMS USED IN SUGAR BOILING.**

**Syrup:** The clarified juice evaporated to a density of 55 - 60 Bx.

**Grain:** The sugar crystals in the masscuit. This word is also used as a verb, to indicate the process of forming the crystals.

**Proof:** That stage of the process when the liquor has been concentrated to a state of sugar saturation with sugar.

**Boiling to Grain:** The boiling of strike in which the sugar is crystallized in the vacuum pan.

**Boiling Blank, or to String Proof:** The boiling of a strike in which the crystallization of the sugar is left to subsequent operation.

**Masscuit:** The thick mixture of the sugar crystals and the mother liquor; also, where strikes are boiled blank or to string proof, the heavy material ready for subsequent crystallization of the sugar, is
called massacuite. The word is also, for convenience, applied to the material in the pan at all stages of the process. The massacuite, are numbered serially, according to their origin, beginning with that from syrup, which is called first massacuite.

Mixed Massacuite: A massacuite formed partly of syrup and partly of molasses. The molassed is said the "boiled in".

Strike of Sugar or Massacuite: The completed massacuite from a boiling is called a strike and the operation in called "boiling a strike of sugar or massacuite". The word strike is also applied, in the same way, to molasses.

Cut: The part of the strike that is left in the pan as a nucleus on which to boil another strike. This is used also as a verb. Many sugar makers use the word cut to indicate the portion of the strike that removed from the pan.

Final Molasses: The liquid residue from which more sugar cannot be profitably extracted.

METHODS OF SUGAR BOILING

Grained Strikes: The syrup is evaporated until it becomes super-saturated and deposits tiny crystals of sucrose. When this stage is reached fresh syrup is admitted gradually and evaporation continued so that the sugar in the syrup added deposits on the crystals already formed. This process is continued until the crystals grow to the size required. In the latter stages of the boiling the supply
of fresh syrup is stopped and the contents of the pan evaporated so the final massaçuite has an average water content of 7%.

The heating is then stopped, and air admitted to normal pressure and the contents are discharged to be cured.

Cut Strikes:

When a cut strike is to be boiled, a part of the massaçuite from a previous strike is left in the pan, and its crystals take the place of the grain that otherwise have to be formed. This method produces a very large-grained sugar.

Blank strikes or Crystallization at Rest:

In sugar boiling the terms "string strikes", "smooth strikes", and "fellied strikes" have practically the same meaning a blank strike. In boiling the blank strikes, the molasses are first heated to dissolve the fine crystals it usually contains. It is then drawn into the pan and concentrated under as high a vacuum as can be obtained with the apparatus. The evaporation is usually stopped when a test sample held between the thumb and a finger produces a strong thread when these digits are separated. The test is often made by pouring the test sample from the proof strik and noting the appearance of the "string" when it breaks. The strikes boiled to a "string proof" are run into small sugar wagons or into large tanks and are placed in a hot-room and are allowed to remain there for a week or more at a temperature of about 38°C. This time depends on the purity of the molasses. This method is termed "crystallization at rest" to distinguish it from the more modern method of "crystallization at motion" which will be described later.
The products made from blank strikes are termed "molasses sugars". These sugars have a low polariscopic test, which usually ranges from 80° - 90° V°. Two grades of molasses sugars are usually made, namely seconds and thirds. The third sugar is boiled as the close of one grinding season and left until the beginning of the next or for a period of 2 to 3 months, before centrifuging.

Crystallization in Motion

This method does not increase the yield of crystallized sugar but produces more uniform crystals in a shortened time than when employing crystallization at rest.

The crystallizer is a horizontal cylindrical tank with an inlet and outlet doors and is provided with a spiral stirrer.

The blank strike after boiling is run into the crystallizer and is stirred slowly and continuously till no more sugar deposits on the crystals.

Crystallization in motion is used when boiling molasses with masses using the crystals of the last a nucleus. After boiling to the proper concentration the strike is run into the crystallizer and is stirred while it cools gradually. The sugar in the molasses deposit on the crystals already present, as the motion keeps the crystals in contact with all the different parts of the solution. So by this method the production of new small crystals, called false grain, is avoided as their presence offers many difficulties afterwards as they clog the holes in the centrifugal sieve and prevent the complete separation of the crystals from the mother liquor, apart from the loss in sugar as these tiny crystals cannot be
separated and utilized.

To sum up, the crystallization processes, we can say that generally, the operations follow the sequence:—

1. Grained strike where the purest sugar is produced by using pure syrup throughout the whole crystallization.

2. Grained strike too, but here molasses are added towards the end of the boiling and the product is pure sugar too. This procedure is adopted until the purity of the resulting molasses falls from about 70 to 50.

3. The cut strikes are employed only in special cases where large sugar crystals are desired.

4. Blank strikes and crystallization in motion are employed to extract the last amounts of crystallizable sugar from the molasses resulting from previous operation. The yield is brown sugar polarizing 65° - 90°, and is used in sugar refineries. The exhausted molasses have a purity of about 28.
The operations now to be described have for their object the mechanical separation of massacuite into crystallized sugar and molasses. It is evident that the most advantageous way to affect this separation is that in which the maximum of crystallized sugar is obtained at once as first product, because of portions of it become again mixed with the molasses, they can only be recovered after much trouble and expense (and not even than entirely) in the less valuable after products. It has been proved that the curing is most easily affected, and with a minimum of loss, when the sugar crystals are well developed, and mother-liquor lumpid and free from so-called "false" grain.

Separation of Sugar From Syrups

The separation of sugars from syrup is accomplished by centrifuging the massacuite in a centrifugal machine. The machine is simply a short, wide brass cylinder with perforated walls lined from the inside with a fine brass screen in order to keep the crystals from passing out of the cylinder. It is suspended by means of a steel rod from the bottom and is enclosed in a cast-iron shell. It is usually driven by means of a belt at a speed of 1200 r.p.m. In operating the centrifuge, it is filled with massacuite about one third full and set in motion, whereupon the centrifugal force will drive the mass against the walls of the machine and force the syrup out through the pores in the screen, while the sugar crystals remain clinging to the inner surface of the screen. The last trace of syrup adhering to the crystals is worked off by sprinkling water while the machine is
still in motion. The syrup produced by washing is called "wash syrup". Wash syrup is diluted to about 60 Bx., filtered and used for boiling white sugar mixed with thick syrup.

**Drying and Bagging of Sugar**

When the washing is completed, the sugar is dumped into a trough situated directly below the centrifuge. The sugar in the trough is forwarded by means of a scroll to one end of the trough where it is transferred by means of a bucket elevator, up to a sugar storage box located on the upper floor. The storage box is provided with a ropper which directs the sugar into a sugar drying machine called a "granulator". A granulator is simply a large, long cylinder made of sheet-iron supported on a series of roller-wheels, inclining toward the outlet end,(at the bottom) and rotated by means of a wheel gear. The interior of the granulator is heated by a steam pipe passing through the center of the cylinder throughout the entire length. At the outlet end, a brass screen is attached to the cylinder so that any lumps of sugar may be kept from going into the bagging room. The sugar is admitted from the storage box at such a rate that the sugar coming out from the granulator is perfectly dry. The sugar is then received in the bagging room where it is weighed and bagged, ready for transportation.

**"Bluesing" of Sugar**

When the sugar is quite centrifuged and washed, it is sometimes blued in order to neutralize the last traces of yellow color. Sometimes a suspension of ultramarine blue in water is drawn into the vacuum pan at the graining point in order that
the nucleus of each crystals may contain sufficient blue to neutralize the yellowish tint of crystals when fully grown. In other cases, a little of a little dilute suspension is poured into the centrifuge after the wash water is separated. The finely divided ultramarine blue remains between the crystals importing to them a bluish tint which pleases the eye of the purchaser. Some employ aniline blue or indanthrene but ultramarine is the most universal dye for white sugar in refineries, as also in factories where white sugar is made directly from cane juice.

Lump Sugar

Instead of sugar in loose crystals, some factories make sugar in lumps, resembling the lump sugar of the refineries.

This class of sugar is made from fine graned massecuites, intended for white sugar and cured as usual, The moist white sugar is mixed with pure syrup and treated in special centrifuges, having small boxes attached to the interior of the drums, the bottom of which consists of the centrifuge gauze. The mixture of crystals and syrup fills the boxes, the syrup escapes, and the sugar is heated with wet steam, which causes the crystals to stick together in a hard lump. As soon as the syrup escaping is colorless, the sugar is discharged in the form of square white lumps which now only need drying.

In the same way slabs of sugar may be made which are cut into cubes by means of an automatic scissors.
CHAPTER III.

THE MANUFACTURE OF BEET SUGAR.

The Plant

The several varieties of sugar-beets now known have been derived by selecting roots that possessed valuable sugar producing qualities. All the varieties are similar to those of the common garden beet, or "Beta Vulgaris".

The most favorable climate condition for the sugar-beet are found where the mean summer temperature is 70°F. It requires an abundance of sunshine for its proper development as a source of sugar.

The question of rain-fall is also of great importance. Summer-rains are very desirable, but heavy autumn rains, especially following a dry summer, are extremely unfavorable to the sugar content of the beet. Late rains cause a second growth of the plant at the expense of the sugar. Cold winter without alternate freezing and thawing are desirable for the proper keeping of the roots in storage preparatory to the manufacture.

A typical beet, so far as the shape is concerned, is shown in Fig. 1. This beet is large and tapering, with only a small neck, or crown and with broad, spreading foliage. These conditions of shape facilitates harvesting. The long top root penetrates the soil deeply in search of moisture; the spreading leaves shade the adjacent soil, thus conserving the moisture and preventing the growth of weeds; and the small neck, which must be removed in harvesting, results in little wash.
Beets weighing more than 3 pounds are usually of inferior quality, but this depends more or less on the variety of seed, the suitability of the soil and the method of cultivation. As a general rule, however, the larger beets are poorer in quality, so far as the sugar content is concerned, than the smaller or medium sized ones.

Composition of the sugar beet.

The sugar beet usually contains from 5 to 5 percent, or more of "marc" or matter that is insoluble in water. As a rule, only two sugars, sucrose and raffinose are present in the nature beet, though it may contain invert sugar, which is always found in the immature root.

The following table '1' gives the percentage composition of the varieties of sugar beet:

<table>
<thead>
<tr>
<th></th>
<th>French Variety</th>
<th>German Variety</th>
<th>Czecho-Slovakia Variety</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of sucrose</td>
<td>17.0</td>
<td>16.7</td>
<td>18.3</td>
</tr>
<tr>
<td>Density of the juice in Ex.</td>
<td>21.3</td>
<td>20.5</td>
<td>22.1</td>
</tr>
<tr>
<td>Percentage of reducing sugars in the juice</td>
<td>0.26</td>
<td>0.29</td>
<td>0.24</td>
</tr>
<tr>
<td>Percentage ash in 100 cc.</td>
<td>0.80</td>
<td>0.71</td>
<td>0.61</td>
</tr>
<tr>
<td>Apparent purity</td>
<td>37.10</td>
<td>36.60</td>
<td>35.10</td>
</tr>
<tr>
<td>Period of growth in days</td>
<td>186</td>
<td>175</td>
<td>212</td>
</tr>
</tbody>
</table>

In addition to sucrose, raffinose and chlorophyll, the beet contains a large variety of compounds namely:

Organic acids, Oxalic, Formic, Citric, Malonic, Succinic, Aconitic, Tricarbalicylic, oxocitric, maleic, and tartaric acids.

Nitrogenous bodies: Betaine, asparagine, glutamine, L-leucine, legumine, tyrosine, xanthine, guanine, hypoxanthine,
adenine, and coniterine.

Non-Nitrogenous bodies: Lecithine, and pectose.

Mineral constituents: Salts of potassium, sodium, rubidium, vanadium, calcium, magnesium, iron and manganese.

The bases are combined with hydrochloric, sulfuric, phosphoric, nitric and silicic acids, and the organic acids named in the preceding list.

Raffinose is present in small quantities and accumulates in the molasses. This sugar forms needle shaped crystals and is not injurious in the manufacture.

The greatest concentration of sugar in a beet root is around the middle and is about 17%, the top contains about 14% and the end about 15%.

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The sixth leaflet of the Department of Industrial Research.
THE MANUFACTURING PROCESSES.

The various stages of the manufacture of beet sugar may be outlined as follows:

1. Washing the beets
2. Weighing the beets
3. Slicing the beets
4. Extracting the juice by the diffusion process
5. Purification of the juice
6. Concentration of the juice to a syrup
7. Crystallization of the sugar
   a. In the vacuum pan
   b. Crystallization at rest
   c. Supplemental crystallization with motion
8. Curing the sugar.

Washing the Beets

On receiving the beets in the factory they are carried up from the first floor into a beet washing machine by means of a spiral lift, called a "Beet Scroll". The beet washer is simply a rectangular tank provided with paddles attached to a shaft which agitates and forwards the beets from one end of the washer to the other. On reaching the further end of the washer, the beets are lifted by means of a wider paddle and thrown into a bunkets elevator called "Beet Elevator", which carries the beets up to the upper most floor of the factory building.

Weighing the Beets:

On arriving at the top of the elevator, the buckets empty the beets into an automatic scale. The automatic scale is
usually so arranged as to weigh 1000 lbs. in one weighing. When filled with the required quantity of beets, the scale automatically closes the bottom of the hopper and empties beets into another hopper which directs them into the beet slicing machine.

**Beet Slicer:**

At the present time the diffusion process has replaced all others in the extraction of the juice from beets. The first step in this process, after the beets have been thoroughly washed and cleaned, is to cut the roots into thin slices termed "Cossettes", and it is of great importance that these be of uniform thickness. The cossettes are usually 1/8 inch thick and 5/8 inch wide.

A beet slicer consists of a cylinder for holding the beets and a circular disk provided with about 20 rectangular openings towards the rim for receiving knife-holders. Knife-holders are rectangular frames exactly fit in the openings. The knives are fixed on the holders by means of bolts. The knives blades are of such a form that the cossette has a V shaped cross-section. The beets are piled in the cylinder and press on the knives by their own weight and as the disk which holds the knives is revolved, the beets are sliced and received on a belt conveyor called a "Cossette Conveyor" and are sent to the diffusion batteries.

**Extraction Of The Juice**

The juice was formerly extracted from the beets by means of hydraulic press. This method, however, has been entirely rep-
laced by the diffusion process. This process was devised by Jules Robert, in France, and received its first practical application in Austria.

The extraction of the juice from beets depends on the phenomenon known as Osmosis or "Diolysis." Beet roots consist of a great number of cells. These cells are elongated their diameter varying from 0.014 mm. to 0.022 mm. and their length from 0.054 mm. to 0.089 mm. The cell walls are porous and consist mainly of cellulose and pectin matter. The substances dissolved in the cell-sap, can be divided into two kinds according to crystallizability and deffusability through a membrane; the first kind is the crystalloids and the second the colloids. The former pass through a membrane more readily than the latter. Sugar and salts belong to the crystalloids; albumins to the colloids.

If we surround beet slices composed of a great number of cells, whose contents are all nearly alike, with water; an water-change of water and soluble matter will take place. First between the surrounding water and the outermost row of cells. When the compositions of the contents of the first row of cells becomes different from that of the second row, then the soluble part in the second row pass to the first row and to the water, and so on from the different rows until the composition of the surrounding water becomes like the composition of the cell-sap after diffusion, then no more diffusion takes place as equilibrium is established. For this reason beets are sliced into thin slices so that this stage of equilibrium is quickly established. There are, however, in sliced beets, about 6.4% of tart and
bushed cells. The contents of such cells are simply washed away.

Temperature is an important factor in the process of diffusion. Although its influence varies according to different substances, a higher temperature always causes more or less rapid diffusion. The diffusion of sugar from beet chips does not take place in cold water but begins at 50 °C. The following explanation has been offered for the necessity of heating diffusion water: the outer cell wall is lined from the inside by a layer of protoplasm which resembles the cellulose wall in structure although it differs in chemical composition. The protoplasm wall is much finer and the sugar molecules are too large to pass easily through it. Heating kills the protoplasm and changes the structure completely and it becomes full of holes so it will no more hinder the passage of sugar molecules. The temperature of the diffusion juice should be raised to a point just enough to soften the cosssets and not to scorch them, so to speak, as this will render the cosssettes too soft and cause them to lie one on another, closing the screen at the bottom of the diffuse, and obstructing the circulation of the juice. Another objection to too high temperature is that it causes more non-sugars to be diffused and makes the process of crystallization more difficult.

A suitable temperature for the diffusion process varies according to the quality of the beets; usually it is kept within the limits between 78° and 85 °C. when the beets are round and the purity of the juice is high, the temperature

After drawing a quantity of juice from the ninth cell, the first diffuser is cut out of the series and is emptied. Usually
may be kept as high as permissible. When the beets are unsound, that is, fermented, unripe, or frozen, the temperature must be kept at the lower limit.

**Diffusion Apparatus.**

The apparatus employed today for the extraction of the juice from beets is called a "Diffusion Battery" consisting of several Diffusers. The batteries commonly used consists of 10, 12, or 14 diffusers. A usual form of a diffuser is a cylinder ending with cones, and provided with powerful covers. The lower cone and cover are lined with screens so that the cossettes may not pass out of the diffuser. The lower cover is opened or closed by means of hydrolic pressure; after being closed, it is held up tight by means of a latch. Each diffuser is provided with a heating chamber called a "Calorisator", and each diffuser is provided with water pipes, valves for admitting water or juice or drawing water or juice.

In beginning the work, the first diffuser is filled with fresh cossettes, and then warm water is passed into it through the pipe at the bottom, while air and gases are vented by means of a cock in the cover. While the first diffuser is being filled with water, the second is receiving cossettes, and so on. When the first diffuser is filled with water, or as it may now be termed, juice, this juice is passed through the heater of the first diffuser into the heater of the second diffuser and into the second diffuser; and when this is filled the juice is passed to the third diffuser and so on. When nine cells of a battery, made up of twelve cells, have been filled, and measured quantity of juice is drawn from the last, the circulation of the liquid
is being maintained by passing air or water into the first
diffuser of the series.

After drawing a quantity of juice from the ninth cell,
the first diffuser is cut out of the series and is emptied.
Usually all the diffusers but three are in operation at the
same time. After cutting off the first diffuser, a new one,
filled with fresh cossettes is attached last and the second
diffuser takes the place of the first and so on, the cossettes
in each diffuser remain in contact with the diffusion juice of
various densities for an average of about 1 hour and 20 minutes,
or longer, the concentration of the juice increases from diffuser
to diffuser, that of the first vessel contains only a small
fraction of a percent of sucrose, and the juice of the last cell
has a density of about four-fifths that of the normal juice.
Under favorable working conditions, the exhausted cossettes
contain from 0.15 to 0.25 \% of sucrose, in terms of the weight
of the beets.

**PURIFICATION OF THE JUICE**

**Straining or Depulping, the Juice**

As it is drawn from the diffusion battery, the juice
contains large quantities of minute fragments of beet pulp.
These must be removed prior to the clarification, since the
pectin bodies would be decomposed, and at least in part, be
rendered soluble. The straining or depulping is accomplished
by filtering the juice by a fine metal screen or perforated
metal sheet.

From now on, that is, the clarification, concentration,
crystallization, and curing of the juice and sugar are exactly as described in the chapter on cane-sugar manufacture.
CHAP. IV

SUGAR REFINING.

The second and third grade sugar i.e. sugar produced from mixed molasses and from molasses alone, is not fit for direct consumption and should undergo refining to be converted into white sugar.

The important point in sugar refining is the bleaching of the syrup, made by dissolving the raw sugar, by decolorizing charcoal and the subsequent crystallization of the pure syrup produced.

If the sugar is not very impure, then proper washing will remove the coating of impurities and leave the pure crystals behind.

Sugar Refining Process:

The centrifugal sugar is mixed with syrup in a "Magma Machine" so as to form a magma of about the consistency of massecuite. The syrup softens the coating of impurities on the crystals and prepares them for washing in the centrifuge. The crystals are thoroughly washed in the centrifuge and the resulting sugar is very light straw color and of a polariscope test of about 99° C.

If pure sugar is derived, this washed sugar is dissolved in the melters forming a solution containing about 50% of sugar. The melted sugar is limed to slight alkalinity in the blow up tanks, and then sufficient phosphoric acid is added to combine with the lime. After thoroughly blowing up the syrup with
steam and skimming it, it is filtered usually through bag filters.

The wash water and the syrup of the magma is pumped to a separate blow up tanks as this syrup contains most of the impurities.

From the bag filters, it is conveyed to a clear filter, where it is passed under pressure through decolorizing charcoal. The resulting colorless liquor is boiled in vacuum pans and the crystals are cured in the centrifuge as usual. The molasses produced are boil again and when the sugar produced is no more pure, it is heated again as was described in the beginning of the process, i.e., melted blow-up and decolorized etc....
PART II.

THE MANUFACTURE OF

GLUCOSE
Hydrolysis Products Of Starch:

Starch is a highly condensed hercule carbohydrate of the formula \((C_6H_{10}O_5)_n\), consisting of approximately 100 anhydride groups which can be resolved by suitable hydrolytic agents into as many equivalents of dextrase, provided the hydrolysis is sufficiently prolonged. Dilute acids will produce complete hydrolysis, the rate depending on the kind of acid and varying approximately as the concentration, but increases rapidly with rise of temperature.

The process of hydrolysis, or conversion of starch manifests itself by characteristic chemical and physical changes. The thick starch paste loses its colloidal nature and becomes more limpid, the concentration of the solution increases, and the solution becomes distinctly sweeter in taste.

If tested with a very dilute aqueous solution of iodine the deep blue color given by the original starch past changes as the hydrolysis proceeds, passing into violet then to a rose red which in turn changes to a reddish brown which grows lighter and lighter until just before complete hydrolysis it disappears altogether. A few drops of the solution poured into alcohol gives a copious white precipitate during the early stages of conversion; as the hydrolysis continues the amount of precipitate becomes less until it disappears near the end.
Glucose.

The term "glucose" as used to define this product must not be confused with dextrose or the pure granulated mono-saccharide, but has reference to a special commercial syrup which is always sold under this name. The name "corn-syrup", which has been suggested fits better, as is the German "starkzucker Syrop." It is a thick, viscid syrup, practically colorless and clear, or of light amber tint, and is the product of partial hydrolysis of starch. Its composition varies somewhat but the average product has a specific rotation about 140°, with a Fehling reducing value of about 47% that of dextrose.

The above corresponds to a composition peculiarly well adapted to the commercial requirements of glucose which are: that it be practically "neutral" (flavorless) sweet syrup, uncrystallizable when highly concentrated and capable of forming uncrystallizable mixtures with cane sugar when the latter is added in large proportions. The syrup must also be capable of being refined so that it is practically colorless. This properties are achieved by hydrolysing starch only so far that at least 25% of dextrin still remains as such. If less dextrin is present the product tends to crystallize when highly concentrated and it will not prevent the "graining" of its mixture with cane-sugar. If more dextrin is present the product will be less sweet and looks cloudy due to the less soluble dextrin.
Manufacture of Glucose.

A suspension of starch in water, having a density of about 20° Bé, is mixed with an acid, hydrochloric or sulfuric are the most generally used acids, the proportion of the acid is from 2 - 5 % on the basis of the dry starch.

The mixture is then led into a converter i.e., a vessel capable of withstanding internal pressure to about 6 atmospheres. The mixture is heated by indirect steam in coils and is boiled under atmospheric pressure for about 10 minutes and then the converter is closed and the pressure raised to about 2 atmospheres i.e., 30 lbs./in². A test sample is drawn from time to time and tested with iodine, when the iodine gives only a faint brown color, in about 30 minutes, the steam is shut and the pressure released quickly so that the escaping gases will carry any smell due to the hydrolysis of glutines always present in the starch used in the manufacture of glucose. The batch is next cooled and the acid neutralized by using sodium carbonate if the acid used was hydrochloric or calcium carbonate if sulfuric acid was used.

The impurities which separate in the neutral solution are filtered off in a filter press and the resulting clear liquid is concentrated in under reduced pressure to add a density of 52° Bé.

On concentrating the solution the dissolved calcium sulfate become less and less soluble and separates out, this is filtered off and the thin syrup is sent to be decolorized.

The decolorization is affected either by mixing the worm syrup with 2 % of decolorizing charcoal stirred and then filtered
or is filtered through charcoal filters as was described in the past dealing with sugar refining (Part I Chap. IV)

The colorless syrup is next concentrated to the final concentration usually 45° Bé, in a vacuum pan. The resulting syrup must be clear and colorless and shows no crystallization on standing.

I made many trials to prepare glucose from starch and quite a number of them were successful.

The first difficulty I met with was the convertor. I used a pressure cooker instead but as the maximum pressure reached was 20 lb/in² and I had no means of drawing test samples to follow the progress of hydrolysis; I tried different batches and heating for different times I started from 2 hrs. and decreased the time 10 minutes at a time.

Next came the question of acid to be used. I had to use a higher proportion so as to affect conversion in a reasonable short time due to the low pressure, as increasing the time of heating resulted in a bitter almost black mass.

For these reasons the use of hydrochloric acid was unpractical because of the relatively high concentration of sodium chloride, in the final product, in fact the samples prepared in this way had a stronger salty taste than sweet, so the acid used was sulfuric acid and the best proportion used was found by experience to be 7 cc. of the conc. H₂SO₄ for every ½ kg. starch and the amount of water was 2½ l.t.

This mixture was boiled under atmospheric pressure for
10 mins. the starch was mixed with part of the water to form a thin paste and was added to the boiling acidulated water at such a rate that boiling did not stop in order to prevent lump formation.

The mixture in a copper kettle, was next put in the pressure cooker and heated to the maximum pressure obtained i.e. 20 lb. /in². The first time the heating was kept for 2 hrs. at the highest pressure and the result was a black bitter solution. Then on decreasing the time of heating, the right period of heating was 30 mins. after the pressure reaches the 20 lb. mark.

Calcium carbonate was used for neutralization and no difficulties were met in filtration as the precipitate was flocculant and settled quite rapidly.

The decoloration was affected in two stages, the first directly after neutralization and filtration and 1% of "Darco" was used at a temperature about 30° - 40° C and the mixture was stirred for 1/2 hr. and then filtered, this gave a practically water clear and colorless solution.

Concentration was made in an ordinary round-bottom 2 lit. pyrex flask and the vacuum was obtained by the use of an ordinary suction pump which gave a vacuum, even more than necessary, which caused the solution to boil between 40° - 45° C.

The volume of the syrup was reduced from about 2½ lit. to 1 lit. and was mixed with 1% of "Darco" and kept warm for ½ hr. and then filtered with suction, this removed the dark yellow color and the precipitated calcium sulfate. The resulting syrup
was next concentrated again to final volume of about 400 cc. which gave a very pale yellow and clear syrup of a density about 40° Be'.

The earlier samples, which were hydrolyzed for a period of 1 hr. showed crystallization of standing and in a months time the whole became one solid mass of glucose hydrate which were dried in a desiccator and pulverized, with diffusely, to a powder.
PART III

GENERAL METHODS FOR

SUGAR ANALYSIS
CHAPTER 1

Qualitative Analysis.

Probably no other class of organic compounds gives such a variety of reaction or form so large a number of chemical derivatives as the sugars. Owing to the great extent of the field only the most important reactions will be mentioned here.

For convenience in identification, sugars will be classified under two general groups:

1. Reducing Sugars
2. Non-reducing sugars

The reducing sugars are distinguished by the fact that they form a precipitate of cuprous oxide when heated with alkaline Fehling solution while the non-reducing sugars do not form a precipitate or form it to a very slight extent and after prolonged boiling. The reducing sugars constitute by far the largest group. The mono-saccharides and many of their derivatives reduce Fehling solution. Most of the disaccharides including maltose and lactose exhibit this property. The best known nonreducing sugars are sucrose, α-maltoside, raffinose, and trisaccharide.
GENERAL REACTIONS OF REDUCING SUGARS

The general tests for reducing sugars may be divided, for convenience, into the following general groups of reactions.

I. Reducing reaction with solutions of metallic salts or of organic compound.

II. Color reactions with alkalies, acids, phenols, and other organic compounds.

III. Hydrozone and osazone reactions with phenyl-hydrozone and its substituted derivatives.

IV. Miscellaneous reactions.

1. Reducing reactions of Sugars:

The simple sugars and certain of the disaccharides have the property of reducing alkaline solutions of many metallic salts such as those of copper, silver, mercury and bismuth. This reaction which is common to most aldehydes is due to the reduction of the metallic base and precipitating it in the form of the suboxide or the metallic form.

In the simplest case the aldehyde groups is oxidized to the carboxyl group.

If an alcohol group is also present further oxidation converts it into the carbonyl and further into the carboxyl as in glycol aldehyde

\[ \text{H}_\text{a} = \text{COH} + 2\text{Ag}_2\text{O} \rightarrow \text{COOH} + 6\text{Ag} + \text{H}_2\text{O} \]

\[ \text{HC} = 0 \rightarrow \text{COH} \]

This oxidation, in the case of the higher mono-saccharides, is usually attended by breaking down of the carbon chain as
by the oxidation of glucose in ammoniacal silver solution.

\[ C_6H_{12}O_6 + 9Ag_2O \rightarrow 3 \left( COOH \right)_2 + 18Ag + 5H_2O \]

The reaction between sugars and alkaline salts of metals as carried out, gives rise to a number of monobasic and dibasic acids (formic, oxalic, etc.) in varying proportions according to the conditions of the experiment. It is not possible, therefore, to express the reaction by chemical equations in a very general way.

The most common of the alkaline salt solutions employed in testing sugars are those of copper. The sulfates and acetates of copper are the salts most generally used, and sugar literatures is filled with modifications for making the tests, only a few of the important solutions will be described here.

**Fehling's Copper Solution:**

This is the more common chemical reagent employed in testing sugars. As ordinarily prepared, the reagent consists of two solutions; solution 'A' containing 34.64 grms. \( CuSO_4 \cdot 5H_2O \), in 500 cc. water and 'B' containing 173 grms. \( MoO_4\cdot H_2O \), salt and 50 grms; sodium hydroxide in 500 cc. water.

By mixing equal volume of A and B, and boiling it with a sugar solutions a brick-red precipitate of cuprous oxide forms if reducing sugars are present. The intensity of coloration and amount of precipitate is proportional to the amount of sugar present. The test is sensitive to about 0.01 mg. of glucose per l. cc.

When solutions A and B are mixed and cupric hydroxide is formed which forms a complex with the hydroxy acid present thus
by the oxidation of glucose in ammonical silver solution.

\[ C_6H_{12}O_6 + 9Ag_2O \rightarrow 3(\text{COOH})_2 + 18Ag + 5H_2O \]

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By mixing equal volume of A and B, and boiling it with a sugar solution a brick-red precipitate of cuprous oxide forms if reducing sugars are present. The intensity of coloration and amount of precipitate is proportional to the amount of sugar present. The test is sensitive to about 0.01 mg. of glucose per 1 cc.

When solutions A and B are mixed and cupric hydroxide is formed which forms a complex with the hydroxy acid present thus
decreasing the concentration of the cupric ions below the requirements of the $K_{sp}$ for cupric-hydroxide. When the solution is boiled with a reducing sugar the free cupric ions are removed in the form of the insoluble cuprous oxide which causes the complex to furnish more free cupric ions and so on.

\[
\text{COONa} \quad \text{COONa} \\
\text{HC=OH} \quad \text{HC=OH} \\
\text{COONa} \\
+ \quad \text{CO(OH)}_2 \\
\leftarrow \quad \text{Cu} + 2\text{H}_2\text{O} \\
\text{HC=O} \\
\text{COONa}
\]

Products obtained by heating reducing sugars with Fehling's soln.

The chemical reactions which take place in the oxidation of sugars by means of Fehling's solution is exceedingly complex. Nef 'l' who made the first systematic studies in this field, found that in l-arabinose the oxidation proceeds along three separate lines

1. From 10 to 25% are oxidized to form pentonic acids

\[\text{C}_5\text{H}_{10}\text{O}_5 + O \rightarrow \text{C}_5\text{H}_{10}\text{O}_8\]

2. From 35 to 45% of sugars are oxidized to form formic, trioxybutyric acid.

\[\text{C}_5\text{H}_{10}\text{O}_5 + 20 \rightarrow \text{HCOOH} + \text{C}_4\text{H}_6\text{O}_5\]

3. From 30 to 38% of sugars are oxidized to form formic and glycolic acids.

\[\text{C}_5\text{H}_{10}\text{O}_5 + 30 \rightarrow \text{HCOOH} + 2\text{C}_2\text{H}_4\text{O}_3\]
With the hexose sugars, d-glucose, d-mannose, and d-fructose. Nef obtained analogous reactions with formation of carbonic formic, glycolic, glyceric, trioxobutyric, and hexenic acid. The formation of all these products has been explained on the basis of the fact that the reducing sugars are not stable in alkaline solutions, but undergo molecular rearrangement and are partly broken up into compounds with shorter carbon chain. The cupric oxide in Fehling's solutions oxidizes the reaction products, with the formation of the acids named above. The primary effect of alkalies on sugars will be described more fully later.

**Benedicts' Copper Solution**

Instead of the sulfate- tartrate, solutions, or other copper solutions have been employed in testing for sugar.

Benedicts' reagent is '2' prepared by dissolving 173 gm. of sodium citrate and 100 gm. of anhydrous sodium carbonate in about 800 cc. of water and 17.3 gms. CuSO₄.5H₂O in about 100 cc. water and making the volume to 1 lit. The solution have an advantage over Fehling's solution and not being reduced by creatine, creatinine, uric acid, or similar substance so it is especially adapted for testing of urine.

**Badfords' Solution '4'**

The reagent contains 13.3 gms. crystalline cupric acetate (neutral) in 200 cc. water and 5 cc. of 38 % acetic acid. Mono-saccharides only reduce this solution but not disaccharides such as lactose.

'1' Ann. 357, 214, 312, (1907) 
'2' J. Bid. Chem. 5, 485 (1909) Samson J. Am. chem. Soc. 61, 2389 (1939) 
'3' Ber., 15, 1885, (1882); 16, 921 (1883)
Tellurin's Silver Solution

The most sensitive of metallic - salt solutions for detecting sugars is ammonical silver solution, first employed by Tollens and hence usually known as Tollens reagent. This is prepared by dissolving one part of silver nitrate in 10 parts of water; a second solution is then made containing, one part sodium hydroxide in 10 parts of water.

Before making the test equal parts of the two solutions are mixed and then ammonia is added drop by drop until the precipitate of silver oxide is completely dissolved. A solution containing one part glucose in 1000 parts of water will cause a strong reduction of Tollén's reagent in the cold, a mirror of silver being deposited within 5 minutes. A solution containing one part glucose per 100,000 parts water also produce a perceptible reduction in the cold but the solution should be kept for 1 to 2 days.

Tollén's silver reagent is also reduced by all aldehydes; it is affected not only by the sugars which reduce Fehling's solution but also by sucrose, raffinose, and all other soluble carbohydrates.

Even the alcohol derivatives of the sugars produce reduction, glycerol, for example, causing the formation of a silver mirror. The reductors with which ammonical silver solution is reduced by soluble organic non-sugars has proved a serious objection against the use of this reagent in ordinary analytical work.

'2' J. Biol. Chem. 5, 485 (1909); Samuelson J. Am. Chem. Soc. 61, 2387 (1939).
'3' Ber., 15, 1655 (1882); 16, 921 (1883).
Reduction of Nitro-Compounds:

Among the reagents used for detecting sugars on the basis of thin reducing effect, are also included a number of organic compounds.

**Picric acid test:** Picric acid \( C_6H_4(NO_2)_3\cdot OH \) is reduced to amino-nitro-phenol \( C_6H_5(NO_2)_2\cdot NH_2\cdot OH \), which has a deep red color. The reaction first observed by Bau n '4' has been widely used for detecting sugars in boiler feed water. The water sample is boiled with few drops of hydrochloric acid to invert the sucrose and 2 to 3 drops of an alcoholic solution of picric acid is added. The reaction gives positive results at a sugar concentration of 1:5000.

**O-Dinitrobenzene test.** This is carried out as follows according to Bose '5', One drop of 1% solution of the reagent in alcohol is mixed with 2 cc. of 25% solution of sodium carbonate and 1 cc. of the sugar solution is added. Upon heating the mixture for 15 to 20 secs, a deep violet color appears which fades gradually. The reaction is very sensitive, permitting the detection of 6 parts per 1,000,000 of glucose, fructose, galactose, mannose, and lactose or 3 parts per 1,000,000 of arabinose. Non-reducing sugars do not give the test.

Other nitro compounds such as m-dinitrobenzene, m-ethynitro benzaldehyde and dinitro salicylic acid, give similar reactions.

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'4' Z. anal. Chem. 4, 185 (1865).
'5' Z. anal. Chem. 87, 110 (1932).
II. COLOR REACTIONS OF SUGARS WITH ALKALIES, ACIDS, PHENOLS, ETC.

As a second general reaction of reducing sugars may be mentioned certain color effects which nearly all soluble carbohydrates give when, brought into contact with different reagents. The reagents employed may be divided into three groups:

a. Alkalies
b. Concentrated mineral acids.
c. Phenols and other organic compounds.

Color Reactions of Sugars with Alkalies:

All reducing sugars have the property of coloring solutions of the hydroxides of alkalies and alkaline earths metals yellow, the applications of heats darkens the color to brown. The exact nature of the coloring matter formed is not understood. Considerable oxygen is absorbed during the reaction, and various products of an acid nature are among the substances formed.

Products Obtained by Heating, Reducing Sugars with Alkalies:

Lactic acid is produced in considerable amount by the action of alkalies on many reducing sugars such as xylose, arabinose, glucose and fructose. The presence of calcium lactate in certain sugar-cane molasses is explained by the action of an excess of lime during clarification upon the reducing sugars of the juice. Formic, acetic, and oxalic acid have also been found.

Nef '6' has developed a theory explaining the formation of these various reaction products. It is based on the suggestion originally advanced by Wobe and Neuberg, that sugars may react as enediols.

The alkalie causes a splitting of the ring, the aldehyde
or keto form is produced and the enediols are formed by the migration of hydrogen atoms. D-glucose, for instance, may form the following three isomers:

\[
\text{CH}_2\text{OH} - \text{CHOH} - \text{CHOH} - \text{CHOH} - \text{CHOH} (1,2 \text{ enediol}) \\
\text{CH}_2\text{OH} - \text{CHOH} - \text{CHOH} - \text{COH} - \text{CHOH} - \text{CH}_2\text{OH} (2,3 \text{ enediol}) \\
\text{CH}_2\text{OH} - \text{CHOH} - \text{COH} - \text{COH} - \text{CHOH} - \text{CH}_2\text{OH} (3,4 \text{ enediol})
\]

Increase in the alkali concentration shifts the equilibrium from the 1,2 enediol toward the 3,4 enediol.

These reactive substances undergo further transformation in various ways, such as:

1. **Splitting of enediols:**
   When the enediols split at the double bond they form the corresponding aldehydes. The resulting pentoses, tetroses and trioses may form again the enediol, split further into corresponding aldehydes. In the presence of an oxidizing agent this form the corresponding acid. This is the cause of the reduction of Fehlin's solution and other veloted reagents.

2. **Shifting of a hydrogen atom:**
   By the shifting of the hydrogen atom of glucose 1,2 enediol, it may change into either fructose or mannose as all the three sugars have the same spatial configuration in the last four carbon atoms counting the carbon atom of the carbonyl group of glucose as No. 1.

The action of alkalies on reducing disaccharides is the same, namely the formation of enediols although the reactions

'6' Ann. 576, 1 - 119 (1910)
following that are much more complicated but yet they give the aldehyde groups by breaking at the double bond which reduces Fehling's solution.

**Color Reactions of Sugars with Mineral Acids**

Treatment of solutions of sugars with concentrated mineral acids gives rise to a number of decomposition products with a development of a color the intensity of which depend on the kind of sugar, the concentration of the acid used and the temperature. By heating a sugar solution with concentrated hydrochloric or sulfuric acid a brown color develops which is due to the formation of an insoluble so-called humus substances of relatively high carbon content (C = 62 - 67 % and H = 3.5 - 4.5 %) the percentage carbon and depth of color increasing on the concentrations of the acid used.

In addition to the insoluble humus, a number of soluble and volatile compounds are produced, such as formic acid, levulinic acid, furfural, methyl furfural, hydroxymethyl-furfural, and a number of dextrin like condensation or reversion products.

The ketones are much more easily decomposed by strong mineral acids than aldoses, and their solutions give rise to color reactions with correspondingly greater faintness. This offers a mean of distinguishing between ketoses and aldoses or detecting ketoses in the presence of aldoses.

If concentrated sulfuric acid is added to a cold solution of a sugar in a test tube so that the acid will flow on the sides of the test tube and form a layer at the bottom, a rose-
color to brown ring is produced as the junction if the solution contains a ketose such as fructose or sorbose or if the sugar gives rise to a ketose on hydrolysis such as, sucrose, raffinose or inulin. With glucose, lactose, maltose and the aldoses in general the color will develop only slowly or not at all.

As if a few of the sugar crystals are placed on a wad of asbestos moistened with concentrated hydrochloric acid in a test tube, the crystals will be colored violet in a short time if the sugar is a ketose or gives a ketose on hydrolysis. Although the latter sugars give the test a little more slowly. Aldoses develop only a bight yellow color in 2 to 3 hours.

Color Reactions of Sugars with Phenols.

The most distinctive color reactions of the sugars are those obtained by treatment with different phenols in the presence of concentrated sulfuric or hydrochloric acid. The development of color in this case is due to the formation of condensation products between the phenol derivatives and the decomposition products obtained from the sugars, particularly furfural and its derivatives. α-Naphthol, thymol, resorcinol, orcino, naphthoresorcinol, phloroglucinol, cresol, and lysol are among the more important phenolic compounds used for making color reactions with sugars. A typical example of the condensation product between furfural and the phenols is di-α-naphthafurylmethane, C₂₃H₁₈O₃, which was obtained by Bredereck from α-naphthol and furfural. It is colorless, but dissolved in concentrated sulfuric acid with a deep violet color.

The color reactions with the phenols are performed in various ways, the test with α-naphthol, Molisch test, for example,
which is perhaps used more frequently than any of the others, is made as follows: 1 to 2 cc. of the sugar solution is heated in a test tube with 1 to 2 drops of 10 to 20 percent alcoholic solution of \( \alpha \)-naphthol. A few cubic centimeters of concentrated sulfuric acid are then carefully added so as to flow down the walls of the tube to the bottom.

If sugars containing a ketone group are present a violet ring will form instantly as the junction of the two liquids; in the presence of aldoses a gentle warming of the test tube is usually necessary to bring out the full intensity of the color. The \( \alpha \)-naphthol test, which is of extreme delicacy is frequently employed in sugar houses and refineries in testing the condensation water for presence of sucrose lost by entrainment.

The test with phenols and hydrochloric acid are usually made by warming a few cubic centimeters of the sugar solution with a solution of the phenol in concentrated hydrochloric acid. The coloration thus obtained are usually very brilliant, varying in tint from a bright red to a bluish violet. The colors formed are not permanent, however, they rapidly darken and the clear colored solution soon becomes turbid with the preceipitation of a dark colored condensation product.

Glacial acetic acid may be also used instead of hydrochloric acid to dissolve the phenols employed in these tests. Under these conditions the solution remain clear and can be used directly for spectroscopic examination or for colorimetric comparison.
III. HYDRAZONE AND OSASONE REACTIONS OF REDUCING SUGARS

WITH PHENYL HYDRAZINE AND ITS SUBSTITUTED DERIVATIVES

In many respects the most important of the qualitative tests for sugars are those obtained with phenyl hydrazine and its substituted derivatives. Phenylhydrazine was introduced as a reagent in sugar chemistry by Emil Fischer '7' in 1884; it has been of immense service not only as a means of separation and identification but also in first opening a way to a thorough understanding of the molecular constitution of sugar.

Hydrazone Reaction: The reaction with phenylhydrazine is limited to such sugars as contain a free carbonyl group and proceed two phases with the production of two entirely different classes of compounds. The first phase of the reaction is common to all aldehydes and ketones the oxygen of the carbonyl group combining with the two atoms of hydrogen of the amine group in the phenylhydrazine with the formation of a group of compounds called hydrazones.

The hydrazone reaction is carried out by treating the sugar solution in the cold with a solution of phenylhydrazine in dilute acetic acid. As the sugar solution may be saturated with crystals of phenylhydrazine-hydrochloride with some solid sodium acetate. After the above treatment the hydrazone of the sugars will separate sooner or later as well-defined crystalline compounds, the length of time for separation depending upon the solubility of the hydrazone formed. The phenylhydrazones of mannose, for example, being very insoluble, will separate almost immediately, those of ketones such as fructose, and
rhamnose also deposit readily; the phenylhydrazone of glucose on the other hand, being very soluble in water, may require 1 or 2 days for its précipitation. By filtering off the hydrazones as they are form, and separation of the sugars in mixtures may often be accomplished.

In place of phenyl-hydrazine any of its substituted derivatives may be used for the purpose of précipitating sugars. The substituted phenylhydrazines yield in many cases characteristic hydrazones with sugars and their use in sugar chemistry has been of greater service. Of the various substituted phenylhydrazines the following are among the most important.

Methyl, Ethyl, Amyl, Allyl, Phenyl, Benzyl, p-Bromo and p-Nitro phenylhydrazines and Naphthylhydrazine.

The reactions with the substituted phenylhydrazines are usually carried out in alcoholic solutions, the hydrazones formed being for the most part much less soluble than those of ordinary phenylhydrazine. A table of melting points will then identify the hydrazone of the sugar.

The sugar can be regenerated and separated from the hydrazone either by treatment with concentrated hydrochloric acid, as employed by Fisher, where the hydrazone is changed into phenylhydrazine hydrochloride, which is unsoluble in concentrated hydrochloric acid, and the sugar is set free.

The other method is by refluxing the hydrazone a 50% alcoholic solution of the hydrazone with an aldehyde. Benzaldehyde is used with phenylhydrazones and formaldehyde with the substituted hydrazones. The hydrazones of the aldehydes being unsoluble are removed and the sugar removed by crystallization.

‘7’ Ber, 17, 579 (1884).
Asazone Reaction:

While the hydrazone reaction is of permanent value in the isolation of sugars, the asazone test with phenylhydrazine is usually of more qualitative significance owing to the greater insolubility of the osazone in water and the consequent greater rapidity and ease of thin separation as compared with the hydrazones.

The reaction is carried out by heating in a boiling water both a solution of the sugar with excess of phenylhydrazine, and acetic acid. First the hydrazone is formed then the hydroxyl group of the carbon atom is oxidized into a carbonyl group on the expense of one molecule of phenyl-hydrazine which is reduced to aniline and ammonia. A third molecule of phenyl-hydrazine then reacts with the new carbonyl group to form the osazone. 

The time of formation of the osazone, crystal structure, and melting point are used to identify the sugar.

One of the important limitations of the use of the osazones in identifying sugars is that, while sugars which differ only in the spacial arrangement of the first two carbon-atoms such as d-glucose, d-fructose and d-mannose, will yield identical osazones.

Methylphenylhydrazine forms osazones readily with ketones but not with aldoses so it will partly help in eliminating the above mentioned limitation of used along with phenylhydrazine.
REACTIONS OF THE NON-REDUCING SUGARS

The comparatively small number of sugars which do not reduce Fehling's solution all belong to the higher di-, tri- and tetra-saccharides and include sucrose, dfructose anhydride, raffinose and other rase sugars, some of them not completely studied yet. The soluble polysaccharides such as, dextrin, inulin, and glycogen, although not classified as sugars, are sometimes included, for convenience, in the group of non-reducing sugars.

A free aldehyde or ketone group, to which the reducing sugar owe their peculiar reactivity, is lacking in the non-reducing sugars so they do not reduce Fehling's solution and the related reagents, not from hydrazones and osazones or react with dilute alkaline or show other reactions typical of reducing sugars.

The non-reducing sugars give many of the color reactions with phenols. But as previously explained these reactions are not given by the non-reducing sugars as such, but by the reducing sugars derived from them due to the hydrolytic action of the acid used in making the reaction.

Some of the special tests which may be used to detect the most commonly encountered non-reducing sugar, sucrose, are described here:

Raybin's Test '8'

If 40 to 50 mg. of sucrose are dissolved in few cubic centimeters of 0.05 N sodium hydroxide solution, is shaken at about \( \theta \) 10°C in a stoppered test tube with 7 to 10 mg. of diazourasil, until the latter dissolved, a blue-green
color develops in few minutes. In the presence of soluble magnesium salt and stable blue precipitate forms.

This test is not given by glucose, fructose, or most other sugars, but by sugars characterized by the fructofuranose-glucose linkage such as raffinose.

Pictet Test for Sucrose. Pictet '9' observed that a mixture of cold, saturated solutions of sucrose and of copper sulfate in water, after standing for several hours, deposits microscopic needles of a double salt $C_{12}H_{22}O_{11} \cdot CuSO_4 \cdot 4H_2O$. This reaction is not given by any of the other common pentoses, hexoses, di and tri-saccharides or by the synthetic sucrose isomers. But in mixtures, at least 10% of sucrose must be present to produce a positive test.

Schlemmeis' Test for Sucrose in the Presence of Inverted Sugar

This is based on the fact that reducing sugars are destroyed by boiling with milk of lime, and do not give the usual sugar reactions after this treatment. 20 cc. of the solution to be tested containing not over 2% of total sugar is mixed in a test tube with 2 cc. of 10% milk of lime and the tube is placed in boiling water bath for 10 - 12 minutes. The tube is now centrifuged, the supernatant liquid precipitated off and tested with naphthol or thymol. If the color reaction cannot be readily seen because of the yellow tint of the treated sugar solution, a dichromate filter is used for the observation.

This is easily accomplished by placing the test tube in which

8' J. Am. Chem. Soc. 55, 2603 (1933); 59, 1402 (1937).

the α-naphthol test is carried out, in a beaker filled with a 0.25 to 0.5 % solution of potassium dichromate to make the color of the treated sugar solution. Under these conditions a positive test for sucrose may be obtained at a concentration of 0.001 % Raffinose and reversion products of reducing sugars also give this test, also commercial glucose which always contain reversion products. The test is therefore specific only in the absence of these other carbohydrates.
SYSTEMATIC PROCEDURE FOR THE DETECTION OR IDENTIFICATION OF CARBOHYDRATES

In order to facilitate the identification of carbohydrates which the chemist may encounter, various schemes for a systematic procedure have been developed. The identification may be based on chemical reaction, on fermentation with yeast, fungi, or bacteria, or on the effect produced by enzymes. The example given here is based on chemical reactions.

Identification by Chemical Reactions.

Dean Jackson and Ballard '10' have compiled a table of chemical reagents used for the detection of carbohydrates and another table showing the tests given with these reagents by a number of carbohydrates. These are reproduced in tables I, and II. The meaning of the figures signs in table II are explained under "Remarks" in table I, and that of the abbreviations in a footnote below table II.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Solvent</th>
<th>Amt. of reagent</th>
<th>Amt. Percent</th>
<th>Time heated at 100°C</th>
<th>Elapsed time before reading</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Water</td>
<td>H₂O</td>
<td>(l)</td>
<td>3</td>
<td>1-5</td>
<td></td>
<td>Cellulose insoluble</td>
</tr>
<tr>
<td>b. I + II</td>
<td>H₂O</td>
<td>3</td>
<td>1</td>
<td>1-5</td>
<td></td>
<td>Heat to expel free iodine; cool.</td>
</tr>
<tr>
<td>c. Nylander's</td>
<td>3% NaOH</td>
<td>(l)</td>
<td>6</td>
<td>1-5</td>
<td></td>
<td>Reagent catalyzed with AuCl₃; (+) br. ppt's; (wh) white ppt's</td>
</tr>
<tr>
<td>d. Bisch's</td>
<td>H₂O</td>
<td>3</td>
<td>30</td>
<td>30 +</td>
<td></td>
<td>Pentoses brown; hexoses etc. blue</td>
</tr>
<tr>
<td>e. Orinol</td>
<td>H₂O</td>
<td>(l)</td>
<td>1</td>
<td>1-5</td>
<td></td>
<td>See table II</td>
</tr>
<tr>
<td>f. Phloroglucinol</td>
<td>H₂O</td>
<td>1</td>
<td>1-5</td>
<td>1-5</td>
<td></td>
<td>See table II; boiling gives CHR, with pectin, gum arabic and gum tragacanth</td>
</tr>
<tr>
<td>g. Na₂CrO₄</td>
<td>H₂O</td>
<td>10</td>
<td>1</td>
<td>1-30</td>
<td></td>
<td>Simple sugars green; pectin, red solution</td>
</tr>
<tr>
<td>h. Sodium metropolynsode</td>
<td>H₂O</td>
<td>1</td>
<td>1 + 2</td>
<td>1-30</td>
<td></td>
<td>Add NH₄OH and heat; (+) green while hot; arabinose green without NH₄OH (+) immediate decolorization; (*) decolorization in 1 hr.</td>
</tr>
<tr>
<td>i. KI₀₄</td>
<td>H₂O</td>
<td>0.1</td>
<td>1</td>
<td>1-30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>j. Febling's</td>
<td>H₂O</td>
<td>(l)</td>
<td>60</td>
<td>1</td>
<td></td>
<td>Add FeCl₃; brown, negative; green, positive; maltose, blue.</td>
</tr>
<tr>
<td>k. K₂Fe(CN)₆</td>
<td>H₂O</td>
<td>0-5</td>
<td>1</td>
<td>1-5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>l. picric acid</td>
<td>H₂O</td>
<td>sat.</td>
<td>1</td>
<td>1-5</td>
<td></td>
<td>Add NH₄ numbers indicate depth of color (red).</td>
</tr>
<tr>
<td>m. H₂SO₄</td>
<td>H₂O</td>
<td>50</td>
<td>1</td>
<td>1-30</td>
<td></td>
<td>Yellow to amber to brown to black. Number = depth of color</td>
</tr>
<tr>
<td>n. HClO₄</td>
<td>H₂O</td>
<td>60</td>
<td>1</td>
<td>1-30</td>
<td></td>
<td>Yellow to amber to brown to black. Number = depth of color.</td>
</tr>
<tr>
<td>o. H₂SO₃</td>
<td>H₂O</td>
<td>50</td>
<td>1</td>
<td>1-30</td>
<td></td>
<td>Yellow to amber to brown to black. Number = depth of color.</td>
</tr>
<tr>
<td>p. HCl</td>
<td>H₂O</td>
<td>20</td>
<td>1</td>
<td>1-30</td>
<td></td>
<td>Yellow to amber to brown to black. Number = depth of color.</td>
</tr>
<tr>
<td>q. KH₃(+)</td>
<td>H₂O</td>
<td>3</td>
<td>1</td>
<td>1-30</td>
<td></td>
<td>Nos. = depth of color; pectin yellow.</td>
</tr>
<tr>
<td>r. (NH₄)₂MoO₄</td>
<td>H₂O</td>
<td>5</td>
<td>5</td>
<td>50</td>
<td></td>
<td>Add carbohydrate, then ½ the vol. cons. H₂SO₄ Nos = depth of color; rhamnose green.</td>
</tr>
<tr>
<td>s. HNO₃</td>
<td>H₂O</td>
<td>25</td>
<td>1</td>
<td>1-30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t. H₂S₂O₃</td>
<td>50% H₂SO₄</td>
<td>10</td>
<td></td>
<td>1-30</td>
<td></td>
<td>Nos = depth of red.</td>
</tr>
<tr>
<td>Reagent</td>
<td>Solvent</td>
<td>Amt. of reagent</td>
<td>Time heated at 100°C</td>
<td>Elapsed time before reaction</td>
<td>Remark.</td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>---------</td>
<td>-----------------</td>
<td>----------------------</td>
<td>-------------------------------</td>
<td>---------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>a. Water</td>
<td><em>H₂O</em></td>
<td>1</td>
<td><em>n</em></td>
<td><em>n</em></td>
<td>Cellulose insoluble</td>
<td></td>
</tr>
<tr>
<td>b. I + HI</td>
<td><em>H₂O</em></td>
<td>3</td>
<td>1</td>
<td>1-5</td>
<td>Heat to expel free iodine; cool.</td>
<td></td>
</tr>
<tr>
<td>c. Nylander's</td>
<td><em>H₂O</em></td>
<td>3% <em>NaOH</em></td>
<td>1</td>
<td>1-5</td>
<td>Reagent catalyzed with AuCl₃; (+)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>bk. ppt’s; (wh) white ppt’s</td>
<td></td>
</tr>
<tr>
<td>d. Bischof’s</td>
<td><em>H₂O</em></td>
<td>5</td>
<td>30</td>
<td>30+</td>
<td>Pentoses brown; hexoses etc. blue</td>
<td></td>
</tr>
<tr>
<td>e. Orzinol</td>
<td><em>H₂O</em></td>
<td>1</td>
<td>1-5</td>
<td></td>
<td>See Table II</td>
<td></td>
</tr>
<tr>
<td>f. Dihydroxyethylglycol</td>
<td><em>H₂O</em></td>
<td>1</td>
<td>1-5</td>
<td></td>
<td>See Table II; boiling gives CHR, with</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>pectin, gum arabic and gum tragacanth</td>
<td></td>
</tr>
<tr>
<td>g. Na₂CrO₄</td>
<td><em>H₂O</em></td>
<td>10</td>
<td>1</td>
<td>1-60</td>
<td>Simple sugars green; pectin, red solution</td>
<td></td>
</tr>
<tr>
<td>h. Sodium metaperiodate</td>
<td><em>H₂O</em></td>
<td>1</td>
<td>1</td>
<td></td>
<td>Add NH₄OH and heat; (+) green while heat;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>arabinose green without NH₄OH (+) immediate decolorization; (‡) decolorization in 1 hr.</td>
<td></td>
</tr>
<tr>
<td>i. MnO₂</td>
<td><em>H₂O</em></td>
<td>0.1</td>
<td><em>n</em></td>
<td>1-60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>j. FeCl₃</td>
<td><em>H₂O</em></td>
<td>0-5</td>
<td>1</td>
<td>1-5</td>
<td>Add FeCl₃; brown, negative; green,</td>
<td></td>
</tr>
<tr>
<td>k. K₂Fe(CN)₆</td>
<td><em>H₂O</em></td>
<td>sat.</td>
<td>1</td>
<td>1-5</td>
<td>positive; melrose, blue.</td>
<td></td>
</tr>
<tr>
<td>l. picric acid</td>
<td><em>H₂O</em></td>
<td>sat.</td>
<td>1</td>
<td>1-5</td>
<td>Add NH₃ numbers indicate depth of</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>color (red)</td>
<td></td>
</tr>
<tr>
<td>m. <em>H₂SO₄</em></td>
<td><em>H₂O</em></td>
<td>50</td>
<td>1</td>
<td>1-60</td>
<td>Yellow to amber to brown to black. Number</td>
<td></td>
</tr>
<tr>
<td>n. HCIO₄</td>
<td><em>H₂O</em></td>
<td>60</td>
<td>1</td>
<td>1-60</td>
<td>- depth of color</td>
<td></td>
</tr>
<tr>
<td>o. <em>H₂SO₄</em></td>
<td><em>H₂O</em></td>
<td>50</td>
<td>1</td>
<td>1-60</td>
<td>Yellow to amber to brown to black. Number</td>
<td></td>
</tr>
<tr>
<td>p. <em>HCl</em></td>
<td><em>H₂O</em></td>
<td>20</td>
<td>1</td>
<td>1-60</td>
<td>- depth of color</td>
<td></td>
</tr>
<tr>
<td>q. <em>KOH</em></td>
<td><em>H₂O</em></td>
<td>5</td>
<td>1</td>
<td>1-60</td>
<td>Nos. - depth of color; pectin yellow.</td>
<td></td>
</tr>
<tr>
<td>r. (NH₄)₂<em>H₃O</em></td>
<td><em>H₂O</em></td>
<td>5</td>
<td>1</td>
<td>1-60</td>
<td>Add carbohydrate, then titr with the vol.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>con. <em>H₂SO₄</em> Nos = depth of color; xylosene green.</td>
<td></td>
</tr>
<tr>
<td>s. <em>MgO</em></td>
<td><em>H₂O</em></td>
<td>25</td>
<td><em>n</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t. <em>Fe₂SO₄</em></td>
<td><em>H₂O</em></td>
<td>50% <em>H₂SO₄</em></td>
<td>10</td>
<td>1-60</td>
<td>Nos = depth of red.</td>
<td></td>
</tr>
<tr>
<td>Reagent</td>
<td>Solvent</td>
<td>A wt. of reagent</td>
<td>Time heated at 100°C</td>
<td>Elapsed time reagent</td>
<td>Remark</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
<td>------------------</td>
<td>----------------------</td>
<td>----------------------</td>
<td>---------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>u. Ni$_2$O$_4$</td>
<td>(8)</td>
<td>Percent</td>
<td>Mins.</td>
<td>Mins.</td>
<td>Nos = relative speed of decolorization of Ni$_2$O$_4$</td>
<td></td>
</tr>
<tr>
<td>v. Heroin</td>
<td>H$_2$SO$_4$</td>
<td>1</td>
<td>#</td>
<td>15</td>
<td>Mostly amber to brown black (9)</td>
<td></td>
</tr>
<tr>
<td>w. Cadene</td>
<td>H$_2$SO$_4$</td>
<td>1</td>
<td>#</td>
<td>15</td>
<td>Mostly rose to brown black (10)</td>
<td></td>
</tr>
<tr>
<td>x. AgNO$_3$</td>
<td>Dil. NH$_2$OH</td>
<td>1</td>
<td>1</td>
<td>1-60</td>
<td>(+) black ppt's; or silver mirror; some give brown solution</td>
<td></td>
</tr>
<tr>
<td>y. p-tolylhydrazine</td>
<td>Dil. HCl</td>
<td>1</td>
<td>#</td>
<td>1-60</td>
<td>Boiling gives for (+) dichromate color; levulose and sucrose give color and Ppt.</td>
<td></td>
</tr>
</tbody>
</table>

1. Mono-, di-, and tri- saccharides are sweet and soluble in cold water. Polysaccharides, dissolve in hot water. Starch, pectin, glycogen, and gum tragacanth dissolve in hot water to give opalescence and foam. Gum arabic gives clear solution and foam. Dextrin an inulin give clear solution but no foam.

2. Nylander's reagent: 2 g. of bismuth subnitrate and 4 g. of Rochelle salt dissolved in 100 g. of 8% NaOH

3. Prepare 10% solution of diphenylamine in alcohol and add 1 volume to 4 volumes of acetic acid and 5 volumes of HCl.

4. To 5 cc. of saturated aqueous solution of arecolnol, add 50 cc. of HCl and dilute to 90 cc.

5. To 0.1 g. of phloroglucinol in 10 cc. of alcohol, add 50 cc. of HCl and dilute to 90 cc.

Solution of arecolnol or phloroglucinol in dil. HCl, will indicate carbohydrates containing levulose.
6. Fehling's reagent. (1) solution of 36.44 g. CuSO₄·5H₂O in water diluted to 500 cc. (2) solution of 125 gr. KOH and 173 g. of sodium potassium tartrate in water diluted to 500 cc.

7. Usually called Moor's test.

8. Prepare solutions (a) 10 gr. of NiSO₄·7H₂O in 1000 cc. of H₂O and (b) 4.8 gr. of K₂S₂O₃ and 6 gr. of KOH in 1000 cc. H₂O. Use equal parts and let stand. Black Ni₂O₃ precipitates. Carbohydrated decolorize this.

9. With varied times and temperatures, carbohydrates give changing colors. A check with a known carbohydrate and revealing and desirable. Perhaps in these reactions, formoldehyde is split off, because color closely follows Kobert's test for codiene etc.
| Carbohydrate    | a | b | c | d | e | f | g | h | i | j | k | l | m | n | o | p | q | r | s | t | u | v | w | x | y |
| 1. Cellulose    |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 2. Starch       |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 3. Double Starch|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 4. Glycogen     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 5. Maltose       |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 6. Maltose       |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 7. Maltose       |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 8. Maltose       |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 9. Maltose       |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 10. Maltose      |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 11. Maltose      |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 12. Maltose      |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 13. Maltose      |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 14. Maltose      |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 15. Maltose      |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 16. Maltose      |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 17. Maltose      |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 18. Maltose      |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 19. Maltose      |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 20. Maltose      |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 21. Maltose      |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |

Abbreviations: Am= Amber; Bk, black; Br, blue; Br, brown; Ch, cherry; G, green; Or, orange; P, purple; R, red; R, rose; V, violet; Wh, white; Y, yellow.
CHAPTER II

QUANTITATIVE ANALYSIS.

The methods used for the quantitative determination of sugar fell in two different groups:

1. Physical methods.
2. Chemical methods.

Physical methods for the Quantitative Determination of Sugars:

There are many methods in use which depend on the physical properties of sugars and sugar solutions, such as specific-gravity, viscosity, optical activity, etc.

Of these, the methods based on the optical activity of sugar solutions are by far the most important, in accuracy and in the scope of application.

All sugars are optically active due to the presence of unsymmetrical carbon atoms in the molecule, and the degree to which the plane polarized light is rotated in a sugar solution depends, other factors remaining the same, on the kind of sugar and the concentration of the solution.

The polarizing power of sugars is expressed as specific-rotation, or specific rotary power, by which is meant the calculated angular rotation which a solution, containing the mass of 1 gr. active substance in a volume of 1 cc. and is polarized in a tube 1 dm. long, gives to the plane of polarized light. The specific rotation \((\alpha)\), can easily be calculated from the angular rotation "\(\alpha\)" of the substance by means of the equation:

\[
(\alpha) = \frac{100\,a}{c \times l}
\]

In which \(c\) is the concentration of the substance (grammes mass in 100 cc. of solution) and \(l\) the length of the observation tube in decm.
As the angular rotation depends on the temperature and on
the wave-length of the light used, so the standard is set at
20°C and the light of sodium (the line D in the spectrum) so
the specific rotation is written as:

\[
\frac{[\alpha]}{D} = \frac{100a}{c \times l}
\]

The following table '1' gives the specific rotations of
some of the common sugars:

<table>
<thead>
<tr>
<th>Sugar</th>
<th>([\alpha]/D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabinose</td>
<td>+104.5</td>
</tr>
<tr>
<td>Xylose</td>
<td>+19.0</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>+8.5</td>
</tr>
<tr>
<td>Galactose</td>
<td>+80.5</td>
</tr>
<tr>
<td>Glucose</td>
<td>+52.5</td>
</tr>
<tr>
<td>Fructose</td>
<td>+92.5</td>
</tr>
<tr>
<td>Invert Sugar</td>
<td>+20.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+66.5</td>
</tr>
<tr>
<td>Lactose</td>
<td>+52.5</td>
</tr>
<tr>
<td>Maltose</td>
<td>+152.5</td>
</tr>
<tr>
<td>Raffinose</td>
<td>+104.4</td>
</tr>
</tbody>
</table>

The above formula for the specific rotation can be modified
in the following way:

\[
\frac{[\alpha]}{D} = \frac{100a}{p \times d \times l}
\]

Where \(p\) is parts by weight of the substance in 100 parts
by weight of solution and \(d\) is the density of the solution.

Solving the above equation for \(p\) we get

\[
p = \frac{100a}{[\alpha] \times d \times l}
\]

which gives us a simple way to calculate the percentage of
a sugar in its solution (in the absence of other optically active
substances) for knowing the angular rotation of the given solution,
the specific rotation of the sugar, the density of the solution and the length of the observation tube.

The following formulas \( 1 \) are given for calculating the concentration (grms. per 100 cc.) of different sugars from the angular rotation (\( \alpha \)) in a 2-dm. tube

1. Arabinose \( c = 0.4785 \alpha \)
2. Xylose \( c = 2.6316 \alpha \)
3. Glucose \( c = 0.9470 \alpha \)
4. Fructose \( c = 0.5405 \alpha \) (left degrees)
5. Galactose \( c = 0.6173 \alpha \)
6. Sucrose \( c = 0.7519 \alpha \)
7. Maltose \( c = 0.3623 \alpha \)
8. Lactose \( c = 0.9524 \alpha \)
9. Raffinose \( c = 0.4785 \alpha \)

The different kinds of polarimeters are adopted to the examination if all optically active substances; in sugar chemistry however, a modified form of a polarimeter, called a Saccharimeter, is used. For convenience the scale of the saccharimeter instead of expressing angular rotation is replaced by one graduated according to percentage.

Owing to the many difficulties and inconveniences connected with the use of sodium or other monochromatic light in practical work, the French physicist Soliel was led in 1845 to devise a means by which ordinary light or lamplight could be used for measuring the optical rotation for sugar solutions. This invention known as the "Quartz-Wedge Compensator", is the characteristic feature of most saccharimeters.

In the quartz-wedge saccharimeter, the polarizer and the analyzer are both stationary; the rotation of the sugar solution is measured by shifting a wedge of optically active quartz between

\[ 1 \] Sugar analysis. Browne and Zerhan. P. 265
\[ 2 \] Sugar anal. B. and Z. P. 295.\]
the solution and analyser until the rotation of the wedge system at a certain thickness exactly neutralizes or compensates the rotation of the sugar solution. By means of a scale attached to the quartz wedge the rotation of the sugar in solution is measured in percentage.

The selection of quartz for compensation is based upon the fact that it has almost exactly the same rotation dispersion as cane sugar; i.e., a section of quartz and cane sugar solution of equal rotation for light of one wavelength will have very nearly equal rotation for light of all other wavelengths. The small disturbances due to the slight difference in rotation dispersion between cane sugar and quartz are eliminated by a dichromate filter.

**The Sugar Scale and Normal Weight of Saccharimeter:**

The 100 point of a saccharimeter scale is usually based upon the rotation of a definite weight (the so-called normal weight) of chemically pure sucrose dissolved in water to 100 mm. at a specific temperature and polarized at the same temperature in a 200 mm. tube.

The greatest confusion has prevailed in saccharimetry in the past and unfortunately still prevails, not only to the size of the normal weight of sugar to be taken for a specified scale, but also as to the conditions of volume and temperature under which its normal weight is to be polarized.

**French Sugar Scale:**

The 100 point of the sugar scale employed upon saccharimeters of French manufacture was originally based upon the rotation in
sodium light of a plate of dextrorotary quartz 1 mm. thick cut exactly perpendicular to the optical axis.

Owing either to mistakes of polarimetric measurements or to defects in the quartz (through natural imperfection or mistake in cutting), the rotation of the 1 mm. plate has been given different values from time to time, the result ranging from + 20.98 to 22.67 and the normal weight of sucrose for such a saccharimeter from 16.000 grm. to 16.471 grm.

The value accepted now is 16.29 grm.

**Ventzke or German Sugar Scale**

The sugar scale most generally used outside of France and the one employed upon all German saccharimeters is that of Ventzke. This scale as originally devised by Ventzke, was based upon the rotation of a solution containing 25% of sucrose by weight but this was later changed to that of a solution of 1.1 sp. gr. 17.5°C read in a tube 234 mm. long. It was found later that it is inconvenient to base the normal weight upon the sp. gr. of a solution and the amount of sugar contained in this solution was adopted as the normal weight. This weight was determined to be 26.048 gm. weighed in air with brass scales and dissolved at 17.5°C to 100 cc. The length of the tube was reduced to 200 mm.

With the introduction of the Mohr cubic centimeter (the volume of 1 grm. of water at 17.5°C weighed in the air with brass weights), the normal weight was recalculated to be 25.9872 grm.

The standard based on the Mohr cubic centimeter was abandoned later and the temperatures changed to 20°C and this necessitated the recalculation of the normal weight and the new value was found to be 26.0000 gms. which was changed later to 25.0000 gr.
and was called the Bates-Jacobson scale.

The adopted normal weight for the Ventzke scale now is 26.026 grm.

The Bidecimal Sugar Scale.

In 1896, it was proposed to adopt a so-called international sugar scale, based upon the normal weight of 20 grm. Among the advantages proposed for such a scale are:

1. It is a compromise between the French and the German scales.

2. Calculations are simplified by use of decimal weights.

3. Aliquots of 50, 25, 20, 10, and 5 cc. of the 100 cc. solution contain even gram quantities.

4. The normal weight, its fraction, and its multiples are always available in one-piece unit.

The saccharimeter used in this university has such a scale and its normal weight is defined as "20 gm. of pure sucrose dissolved in 100 ml. of solution and polarized in a 200 mm. tube at 20° C."

The following table gives the normal weight to Ventzke scale for various sugars:

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Normal weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>26.026 gr.</td>
</tr>
<tr>
<td>Glucose</td>
<td>32.398% gr.</td>
</tr>
<tr>
<td>Fructose</td>
<td>12.416 gr.</td>
</tr>
<tr>
<td>Invert Sugar</td>
<td>26.333 gr.</td>
</tr>
<tr>
<td>Lactose (+H₂O)</td>
<td>32.972 gr.</td>
</tr>
<tr>
<td>Maltose</td>
<td>13.528 gr.</td>
</tr>
<tr>
<td>Raffinose (+5H₂O)</td>
<td>16.574 gr.</td>
</tr>
<tr>
<td>Raffinose (Anhyd.)</td>
<td>14.062 gr.</td>
</tr>
</tbody>
</table>
For the Bates-Jacobson scale with the normal weight of sucrose as 26.000 gr. the above values are multiplied by 0.999, and for the Bidecimal scale by 0.763.

"1" Sugar Anal. B. and Z. p 298.

CHEMICAL METHODS FOR THE QUANTITATIVE DETERMINATION OF SUGARS

The principal chemical methods for determining sugars are based upon the property which all aldehydes and ketones have of reducing alkaline solutions of certain metallic salts.

Of the various alkaline solutions of metals, those of copper are employed most generally in sugar analysis.

Fehling in 1848 first worked out the details of the alkaline copper methods, as they now stand, and the copper sulfate and alkaline tartarate reagent had since been called by his name.

All the different methods using alkaline solutions of metallic salt are based on the same principle i.e. the oxidation of the sugar by the metallic ion which is reduced in its turn to the free metal or to the lower insoluble oxide; but they differ in the way the quantity of sugar used is calculated. Some are based on the weight of the cuprous oxide (copper solutions) formed which bears a relation to the quantity of reducing sugars used and can be formed from given tables, and others find the quantity of unreduced copper from which, the original quantity of copper, the part reduced is formed which gives the quantity of sugar used.

The first method is gravimetric and being slow is not fit for routine work or when many samples have to be analyzed as it
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\[ \text{Sugar Anal. B. and Z. p 298.} \]

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The first method is gravimetric and being slow is not fit for routine work or when many samples have to be analyzed as it
was the case with my work, so naturally I chose a method based on the second way. The simplest way to determine unreduced copper is volumetrically although it can be found by electrolysis or gravimetrically. Again I wanted a method which, quick with a short heating period and at a temperature easily obtainable with the equipment I had; and in my work I had to analyze mixtures of sugars which almost all of them were composed of sucrose, glucose, and fructose, so a solution which had equal reducing power towards glucose and fructose and which would permit the quantitative determination of fructose in the presence of glucose was necessary.

These properties were found in the modified Luff-Schoorl method.**

*Modified Luff-Schoorl Method* 

The copper reagent is prepared as follows:

1. 388 g. of crystalline sodium carbonate (Na₂CO₃·10H₂O) or its equivalent of the anhydrous salt are dissolved in 300 - 400 cc. of lukewarm water in 1 lit. volumetric flask and to this is added with stirring a solution of:

2. 50 g. Citric acid in 50 cc. of water, after all effervescence has ceased add gradually with constant vigorous shaking a solution of:

3. 25 g. crystalline copper sulfate (CuSO₄·5H₂O) dissolved in 100 cc of distilled water. Make up to volume and let stand for few days; decant the clear portion and store; the solution will keep indefinitely and shows no auto-reduction even upon boiling.

Place 25 cc of this reagent and 25 cc, of the sugar solution...

---

(1) *Z. Ang. Anal. B. and Z.* P. 832
a 300 cc. erlenmeyer flask, add few pieces of pumice stone and attach a water-cooled reflux condenser. Heat over a free flame so that the contents will start to boil in about 2 min. then heat over an asbestos centered wire gauze and let it boil gently for exactly 10 mins. longer. Remove the flame and cool immediately in running water. Add 5 gm. of potassium iodide and then add slowly with stirring 20 cc. of 25 % hydrochloric acid. Titrate the liberated iodine with standard sodium thiosulfate solution using starch indicator towards the end. Run a blank and with 25 cc. of the reagent and 25 cc. of distilled water following the same procedure exactly. The difference between the two titrations is equivalent to the reduced copper and to the quantity of reducing sugars present which is found from the following table:

<table>
<thead>
<tr>
<th>Vol. cc.</th>
<th>Glucose Fructose Invert</th>
<th>Ahydrous Lactose</th>
<th>Amydrous Maltose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mg.</td>
<td>Mg.</td>
<td>Mg.</td>
</tr>
<tr>
<td>1</td>
<td>2.4</td>
<td>3.6</td>
<td>3.9</td>
</tr>
<tr>
<td>2</td>
<td>4.8</td>
<td>7.5</td>
<td>7.8</td>
</tr>
<tr>
<td>3</td>
<td>7.2</td>
<td>11.0</td>
<td>11.7</td>
</tr>
<tr>
<td>4</td>
<td>9.7</td>
<td>14.7</td>
<td>15.6</td>
</tr>
<tr>
<td>5</td>
<td>12.2</td>
<td>18.4</td>
<td>17.6</td>
</tr>
<tr>
<td>6</td>
<td>14.7</td>
<td>22.1</td>
<td>19.2</td>
</tr>
<tr>
<td>7</td>
<td>17.2</td>
<td>25.2</td>
<td>23.5</td>
</tr>
<tr>
<td>8</td>
<td>19.4</td>
<td>29.5</td>
<td>27.5</td>
</tr>
<tr>
<td>9</td>
<td>22.4</td>
<td>33.2</td>
<td>31.5</td>
</tr>
<tr>
<td>10</td>
<td>25.0</td>
<td>37.0</td>
<td>35.5</td>
</tr>
<tr>
<td>11</td>
<td>27.6</td>
<td>41.3</td>
<td>39.5</td>
</tr>
<tr>
<td>12</td>
<td>30.3</td>
<td>44.6</td>
<td>43.5</td>
</tr>
<tr>
<td>13</td>
<td>33.0</td>
<td>48.4</td>
<td>47.5</td>
</tr>
<tr>
<td>14.</td>
<td>35.7</td>
<td>52.2</td>
<td>51.6</td>
</tr>
<tr>
<td>15</td>
<td>38.5</td>
<td>56.0</td>
<td>55.7</td>
</tr>
<tr>
<td>16</td>
<td>41.3</td>
<td>59.9</td>
<td>59.8</td>
</tr>
<tr>
<td>17</td>
<td>44.2</td>
<td>63.8</td>
<td>63.9</td>
</tr>
<tr>
<td>18</td>
<td>47.1</td>
<td>67.7</td>
<td>68.0</td>
</tr>
<tr>
<td>19</td>
<td>50.0</td>
<td>71.7</td>
<td>72.2</td>
</tr>
<tr>
<td>20</td>
<td>53.0</td>
<td>75.7</td>
<td>75.5</td>
</tr>
<tr>
<td>21</td>
<td>56.0</td>
<td>79.8</td>
<td>80.9</td>
</tr>
<tr>
<td>22</td>
<td>59.1</td>
<td>83.9</td>
<td>85.4</td>
</tr>
<tr>
<td>23</td>
<td>62.2</td>
<td>88.0</td>
<td>90.0</td>
</tr>
<tr>
<td>24</td>
<td>65.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The same table may also be employed if larger or smaller quantities of the copper reagent than the 25 cc. specified are used. As for example, 50 cc. reagent plus 50 cc. sugar solution. The titer found is divided by 2, the corresponding milligrams of sugar found from the table, and the result multiplied by 2. Thus a titer of 20 under these conditions indicate 25.0 x 2 or 50 mg. of glucose and not 53.0 mg. The same thing is applied if smaller volume is used, namely find the milligrams of sugar which correspond to 25 cc. of reagent and then convert to the volume used.

Two points should be borne in mind, the final volume of reagent plus sugar solution must always be twice the volume of the reagent, use distilled water to accomplish this point, and second, after boiling the solution should show a blue color indicating the presence of unreduced copper, if no blue color is present, repeat the determination using smaller volume of sugar and make up for the difference with distilled water.

Classifying Agents

A clarifying agent is a substance added to the solution of the sugar or the sugar containing material which causes the coagulation and the precipitation of the gummy, albuminoids and waxy materials present, which results in a solution clear and easy to filter.

The most used clarifying agents are:

1. Lead subacetate. It is prepared by mixing 300 gr. lead acetate and 100 gr. lead oxide (litharage) in one liter of water stirred and heated gently for about 2 hours. It is left to settle and the clear solution decanted.
The same table may also be employed if larger or smaller quantities of the copper reagent than the 25 cc. specified are used. As for example, 50 cc. reagent plus 50 cc. sugar solution. The titer found is divided by 2, the corresponding milligrams of sugar found from the table, and the result multiplied by 2. Thus a titer of 20 under these conditions indicate 25.0 x 2 or 50 mg. of glucose and not 53.0 mg. The same thing is applied is smaller volume is used, namely find the milligrams of sugar which correspond to 25 cc. of reagent and then convert to the volume used.

Two points should be kept in mind, the final volume of reagent plus sugar solution must always be twice the volume of the reagent, use distilled water to accomplish this point, and second, after boiling the solution should show a blue color indicating the presence of unreduced copper, if no blue color is present, repeat the determination using smaller volume of sugar and make up for the difference with distilled water.

**Clarifying Agents**

A clarifying agent is a substance added to the solution of the sugar or the sugar containing material which causes the coagulation and the precipitation of the gummy, albuminoids, and waxy materials present, which results in a solution clear and easy to filter.

The most useful clarifying agents are:

1. Lead subacetate. It is prepared by mixing 300 gr. lead acetate and 100 gr. lead oxide (litharge) in one liter of water stirred and heated gently for about 2 hours. It is left to settle and the clear solution decanted.
2. Neutral lead acetate: It is a saturated solution of lead acetate in water.

3. Dry basic lead acetate: (Horne Powder) It is obtained as a dry powdered salt and contains 72.3% Pb, which corresponds to the composition of 3Pb(C₂H₃O₂)₂ · 2PbO.

4. Alumina cream: Prepare a cold saturated solution of potassium aluminium alum in water. Add conc NH₄OH with constant stirring until the solution is alkaline to litmus; let the precipitate settle and wash by decantation with water until the wash water gives only a slight test for sulfate ions with BaCl₂ solution. Pour off the excess of water and store the residual cream in a stoppered bottle.

Clarifying agents are added before completing to volume except the dry Horne powder which is added after completing the volume. As the basic lead acetate precipitates the fructose as lead fructoseate, therefore in the presence of reducing agents to be determined the use of neutral lead acetate or alumina cream for clarifying is imperative.

Hints on Clarification and Filtration

After the addition of the clarifying agents (about 3 or 4 cc. per 100 cc. of the solution) add one drop of ether to clear the foam before completing to the mark. Shake thoroughly and let stand for about 10 mins. Pour into a filtered filter paper capable of holding at least half the quantity at one time, collect the clear filtrate after rejecting at least the first 25 cc., the adsorption power of filter paper to water and sugars in not proportional to the composition of the solution.

If lead solutions has been used for clarification, the excess
lead should be removed by addition of dry, powdered anhydrous sodium carbonate to the filtrate and refiltering; as the excess of lead will cause a change in the rotation of the sugar solution and will react with the copper sulfate to form the insoluble lead sulfate and with the hydrochloric acid used in inversion forming the precipitate lead chloride.
1. In the Absence of other Sugars:

Weigh out one normal weight exactly, transfer to a 100 cc. volumetric flask, add about 70 cc. water, stir to dissolve, add the proper clarifying agent, complete to the mark and polarize in a 200 mm. tube. Reading is percentage sucrose.

2. In the Presence of Mono-Saccharides:

Prepare a normal solution as directed above and find direct polarization reading. Invert part of the solution as follows:

Transfer 50 cc. of the normal solution to a 100 cc. vol. flask, add 25 cc. water and 5 cc. conc. HCl, while shaking, heat in a water bath at such a rate that the internal temperature reaches 67° - 69° C in 2 - 2½ mins. Keep at that temperature for 7 mins. longer. Cool, rinse in the thermometer, make to volume and polarize in a 200 mm. tube. This gives the "Invert reading", calculate percentage sucrose in the original sample from the following formula.

\[
S = \frac{100D}{142.65 - \frac{20}{t}}
\]

where \( S \) = percentage sucrose

\( D = \) Direct polarization - 2 invert polarization

\( t = \) Temperature of polarization in degrees centigrade.

It is very important that the two polarizations should be made at the same temperature although not necessarily 20° C, but it is preferrable to be near 20° C.
This method depends on the change in polariscope reading due to the inversion of the sucrose; as the mono-saccharides suffer no change at the used concentration of acid and at the used temperature, then any change in the rotation is due to the invert sugar produced.

In calculating \( D \) in the above formula, twice the invert reading was taken because the invert solution is \( \frac{1}{2} \) normal. If the invert reading is negative then it is added to the direct reading, of course.

**THE QUANTITATIVE DETERMINATION OF GLUCOSE**

1. **In The Absence Of Other Sugars**

   When only glucose is known to be present, then it can be determined either by the use of a saccharimeter or chemically.

   a. For the determination by the saccharimeter, follow the procedure given under the determination of sucrose (Method-1) using the normal weight of glucose as given in the beginning of this chapter.

   b. For determining glucose chemically, prepare a solution containing about 0.5 - 1 percent of solid matter and follow the Modified Luff-Schoorl method, after proper clarification.

2. **In The Presence Of Non-reducing Sugars**

   Follow the procedure given above (method 1, b.)

3. **The Determination: Commercial Glucose in Other Sugar Products**

   Method of A.O.A.C. 'l'

   Prepare an inverted half-normal solution of the substance, do not cool after inversion, make neutral to phenol-phthaline 'l' Methods of Analysis, A.O.A.C. 5th ed; p 498, 1940.
with sodium hydroxide solution, slightly acidify with dilute HCl (1+5) and treat with 5 to 10 cc. of alumina cream before making to the mark. Filter and polarize at 87°C. in a 200 mm. jacketed metal tube, preferably silver. Calculate the quantity of commercial glucose solid polarizing 481 V by the formula

\[ S = \frac{200 R}{195} \]

where \( S \) is the quantity of glucose as described above, and \( D \) the saccharimetric reading.

The normal weight here refers to that of sucrose and not to glucose.

The cause for using a high temperature here is because of the fact that invert sugar is optically inactive at 87°C. Because the rotation of fructose decreases rapidly with rise of temperature so that at 87°C the less rotation due to fructose is compensated by the dextro-rotation due to the equivalent amount of glucose. So this method of high temperature polarization eliminates the effect of any present sucrose or invert sugar or originally present.

This method is used to detect the adulteration of honey and other similar products with commercial glucose.

**THE QUANTITATIVE DETERMINATION OF FRUCTOSE**

1. If it is the only reducing sugar present, then the procedure is that described for the determination of reducing sugars or as described for glucose. (1, b.)

2. In The Presence Of Other Sugar:

The selective determination of fructose in a mixture of
sugars is carried either chemically or physically. This method depends on the property of fructose of suffering a big change in the rotation with change in temperature.

Prepare a normal solution, clarify, filter, and find the direct polarization at 20°C in a 200 mm. tube and polarize again at 87°C in a 200 mm. tube also. Calculate the weight of fructose in the normal weight by the formula:

\[ F = \frac{R_{20} - 1.0315}{2.3919} \cdot R_{87} \]

where \( F \) is the weight of fructose in the normal weight
\( R_{20} \) is the direct polarization at 20°C.
\( R_{87} \) is the direct polarization at 87°C.

b. _Chemically_

This method depends on the fact that, fructose being a ketose (the only common ketose in ordinary sugar analysis) is more difficult to oxidize than other common sugars which are aldoses. The oxidizing agent is sodium hypochlorite.

**Kolthoff - Kruisheer Method For Determining Fructose 'l'**

**Reagents:**

1. 4 N. Sodium hydroxide solution
2. 4 N. Sulfuric acid solution
3. Iodine solution containing 15 grm. iodine and 15 grm. potassium iodide in 100 cc.
4. 20 percent sodium sulfite solution
5. 2 percent sodium sulfite solution

Procedure:

Dissolve a suitable quantity of the product representing from 1.75 and 3.5 grms. total solids, in water in a 100 cc. vol. flask, clarify, complete to volume and filter. Pipette 25 cc. of this solution into another 100 cc. vol. flask, add 25 cc. water and then 5 cc. of 4 N. sodium hydroxide. Run in at once 16 cc. of the iodine solution or enough to impart a distinctly brown color to the solution. Let stand for 5 to 7 mins. and add 3 cc. of 4 N. sulfuric acid. Remove the excess iodine first with 20 percent sodium sulfite solution, and then carefully with 2 percent sodium sulfite solution. Add starch indicator towards the end and continue the titration till the blue color is discharged. Add 4 N. sodium hydroxide till the solution is slightly acid to methyl orange. Complete the volume and determine the fructose in a 25 cc. aliquot by the modified Sufi-Schoorl method. Calculate the percentage of fructose in the original sample.

In the presence of sucrose it is advisable to first invert the solution, neutralize, and determine the fructose after inversion. The fructose produced by the inversion of sucrose is 52.63 percent of sucrose, as found by double polarization.

ANALYSIS OF MIXTURES OF SUGARS

The most important mixtures of sugars encountered in everyday analysis are those composed of sucrose, glucose, and fructose.

1. Sucrose and any one of the other two sugars present a
very simple mixture the sucrose being determined by double polarization and the other reducing sugar by the copper-solution.

2. A mixture of glucose and fructose can be analysed by finding the total reducing sugar in the original sample and after the oxidation of glucose as described in the method for determining fructose selectively. The difference between the two values represent the glucose. From this point, it is easy to understand why the copper reagent employed must have the same oxidizing power towards glucose, and fructose.

3. A mixture of the three sugars together can be analyzed by combining the above mentioned two procedures, i.e. Sucrose, by double polarization, fructose by the hypo-iodite method, and glucose by difference.

MOISTURE IN SUGAR CONTAINING PRODUCTS

The water content in a sugar containing product can be determined either by drying a sample at 100°C in an oven under reduced pressure or by extracting the water with a volatile immiscible solvent. Generally Xylol is used but as there is the danger of fructose decomposing at the boiling point of xylol 138°C, toluene, with the lower boiling point of 110.6°C is used instead, otherwise the procedure is exactly as described in books on commercial quantitative analysis.
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