

*Citation:*

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If, on the other hand, we could further the hydrolysis, and thus accelerate the "transformation in time" which showed itself in many cases, a positive proof would be given.

Since, however, traces of NaOH already form a fine granular precipitate, such an experiment seems to be excluded from measurement.

The chemical complications, which in particular for iron-salts are so numerous, constantly impede the investigation of these salts.

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**Microbiology.** — "*A microsaccharimeter*". By Miss. H. J. VAN LUTSENBURG MAAS and Prof. G. VAN ITERSSEN JR. (Communicated by Prof. M. W. BEIJERINCK.)

In the conscientious and extensive work of A. J. KLUYVER: "Biochemische suikerbepalingen" <sup>1)</sup> (Biochemical Sugaranalysis) a fermentation-saccharimeter is described, which enables us to quantitative fermentations under perfect sterile circumstances. The quantities of the different fermentable sugars, possibly at the same time present, are to be calculated from the quantities carbonic acid, produced in such an apparatus from a fixed volume liquid by different ferments.

The rich material, which KLUYVER published, shows in a convincing way, how this apparatus gives a most satisfactory and at the same time simple solution for the problem of quantitative sugar-determinations by means of the fermentation-method. Such a solution has in spite of the researches of many predecessors never been found.

The application of this method in studying biological questions, from which KLUYVER gives already some interesting examples, promises most important results.

By no means the fermentation-saccharimeter, whose description will follow, will be able to supersede the apparatus, used by KLUYVER. The latter will always be preferred when accuracy is required and a sufficient quantity of the sugars is to be had. The reason why, will be explained later, we only mention it here, because the applicability of the here described method is justified by the results, found with the apparatus of which KLUYVER gives the description.

In the first place some remarks may follow on this last apparatus and the limits of what can be attained will be indicated.

<sup>1)</sup> Published by E. J. BRILL at Leiden, 1914.

In the current form the apparatus wants about 1 cc. liquid to ferment. By taking a smaller size this volume can be reduced to 0.5 cc., but the accuracy of the reading diminishes (influence of the convex mercury meniscus). For constructive and practical reasons it seems impossible to reduce the size more.

The quantity of fermentable sugar, used in the apparatus of ordinary size is about 40 mgr. (corresponding with 10 cc. CO<sub>2</sub>) and should not be less than 4 mgr. The last limit is determined by the circumstance, that almost an equal volume of the carbonic acid as is produced from this quantity of sugar by the fermentation is soluble in 1 cc. of the sufficiently fermented liquid under average barometric pressure and at the temperature of the room. By applying a manipulation, viz. the addition of a known; small quantity fermentable sugar, it is possible to determine smaller quantities of sugar with this apparatus, but the analysis is not very accurate in that case. With the developing of small quantities of carbonic acid the influence of the factor, which is to be charged for the gas, dissolved in the fermented liquid, is comparatively very large, and just this factor is by the changing composition of that liquid always somewhat uncertain.

The inoculation-material for this apparatus is a small quantity of yeast, which is taken from a tube-culture with the aid of a thick platinum needle. That yeast quantity is so chosen by KLUYVER, in connection with a research of JODLBAUER, that the proportion yeast to sugar is about 1 : 2. The influence of the autofermentation is cancelled. With this yeast-concentration the fermentable sugars have usually completely disappeared after about 40 hours (when raffinose is present the time, necessary for the fermentation is much longer). This long fermentation makes it necessary to sterilize carefully the apparatus, the fermenting mixture and the mercury, shutting off the liquid.

For many biological researches a method for quantitative sugar-analysis would be desirable for quantities smaller than 1 cc. and often it will be necessary to estimate smaller portions than 4 mgr. with greater accuracy than is possible with the aid of the manipulation in the apparatus referred to. Therefore now an apparatus will be described with which this purpose can be reached. The quantities of sugar, which can be fermented are between 3.5 mgr. and 0.1 mgr. and a drop of 0.010 cc. will be sufficient to perform the analysis, though it is preferable to take a larger quantity of the liquid. Moreover it will be evident that with this new method the fermentation will be much sooner finished than with the old one, so that no sterile circumstances are necessary.

§ 1. *Description of the microsaccharimeter.*

The principal part of the microsaccharimeter (see the Plate , fig. 1) is made of a capillary tube of thick glass, with an inner diameter of about 2.8 mm. At a short distance from one of the ends this tube has been blown to a cylindric reservoir, the content of which is about 1 cc. and with a short neck. The other end of the tube has been enlarged to a funnel, of a content of about 1.5 cc. The length of the glass apparatus, measured from the point on the utmost right to the utmost left is about 35 cm. (in connect with the usual dimensions of the thermostats). The capillary tube has such a curve under the reservoir that the longest, straight part (see fig. 1) mounts to about half way the reservoir. This long straight part has been calibrated; the scale-division is in parts, each from 0.01 cc. The zero-point is as near as possible to the downward directed curve and the division continues till the upward bent. With the here mentioned dimensions this division will go to about 0.9 cc. and it is desirable, that it should not be much shorter. The dimensions are for the rest so chosen, that the content of the reservoir is a bit smaller than that of the bent and straight part of the capillary tube and the funnel together, a circumstance, which is to be observed by the constructor of the apparatus. The finishing of the neck of the small reservoir is to be done carefully. The opening of that neck is upward somewhat enlarged in a conical form, while also from the very short, narrow part of this opening downward a very regular conical transition must be (see fig. 3).

The glass apparatus is placed on a small stand (see fig. 2), made from a wooden platter ( $5.5 \times 38$  cm<sup>2</sup>), on which a wooden block has been fastened, that bears a cork clamp. This clamp is made from a conical cork of good quality (largest diameter 4 cm., high 3.5 cm.). This cork has on the short side a groove, which continues to some distance from the large side. The curved part of the capillary tube fits in this groove. The cork is further on at two sides filed parabolical (see fig. 2).

The glass apparatus is to be fixed in every desired stand by a brass pin with winged nut, fitting in the cork just above the curve of the capillary tube. By removing the brass pin the glass apparatus can be taken from the stand, which is necessary to clean it. To sterilize the apparatus is superfluous, but it should be dried carefully.

The two mercury levels, being after the fermentation in the reservoir and in the divided part of the capillary tube, can be placed on the same height by different simple ways. A rather good method

is by using an apparatus as shown in figure 4. This apparatus beats a pin, which can be moved over a vertical stand. The pin reaches before the microsaccharimeter, when the stand is placed behind it. To compare the mercury levels all is placed on an exactly horizontal table and by removing the apparatus over this table and changing the angle between the capillary tube and the platter, the two levels can be brought on exactly the same height.

For using this microsaccharimeter we want (besides different ferment-cultures): dried and cleaned mercury, paraffin with a melting-point of about 55° C., red sealing-wax of superior quality, some metal spatulas, a platinum spatula in a needle-holder, a number of dropping syringes, some capillary tubes (diameter 1 mm.), some small sterile glass tubes with cotton-wool stops and sterile main-water.<sup>1)</sup>

### § 2. *Preparation of the yeasts.*

For a quantitative analysis with the microsaccharimeter the yeast is to be submitted to a very simple preparation. The yeast quantity, used in this apparatus is in proportion to the quantity of sugar rather large. So the volume of the carbonic acid, developed out of the glycogen present in the yeast can often be very important compared to the gas, produced by the fermentation of the sugar. This difficulty is to be prevented; before bringing the yeast in the apparatus, it is made free from glycogen by auto-fermentation.

The different ferments are the best cultivated in the ordinary culture-tubes on the surface of malt-gelatine. When tubes of a large size are used, one contains enough yeast to do at least six quantitative determinations with the microsaccharimeter. With the aid of a sterile platinum spatula the yeast is to be carefully taken from the gelatine-surface and divided in some cc. sterile main water in a glass tube stopped with cotton wool. Then the tubes with the different yeasts are placed in a thermostat at 30° C. With the aid of the iodine reaction it can be settled that under these circumstances all the glycogen has disappeared by auto-fermentation after four hours.

After this preparation the yeast has sunk to the bottom of the tube and the water, standing above, can easily be taken away with a dropping syringe. For this no sterile syringe is wanted, but for each other kind of yeast a new or cleaned one is to be used.

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<sup>1)</sup> The microsaccharimeter is to be had at J. C. Th. MARIUS, Lim., Utrecht the ferment cultures at the "Centralstelle für Pilzkulturen" at Amsterdam

In the water above the yeast but little carbon dioxide is dissolved. In the following calculation the water has been supposed to contain no carbonic acid, so a small, practically to be neglected, mistake is made. This mistake is completely to be avoided by refreshing the water above the sunken yeast, which too should be taken away with a dropping syringe. The sunken yeast is divided in the remaining water and brought into the saccharimeter with a capillary tube.

### § 3. *Method of using.*

In the first place the microsaccharimeter is to be filled with a certain quantity of dried, cleaned mercury.

The nut-wing is unscrewed and the glass apparatus placed as fig. 2 shows. The filling is done by the funnel, with a dropping syringe. When the funnel is full, it is carefully raised; the mercury streams to the reservoir *A* and remains partly in the capillary tube. The next lowering of the funnel makes the mercury stream partly back. By addition or removal of mercury the quantity can be taken so, that the reservoir with its neck and the capillary tube is filled as far as or just past the zero-point. To control this, the funnel is raised till the mercury reaches the border of the opening of the neck; the mercury will then be adjusted at zero, or between the first marks. After the apparatus has been filled with mercury, it is placed in the original position and with the aid of a metal spatula a bit of paraffin, melted on that spatula, is spread on the polished surface of the neck.

Only a thin cover is wanted, but it should reach the border of the opening; it is even to be preferred to cover the inner-wall of this neck over a short distance, but it is not necessary.

Now the nut-wing is fastened, but so, that the glass apparatus can still be moved in the cork and remains in every required position, when released. Then the apparatus is placed so that the tangentplane to the mercury level in the neck coincides with the paraffin cover. Therefore the eye is kept in the tangent-plane to this surface and the funnel is to be raised till the mercury meniscus can just be seen. In this phase the *first reading* of the mercury in the calibrated tube is made.

By the action of the capillarity in that calibrated tube a strongly convex meniscus is formed and the position of the utmost tangent-plane is to be read without difficulty with the naked eye up to in tenth parts of the calibration.

Next a drop of the fermenting liquid is brought on the mercury,

and on the surface of the neck with the aid of a dropping syringe. The size of this drop is to be regulated by the quantity of sugar to be fermented. This quantity ought not to surpass 3.5 mgr. and practically not to sink under 0,1 mgr. The concentration of the solution should be more than 0,4%. Good results are to be had with a 3% sugar solution, from which it is best to take drops of 0,06—0,08 cc.

After this the funnel is carefully lowered; thereby the drop of liquid is drawn into the apparatus. This can be performed without any loss of liquid, if only the paraffin cover have been laid down in the right way on the neck of the reservoir. Should however any liquid be left behind, then this has to be removed with a small piece of filter-paper.

The meniscus of the solution in the upper part of the neck will be convex, especially when some paraffine had been brought along the inner side of the neck. This shape of the meniscus can be obtained in the best way by making the solution rise from a lower part of the neck up to the top, taking care however, not to have it lowered under the narrow part. In this way it is possible to bring the tangent-plane at the meniscus on the level of the upper surface of the neck. After fixing the apparatus in this position, the 2<sup>nd</sup> reading of the meniscus of the mercury in the straight capillary glass tube is made.

The difference between the two first readings gives: the volume of the sugar solution to an accuracy of 0,001 cc.

Now again the liquid is a bit lowered, but not so far as to reach the narrow part. Then, with a thin capillary glass tube, we add a drop of yeast-suspension, which has been prepared previously as already indicated. The drop is carefully thrown into the apparatus, until the meniscus, which again will be convex, reaches the same level as mentioned before. Now the 3<sup>d</sup> reading is taken.

The difference between the 3<sup>rd</sup>. and the 2<sup>nd</sup>. reading gives: the volume of the added yeast-suspension. Care must be taken that to 1 part of sugar about from 5 to 8 parts of yeast be added (weighed in living state)<sup>1)</sup>. With sugar concentrations of about 3 per cent and with suspensions of yeast, prepared in the described way, this can be done by taking the volume of the suspension nearly the same as that of the sugar solution.

After this 3<sup>rd</sup>. reading the liquid is allowed to go down to the narrow part of the neck; there the meniscus will be decidedly con-

<sup>1)</sup> Just by the choice of these large quantities of yeast, the fermentation-time is much reduced, compared with the duration of the analysis, made by KLUYVER.

cave. Then one melts some paraffine on a small metallic spatula and lets it flow along the inner side of the neck on the surface of the liquid. In this way it is possible to fill up the whole upper part of the neck with paraffin, without any difficulty and with a startling result. No air bubble ought to be present between the liquid and the paraffin, but no difficulties will arise, should a bubble be present, provided its volume is small compared with that of the carbon dioxide, evolved by the fermentation. Once the paraffin solidified, the 4<sup>th</sup> reading is made.

After this the apparatus can be sealed definitively, for which sealing-wax was used, as paraffine shrinks, when it solidifies and easily gets loose from the glass. The application of the sealing-wax is as follow. At the outer side of the round upper part of the neck, a ring of paraffin is taken away with a small knife. Care should be taken, not to damage the stop of paraffin, which seals off the liquid in the neck. Now on a small metallic spatula some sealing-wax is liquefied by heating and the melted wax is put on the part of the neck, from which the paraffin had been taken away. Not before the wax is well fixed on the glass, a drop of liquefied wax is put on the paraffin stop. Now the whole closure can be perfected by adding more sealing-wax.

This done, one puts the apparatus in the thermostat of 30° C., fixing it in the position of figure 2. The fermentation will be completely finished within 6 hours<sup>1)</sup>. This time past, the apparatus is taken from the thermostat and fastened in such a position, that the mercury in the capillary tube and in the reservoir are on about the same level. Two hours are quite sufficient to have the apparatus cooled to the temperature of the air. The 5<sup>th</sup> reading is then made, but not before the mercury in the tube and in the reservoir is carefully placed on the same level. This can be done, as already indicated, by means of the small auxiliary apparatus, described in § 1. As the same time the temperature of the air and the barometer are read. Now all *data*, necessary for the calculation of the analysis are known.

In the experiments, dealt with in § 5 of this communication, the preparation of the yeast took place in the morning; the micro-saccharimeters were put into the thermostat at about 3 o'clock in the afternoon and were taken out of the thermostat in the evening of the same day. The last reading was made next morning.

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<sup>1)</sup> Till now, we did not yet study the fermentation of raffinose with this apparatus, it seems possible, that this sugar will ask a longer time to ferment completely.



§ 4. *How to calculate the results.*

The difference between the 5<sup>th</sup> and the 4<sup>th</sup> reading gives: the volume of the gaseous carbon dioxide, present in the apparatus at the end of the fermentation. Another portion of carbonic acid however is retained by the liquid and this portion too has to be taken into account. Now KLUYVER observed, that when sugar is fermented in yeast-extract at 15° C. and 760 mm., in 1 cc. of the liquid a quantity of carbonic acid is left behind which has a volume of 1.2 cc. at 0° C. and 760 mm. (provided that supersaturation of the liquid is avoided). By BOHR and BOCK however it was pointed out that at 15° C. and 760 mm. pure water retains a volume of carbonic acid, which after reduction to 0° C. and 760 mm. amounts to 1.019 cc. The fact that KLUYVER found more, can be explained by the special nature of his liquid.

Though in our experiments the carbon dioxide was not dissolved in pure water, as every fermented liquid retains alcohol, yet our liquid approaches more to pure water than yeast-extract. It is very probable, that under these circumstances the foresaid number falls between the two numbers, mentioned above. Moreover our readings were made at temperatures between 17° and 20° C. Now the solubility of carbon dioxide diminishes rather rapidly, when the temperature rises. After BOHR and BOCK the foresaid volume becomes 0.878 cc. at 20° C.

For these reasons we assume the forementioned volume, under the conditions of our experiments, to be 1 cc. This simplifies the calculation.

The total volume of carbon dioxide of 0° C. and 760 mm. now can be found by reducing at first the gaseous carbon dioxide to that temperature and pressure, which may be done quite efficiently by means of a table, published by KLUYVER<sup>1)</sup>. This done, the volume of all the liquid is to be added (viz. the difference between the 3rd. and the 1th. reading).

KLUYVER made a large number of determinations of the volume of carbon dioxide (reduced to 0° C. and 760 mm.), obtainable with 6 different species of yeasts from 40 mgr. of 8 different sugars in the apparatus, used by him (see table XXVIII of his publication). Then also the number of milligrammes of sugar, equivalent to 1 cc. of CO<sub>2</sub> at that temperature and under normal pressure were known. We have limited our experiments for the

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<sup>1)</sup> l. c. p. 61.

yeasts to *Saccharomyces cerevisiae* (press-yeast), *Torula dattila* and *Torula monosa*, and for the sugars to glucose, fructose, saccharose and maltose. Especially the quantitative determination of these sugars, separately or as mixtures will be required in biological research work. Now these determinations are possible with the 3 yeasts mentioned, with this exception alone, that glucose and fructose are always found together. The first of the 3 yeasts is capable to ferment the 4 sugars, the second the monoses and saccharose and the last ferments only monoses.

Now KLUYVER established, that out of the 4 mentioned sugars in his apparatus nearly the theoretical quantity of carbon dioxide is produced. Certainly in our microsaccharimeter we may expect no smaller quantity of this gas, as reproduction of yeast is practically impossible within the 6 hours of our experiments, whilst under the circumstances of KLUYVER some reproduction may be expected. Therefore we took the theoretical value to make our calculations. This means, that we supposed a yield of 1 cc. of carbon dioxide (of 0° C. and 760 m.m.) to be equivalent to 4.05 mgr. of absolutely dried hexose (respectively to 4.45 mgr. hexose-hydrate, containing 1 H<sub>2</sub>O) and to 3.85 mgr. of absolutely dried bihexose (respectively to 4.05 mgr. of bihexose-hydrate, containing 1 H<sub>2</sub>O).

#### § 5. Numerical illustration.

Here follow some examples of determinations, which we performed with the microsaccharimeter. We give only a small number of applications of this apparatus on the analysis of natural products, as we intend to publish a more detailed communication on this subject later on. Here we principally mention the results of fermentations with sugar solutions; we took the most pure sugars to be got. Thus, with the numbers published here, we intend to demonstrate the applicability of the method.

1. A 3 per cent. solution of glucosehydrate was fermented by *Torula monosa*. The readings were successively: 0,012; 0,070; 0,128; 0,133 and 0,436 cc. The last reading was made at 19° C. and under a pressure of 767 m.m.

The gaseous carbon dioxide, present in the apparatus after the fermentation, was 0,303 cc. After reduction to 0° C. and 760 mm. this becomes 0,286 cc. The volume of the liquid in the apparatus is found to be 0,116 cc. Thus, the whole volume of carbon dioxide of 0° and 760 mm., obtained by the fermentation, may be supposed to be 0,402 cc.

This carbon dioxide is equivalent to  $0,402 \times 4,45 = 1,78$  mgr. of glucosehydrate. Originally the apparatus received 0,058 cc. of liquid, corresponding to 1,74 mgr. of glucosehydrate.

2. With *T. monosa* we fermented a 1 per cent. solution of glucosehydrate. The successive readings were: 0,020; 0,030; 0,041; 0,053; 0,059 cc. The last reading was taken at 19° C. and 760 mm. The quantity of gaseous carbon dioxide in the apparatus after fermentation is found to be 0,006 cc.; after reduction to 0° C. and 760 mm. this volume remains the same. The volume of liquid was 0,021 cc. Consequently the total amount of carbonic acid of 0° and 760 mm. may be assumed to be 0,027 cc. This gives  $0,027 \times 4,45 = 0,12$  mgr. glucosehydrate, whereas we took 0,10 mgr.

3. In a similar way we obtained the following results by fermenting other solutions of glucosehydrate with *T. monosa*. The two corresponding numbers are placed one beneath the other.

|        |      |      |      |      |      |      |      |      |      |      |
|--------|------|------|------|------|------|------|------|------|------|------|
| Taken: | 1.89 | 1.77 | 1.41 | 1.35 | 1.59 | 1.59 | 1.83 | 1.74 | 1.89 | mgr. |
| Found: | 1.74 | 1.78 | 1.41 | 1.38 | 1.57 | 1.64 | 1.81 | 1.78 | 1.86 | mgr. |
| Taken: | 2.01 | 0.34 | 0.30 | 0.21 | 0.19 | 0.10 | 2.16 | 1.71 | 0.84 | mgr. |
| Found: | 1.99 | 0.38 | 0.29 | 0.26 | 0.22 | 0.12 | 2.20 | 1.65 | 0.93 | mgr. |

4. Solutions of glucosehydrate, fermented by *T. dattila* gave the following results:

|        |      |      |      |      |      |      |      |      |      |      |      |      |
|--------|------|------|------|------|------|------|------|------|------|------|------|------|
| Taken: | 1.29 | 2.67 | 1.62 | 2.04 | 1.68 | 1.32 | 1.89 | 1.56 | 1.65 | 1.98 | 1.89 | mgr. |
| Found: | 1.54 | 2.65 | 1.74 | 1.94 | 1.62 | 1.34 | 1.93 | 1.60 | 1.78 | 1.93 | 1.65 | mgr. |

5. In the same way we found by fermenting solutions of glucosehydrate with *S. cerevisiae* (press-yeast):

|        |      |      |      |      |      |      |      |      |      |
|--------|------|------|------|------|------|------|------|------|------|
| Taken: | 1.56 | 1.83 | 2.13 | 2.13 | 2.04 | 1.80 | 2.31 | 1.50 | mgr. |
| Found: | 1.62 | 1.90 | 2.18 | 2.14 | 2.20 | 2.16 | 2.38 | 1.82 | mgr. |

6. Quantitative determinations of fructose by fermenting with *T. monosa* gave us:

|        |      |      |      |      |      |      |
|--------|------|------|------|------|------|------|
| Taken: | 1.65 | 1.68 | 1.26 | 2.01 | 2.25 | mgr. |
| Found: | 1.58 | 1.69 | 1.23 | 1.97 | 2.10 | mgr. |

7. From similar determinations of fructose, fermented by *T. dattila* resulted:

|        |      |      |      |      |      |      |      |
|--------|------|------|------|------|------|------|------|
| Taken: | 1.47 | 1.62 | 0.99 | 1.44 | 1.89 | 1.50 | mgr. |
| Found: | 1.67 | 1.88 | 1.12 | 1.44 | 1.88 | 1.64 | mgr. |

8. The results of fermenting fructose with *S. cerevisiae* were these:

|        |      |      |      |      |      |      |      |
|--------|------|------|------|------|------|------|------|
| Taken: | 1.56 | 1.68 | 1.80 | 1.56 | 1.41 | 1.68 | mgr. |
| Found: | 1.51 | 1.81 | 1.80 | 1.60 | 1.15 | 1.63 | mgr. |

9. Saccharose, fermented with *T. dattila* gave:

Taken: 1.65 1.80 1.68 2.49 2.19 2.31 2.25 0.78 2.13 1.31 1.59 mgr.

Found: 1.66 1.76 1.76 2.47 2.27 2.20 2.17 0.82 2.13 1.38 1.67 mgr.

Taken: 1.86 1.71 1.62 1.59 2.31 1.74 1.31 1.65 1.77 1.77 2.10 1.83 mgr.

Found: 1.97 1.73 1.68 1.58 2.21 1.80 1.38 1.64 1.81 1.83 2.17 1.95 mgr.

10. Solutions of saccharose with *S. cerevisiae* gave the following numbers:

Taken: 1.41 1.53 2.19 2.25 2.70 1.23 2.13 1.50 1.59 1.59 mgr.

Found: 1.72 1.62 2.25 2.35 2.53 1.34 2.16 1.70 1.62 1.75 mgr.

Taken: 1.65 1.80 1.47 mgr.

Found: 1.93 1.90 1.59 mgr.

11. Solutions of maltose, fermented with *S. cerevisiae*:

Taken: 1.26 1.80 2.46 2.31 1.83 1.65 2.52 1.86 1.68 1.35 1.38 mgr.

Found: 1.38 1.72 2.32 2.32 1.72 1.52 2.42 1.66 1.55 1.30 1.51 mgr.

12. With a solution, containing 3 per cent. glucosehydrate, 3 per cent. saccharose and 3 per cent. maltosehydrate, we undertook three fermentations, viz. with *T. monosa*, *T. dattila* and *S. cerevisiae*. With *T. monosa* the carbonic acid obtained from 0,045 cc. of the solution was 0,326 cc., with *T. dattila* 0,633 cc. from 0,045 cc. and with *S. cerevisiae* 0,932 cc. from 0,043 cc.; all gasvolumes being reduced to 0° C, and 760 mm. From these numbers we calculate that of 1 cc. of the solution 7,1; 14,1 and 21,7 cc. of carbon dioxide will be obtained by each of the 3 yeasts. Consequently there were obtained 7,1 cc. from monoses (here from glucose-hydrate), 7,0 cc. from saccharose and 7,6 cc. from maltosehydrate. This means a composition of the solution of 3,1 per cent. of glucosehydrate, 2,7 per cent. of saccharose and 3,1 per cent. of maltosehydrate.

13. Other determinations with solutions of the same composition gave the following results:

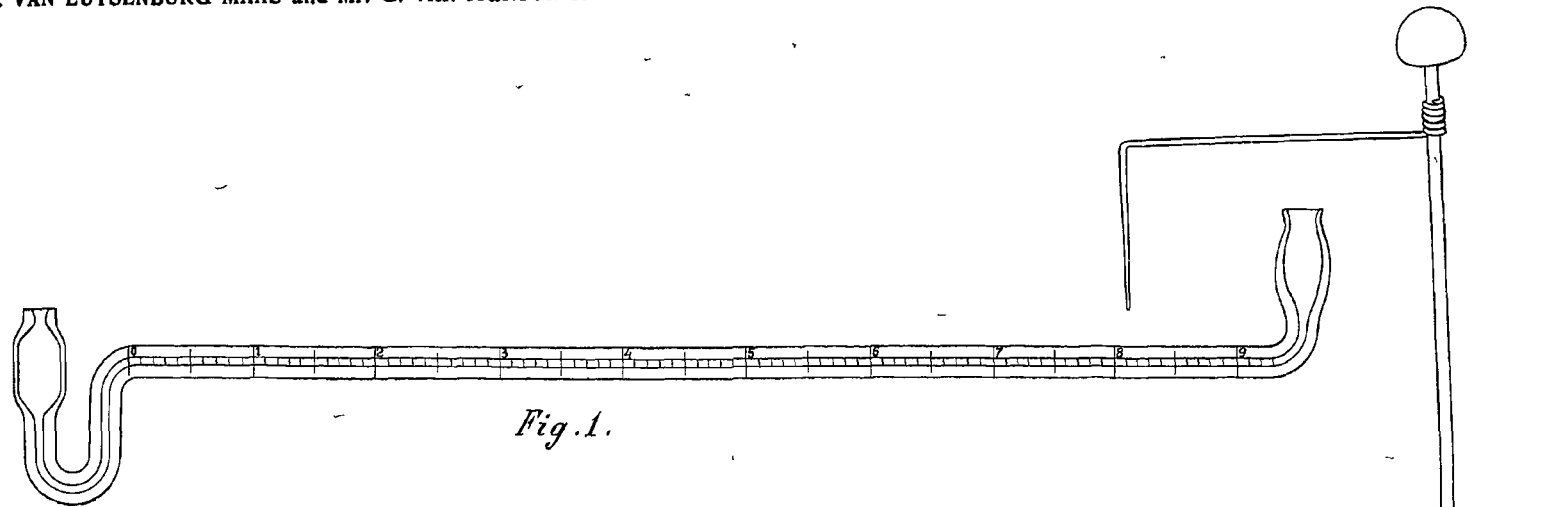
2,85 % glucosehydraat; 3,2 % saccharose; 2,7 % maltosehydrate.

2,98 % „ 3,19 % „ 2,84 % „

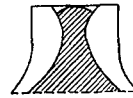
The three last numbers were calculated from the results of analysis, made in triplo.

14. Juice, pressed from a slice of orange, was diluted with water to the threefold of the original volume and the diluted juice was fermented with the three different yeasts. One cc. of this liquid practically gave the same amount of carbon dioxide, when fermented with *T. dattila* and with *S. cerevisiae*, so that maltose was absent. The composition of the undiluted sap was calculated as to be: 2,6 per cent of monoses and 3,1 per cent. of saccharose.

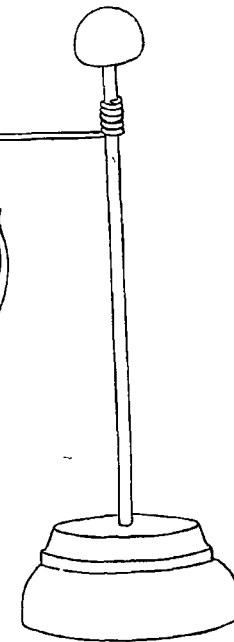
Miss H. J. VAN LUTSENBURG MAAS and Mr. G. VAN ITERSON Jr.: "A microsaccharimeter."



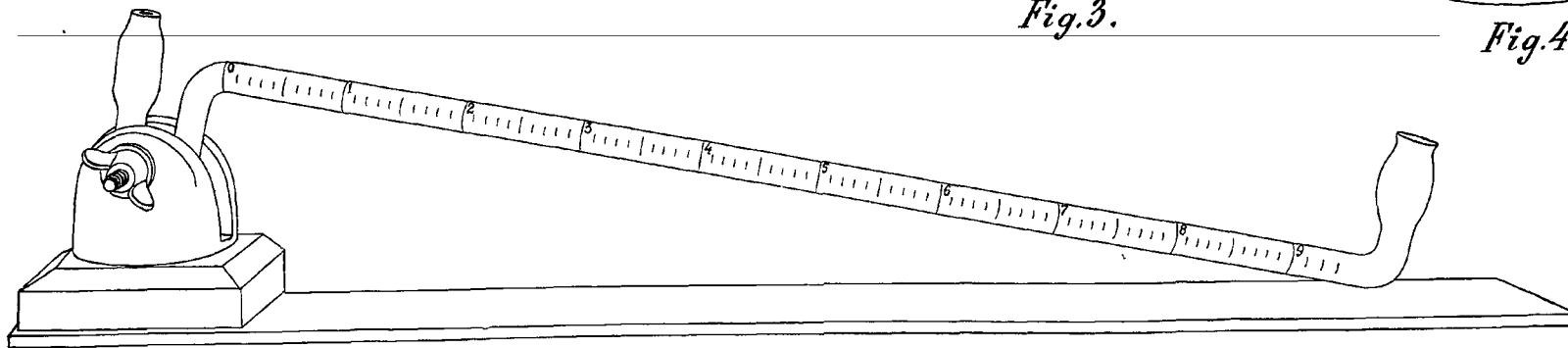
*Fig. 1.*



*Fig. 3.*



*Fig. 4.*



*Fig. 2.*

15. Nectar from *Nicotiana affinis*, after dilution to about the threefold of the original volume, was fermented with *S. cerevisiae*. Two drops from the same flower were brought into two small platinum scales and herein the water was added. (The weighings were made with a torsion-balance, accurate to 0,1 mgr.). The two analyses, made separately gave as results 33,9 and 34,4 per cent. of sugar in the undiluted nectar; the sugar being calculated as hexose.

In studying the numbers published here, one will see, that on the whole the results obtained with the microsaccharimeter, were quite satisfactory. Add to this, that sugar determinations by chemical analysis too are of no great accuracy, whilst here we took only a few milligrammes of sugar. For the study of a large number of biological problems the accuracy that was reached here, certainly will be quite sufficient.

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*Delft, July 1915.*

#### EXPLICATION OF PLATE.

- Figure 1. Longitudinal section of the glass apparatus of the microsaccharimeter.  
 Figure 2. General view of the microsaccharimeter (the glass apparatus, fixed in the cork clamp).  
 Figure 3. Longitudinal section of the neck (enlarged) of the microsaccharimeter, filled with mercury, as for the 1th. reading.  
 Figure 4. Auxiliary apparatus, which may be used to place the mercury in the tube and the reservoir on the same level.

**Chemistry.** — “*Investigations on the Temperature-Coefficients of the Free Molecular Surface-Energy of Liquids between  $-80^{\circ}$  and  $1650^{\circ}$  C.*” **X.** *Measurements Relating to a Series of Aliphatic Compounds.* By Prof. F. M. JAEGER and Dr. JUL. KAHN.

§ 1. For the purpose of comparison of the variations, which occur in the values of the molecular surface-energy of several derivatives of the aliphatic series, when simple substitutions have been made in them, it appeared necessary also to investigate in detail the surface-tension and its temperature-coefficient of the following compounds: *Ethyl-iodide, Ethylene-chloride, Ethylidene-chloride, Acetylene-tetrachloride, Acetylene-tetrabromide, Epichlorohydrine, Carbonbisulphide, Methylalcohol, Formic Acid, Mono-, Di- and Trichloroacetic Acid, Levulinic Acid, Nitromethane, Bromonitromethane, Capronitrile, Dimethylsuccinate, Diethylbromoisosuccinate, and Acetylacetone.*