THE ESTIMATION OF THE DIASTATIC POWER OF MALT, ETC.

BY W. J. SYKES, M.D., AND C. A. MITCHELL, B.A.

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AMONGST the different items which have to be ascertained in the examination of malt, the determination of its diastatic power is one of considerable importance, since the requirements of manufacturers in this direction vary considerably, according to the nature of the article they wish to produce.

Regarded from a diastatic point of view, malts may roughly be divided into four classes, in a gradually descending scale :

Green Malt.—This is barley fully germinated, and ready for placing on the drying-kiln. Since malt invariably suffers a loss of diastatic power during the drying process, however carefully and in whatever way this may be conducted, some

* See the 22nd and 23rd Annual Reports of the Local Government Board—Supplement containing the Report of the Medical Officer.

distillers, in order to avoid this loss, make use of malt in this form. By doing so they not only avail themselves of malt possessing the highest possible diastatic power, but at the same time save the cost of drying.

Air-dried Malt.—This is green malt dried at the ordinary temperature in a current of air. It is mostly used for experimental purposes, such as the preparation of diastase, etc.

Distillers' Malt.—In this the germination of the barley is pushed so far as to develop the diastatic power of the grain to its maximum degree. The green malt so produced is then dried slowly and carefully on the kiln, where it is never allowed to reach a temperature higher than 120° to 125° F. In this way loss of diastatic power is reduced to the minimum consistent with the production of a staple article.

Brewers' Malt.—In this the germination is not carried so far as in the before-mentioned malts, consequently the diastatic power is not developed to the fullest extent. Though in the initial stages of the drying process the heat is applied very slowly and gradually, towards its termination the temperature is allowed to rise, for pale malts, to 180° F., and for high-dried malts to as much as 210° to 220° F. The malt in these latter stages undergoes a sort of cooking process, which largely develops its flavour and aroma. During this portion of the drying much of its diastatic power is lost, but with malts intended for the brewing of beer, it is necessary that such should be the case.

From time to time various processes have been proposed for the estimation of the diastatic power of malt, many of which are of a more or less crude and unsatisfactory nature. The first really efficient process was that devised by Kjeldahl (*Comptes rendus des Travaux de Carlsberg Lab.*, 1879). In a series of investigations on the action of diastase on starch, he had discovered that in such transformations the amount of reducing substance formed was strictly proportional to the amount of diastase, so long as the amount produced in the mixture was not allowed to exceed 45 per cent. of the total possible yield. In other words, to comply with what is now known as "Kjeldahl's law of proportionality," the diastase must not be present in a quantity greater than will convert a little less than half the starch present.

For the actual determination, 10 grammes of the malt, after being finely ground, were extracted with 400 c.c. of water for six hours; 15 c.c. of the bright filtrate from this were added to 200° c.c. of a 3 to 4 per cent. solution of soluble starch, heated to a temperature of from 57° to 59° C., at which temperature the whole was kept for twenty minutes. The amount of reducing substance reckoned as maltose was then determined either gravimetrically, volumetrically, or colorimetrically, and from this the amount of reducing substance contained in the original soluble starch solution was deducted. The diastatic power was considered to equal 100, when 15 c.c. of malt-extract of the above strength and under the above conditions yielded an increase of cuprous oxide equivalent to 1 gramme of maltose. The soluble starch used in Kjeldahl's process was prepared by allowing an aqueous extract of malt to act upon gelatinized starch at a high temperature (180° F.). In such a case, those portions of the diastase, which convert the starch into bodies capable of reducing Fehling's solution, are almost completely destroyed, and only the liquefying portion of the diastase remains intact—that is, the portion which converts gelatinized starch into its soluble

modification. However carefully the operation may be performed, some reducing bodies are invariably produced, hence it is necessary to determine their amount in each fresh batch of solution employed; and as soluble starch solution is unstable, it must be prepared as wanted, and this is a distinct inconvenience.

Some ten years later C. J. Lintner discovered that soluble starch could be readily prepared in a stable form by the limited action of various acids upon starch, a fact which he practically applied to the simplification of Kjeldahl's process.

Lintner's soluble starch is prepared by allowing potato starch to stand under 7.5 per cent. hydrochloric acid for seven days at the ordinary temperature, or for three days at a temperature of 104° F. The same result can be obtained, according to Brown and Morris (*Trans. Chem. Soc.*, 1889, p. 450), in twenty-four hours, at the ordinary temperature, by employing 12 per cent. hydrochloric acid. The starch is then washed until free from acid, dried in the air, and preserved for use. Under this treatment the starch completely loses its power of forming a paste; and though it is almost insoluble in cold water, is readily soluble in that medium at temperatures over 60° or 70° C., from which it again very slowly separates on cooling. On microscopic examination no change can be detected in the appearance of the starch granules either with ordinary or with polarized light. Its solution has not the slightest reducing action upon Fehling's solution.

For the estimation of the diastatic power of a substance, a 2 per cent. solution of this soluble starch is employed. It is best prepared by placing the required amount of water in a flask, and the weighed quantity of soluble starch in a smaller beaker. Sufficient water is poured from the flask on to the soluble starch to form a mixture of the consistence of thick cream, the remainder of the water in the flask brought to near its boiling point, and the well-stirred contents of the beaker poured in, when the soluble starch dissolves immediately. A little of the hot solution is poured into the beaker to dissolve any remnants of starch, and returned to the contents of the flask, which are then allowed to cool.

Twenty-five grammes of malt are finely ground in a coffee-mill and stirred into 500 c.c. of water. The mixture is allowed to stand for six hours, with an occasional stir. The solution is filtered through a dry filter-paper, the first cloudy portions rejected, and about 50 c.c. of the bright filtrate (malt-extract) collected.

Ten test-tubes, of a capacity of about 20 c.c., are taken, and 10 c.c. of the cold 2 per cent. soluble starch solution pipetted into each. To the first of these is added 0.1 c.c. of the malt-extract, to the second 0.2 c.c., and so on, so that the tenth test-tube receives 1.0 c.c. After each tube has been well shaken, the whole are allowed to stand for one hour in a water-bath kept at a temperature of 70° F. At the expiration of this period, 5 c.c. of Fehling's solution are added to each, the tubes well shaken, and immersed in boiling water for twenty minutes, after which they are taken out and examined. It will perhaps be found that the contents of one of the tubes are colorless, in which case the amount of malt-extract added to this tube is taken for the subsequent calculation. Oftener the contents of one tube are yellow, the next one blue; in this case the average of the amounts added to the two tubes is taken.

The diastatic power is calculated on the basis that when 0.1 c.c. of malt-extract

of the above strength added to 10 c.c. of 2 per cent. soluble starch solution, and treated in the above manner, yields just sufficient reducing substance to exactly reduce 5 c.c. of Fehling's solution, the diastatic power of the malt yielding the extract shall be taken as 100. The diastatic power of any other malt is found by a sum in inverse proportion. For instance, if in such a determination the end point is found to lie between the sixth and seventh tube, the amount of malt-extract to be taken would be $0.6 + 0.7 \div 2 = 0.65$, and $\frac{100}{0.65} \times 0.1 = 15.55$. This figure must then be calculated upon water-free malt : supposing the malt contained 4.5 per cent. of moisture, then $\frac{100}{95.5} \times 15.55 = 16.23$ expresses the diastatic power of the malt on Lintner's scale.

In the examination of green malts, where the diastatic power often exceeds 100, the malt-extract is diluted with an equal volume of water before being added to the soluble starch tubes. The result is then multiplied by 2.

For determining the diastatic power of precipitated diastase, a solution of 0.03 grammes of the substance in 25 c.c. of water takes the place of the malt extract. Consequently, the energy of a precipitated diastase is considered as 100 when 0.12 milligramme yields just sufficient reducing substance to decolorize 5 c.c. of Fehling's solution under the above experimental conditions. This quantity was fixed upon because it represented the power of the most energetic diastase which had been then obtained. In recent times Osborne (ANALYST, xx. 232) has prepared a diastase six hundred times as energetic as this. In examining such specimens, the diastase solution has to be made correspondingly weaker ; the right proportion is found by preparatory trials of solutions of various strengths, in which 0.1 and 1.0 of the solution are respectively added to 10 c.c. soluble starch solution. When one of these after treatment with Fehling's solution shows over, and the other under, reduction, the solution of diastase is of the proper strength. The degree of dilution must obviously be taken into account in the subsequent calculation.

For conducting experiments of this kind, the star-apparatus of Reischauer, illustrated in the accompanying engraving, is exceedingly convenient. A special pipette, graduated so as to deliver quantities gradually increasing from 0.1 c.c. to 1.0 c.c. by 0.1 c.c., is also useful.

It is obvious that in the above method there are only ten definitely fixed points, and consequently a definite result can only be obtained in the operation when one of the tubes is exactly decolorized, and this rarely happens in practice. Should the end point lie between two tubes, in order to obtain a more approximate result the operation must be repeated with gradually increasing quantities of malt-extract, commencing with the quantity which caused incomplete reduction,

and ending with that which produced over-reduction. Should the end-point again fall between two tubes, the operation has to be performed a third time, and so on.

In the determination of the diastatic power of brewers' malts, which range from 25 to 45, since the end-point does not fall between the first and second tube, the numbers found are, as a rule, sufficiently approximate for practical purposes, and in any case do not require more than one repetition of the operation. With distillers' malts the case is different; the end-point generally falls between the first and second tube, and between these there is a wide gap—from 50 to 100.

In order to avoid several repetitions of the operation, and to obtain the desired result in one determination, we suggest a slight modification, which to some extent combines the methods of Kjeldahl and Lintner. The soluble starch solution, malt-extract, and diastase solution, are all prepared in the manner mentioned before. Instead, however, of employing a number of tubes, we make use of one small wide-mouthed flask, of a capacity of about 200 c.c. Into this 100 c.c. of the soluble starch solution are placed, 1.0 c.c. of the malt-extract added, the whole well shaken, and allowed to stand at a temperature of 70° F. for one hour; 50 c.c. of Fehling's solution are then added, the flask, with its mouth covered with a watch-glass,* placed on wire-gauze over a Bunsen-flame, and heated up to 98° C. It is then removed from the flame, and immersed in boiling water for seven minutes. It has been found experimentally that by proceeding in this way the contents of the flask are submitted to conditions of temperature and time closely approximating those of the test-tubes employed in Lintner's method. The cuprous oxide is collected in a Soxhlet filter-tube, reduced in hydrogen, and weighed. The weight of the copper found, divided by 0.438 (the quantity of copper contained in 50 c.c. of Fehling's solution), and multiplied by 100, gives the diastatic power. In this way a determinate figure is obtained for any diastatic power between 0 and 100 by one operation.

In the following table we give the results of a number of experiments which were made with a view to determining how the figures obtained in this way agree with those obtained by Lintner's method:

Malt.	Moisture per cent.	Extract lbs. per quarter.	Diastatic Power.		Remarks.
			Lintner's Method.	Sykes and Mitchell.	
1. English ...	—	—	5	3.3	Old musty sample.
2. Smyrna (Black and White, 1892)	—	—	10	11.2	
3. Foreign ...	3.8	85.34	12	15	
4. Syrian ...	6.7	78.96	12	15.7	
5. English ...	12.7	—	17	22	Old sample.
6. Syrian ...	4.01	85.0	19	22.7	
7. English ...	4.17	90.72	21	22.8	Said to be low dried.
8. Yorkshire (1892)	10.1	—	25	24	
9. Foreign ...	7.3	77	40	38.7	
10. English ...	4.7	94	77.2	83	Dried at 125° to 130° Fahr.

* Bearing in mind Kjeldahl's experiments (ANALYST, xx. 227), in some cases a current of hydrogen was passed through the flask during the heating process, in others the flask was simply covered with a watch-glass. This latter precaution was found sufficient to prevent the disturbing influence of the atmospheric oxygen.

The time required for making an estimation, amounting to about eight hours in all, is often a very inconvenient one; but we have found that the malt-extract may be filtered after the six hours, and the filtrate left until the next day without affecting the result. This is shown by the results given in the following table:

Sample.	Estimation immediately after filtering.				Estimation next day.	
	Lintner's.		S. and M.		Lintner's.	S. and M.
3	12	...	15	...	14	16
5	17	...	22	...	16	18
6	19	...	22·7	...	19	—

From these examples it will be seen that the figures obtained by our method agree fairly well with those obtained by Lintner's. It is probable that the former are the more accurate, since under the conditions of the process Kjeldahl's law of proportionality is strictly adhered to, and the figures obtained by Lintner's method are often the average between two tubes.

As the whole process is to a great extent of an empirical nature, the results obtained by either modification would possess the same utility for practical purposes. We can, however, fairly claim that a closer result is obtained in a shorter time by our method, and that it is especially useful when malts having high diastatic powers have to be dealt with.

DISCUSSION.

Mr. A. R. LING said that he was glad to hear that the authors regarded the method as a purely empirical one, and, in his opinion, it must remain so. It had been stated by several observers—and his own experiments in conjunction with Mr. J. L. Baker bore out that statement—that when some malts were made to act on starch paste, a certain amount of glucose was found among the products of the transformation, and, in the case of these malts, of course, Kjeldahl's law of proportionality—on which the method of determining diastatic powers was based—would not hold. In the method as usually carried out the diastatic solution (malt extract) was allowed to act on the solution of soluble starch at practically the ordinary temperature. But, inasmuch as the mashing temperature adopted in brewing was considerably above this, he thought that the determination of the diastatic capacity of a malt at the particular temperature at which it was to be afterwards mashed would perhaps furnish information of greater practical utility. He had used an ordinary titration method for determining the diastatic power of malt, and found that it yielded results which compared favourably with those obtained by Lintner's method. With regard to malts of very high diastatic power, in Lintner's method the value indicated between the first two tubes—containing 0·1 and 0·2 c.c. of malt extract respectively—was 100–50; and he should like to know the accuracy which could be attained by the author's method with malts of this class.

Mr. A. C. CHAPMAN asked if the authors could give any information as to the working of the process in connection with distillers' malts of very high diastatic power, viz., higher than 77, which was the highest mentioned in the table, as he understood that the process had been devised more particularly to meet the requirements of such cases. In the examples given the agreement of the numbers with those obtained by Lintner's method was certainly good. He also pointed out that if it were permissible to allow the aqueous malt extract to stand overnight in this case, the same might be

said of Lintner's method, for the experiments were up to this point carried out in practically the same manner in the two methods.

The suggested correction for the reducing substances in the aqueous malt extract had always appeared to him to be unnecessary in view of the limits within which it was possible to reach the differences between the various tubes.

With regard to the estimation of the amount of copper obtained, he had obtained perfectly satisfactory results by weighing the cuprous oxide direct, which was of course much simpler and quicker than reduction in hydrogen. He used a platinum Gooch's crucible, and filtered off and washed the oxide with the aid of a filter pump. The time occupied in filtering, washing, drying and weighing, did not amount to many minutes.

Dr. SYKES, in reply, said that although the method they had described, as with all others on the same principle, could not be said to be an absolute measure of the diastase, still, since Lintner's figure was usually an estimate between two tubes, he thought the figures they obtained were more determinate.

With regard to the reducing bodies formed, he thought it was exceedingly probable that they did vary materially in different cases, but, as the whole subject of the transformation of starch under the action of diastase was still involved in considerable obscurity, it was impossible to say what the differences were.

Mr. Ling's proposal to conduct the diastase determinations at the temperature at which the malt would be used in actual practice, was certainly a point worthy of consideration. It might probably be found that such a course would yield results more in accordance with the behaviour of the malt in the mash-tun.

With regard to Mr. Chapman's inquiry about distillers' malts, such samples seemed somewhat difficult to meet with, and the figures referred to in the tables were the highest they had been so far able to obtain.

There was no objection to letting the filtered malt extract remain overnight when Lintner's process was employed.

They had estimated the copper by reduction in hydrogen, because the almost universal consensus of opinion appeared to favour the view that this was the most exact method. If no oxidation of the cuprous oxide took place in Mr. Chapman's method, then, by an alteration of the factor, it would serve equally well.