MEETING OF THE LONDON SECTION HELD AT THE CHARING CROSS HOTEL, STRAND, W.C.2, ON MONDAY, NOVEMBER 10TH, 1930.

MR. H. W. HARMAN (in the Chair).

The substance of the following paper was given in an Address and discussed under the title :—

THE PRACTICAL APPLICATION OF THE RESULTS OF RESEARCH TO THE PRODUCTION OF MALT AND WORT.

BY L. R. BISHOP, M.A., PH.D.

THE study of wort composition provides a very wide range of problems of such complexity that the greatest caution is necessary before the results can be claimed to be of practical application. Among these problems that connected with the nature of the nitrogenous constituents of wort, their variation under different malting and mashing conditions and their availability as yeast nutrients is not the least important. An investigation of the nitrogenous constituents of wort with particular reference to the nutrition of yeast was undertaken by the late Professor S. B. Schryver and Miss E. M. Thomas with the objects outlined in an earlier Address to this Section (this *Journ.*, 1928, 532). The interruption of this work by the much regretted death of Professor Schryver prevented the publication of a finished Report, but sufficient data had been obtained to make it desirable to collect them and consider the results with some of my own work at Rothamsted. The present occasion offers an opportunity to give an account to members of the Institute of what has been done, in order to show that the subject is receiving its due share of attention and to have the benefit of their practical opinions at this stage. The data disclose broad regularities in the nitrogenous composition of wort analogous with that of barley (this *Journ.*, 1928, 101), and indicate similar regularities in the carbohydrate composition. They suggest interesting applications in an analytical method for determining the degree of modification of malt and the amount of yeast feeding material available. In addition they appear to be sufficiently definite and suggestive of further lines of investigation to make it desirable to collect them in an accessible form.

The present writer felt himself in a somewhat difficult position when he agreed to collate such material as was available, and it will be obvious that his discussion of results, obtained largely by others, must be of a very different nature from a Report such as Professor Schryver would have written had he been able to carry the research through to a conclusion. It will therefore be clearly understood that the object is to present suggestions as to the possible applications of these results, and to indicate such further lines of investigation as appear to arise from them.

All the nitrogen analyses referred to were carried out by Miss Thomas according to methods described by S. B. Schryver and E. M. Thomas (this *Journ.*, 1929, 571). These analytical methods are not entirely satisfactory, and will no doubt be modified when the investigation of wort composition is continued. The results are, however, comparative and reveal many points of interest, but it may be noted that the Van Slyke method employed, measures only certain of the nitrogen groups which occur in amino acids. The peptide values are lowered also by the interaction of amino acids with the carbohydrates present during the acid hydrolysis employed in the estimation. The total amount of nitrogen in compounds with peptide linkages would probably be about 50 per cent. greater than that recorded as "peptide nitrogen," and this would account for some of the nitrogen recorded as "undetermined nitrogen." All the analyses were made on bright worts from which the coagulable proteins had been removed by boiling for 15 minutes and filtering.

In addition to the analyses which serve as the basis of discussion in this paper, Miss Thomas had made a number of others on bright boiled worts from English malts

mashed at 150° F. at the rate of 160 grms. in 700 cc. of distilled water, the volume being finally made up to one litre. Considerable variation in the distribution of nitrogen was found but no attempt is here made to correlate it with variations in the malts or mashing conditions. The extreme figures for each nitrogen group and the average for twelve analyses are given in Table 1.

TABLE I.

Variation and average nitrogen distribution in twelve worts.

Nitrogen groups.	Percentages of the total permanently soluble nitrogen.	
	Extreme variation	Average.
Ammonia nitrogen ..	2·3 — 4·4	3·1
Amino nitrogen ..	15·5 — 20·0	17·8
Amide nitrogen ..	7·2 — 9·2	8·1
Peptide nitrogen ..	20·9 — 33·9	28·2
Undetermined nitrogen	37·1 — 46·8	42·8

Percentages of the total permanently soluble nitrogen, varying between 53 and 63, were thus allotted to the various groupings determined. The residual nitrogen, ranging between 37 and 47 per cent. of the total wort nitrogen, is referred to as "undetermined nitrogen." A proportion of this is due to the nitrogen of amino and peptide compounds not determined by the Van Slyke and hydrolytic methods employed. Another proportion, probably existing in a colloidal state, may have important effects in brewing though it does not appear to be utilised by the yeast during fermentation, as far as can be judged from other analyses made from worts before and after fermentation. The results of these analyses showed that a considerable proportion of the groups determined was removed during fermentation, but, as no estimate can be formed, from the experiments recorded, of the nitrogen excreted from the yeast, no definite information on the actual assimilation by yeast can be deduced at this stage. The matter may be complicated further by the precipitation of nitrogen. The analyses before and after fermentation merely show the balance between assimilation plus precipitation and excretion. On an average one third of the nitrogen in the determined groups was found to be removed during fermentation, but the fraction for the individual groups varied considerably on each side of this. The largest difference before and after fermentation was in the peptide group which was originally present in largest quantity.

Interesting and possibly important results of this work were indicated by a few experiments dealing with the removal of nitrogen from bright boiled and filtered wort by adsorption on various substances. Thus it was found that quantities varying between 14 and 34 per cent. of the total nitrogen of the bright wort were removed by treatment with adsorbent charcoal. Analyses made before and after such treatment revealed no differences in the percentages of nitrogen in the various determined groupings. From this it might appear that the adsorbed colloidal nitrogen was derived entirely from the so-called "undetermined nitrogen." The reduction in "undetermined nitrogen" by treatment with adsorbent charcoal varied between 31 and 50 per cent. of its original quantity.

A further possible application of the experiments in which wort was treated with adsorbent charcoal follows from the fact that during fermentation slightly larger quantities of "determined nitrogen" were removed from the treated wort than from the corresponding untreated wort. In some of the fermentation experiments the quantity of "undetermined nitrogen" removed, I suggest, by deposition or adsorption on the yeast, was also approximately equal to that removed from the same wort by adsorption methods. This naturally raises interesting speculations as to whether yeast becomes coated and as to the effect of this on fermentation. It is hoped to investigate the suggestion that the coating of yeast with colloidal nitrogen compounds may be responsible for the rising of yeast in top fermentation.

In four fermentation experiments analyses were made at daily intervals with the very striking result that a balance between the nitrogen removed from the wort and that taken up by the yeast could not be obtained at the beginning and end of fermentation: the wort and yeast having been analysed by the Kjeldahl process involving digestion with sulphuric acid, potassium sulphate and copper sulphate. Very close agreement was, however, obtained between removal and assimilation (in which is included precipitation) on the second or third day of fermenta-

tion, as is shown by a typical example of these analyses given in Table 2.

TABLE 2.
Nitrogen removed from wort and gained by yeast.

Days fermented.	Nitrogen removed from wort, grm. per 100 cc.			N. gained by yeast filtered from 100 cc. wort.	Difference.
	N. in groups.	Undetermined.	N. Total N.		
0	0·0310	0·0410	0·1020	0·0025	—
1	0·0037	0·0021	0·0118	0·0033	0·0025
2	0·0119	0·0045	0·0164	0·0146	0·0018
3	0·0160	0·0030	0·0190	0·0189	0·0001
4	0·0175	0·0039	0·0214	0·0203	0·0011
5	0·0185	0·0041	0·0226	0·0195	0·0031

The line for 0 days gives nitrogen originally present in 100 cc wort and in yeast added to 100 cc. wort.

The figures for the wort represent the difference between the nitrogen in the various categories in the original wort (0) and that found on the day specified. Those for the yeast represent the increase in the total nitrogen of the yeast recovered from 100 cc. wort over that in the yeast originally added to this volume, as determined by the ordinary Kjeldahl method. The nitrogen apparently gained by the yeast (including that precipitated as well as assimilated) was less than that removed from the wort in the early and later stages of the fermentation, but the balance was very even on the third day.

The explanation suggested for the apparent discrepancy, following E. I. Fulmer and L. M. Christensen (*J. Phys. Chem.*, 1925, **29**, 1,415) is that in the early and later stages of yeast growth a greater part of the nitrogen may be in the form of ring compounds which it is known cannot be analysed by the usual Kjeldahl methods. At intermediate stages of fermentation these compounds appear to be transformed into others amenable to the analysis used. The low results for yeast nitrogen found after that would apparently correspond to renewed formation of ring compounds. It would appear possible that the amount of these ring compounds may be related to the fermentative activity of the yeast, that they increase as the yeast comes to a resting stage and decrease as it becomes active.

Christensen and Fulmer (*Plant Physiology*, 1927, **2**, 455) devised a modification of the Kjeldahl process, involving digestion with hydrogen peroxide, by which they obtained a complete recovery of the nitrogen in yeast, which was shown by the agreement of the results with those obtained by the Dumas method. Preliminary experiments with this method indicate that these authors were justified in their contention. With yeast the differences between the results of the ordinary Kjeldahl process and those obtained by the Christensen and Fulmer modification are considerable, this suggests that a large proportion of extant figures for yeast nitrogen content may possibly be incorrect. This question is being subjected to a thorough investigation in connection with the Yeast Research.

EXPERIMENTAL.

The results which it is proposed to discuss more fully in this paper were obtained by analysis of boiled filtered worts. 200 grms. of malt, ground and weighed in the manner laid down for the Standard methods of malt analysis (this *Journ.*, 1922, 775) were mashed for one hour with 700 cc. of distilled water at the temperature necessary to give the mashing temperature specified for each experiment. The mash was made up to 1 litre at 60° F., filtered, boiled for 15 minutes, adjusted to volume and again filtered. Determinations were made of the specific gravity, optical rotatory power and reducing sugars, the last by the method of J. H. Lane and L. Eynon (*J. Soc. Chem. Ind.*, 1923, **42**, 32T). The results of this estimation are expressed as "apparent maltose," although other reducing sugars are known to be present. The total solids in the wort were calculated by using the solution factor 4·0. In addition analyses to determine the nitrogen distribution in the wort were made by Miss Thomas by the methods previously referred to.

The malts selected for these experiments were made for the Barley Research with barley from the same pure line of Spratt-Archer seed grown in 1928 at Wellingore (Lincoln Heath) and Rothamsted (Harpenden, Herts.), and from Plumage-Archer barley grown in 1928 at Sprowston (Norwich). These barleys were selected on account of their widely different nitrogen contents, and they were all malted under the same conditions in "stocking." F.112, a six-rowed winter barley raised by Dr. Beaven, and grown in Wiltshire, was also used in some experiments. This barley was malted in a thirty quarter bulk in an ordinary floor malting. The Californ-

ian and Chilean malts were commercial brewing malts obtained from maltsters; 405B, 414B, 404B and 426/9B were bulk malts made for the Institute Researches from Spratt-Archer barleys grown in 1927 and 1928.

A. Analyses of Worts from Different Barleys Mashed at 65° C.

(1) *Total Permanently Soluble Nitrogen.*—Before discussing Miss Thomas's estimations of the separate nitrogen fractions in wort it is first of all necessary to discuss the variations in total permanently soluble nitrogen. Earlier work by H. T. Brown (this *Journ.*, 1909, 169) has shown that for ordinary malting conditions the total permanently soluble nitrogen content of a wort is an approximately constant proportion of the total nitrogen content of the barley or malt, *i.e.*, under normal malting conditions the amount of permanently soluble nitrogen is governed by the nitrogen content of the barley.

The total permanently soluble nitrogen found in a wort varies with the concentration of the mash, a point which will be referred to later. The figures now given refer to the nitrogen found in the wort obtained by the standard method for determination of extract of a malt, after a known volume has been boiled for a quarter of an hour during which the volume was kept approximately constant by occasional additions of water. The boiled wort was cooled, made up to volume, filtered and the nitrogen determined by the Kjeldahl method.

A normally modified "stocking" malted English two-rowed barley mashed under these conditions yields an amount of permanently soluble nitrogen around 35 per cent. of the total nitrogen of the dry barley. In the case of Californian Bay brewing malt, the permanently soluble nitrogen amounts to about 29 per cent. of the total nitrogen of the dry barley. The figures of the Institute of Brewing Research barleys of 1922, already published (*ibid.*, 1924, 969) agree with this in showing about 35 per cent. for a wide range of Plumage-Archer barleys.

The above figures are derived from a study of a very large range of "stocking malts." They are probably rather higher than those given by normally modified bulk floor malts. From the available data, it appears that figures of 33 per cent. for "two-rowed" malts and 28 per cent. for "six-rowed" malts respectively, are more applicable to practical conditions of floor malting.

The figures given above refer to malts which have been modified to the extent usual in practice in this country, but it is known that the percentage of permanently soluble nitrogen can be varied by changes in the malting conditions. The figures obtained for the "stocking" malted Institute of Brewing barleys for 1922, 1923, 1924 and 1925, show considerable variations. The malts of each individual year were made under identical conditions, from a wide variety of barleys and, consequently, if some of the malts of each year were normally modified, others would necessarily be either over- or under-modified. Figures lower than the normal for the permanently soluble nitrogen as a percentage of the total nitrogen of the barley would correspond with under-modification and higher figures with over-modification. The average percentages for all the barleys malted in one year may be taken as an indication of the average modification for that year. These averages are given in Table 3. The analyses of the 1922 barleys from which the averages were calculated have already been published (*ibid.* 1924, 973). The figures available for 1923, 1924 and 1925 are collected in an appendix, page 358.

A statistical examination of the relation existing between the nitrogen content of barleys and the extracts of their malts (*ibid.*, 1930, 421 and *Roth. Mem.* 15) led to the calculation of equations by means of which the extract which should be obtained from any barley could be predicted from the nitrogen content and thousand corn weight by the use of a factor for each of the latter quantities; and it was found that the "nitrogen factor" for any series of barleys of one variety malted under constant conditions, varied with and, consequently, was a measure of these constant conditions or of the resultant degree of modification. The "nitrogen factors" for the "stocking" malts of 1922, 1923, 1924 and 1925 are given in Table 3, with the average for the year of the permanently soluble nitrogen percentage on total nitrogen of the dry barley.

The "nitrogen factors" calculated for the 1922 and 1923 barleys suggested that the malts made in "stocking" from these barleys had an average modification. This is supported by the normal percentage of per-

TABLE 3.

NITROGEN RELATIONSHIPS AND MODIFICATION.

Year.	1922.	1923.	1924.	1925.
N. factor in extract equation	9·0	6·8	5·8	12·0
Average permanently soluble N. as per cent. of total N. (standard mash)	34·4	35·1	33·5	35·9

manently soluble nitrogen which the malts gave (calculated on the total nitrogen content of the dry barleys). In 1924, on the other hand, the corresponding barleys appear to have been undermodified. This is suggested (*a*) by the small nitrogen factor in the extract prediction equation for these barleys, (*b*) by the relatively low average percentage of permanently soluble nitrogen on total barley nitrogen, and (*c*) made more conclusive by a note in the maltsters report (*ibid.*, 1925, 601) stating that "it is probable that another day's flooring would have increased the extracts." The experimental malts made from the 1925 barleys appear to have been somewhat over-modified on the average (*a*) from the large nitrogen factor in the extract prediction equation, and (*b*) from the high average percentage of permanently soluble nitrogen which was obtained in spite of the presence of dead corns in some samples.

Information is thus gained from two quite independent sources of analytical information on the conditions under which the barley has been malted, or of the degree of modification of the malts. The extent to which they agree is indicated in Table 3. The average permanently soluble nitrogen in 1922 and 1925 is almost certainly lowered by the presence of a large number of dead corns in some of the samples.

The suggestion is made that there is a "chemical modification" of the nitrogen compounds in barley which runs approximately parallel with the "physical modification" (cytase action). High nitrogen barleys are more difficult to modify, both chemically and physically: that is, to reach the same degree of modification they need a longer time or a higher flooring temperature than low nitrogen barleys. This is well illustrated in a series of three barleys which were malted under the same conditions in stockings buried in the same piece. These barleys are referred to in Table 4 as "Norwich" (low nitrogen content), "Wellingore" (medium nitrogen content) and "Rothamsted" (high nitrogen content). Physical examination indicated that the Wellingore malt was normally modified, and the Rothamsted malt undermodified.

TABLE 4.

ANALYSES OF BARLEYS AND MALTS.

	Norwich.	Wellingore.	Rothamsted.
Nitrogen per cent. on dry barley	1·242	1·562	1·926
Perm. sol. N. as per cent. total N.	41·3	34·2	32·3
Cold water extract, per cent.	25·2	21·7	22·7
Diastatic power (Lintner)	40·0°	63·5°	77·0°

The permanently soluble nitrogen of the Norwich malt, 41·3 per cent. of the total nitrogen of the barley, is greatly in excess of that previously referred to as corresponding with normal modification, and the percentage of fully grown corns indicated that this was considerably over-modified. The acrospire counts of the Wellingore malt indicated that this was irregularly grown, but the average growth and the permanently soluble nitrogen correspond more closely with a normally modified malt. The permanently soluble nitrogen of the Rothamsted malt suggests its relative under-modification but the value is somewhat lowered by the percentage of dead corns. P. Petit (*Bull. Ecole Brass. Nancy*, 1907, No. 9) gave evidence that there is a relation between the acrospire length and the percentage of permanently soluble nitrogen, and these examples with widely spaced nitrogen contents support the existence of such a relation, but he has since abandoned his studies as he found that the relation varied from year to year.

Certain further considerations arise from Table 4. The cold water extracts here are high in each case corresponding partly to the overgrowth and partly to the fact that this figure is usually high in stocking malts. The permanently soluble nitrogen in the Wellingore and Rothamsted malt is slightly reduced by the dead corns. The malts were cured very lightly indeed and in consequence the diastatic powers were exceptionally high. As the diastatic power increases with the nitrogen content, the

high diastase (before kilning) would tend to raise the matter soluble in cold water in high nitrogen barleys by increasing conversion during germination although the speed of modification is reduced. This will explain why the matter soluble in cold water of the Rothamsted malt is higher than that of the Wellingore. The cold water extract is consequently unreliable as a measure of modification.

The dependence of the permanently soluble nitrogen percentage on the length of time on the floor is shown also by Schjerning's results at normal malting temperatures. See Tables 7 and 8 (pp. 352 and 353).

Mash concentration.—R. Wahl and A. Nilson (*Amer. Brew. Rev.*, 1894, 7, 579) quoted by *Schjerning* (*Compt. rend. Lab. Carlsberg*, 1912, 9, 298), H. Schjerning (*ibid.*) and J. H. Oliver (this *Journ.*, 1929, 191) have already shown that the percentage of permanently soluble nitrogen is affected by the concentration of the mash. In Table 5 a comparison is made of the permanently soluble nitrogen of various malts mashed at the rate of 200 grms. per 700 cc. and made up to 1 litre, and at the standard rate of 50 grms. in 360 cc. made up to 515 cc., allowance being made for the volume of the grains. A greater proportion of the nitrogen is dissolved in the concentrated mashes. The figures given here are regular enough to suggest that a 29 per cent. mash when compared with a standard mash (approximately 14 per cent.) gives an increased amount of wort nitrogen corresponding to an increase of 2 per cent. calculated on the total nitrogen of the barley.

TABLE 5.

PERMANENTLY SOLUBLE NITROGEN IN WORTS FROM 29 PER CENT. AND 14 PER CENT. MASHES.

	Norwich.	Wellingore	426/9B.	Rothamsted	F112
Perm. Sol. N. per cent. on dry malt—					
29 per cent. mash	0·540	0·576	0·518	0·642	0·465
14 per cent. mash	0·514	0·535	0·486	0·622	0·440
Perm. Sol. N. as per cent. on Total N. of barley—					
29 per cent. mash	43·5	36·8	33·2	33·4	35·4
14 per cent. mash	41·3	34·2	31·1	32·3	33·5
Difference	2·2	2·6	2·1	1·1	1·9

The permanently soluble nitrogen of malt as a measure of modification.—None of the usual tests for the degree of modification of malt is entirely reliable, and they fail to demonstrate over-modification. The main point in this section of the paper is the suggestion that *the permanently soluble nitrogen when calculated as a percentage on the total nitrogen content of the barley may be used as a measure of modification.* It is, however, made with certain reservations. The amount of permanently soluble nitrogen present is a balance between the amounts broken down and the amounts resynthesised and this balance is liable to be changed under the influence of many factors, for instance by variations in flooring temperatures, as is illustrated below from the work of Schjerning ; but the limited range of malting conditions ordinarily in use in any particular maltings permits of the elimination of many of these possibilities.

If the percentage of permanently soluble nitrogen on the total nitrogen of the barley proves fairly constant for one malting firm, its value is obvious. *A determination of the total nitrogen when buying barley will give, at that stage, an indication of the amount of permanently soluble nitrogen which there will be in the resulting wort from malt made under conditions normal to that firm* ; and the value of this in indicating the yeast feeding nitrogen is shown later.

Table 6 is given as an example of the working of this prediction in practice. The malts are a set of 34 made by Gilstrap, Earp & Co., Ltd., in bulk, from 1926 barleys of the Institute set. The predicted figure for permanently soluble nitrogen was obtained by dividing the total nitrogen of the dry barley by 3. It might be noted that dupli-

cate analyses of the barley samples have shown that one or two of the largest divergencies indicated in the Table were due to the difficulty of obtaining representative samples of bulks of barley and malt, a most important consideration in analyses.

TABLE 6.

AGREEMENT BETWEEN PERMANENTLY SOLUBLE NITROGEN OBTAINED AND PREDICTED.

1926 *Bulk Malts.*

Perm. Sol. N. as per cent. on Dry Malt.

Place.	Plot.	Calculated.	Found by analysis.	found—calculated.
Dunbar	1	0·51	0·49	— ·02
	2	0·51	0·50	— ·01
Dunmow ..	3	0·53	0·55	+ ·02
	4	0·49	0·53	+ ·04
	5	0·49	0·52	+ ·03
	6	0·52	0·55	+ ·03
Wellingore ..	11	0·45	0·52	+ ·07
	12	0·48	0·51	+ ·03
	13	0·48	0·50	+ ·02
	14	0·44	—	—
Chiselborough ..	16	0·48	0·51	+ ·03
	18	0·49	0·49	0
	20	0·47	0·46	— ·01
	15	0·51	0·48	— ·03
	17	0·49	0·51	+ ·02
	19	0·48	0·47	— ·01
Sprowston ..	25	0·47	0·45	— ·02
	26	0·49	0·50	+ ·01
	27	0·53	0·53	0
	28	0·55	0·48	— ·07
Beverley	29	0·50	0·46	— ·04
	30	0·49	—	—
	31	0·53	0·49	— ·04
	32	0·52	0·50	— ·02
Longniddry ..	33	0·47	0·50	+ ·03
	34	0·46	0·46	0
	35	0·49	0·47	— ·02
	36	0·46	—	—
Rothamsted ..	41	0·51	0·51	0
	42	0·54	0·50	— ·04
	43	0·58	0·50	— ·08
	44	0·52	0·51	— ·01
	45	0·54	0·51	— ·03
	46	0·52	0·53	+ ·01

Further if the malting conditions deviate from the normal this will be revealed by the resulting percentage of permanently soluble nitrogen obtained, which can be calculated from the barley total nitrogen figure already mentioned together with the "soluble protein" figure usual in malt analyses. The extra information should therefore be available with little or no extra trouble.

Malting conditions and the percentage of permanently soluble nitrogen.—Schjerning's figures which show the effect of widely varying malting conditions on the permanently soluble nitrogen (on one barley) are not readily available so they are repeated here. Table 7) (ex. *Compt. rend. Carls. Lab.*, 1910, **8**, 169).

Clearly here the higher the flooring temperature the smaller becomes the percentage of permanently soluble nitrogen on total nitrogen for corresponding amounts of growth (which can be gauged from the proportion of overshot corns).

The amount of moisture in the grain also has an effect on the percentage of permanently soluble nitrogen. It will be seen from Table 8 that normal moisture supply apparently results in the highest percentage of permanently soluble nitrogen.

2.—AMOUNT OF DIFFERENT NITROGEN GROUPINGS IN WORTS.

The preceding considerations suggest that the amount of permanently soluble nitrogen in malt is governed chiefly by the nitrogen content of the barley and by the malting conditions—the flooring temperature, moisture content and degree of modification. The total permanently soluble nitrogen is however a complex mixture of many different nitrogen compounds which play different parts in the subsequent history of the wort and beer. It is therefore necessary to discover how the amounts of the separate nitrogen compounds are affected. As a start in this direction the quantities of several of the nitrogen groupings have been determined in worts from different malts mashed under identical conditions. The results are given in Table 9 and Diagram 1.

In Diagram 1 are shown the results of the present analyses which are given in numerical form in Table 9. Though there are irregularities in the relative quantities of the different groups, the general indication is that each increases with increasing total nitrogen content of the original barley, but the increase in the three smaller groups appears to be relatively slight and is not

TABLE 7.

EFFECT OF TIME AND TEMPERATURE ON THE PERCENTAGE OF PERMANENTLY SOLUBLE NITROGEN ON TOTAL NITROGEN.
(From Schjerning.)

Days on Floor.	4	7	10	12
Temperature 14° C. (57° Fahr.) ..	17·5	28·8	32·4 few over shot	34·1 many over shot
,, 16° C. (60.8° Fahr.) ..	19·3	29·1	32·3 some over shot	—
,, 20° C. (68° Fahr.) ..	24·8	23·7	25·6 many over shot	28·2 many over shot
,, 26° C. (78·8° Fahr.) ..	17·2	19·0 some over shot	21·0 many over shot	—

DIAGRAM 1.
Malts of Different Nitrogen Content Mashed at 65°C.
Analysis of Wort.

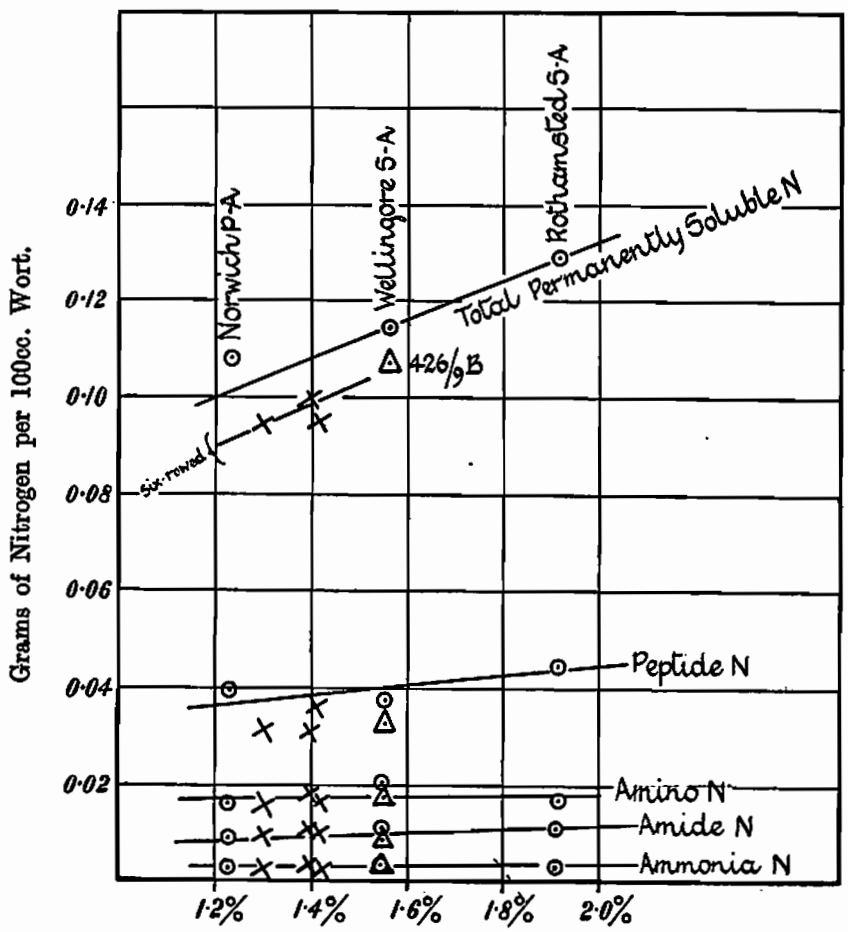


TABLE 8.

EFFECT OF MOISTURE ON PERCENTAGE OF PERMANENTLY SOLUBLE NITROGEN ON TOTAL NITROGEN. (From Schjerning.)

Days on Floor.	4	7	10
Moisture under normal	9·3	27·3	30·3 few over shot
Moisture, normal	17·5	28·8	32·4 few over shot.
Moisture over normal	9·1	26·4	31·9 many over shot.

TABLE 9.

ANALYSES OF WORTS. MASHING TEMPERATURE 65° C.

	Two-rowed barleys			Six-rowed barleys		
	Norwich	Wellingoro	Rothamsted	F112	Califn. Bay Brew	Chilean.
Total Nitrogen, barley	1·242	1·562	1·926	1·313	1·414	1·426
Sp. Gr. wort	1·0583	1·0589	1·0566	1·0571	1·0570	1·0563
Solids per cent.	14·58	14·72	14·15	14·28	14·25	14·07
Maltose per 100cc.	—	—	—	9·19	9·67	—
Maltose per cent. on solids	—	—	—	64·4	67·8	—
$[\alpha]_{D5\cdot66}$	—	—	—	114·1	115·1	—
$[\alpha]_{D4\cdot0}$	—	—	—	118.2	119.3	—
Nitrogen in filtered wort.						
(1) *Grms. per* 100cc.						
Total nitrogen	0·1082	0·1144	0·1288	0·0947	0·0992	0·0950
Ammonia ,, ...	0·0025	0·0023	0·0039	0·0022	0·0019	0·0021
Amino ,, ...	0·0155	0·0197	0·0166	0·0147	0·0170	0·0158
Amide ,, ...	0·0080	0·0100	0·0105	0·0090	0·0102	0·0094
Peptide ,, ...	0·0389	0·0372	0·0446	0·0308	0·0297	0·0360
(2) *As per cent. of total soluble nitrogen.*						
Ammonia N.	2·31	2·01	3·03	2·32	1·92	2·21
Amino N.	14·32	17·40	12·89	15·52	17·14	16·83
Amide N.	7·39	8·74	8·15	9·50	10·28	9·90
Peptide N.	35·95	32·52	34·63	32·52	30·00	37·90
Per cent. N. accounted for	60·0	60·70	58·70	59·90	59·30	66·80
(3) *As per cent. of total solids of wort.*						
Total sol. N.	0·742	0·777	0·910	0·663	0·696	0·675
Ammonia N.	0·017	0·016	0·027	0·015	0·013	0·015
Amino N.	0·106	0·134	0·117	0·103	0·119	0·112
Amide N.	0·055	0·068	0·074	0·063	0·071	0·067
Peptide N.	0·267	0·253	0·315	0·216	0·209	0·256

[For $[\alpha]_{D4\cdot0}$ see foot note to Table 10.

large enough to be proportional to the total nitrogen. The greater part of the change in the total permanently soluble nitrogen comes from the changes in the peptide and undetermined nitrogen. It can be seen also that the six-rowed samples analysed appear to contain about the same amounts of ammonia, amide and amino-nitrogen and that the smaller amounts of total permanently soluble nitrogen which they contain is due to the smaller amounts of peptide and undetermined nitrogen.

B.—Analyses of Worts from Malts Mashed at Different Temperatures.

(1) *Total permanently soluble nitrogen.*—H. T. Brown has already shown (this *Journ.*, 1909, 169), that with varying mashing temperatures there is a broad optimum zone around 50°C. for the maximum amount of permanently soluble nitrogen in worts from a certain malt. The amount of nitrogen in solution falls with higher and lower mashing temperatures and below 30° and above 80°C. remains approximately

TABLE 10.

Analyses of Worts Mashed at Different Temperatures.

Particulars of Bulk Malts Used.

Report No.	Year.	Variety.	Total Nitrogen on Dry Barley.	Mashing Temperatures.
414B	1927	Spratt-Archer	1·44	40°, 55°, and 70°C.
405B	1928	,,	1·38	60° and 70° C.
404B	1928	,,	1·51	45°, 50° and 55° C.
426/0B	1928	,,	1·43	60°, 65° and 70° C.

Analyses of Worts.

Malt.	405B		414B			404B			426/0B		
Mashing temperature. °C.	60°	70°	40°	55°	70°	45°	50°	55°	60°	65°	70°
Specific gravity	1057·9°	1061·0°	1028·5°	1052·3°	1058·7°	1035·9°	1040·9°	1050·4°	1056·1°	1058·1°	1055·1
Solids per cent. (sol. factor 4)	14·48	15·27	7·12	13·08	14·66	8·99	10·24	12·61	14·04	14·54	13·7
Apparent Maltose, grms. /100 cc.	10·0	8·33	4·09	8·98	8·09	5·48	6·48	8·43	90·4	8·85	7·1
Apparent Maltose % on solids	69·0	54·5	57·4	68·9	55·2	61·0	63·2	66·8	64·3	60·8	52·1
$[\alpha]_{D4.0}$	120·8	130·3	69·3	105·8	125·9	100·8	109·9	116·7	120·2	125·0	129·3
$[\alpha]_{D3.86}$	116.4	125.8	66.8	102.0	121.5	97.4	106.0	112.6	116.0	120.8	124.0

Nitrogen in Filtered Worts.

(1) *Grams per* 100cc.											
Total nitrogen	0·1077	0·0990	0·1208	0·1294	0·1140	0·0934	0·0946	0·0950	0·1036	0·1074	0·0934
Ammonia N.	0·0026	0·0023	0·0037	0·0032	0·0031	0·0022	0·0025	0·0024	0·0018	0·0018	0·0020
Amino N.	0·0178	0·0155	0·0180	0·0168	0·0173	0·0169	0·0169	0·0173	0·0157	0·0172	0·0130
Amide N.	0·0090	0·0086	0·0104	0·0105	0·0094	0·0076	0·0075	0·0076	0·0095	0·0083	0·0079
Peptide N.	0·0304	0·0282	0·0317	0·0419	0·0327	0·0240	0·0297	0·0302	0·0323	0·0320	0·0233
(2) *As per cent. total soluble nitrogen.*											
Ammonia N.	2·41	2·32	3·00	2·48	2·75	2·35	2·64	2·53	1·78	1·68	2·40
Amino N.	16·53	15·66	14·90	12·98	15·18	18·09	17·86	18·21	15·15	16·01	15·60
Amide N.	8·36	8·69	8·61	8·11	8·25	8·14	7·93	8·00	7·46	7·73	9·47
Peptide N.	28·23	28·48	26·24	32·38	28·68	26·34	31·50	31·79	31·18	29·80	27·94
Per cent. accounted for ...	55·0	55·1	52·8	56·0	54·9	54·9	59·8	60·5	57·2	55·3	55·4
(3) *As per cent. total solids of wort.*											
Total nitrogen	0·744	0·648	1·696	0·989	0·777	1·040	0·924	0·753	0·738	0·739	0·605
Ammonia N.	0·018	0·015	0·052	0·024	0·021	0·024	0·024	0·019	0·013	0·013	0·014
Amino N.	0·123	0·101	0·253	0·128	0·118	0·188	0·165	0·137	0·112	0·118	0·094
Amide N.	0·062	0·056	0·146	0·080	0·064	0·095	0·073	0·060	0·067	0·057	0·057
Peptide N.	0·210	0·185	0·445	0·320	0·223	0·274	0·290	0·239	0·230	0·220	0·170

Note.—The figures for the conventional $[\alpha]_{D3.86}$ are given for comparison with those calculated with the solution factor 4.0.

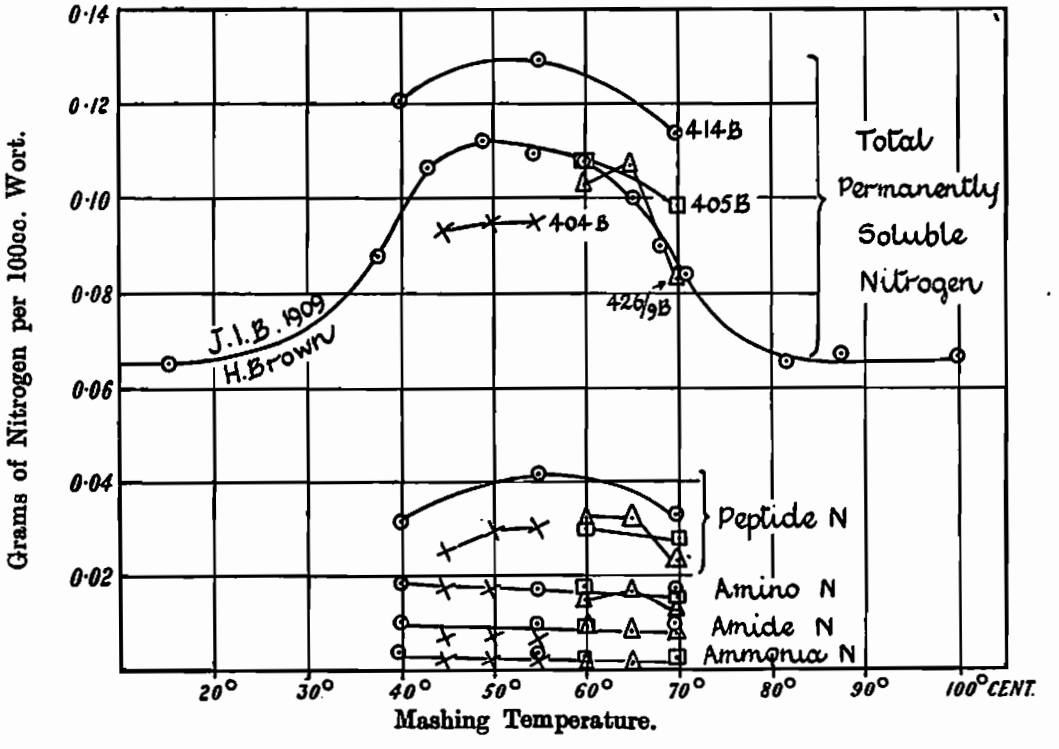
constant at about half the maximum value. His results are given as a dotted curve in Diagram 2 and serve as a connecting link for the present analyses of several malts each of which covers only part of the temperature range. These give curves which appear to run parallel to that given by Brown. The absolute height of the curve at any temperature, e.g., 65°C., varies, as previously concluded, with the nitrogen content of the barley and the malting conditions.

ponsible for the corresponding maximum total soluble nitrogen found at this temperature. The results are given in Table 10 and in Diagram 2.

These results agree in general with those obtained by Schjerning by his own precipitation method (*Compt. rend. Carls. Lab.*, 1912, **9**, 237). He, however, finds, as well as peptic, evidence of tryptic action with an optimum at 45°C., *i.e.*, he finds changes in the amount of simpler compounds, which are not supported by the present results.

DIAGRAM II.

Analysis of Permanently Soluble Nitrogen of Worts from different Mashing Temperatures.



(2) *Amounts of different nitrogen groupings.*—The detailed analyses of the worts indicate that change of mashing temperature has effects analogous to those resulting from the use of barleys with different nitrogen contents. The variations of the two largest groups, peptide and undetermined nitrogen, are clearly evident, but those of the smaller groups are so much smaller that, if any, they may be hidden in the analytical errors of the method. The peptide nitrogen rises to a maximum at 50°C. and is therefore mainly res-

The results of Weiss quoted by Schjerning (*loc. cit.*) a rea apparently in closer agreement with the present ones as Weiss finds the optimum peptic action at 50°C. and a broad tryptic optimum zone. Since the results in this paper show a marked change in the amount of peptide linked nitrogen with no marked change in the amino-nitrogen, they indicate that the active proteolytic enzyme in mashing is the peptase and there is little evidence of peptidase action.

The proportions of nitrogen compounds

on total wort solids naturally vary greatly with the mashing temperature, especially between 50° and 65°C. in which range the amount of nitrogen compounds extracted from the malt is falling while the amount of total extract is rising. This becomes evident from the figures in the last section of Table 10.

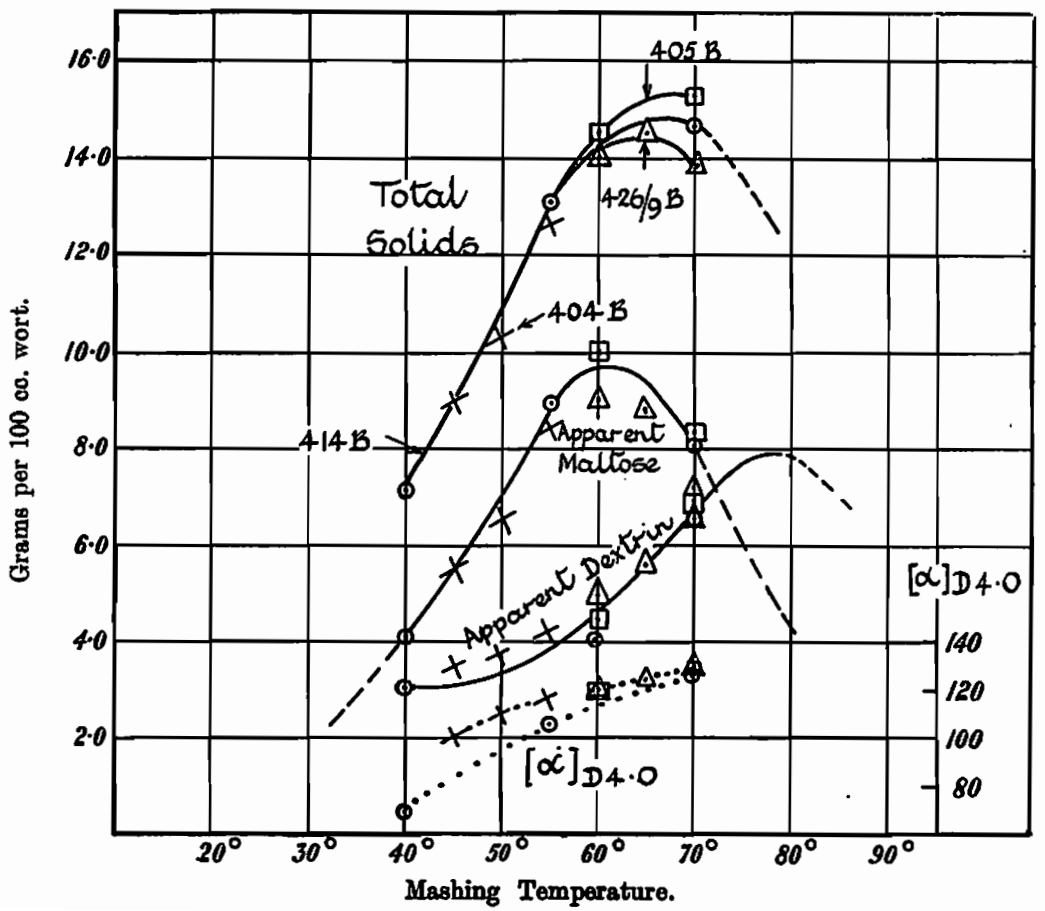
Interpretation of Results.—The methods of analysis employed measure the nitrogen in the form of different groups and not in the form of different compounds. The more complex nitrogenous bodies have some free amino-groups but the greater part of the nitrogen is in the form of peptide linkages. So that the amount of the latter measures the amount of complex nitrogenous compounds. The free amino-nitrogen is a measure of the amino-acids and simpler polypeptides as it comes mainly from these amide groups are present also in the more complex compounds but the amount may be regarded as some measure of the quantity of asparagin present.

Since the main changes identified are in the peptide linked nitrogen it is possible to deduce that these changes are changes in the amounts of the more complicated nitrogenous constituents, chiefly dissolution of insoluble proteins. This holds for the differences between barleys of different nitrogen contents and for the differences produced by changing the mashing temperature.

The following deductions are made from this, but they are not to be regarded as anything more than tentative suggestions. (1) If the yeast feeding nitrogen comes mainly from the less complex compounds it appears

DIAGRAM III.

Changes in Carbohydrates with Mashing Temperatures



that, although this does increase with increasing total nitrogen, the increase is not large, *i.e.*, the amount of yeast feeding nitrogen is less in low nitrogen barleys, especially when calculated on wort solids, but it is not markedly less. The main differences found are in the amounts of the more complex nitrogen compounds which may result in yeast coatings during fermentation and confer on the beer such properties as "palate-fulness" and also tendency to haze.

(2) On the same assumptions it appears that, although six-rowed barleys give less total soluble nitrogen, the amount of yeast-feeding nitrogen is almost the same as that from English two-rowed barleys of the same total nitrogen content. The amount of complex nitrogen compounds is much less in the six-rowed barleys and it may be deduced from these results that they would yield beers with less "body" or "palate-fulness" and less tendency to protein haze.

C. Relation of Carbohydrates of Wort to Mashing Temperature.

Estimations of the specific gravity, reducing sugars and $[\alpha]_D$ were made on the worts used for the study of the effect of mashing temperature on the nitrogen compounds. From these analyses it is possible to make certain broad and approximate generalisations, as with the amounts of the nitrogen compounds in the same worts. The results of the analyses are given in Table X. (p. 354) and in Diagram 3.

It is surprising that, as far as is known to the author, the whole of these general relationships have not previously been demonstrated for malt. H. T. Brown (this *Journ.*, 1909, 169) showed that the total extract obtained from a malt rose to a maximum at a mashing temperature around 65° C., and the present results support this. It is generally accepted that as the mashing temperature is increased the proportion of dextrin increases and the proportion of maltose falls.

The present results show that this belief is only true over a limited temperature range (60°-70° C.). Curves are given in Diagram 3, for "apparent maltose" calculated from the Fehling reducing power and for "dextrin," or the difference between the total solids and the "apparent maltose." From these it appears that below 60° (from 40° to 60° C.) the reducing sugars increase more rapidly than the "dextrin," so that the proportion of "maltose" to "dextrin" *increases* with the increase of temperature over this range.

In view of the presence in wort of reducing substances other than maltose and of extract producing substances other than dextrin and maltose, it is clearly realized that neither of these curves is a correct absolute measure; but it is probable that the reducing substances other than maltose are nearly constant at all mashing temperatures and similarly that the other substances in extract besides reducing sugars and dextrins, are approximately constant in amount so that both curves afford good relative estimates. Even the changes of the nitrogen compounds already discussed are not sufficiently large, relative to the amounts of carbohydrate present, to be important in this connection.

The sharply marked optimum at 60° C. for the amount of "apparent maltose" is noteworthy. The other substances in wort, chiefly dextrins, increase more slowly with mashing temperature and rise to an optimum above 70° C. The rise in $[\alpha]_D$, as might be expected, approximately parallels the rise in dextrin, but is changed from a concave to a convex curve by the effect of the rapid increase of "maltose" over the middle part of the range studied. The curves provide circumstantial evidence for the existence of two sucroclastic enzymic actions in malt: one giving reducing sugars with a sharply marked optimum at 60° C. and the other dextrins with an optimum above 70° C.

It is interesting to note that these optima are similar to those found by C. O'Sullivan for the extracted enzyme acting on soluble starch, whereas T. Chrzaszcz (*Woch. Brau.*, 1910, **27**, 69) found under these conditions a considerably lower saccharifying optimum of 50-55° C., a difference probably due to the absence from his preparations of protective substances.

The range in nitrogen content (Table 10) is small, and the number of examples too few for generalisation, but it may be noted that both above and below a mashing temperature of 150° Fahr. (65·6° C.) the extract is lower, according as the nitrogen increases. The differences appear to be exaggerated above this temperature, and less below it. Similarly there is less "maltose"

at any given mashing temperature the higher the nitrogen of the barley, but the "dextrin" curves show the opposite, *i.e.*, there is more dextrin the higher the nitrogen content and this is supported by the $[\alpha]_D$ curves.

Summary and Conclusions.

The object here has been to collect and discuss the results of investigations on the nitrogenous constituents of wort, which were interrupted at an incomplete stage. In correlation with other work incorporated, this is intended to form the basis for further projected researches. The experiments and discussion are consequently regarded as of a preliminary nature, but the data already obtained seemed sufficient to serve as the

Appendix.

Permanently Soluble Nitrogen Content of Worts from the Institute Barleys of 1923, 1924 and 1925.

1923.

Barley.	Per cent. on dry malt.	Per cent. on total nitrogen of barley.
Barneyhill	0·571	33·3
Cawkwell	0·522	35·1
Wellingore	0·557	38·6
Eyton	0·596	35·1
Chiselborough	0·526	35·1
Orwell Park	0·673	34·8
Walcott	0·633	35·1
Dereham	0·704	35·1
Beverley	0·451	33·7
Harper Adams	0·579	33·2
Rothamsted	0·545	34·0
Woburn	0·540	31·7
Warminster	0·612	41·2
Average		35·1

1924.

Barley No.	Per cent. on dry malt.	Per cent. on total nitrogen of barley.
12	0·499	35·0
13	0·497	34·0
14	0·402	31·8
15	0·411	34·3
20	0·502	35·7
26	0·439	32·3
31b	0·447	30·8
32	0·520	32·7
39	0·522	32·9
40	0·512	31·7
43	0·432	32·4
44	0·455	35·4
53	0·509	33·7
54	0·566	34·8
58	0·539	31·0
59	0·566	33·1
63	0·427	33·0
67	0·486	35·8
69	0·469	35·2
71	0·389	30·9
82	0·528	34·8
109	0·490	36·8
110	0·508	32·9
Average ...		33·5

1925.

Barley No.	Per cent. on dry malt.	Per cent. on total nitrogen of barley.
10	0·656	38·4
13	0·659	39·0
13N	0·656	35·8
20	0·577	37·8
23	0·565	39·0
23N	0·592	37·5
25	0·569	38·5
28N	0·550	39·1
30	0·608	36·3
31b	0·560	38·0
31N	0·592	36·2
33	0·719	32·2
36	0·719	32·3
36N	0·707	30·7
43	0·652	42·2
47	0·563	38·6
47N	0·628	35·3
49	0·484	30·4
52	0·503	33·9
52N	0·497	31·7
54	0·567	35·1
57	0·542	34·0
57N	0·538	34·6
Average ...		35·9

basis for the formulation of certain broad generalisations of practical interest and worthy of note at the present stage.

(1). The amount of permanently soluble nitrogen in the wort from an English malt ranges from about 30 to 40 per cent. of the total nitrogen content of the original dry barley. The percentage varies with and is a measure of malting conditions. For average malting conditions, the value is about 35 per cent., 30-33 per cent. implies under-average modification, and 37-40 per cent. over-average modification. These values are for ordinary malting temperatures (around 16° C.). They are probably lower for higher temperatures. The figures given here are for permanently soluble nitrogen determined in the standard laboratory hot mash, the values are about 2 per cent. higher for a 29 per cent. mash. The percentage found in a number of analyses of malts from 6-rowed barleys was around 29.

It will be noted that these figures are for "stocking malts." The corresponding figures for "bulk malts" would appear from the available data to be about 33 and 28 per cent., respectively, for normal modification.

(2) The higher permanently soluble nitrogen of malts with higher nitrogen content was found to be due largely to increase in the "peptide" and "undetermined" nitrogen. There appears, however, to be a general tendency towards a corresponding but smaller, increase in the nitrogen of the simpler groups.

(3). The nitrogenous composition of a wort varies also with the mashing temperature. The ammonia, amide and amino-nitrogen appear to remain almost constant. The peptide nitrogen and consequently the total permanently soluble nitrogen rise to a broad optimum zone around 50° C.

(4). Simultaneous study of the carbohydrates of wort has given evidence of analogous regularities among these. Other factors being constant, with increasing mashing temperature the amount of "apparent maltose" increases rapidly from 40° C. to a sharply defined optimum at 60° C. and then rapidly falls. Other substances in the wort, chiefly dextrins, rise more slowly to an optimum above 70° C.

(5). The manner in which such results may be employed is outlined. The nitrogen content of the barley will indicate, with given malting conditions, the amount of permanently soluble nitrogen in the final malt wort, and it would appear that under comparable conditions with one variety, the total permanently soluble nitrogen affords some indication of the yeast feeding nitrogen. But on the assumptions made, this would appear to be largely independent of the malting conditions, nitrogen content and variety, being dependent to a great extent only on the amount of malt used.

(6) Six-rowed barleys differ from two-rowed chiefly in the smaller amount of peptide nitrogen which they give, suggesting that, from the nitrogen standpoint, they differ not so much in yeast feeding properties, but chiefly in giving less palate fulness and less tendency to protein haze.

When the nitrogen requirements of yeast are known and when more definite information is available on the nitrogenous constituents which affect the properties of beer, then the present information should be of material assistance in deciding (a) the best nitrogen content for brewing barley and (b) the best malting and mashing conditions.

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November 10th, 1930.