THE INSTITUTE OF BREWING RESEARCH SCHEME.

PREDICTION OF EXTRACT III.

APPLICATION OF THE CARBOHYDRATE REGULARITY PRINCIPLE.

By L. R. Bishop, M.A., Ph.D.

The extract which a barley will yield when made into malt is frequently estimated on the Continent by measuring the extract obtained from the barley; this has some disadvantages.* It is evident that, were it possible, isolation and weighing of the complementary fraction, the "spent grains" of barley, would be an equally satisfactory method but it would probably take as long. The carbohydrate regularity principle (L.R. Bishop and D. Marx (this *Journ.* 1934, 62) suggests that a quick modification is possible.

The earlier method for extract prediction, (L. R. Bishop, *ibid*, 1930, 421; L. R. Bishop and F. E. Day, *ibid*, 1933, 545), virtually amounts to estimating the spent grains. For, in each variety, at any given thousand corn weight the "spent grains carbohydrates" are constant in amount. The variable is the quantity of protein in spent grains together with some carbohydrate which this protein has "sealed up"; it is, therefore, possible to estimate the extract by subtracting from the varietal constant a variable quantity equal to the protein and the carbohydrate it "seals up."

This method is satisfactory provided that the variety of the barley and its characteristic constant are known. Often, however, in practice this is not the case, as barley samples are usually of mixed or unknown origin. With these the carbohydrates of spent grain are variable as well as the proteins and clearly from an estimate of both variables a satisfactory method of extract calculation would result. This would have the distinctive advantage that it could be applied to any sample of barley, irrespective of its variety.

Such carbohydrate estimations are available but are also lengthy and involved. The "carbohydrate regularity" principle, however, suggests a quicker solution of the problem, for it should be possible to measure a definite fraction of the carbohydrates of spent grain which would bear a regular relation to the total and the problem of estimating the extract of any barley sample would be solved. This is what the "insoluble carbohydrate" estimation attempts to do. In it, the varying effects of the proteins and of the carbohydrates of spent grains are measured separately and then added together to estimate the quantity of spent grains and so the extract.

One further possibility is to measure the effects of the carbohydrates and proteins of spent grains in one estimation. This has been attempted in an "insoluble fraction" method which is not sufficiently accurate, though it may prove capable of improvement

SECTION I.

Methods and Results.

The method for the estimation of the "insoluble carbohydrate" has been given in a previous paper (this *Journal*, 1934, 62), it has been applied to 209 barleys of a wide range of origin and character. On the same barleys determinations have been made of nitrogen, moisture and thousand corn weight. The extracts of the corresponding malts have also been determined. The results are given in Appendix II. A short summary of the statistical treatment of the results is given in Appendix I.

A test has also been made of a method based on the estimation of an "insoluble fraction." The details of this method are given in Section 5, page 84.

* The methods used are complicated and that of Seibriger is stated to take 24 hours by Schéele and Svenson (*Svenska Bryggare Föreningens* 1929, 41.). Further, Steenhof (ibid., 1927, 171) has shown that in order to obtain accurate results it is necessary to determine the nitrogen content as well.

SECTION 2.

Discussion.

(a) *Test with six representative varieties.*

The first test made, was to apply the insoluble carbohydrate method to six samples of each of six varieties. Both the samples and varieties were selected to give as wide a range as possible in extract, the extreme range being 87.4 to 103.6 lb. The method was found to work very well, as is demonstrated in the following diagram.

*This very useful type of diagram was devised by Dr. E. M. Crowther, F.I.C.; it allows diagrams to be drawn illustrating the relation between three quantities; this previously needed a solid model.

SIX VARIETY EQUATION FOR EXTRACT PREDICTION.

$$E = 136{\cdot}7 - 8{\cdot}34N - 3{\cdot}17I$$

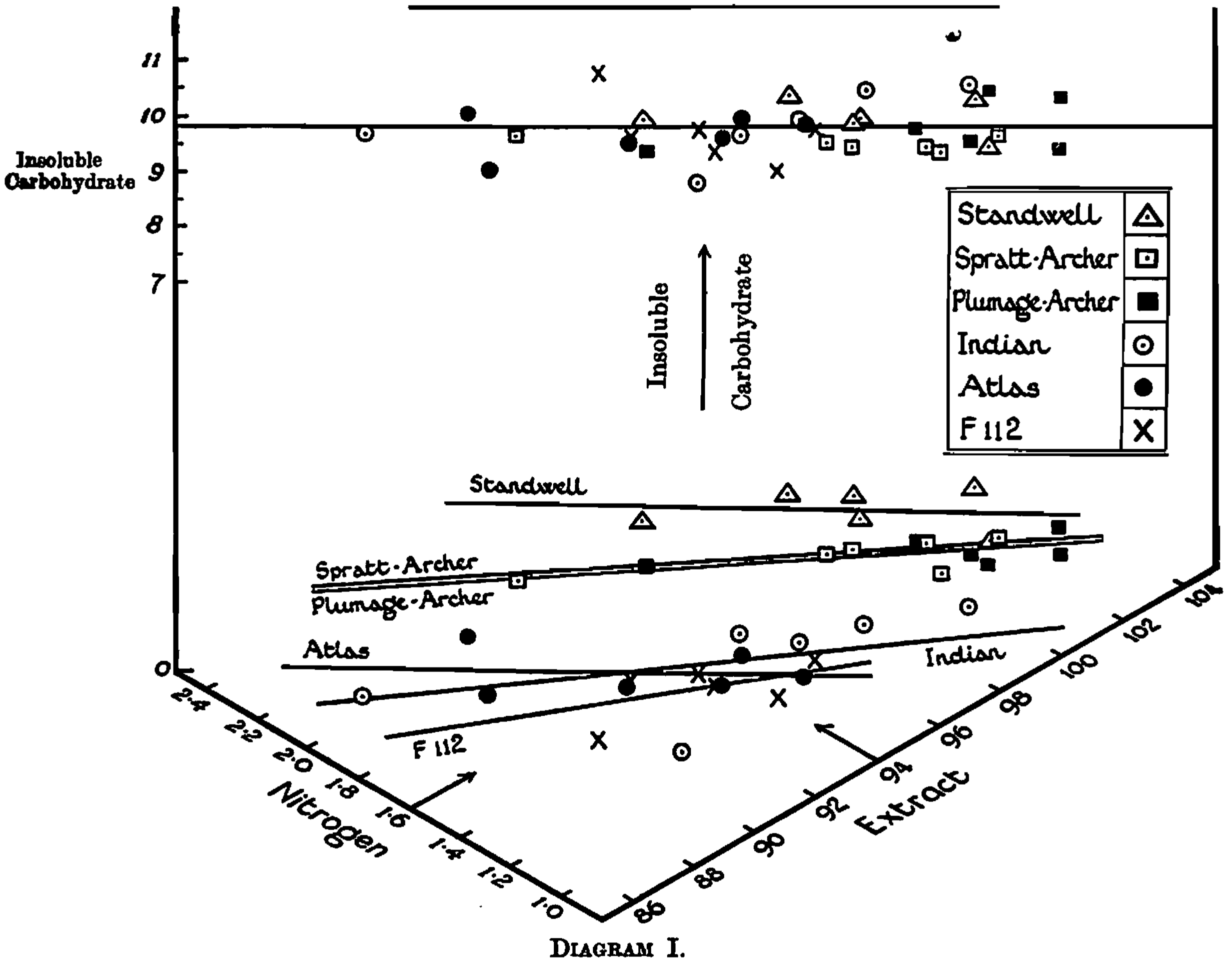

Diagram I.

In Diagram 1* the extract is plotted against the nitrogen content on two axes inclined at 120° instead of the usual 90°. The points at the bottom of the diagram show the relation between nitrogen and extract and the large differences between different varieties. This part of the diagram corresponds to the diagram in a previous paper (L. R. Bishop and F. E. Day, this *Journ.*, 1933, 545). The present method of plotting, however, allows of the addition to each point of a height corresponding to the weight of insoluble carbohydrate obtained. Then, since the low extract varieties yield larger amounts of insoluble carbohydrate and the high extract ones less, the points are all brought close to one line at the top of the diagram. The closeness of approach to this line affords visual proof of the accuracy of the two factors, nitrogen content and insoluble carbohydrate, in accounting for the extracts of the malts.

Together, nitrogen and insoluble carbohydrate account for 91 per cent. of the variation† in extract; the 9 per cent. (error) unaccounted for was found to be much smaller than in any other of the extract calculation methods tried; and the insoluble carbohydrate content alone, proved considerably more accurate than nitrogen content and thousand corn weight, when no allowance was made for the separate varietal characteristics. The relative accuracy is shown in the following diagram (Diagram 2).

The standard error of calculations from nitrogen and insoluble carbohydrate content is still high (± 1·3 lb.) but this is partly

† This word is used instead of the correct statistical term which is variance.

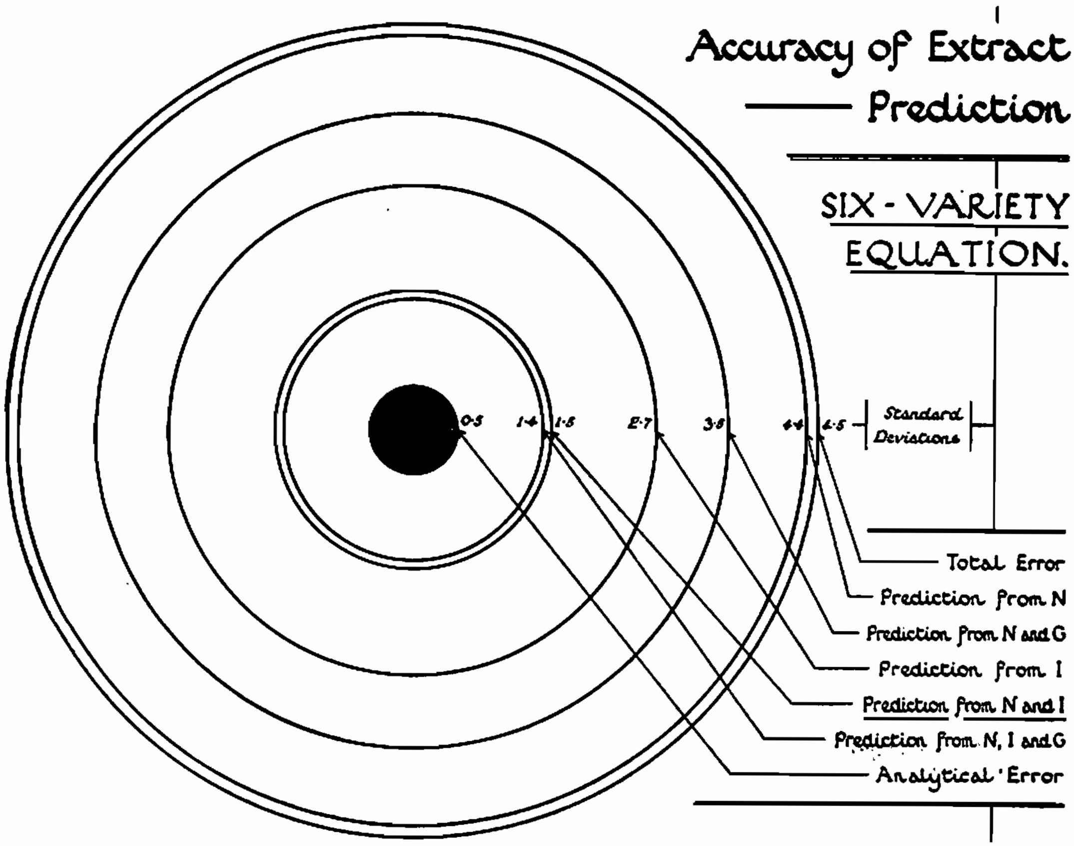

DIAGRAM II.

Attempts to measure the extract, whether by prediction or by analysis, may be represented as marksman's shots aimed at the centre of the bull's-eye of a target. All of the shots are subject to errors and, as in every type of measurement, some individuals are, by chance, more in error than others. A circle can, however, be drawn (with a radius proportional to the standard error) which includes 68 per cent. of the shots. Then the area of this circle (proportional to the square of the standard error) is a measure of the variation (variance) of the results.

This has been done in Diagram 2, where the size of circles shows the closeness with which calculations from the different factors approach the blackened "bull" representing the errors of the analyst in determining the extract. Calculation from the nitrogen content and percentage of insoluble carbohydrate gives the most accurate predictions, and the addition of a factor for the thousand corn weight does nothing to improve the accuracy.

due to the wide range in the physiological condition of the barleys at malting and possibly to variation in the malting treatment. The effect of these factors can be measured by the percentage (P) of the total barley nitrogen becoming permanently soluble in the wort. With this factor taken into account as well the standard error is reduced from ± 1·3 lb. to ± 1·1 lb. The errors still remaining are almost entirely due to some varietal factor still unaccounted for. When this is allowed for by a constant difference between each pair of the varieties the standard error is reduced to the very low figure of ± 0·45 lb. and this remainder may be safely attributed to experimental errors. The equation giving this very accurate result is:—

$$(3)\ E = a + 0{\cdot}1086G + 0{\cdot}223\,P - 1{\cdot}5\,I - 9{\cdot}03\,N.$$

Where "a" is a constant depending on the variety. This equation is comparable with $E = A - 10{\cdot}20N + 0{\cdot}176G$, which has a standard error of $\pm 0{\cdot}95$lb. This equation shows that "I" has a small effect in each variety apart from thousand corn weight. This probably implies, as previously mentioned, that the regularity principle is not absolute but that small varieties in carbohydrate composition occur as the result of seasonal influences.

(b) Practical Tests with English Two-Rowed Barleys.

The question next arose as to how closely such extract calculations hold under commercial conditions. By the kindness of Edward Sutcliffe, Ltd., a number (104) of English barleys were received, together with their analyses as malt and details of temperature and time on the floor and kiln.

The barleys were ordinary commercial samples of unknown variety but a study

ACCURACY OF EXTRACT PREDICTIONS.

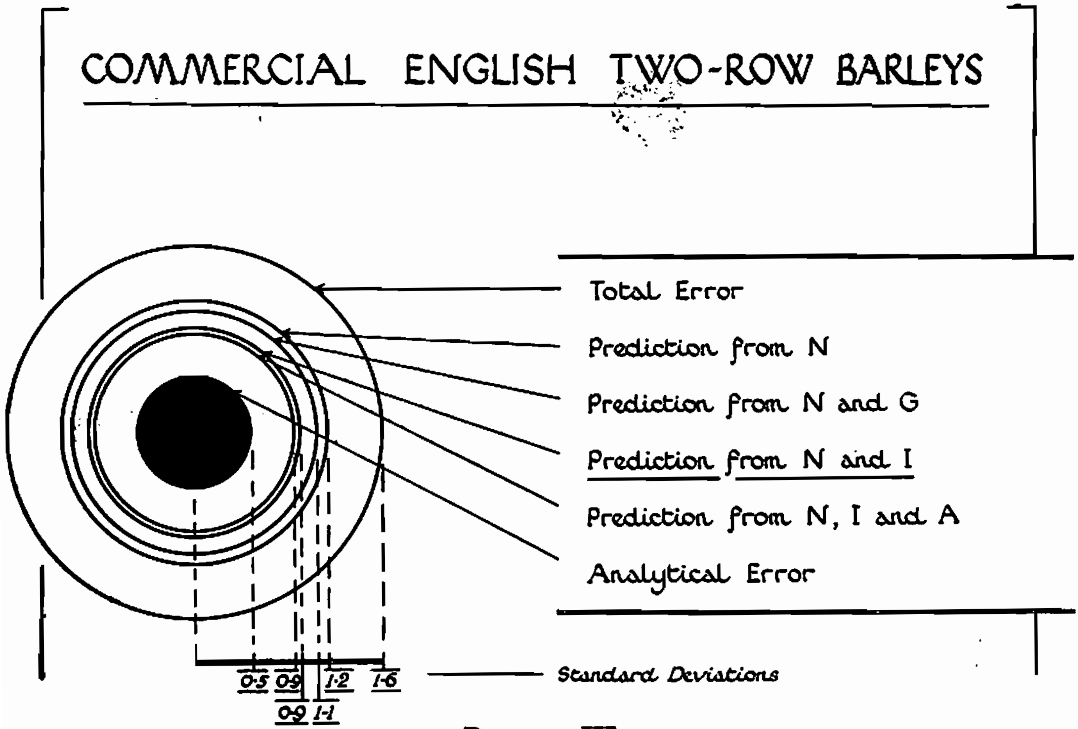

DIAGRAM III.

In Diagram 3, as in Diagram 2, the areas included by the circles are proportional to the errors of extract calculated from the factors named. In order of decreasing accuracy these are nitrogen and insoluble carbohydrate content, followed by nitrogen and thousand corn weight, while nitrogen content alone is slightly more inaccurate. The superiority of nitrogen and insoluble carbohydrate content calculations over those from nitrogen and thousand corn weight, although small, is large enough to be undoubtedly significant.

of the results showed that, when considered from the point of view of extract yield, these English barleys nearly all behaved as one variety, with an average close to that found for Plumage-Archer. The original equation given for this variety (L. R. Bishop, this *Journ.* 1930, 421) was found to fit the results very well (the standard error was ± 1·1) and the average difference from it was only + 0.03 lb. W. J. Mitchell (this *Journ.*, 1932, 241) has shown that this equation holds for Scottish barleys also. Nevertheless there are varieties of two-rowed barleys differing considerably from Plumage-Archer in their extract yields for any given nitrogen content (L. R. Bishop and F. E. Day, this *Journ.*, 1933, 545). Some representatives of such varieties must have been included in this set, so that when barley nitrogen and insoluble carbohydrate contents are taken, the extracts can be calculated more accurately This is shown by the standard errors of the two : that for nitrogen content and thousand corn weight is ± 1.1, while that for nitrogen content and insoluble carbohydrate is ± 0.9 which corresponds to a 10 per cent. increase in accuracy ; an improvement which is significant statistically but may not be large enough to justify the use of the method in practice, especially as English barleys are usually bought in small lots. The relative accuracy of the different calculation methods is shown in Diagram 3.

(c) *Practical Tests with Californian Six-Rowed Barleys.*

Californian barleys on the other hand are bought in large quantities, varietal differences in extract are larger and mixtures often occur. Consequently an extract equation eliminating varietal effects promises to be of more use and such has actually been found to be the case. 47 samples and data relating to them were supplied by Messrs. Hugh Baird and Sons.

The results showed that calculations from

ACCURACY OF EXTRACT PREDICTIONS.

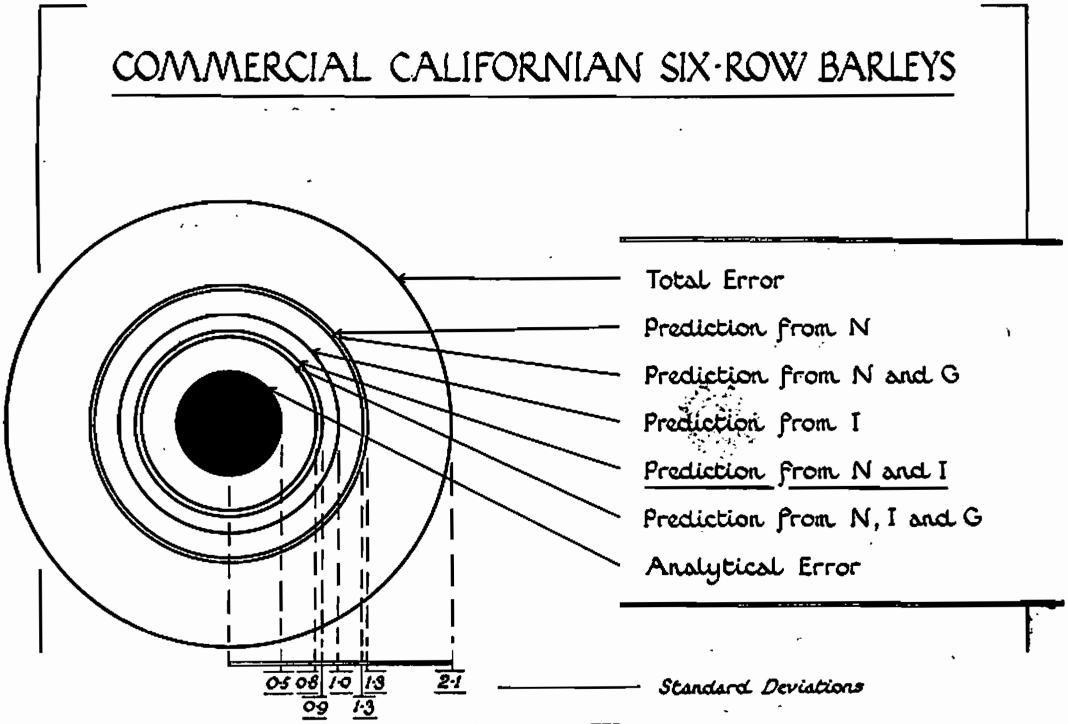

DIAGRAM IV.

The total variability in extract is greater here than with the English two-rowed barleys and the distinctly smaller circles show the marked superiority of insoluble carbohydrate content alone, and still more in conjunction with nitrogen content over nitrogen and thousand corn weight, as a means of calculating the extract.

the nitrogen content and thousand corn weight accounted for 64 per cent. of the variation in extract (standard error ± 1.3 lb.), that the insoluble carbohydrate alone accounted for 76 per cent., and together with nitrogen content for 83 per cent. of the variation ; the standard error for calculations from the latter two (N and I) was ± 0.9 lb. and again, no improvement was effected by considering the thousand corn weight in addition. The relative accuracy of the equations is shown in Diagram 4.

Through the kindness of Hugh Baird and Sons, it was possible to make a short study of the barleys of the previous year. A set of Californian barleys of known varieties supplied by the United States Department of Agriculture, was also studied. Both these sets confirmed the conclusion that nitrogen content and insoluble carbohydrate give, irrespective of variety, a reliable guide to the extracts which Californian barleys will yield as malts.

(*d*) *Combining the results from the Two-Rowed and Six-Rowed Barleys.*

As a further test of the method the results from the two sets of commercial barleys (English two-rowed and Californian six-rowed) were put together and the extracts calculated from the derived equation. Here the varietal differences were considerable. The extreme range was 102.8 to 91.0 lb. and the means of the two sets were 100.2 and 94.5 lb. In consequence of the large varietal differences, nitrogen, or nitrogen and thousand corn weight, proved very inaccurate in accounting for the extracts. The standard error of the N and G calculations was ± 2.1 lb. and they accounted for only 56 per cent. of the variation. The insoluble carbohydrate alone had a much smaller standard error (± 1.3 lb.) and accounted for 84 per cent. of the variation. On the other hand, the nitrogen content and insoluble carbohydrate predicted the extracts with the same error as for the individual sets (± 0.9 lb.)

ACCURACY OF EXTRACT PREDICTIONS.

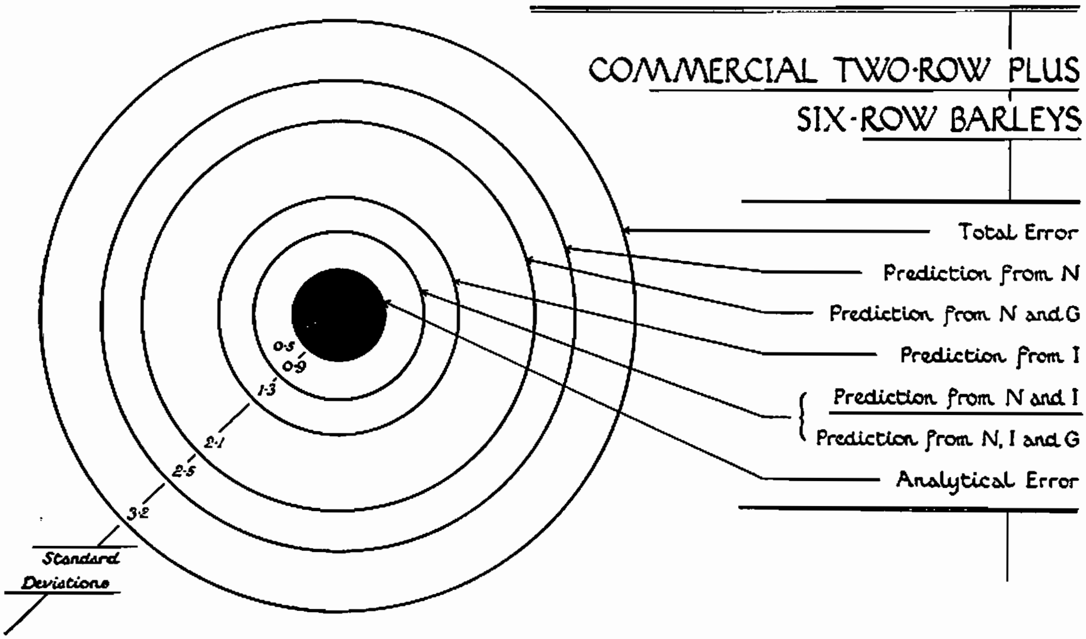

Diagram V.

The relative accuracy of the different extract predictions, is illustrated in Diagram 5.

EXTRACT PREDICTION—TWO-ROW PLUS SIX-ROW RESULTS.
GIVING ALTERNATE CASES ONLY IN TWO-ROW.

$$E = 134{\cdot}7 - 9{\cdot}78N - 2{\cdot}64I$$

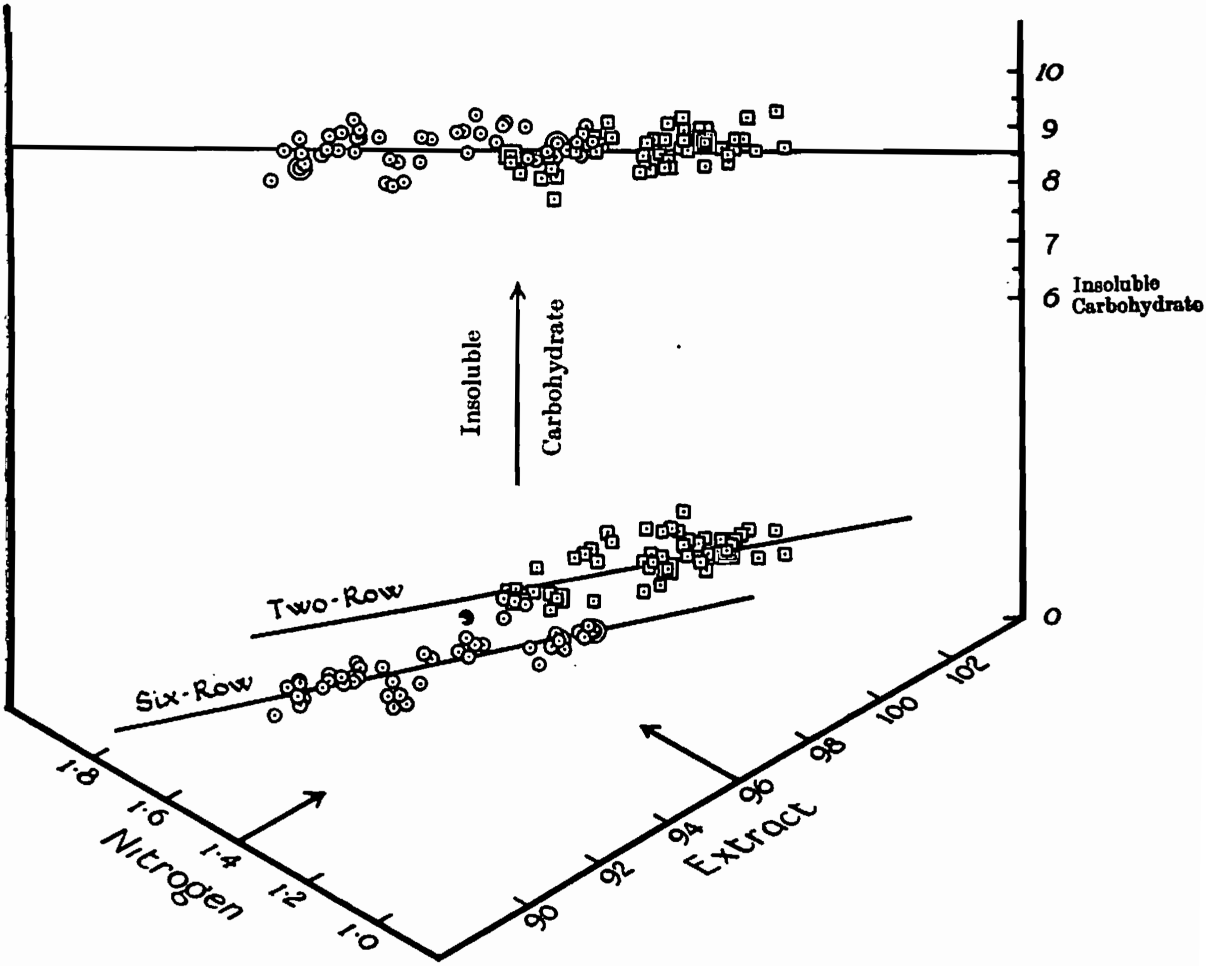

DIAGRAM VI.

Diagram VI makes it clear that the large varietal differences between English and Californian extract yields are completely accounted for by the differences in their carbohydrate composition, as measured by the "insoluble carbohydrate" estimation.

and accounted for 92 per cent. of the variation in extract.

3. Calculation of a "Restricted General Equation."

All the results were combined and one equation calculated from them. They were from the six varieties (Standwell, Spratt-Archer, Plumage-Archer, Indian, F. 112 and Atlas), the commercial two-row and the commercial six-row samples. Equation (1), *i.e.*,

(1) . . . E=134·7 —9·0 N —2·8 I . . .

held accurately for the 187 samples, and the standard error was ± 1·0 lb.

The results from six barley varieties came from stocking maltings, while the commercial barleys and malts represented ordinary bulk maltings. The fact that the standard error was not appreciably increased shows that no disharmony exists between the two. The equation therefore appears to be of fairly general application, although, as shown, the predictions for certain varieties are not very accurate, and the equation has, therefore, been styled a "restricted general equation." It has the same uses as the specific varietal equations given in an earlier paper (L. R. Bishop and F. E. Day, this *Journ.*, 1933, 545), with the added advantage that it can be applied to samples which are of mixed or unknown varietal origin. It would appear to be most useful with Californian samples. The uses are (*a*) as an aid in buying barleys, (*b*) as a check on the efficiency of the malting method, (*c*) as a check on the accuracy of the extract determination, (*d*) for use in the breeding of new varieties.

VARIETAL IRREGULARITIES.

One or two of the six varieties studied showed evidence of small regular deviations between calculated and observed extracts and larger divergences were noted in a few among several odd samples of other varieties. Figures for these samples are given in Table I.

Most varieties appear to agree well, but there are striking exceptions in the case of one sample of Trebi, and regular exceptions in the case of variety B·244 (a new variety bred at Cambridge). Although this variety gives a high extract for a six-rowed barley, yet the calculation predicts a higher extract still. The probable explanation of these divergencies is that the insoluble carbohydrate, as here determined, includes only part of the carbohydrate remaining insoluble in the spent grains of the malt. In most varieties the proportion is similar between the fractions included in and omitted from this estimation. However, the underlying hypothesis is not invalidated, in fact it would suggest that certain varieties would regularly have a different proportion between the measured and the total insoluble carbohydrate.

4. SIGNIFICANCE OF THE FACTORS IN THE GENERAL EQUATION.

It is usually assumed, as Haase (*Woch. Brau.*, 1906, 23, 35) suggested, that the lowering of extract by nitrogen is due simply to insoluble protein replacing carbohydrate in the grain in equivalent amounts. Four years before Beaven (this *Journ.*, 1902, 558) calculated the effect of nitrogen but gave no

TABLE I.

APPLICATION OF RESTRICTED GENERAL EQUATION (1) TO OTHER VARIETIES.

Barley No.	Year.	Variety.	Predicted Extract (1).	Extracts on analysis.	Difference.	Varietal Average.
—	—	July	100·6	101·2	+ ·6	—
—	1928	O.A.C. 21	90·8	92·1	+1·3	—
9 & 10	1928	Trebi	95·4	94·3	—1·1	—2·6
	1930	Trebi	93·6	87·9	—5·7	
	1932	Trebi	96·7	95·8	— ·9	
9 & 10	1931	Californian (variety unknown)	96·0	96·5	+ ·5	—
	1926	Hero	89·1	86·2	—2·9	—
7	1931	Vaughn	88·1	83·5	—4·6	—
	1926	Tennessee Winter	86·0	87·2	+1·2	—
2	1931	Tennessee Winter	96·8	97·3	+ ·5	—
11	1931	Tennessee Winter	94·6	94·0	— ·6	—
	1926	Mariout	87·6	87·5	— ·1	—
24	1931	Mariout	96·1	94·8	—1·3	—
	1926	Coast	84·3	86·8	+2·5	—
	1926	Smooth Awn	92·2	89·4	—2·8	—
199	1927	B.244	98·6	94·6	—4·0	—3·3
200c	1928	B.244	100·6	99·2	—1·4	
131	1929	B.244	97·2	94·0	—3·2	
110c	1930	B.244	102·2	98·7	—3·5	
111c	1930	B.244	101·6	98·1	—3·5	
115c	1930	B.244	101·2	97·4	—3·8	
116c	1930	B.244	100·9	97·5	—3·4	

equation as he realised qualitatively the effect of other factors, such as husk and modification.

The results given here show that simple replacement of carbohydrates by protein can be only a partial explanation, and there is no doubt that some other effect comes into play. In Appendix I. the actual stages are given by which; for comparison with that actually obtained, an equation is derived on the simple protein replacement hypothesis. In this comparison equation (1) has been converted to give " true " extract as a percentage on dry malt. The " true " extract has been obtained by incorporating a correction for the fact that, in the Institute Standard Method, a constant 15 ccs. is allowed for the volume of the grains, whereas in actual fact the volume varies inversely with the extract. The two equations are :—

Actual equation

$$E_2 = 104 \cdot 6 - 7 \cdot 27N - 2 \cdot 23I.$$

Expected equation

$E_2 = 104 \cdot 2 - 4 \cdot 38N - 1 \cdot 04$ (true insoluble carbohydrate).

Where E_2 is the " true " extract as a percentage on dry malt.

The agreement between the constants (104·2 and 104·6) is close, but the actual nitrogen factor (7·27) is nearly twice as large as that expected on the protein replacement hypothesis (4·38), showing that another effect comes into play which may be explained as follows: the extracts were determined by the Institute Standard Method in which the malt is ground in a roller mill with ½ mm. gap between the rolls. With this degree of grinding, some of the potentially soluble carbohydrate is probably "sealed up" in a protein matrix, and this effect increases with increasing nitrogen content. This was shown by comparing the extracts obtained by the standard and by fine grinding from the same malts (Calculated from data of H. Lloyd Hind, this *Journ.*, 1924, 971). With fine grinding more extract was obtained, particularly from the high nitrogen malts, and the nitrogen factor for fine grinding (—4·9) approaches that suggested by the simple protein replacement hypothesis.

The insoluble carbohydrate factor, which should approximate to 1·0, is also much larger (2·23). This is partly explained by the fact already mentioned, that the present method of estimation measures only about half the insoluble carbohydrate corresponding to that in spent grains. The method includes all of the cellulose, but only part of the hemicellulose and lignin of spent grains (see L. R. Bishop and D. Marx, this *Journ.*, 1934, 62). Since the individual carbohydrates are regularly related to the total quantity for each variety in proportions which are similar in most varieties, it is usually sufficient to measure this proportion of the whole and multiply by a constant factor for all varieties. With some varieties, e.g., B.244, the factor required is rather different and this leads to a certain amount of systematic error.

The figures obtained were subjected to a detailed statistical analysis in order to analyse out the effect and interaction of the factors, nitrogen, thousand corn weight and insoluble carbohydrate (see Appendix I p. 86): and the following conclusions were reached.

(a) That nitrogen, alone or with thousand corn weight, is only of use in calculating the extract within the limits of each variety separately. Here, as shown above, its effect is due to the replacement of carbohydrate by insoluble protein, with the further effect that in coarse grinding higher nitrogen is associated with a greater " sealing up " of potentially soluble carbohydrate.

(b) In all varieties the thousand corn weight plays the same part which, as has previously been suggested, may be due to the smaller proportion of insoluble husk in heavier grain. This view is supported by other results given here, since the insoluble carbohydrate, a large part of which comes from the husk, is found to be inversely related to the grain weight.

(c) Insoluble carbohydrate successfully accounts for much of the difference in extract which exists between varieties. This shows that it is owing to differences in their carbohydrate composition that varieties differ in extract yield, the effect of nitrogen being the same for all varieties. The analysis reveals that besides accounting for the differences between varieties, the insoluble carbohydrate estimation accounts also for the small differences between samples of the same variety which are partly produced by thousand corn weight. Consequently, there is little advantage in taking into account the latter as well as insoluble carbohydrate and nitrogen content when calculating extract.

It was found that the nitrogen content varies independently of the insoluble carbohydrate, implying that high nitrogen is not associated with heavier husk, since the insoluble carbohydrate comes mainly from the latter. It follows that what the buyer recognises as " coarse skin " in high nitrogen barleys does not imply a thicker or heavier husk, so that the present results afford confirmation of the similar lack of relation found by Horace Brown, (*Trans. Guin. Research Lab.* 1903-06, p. 134) from direct measurements of husk thickness.

On the other hand the direct effect on extract of the differing huskiness of different varieties of barley has long been recognised. The present study confirms the existence of this by measuring it accurately and shows that it is directly associated with the characteristics of the different varieties.

SECTION 5.

The Total Insoluble Fraction Estimation.

As mentioned in the introductory paragraphs of this paper, the possibility exists of measuring the effects of both protein and carbohydrate on extract in one estimation and the accuracy of one of the possible methods for this has been investigated.

The method used was similar to that for the " insoluble carbohydrate " estimation, except that only one extraction was made with a boiling solution containing 10 per cent. calcium chloride and ½ per cent. hydrochloric acid. This was boiled at a definite steady rate continued for exactly ten minutes, and the insoluble fraction filtered off on balanced filter papers, washed, dried and weighed as in the " insoluble carbohydrate " method. Results can be obtained in 2-3 hours after receiving a sample.

A wide range of barleys was used to test the method. Analysis of the data shows that it is not as accurate as other methods for predicting extract.

The equation obtained was :—

(2) $E=138{\cdot}7-2{\cdot}3\,F \;\ldots\; \pm 1{\cdot}9\ (n=57)$.

Where F=percentage of insoluble fraction on dry barley.

This is too inaccurate for practical use, and is given only as a suggestion of further possibilities.

The insoluble fraction averages 70 per cent. of the spent grains which it estimates, but it was found that a modification of the method gave results almost identical with the corresponding weight of spent grains. The modification consisted in the substitution of ½ per cent. lactic acid for the ½ per cent. of hydrochloric acid in the extracting solution. However, the residues filtered slowly, and the accuracy was no greater, as the same defect was found in both. This defect is similar to and probably an exaggeration of that found in the " insoluble carbohydrate " method, which, it will be recalled, extracts a certain type of carbohydrate which remains insoluble under enzyme action in mashing. As a result, B·244 gives a smaller and Standwell a greater residue than they " should " in both methods.

The method given was selected after extensive trials of various salts, acids, etc., as peptising agents for the carbohydrates of extract.

I have very much pleasure in thanking the following who have given invaluable assistance in this investigation :—

Hugh Baird & Sons, Ltd., Edward Sutcliffe, Ltd., the National Institute of Agricultural Botany, and Messrs. Arthur Guinness, Son & Co., Ltd., who have supplied barleys and malts. Also

Mr. S. F. Weeden, Mr. G. D. Clarkson, Mr. F. E. Day, B.Sc., F.I.C., and Miss D. Marx, M.Sc., who have supplied figures and analyses, and those members of the staff at Rothamsted who have assisted in the statistical-interpretation of the results.

Summary.

The carbohydrate regularity principle has been applied to give practical results in the form of an extract prediction equation, which can be used where the variety of the barley is unknown—a common position in practice. It appears to be particularly accurate and useful with Californian barleys.

The equation for this calculation is :—

$E=134{\cdot}7-9{\cdot}0\,N-2{\cdot}8\,I \ldots \pm 1{\cdot}0$ lb.

Where E=brewers' lb. of extract per quarter of dry malt, determined by the Institute of Brewing Standard Method.

N=*nitrogen percentage on dry barley.*

I=*percentage of " insoluble carbohydrate " on dry barley.*

The equation can be converted to give extract as a percentage on dry malt (E_1), and then is :—

$$E_1 = 102\cdot7 - 6\cdot90\,N - 2\cdot12\,I \ldots \pm 0\cdot8\%.$$

This equation is based on extract figures obtained by coarse grinding and mashing at a constant temperature of 65·55° C. Some adjustment would be necessary for this to agree with the results obtained for extract determined by the "Congress" method.

The size of the protein factor in this equation demonstrates the incompleteness of the original hypothesis of Haase, that protein simply replaces carbohydrate and so exerts a corresponding reduction of extract. While this is responsible for half the observed effect the other half is due to a "sealing up" of carbohydrate by protein, which becomes more marked in high nitrogen barleys. This applies to the Institute of Brewing Standard Method of Extract Determination ; with fine grinding "sealing up" does not occur.

The success of the insoluble carbohydrate factor over a wide range of barleys affords strong support to the "carbohydrate regularity" hypothesis.

A test has also been made of an "insoluble fraction" method which estimates the combined effect of carbohydrates and proteins on extract. It is quick and simple, but gives only an approximate estimate of the extract.

TABLE 2.

REGRESSION EQUATIONS.

No.	Factors.	Equations.	S.E.	V as %.
(a) *Six Varieties.*		n=36		
4	—	E= 97·16=mean	4·33	0
5	N	E=106·05 — 5·83N	4·10	11
6	NG	E= 92·04 — 8·11N +0·459G	3·29	42
7	I	E=119·11 —2·58I	2·79	60
8	NI	E=136·61 — 9·12N —3·00I	1·30	91
9	NIG	E=133·51 — 9·26N —2·86I +0·058G	1·30	91
(b) *English Two-row Barleys.*		n=104.		
10	—	E=100·2 =mean	1·65	0
11	I	E=127·7 —3·67I	1·32	36
12	N	E=118·0 —11·83N	1·17	50
13	NG	E=109·5 —11·99N +0·239G	1·08	58
14	NI	E=135·6 — 9·91N —2·74I	0·93	69
15	NIG	E=131·0 —10·16N —2·46I +0·078G	0·92	70
16	(NG)	Original Plumage-Archer Eqn.	1·12	—
(c) *Californian Six-row Barleys.*		n=47.		
17	—	E= 94·5 =mean	2·07	0
18	N	E=120·4 —16·72N	1·28	63
19	NG	E=109·0 —13·25N +0·147G	1·27	64
20	I	E=137·8 —4·58I	1·03	76
21	NI	E=137·4 — 7·86N —3·25I	0·86	83
22	NIG	E=153·3 —10·62N —3·75I +0·174G	0·83	85
(d) *Two-row plus Six-row barley.*		n=151.		
23	—	E= 98·5 =mean	3·18	0
24	N	E=127·4 —19·10N	2·54	37
25	NG	E=143·5 —19·18N +0·419G	2·12	56
26	I	E=123·0 —3·03I	1·29	84
27	NIG	E=134·1 — 9·62N —2·69I +0·019G	0·91	92
28	NI	E=134·7 — 9·78N —2·64I	0·90	92

APPENDIX I.

Outline of Statistical Evidence.

The methods used in the study are given in Prof. R. A. Fisher's book, "*Statistical Methods for Research Workers*" (Oliver & Boyd), and reference is made to the appropriate section numbers.

The following abbreviations are used :—

E, N, and I as above.

G=weight in grms. of a thousand corns, dry.

S.E.=standard error (see Fisher, § 13).

V=variance accounted for as a percentage of the total variance (see Fisher, § 3).

n=number of barleys and their malts studied.

Section II.

Table 2 gives the calculated regression equations for each set (see Fisher, § 25).

Section 3.

" Restricted general equation "

(29) . . . E = 134·61 —9·045 (±0·495) N — 2·774 (±0·070) I . . . ±0·995 lb. (n=187).

With factors given as rounded values and with the compensating adjustment of the mean, this gives equation (1).

(1) . . . E=134·7 —9·0N —2·8I.

Section 4.

Derivation of an Equation showing the expected Extract Factors on the Haase Hypothesis.

It is more convenient to consider the results in terms of "true" extract as a percentage on dry malt (E_2).

The equation expected *a priori* would be :—

(3) . . . $E_2=[100 - (\text{insoluble matter in barley})] \times \frac{[324+18\times\frac{3}{4}]}{324}$

Where $\frac{[324+18\times\frac{3}{4}]}{324}$ is a correction for the water added in hydrolysis of 75 per cent. of the starch to maltose and 324 and 18 are the equivalent weights of starch and water.

It is known that the loss of carbohydrate by respiration in malting is greater in high nitrogen barleys, but there is a correspondingly greater loss of nitrogen in the rootlets, so that the equation is not appreciably affected by change of nitrogen content during malting. The total nitrogen of the malt is slightly lower than that of the barley, and the relation does not appear to vary with the malting loss or the nitrogen content. [Malt T.N.=Barley T.N. —0·025]. The difference is partly explained by lack of comparability in the different methods employed for the determination of mositure ; that for malt leaves in more water than that for barley. The effect of this on extract is about — 0·5 per cent.

Equation (30) can be expanded by subdividing the insoluble matter :—

(31) . . . $E_2=[100 - (\text{insoluble protein}+\text{true insoluble carbohydrate})]\times\frac{337\cdot5}{324}$

The effect of enzymatic attack on carbohydrates is indirectly included, since the theoretical concept of the "true" insoluble carbohydrates includes all such that are insoluble after malting and mashing.

Taking a conversion factor of 6·0, the protein can be stated in terms of nitrogen.* The proportion of the total nitrogen which becomes soluble must also be allowed for. It has been shown (L. R. Bishop, this *Journ.*, 1931, 345) that with two-row and six-row barleys that 35 per cent. and 27 per cent. respectively of the nitrogen becomes soluble in the wort. Hence 65 per cent. and 73 per cent. remain insoluble, and the expected insoluble protein in terms of total nitrogen is 3·9 (=6·0×0·65) and 4·4 (=6·0× ·73). The mean figure is put at 4·2.

This gives the following anticipated equation :—

(32) . . . $E_2=[100 - 4\cdot2N - 1\cdot0\times(\text{true insoluble carbohydrate})]\times\frac{337\cdot5}{324}$

giving $E_2=104\cdot2 - 4\cdot38N - 1\cdot04\times(\text{true insoluble carbohydrate})$.

The actual equation obtained (29), when

* As stated in a previous paper, the conventional factor of 6·25 is definitely too high for barley proteins. The factor 5·7 is used in America for wheat proteins, (D. Breeze Jones, Circular 183, U.S. Dept. Agric., 1931) and this is definitely too low, being correct only for the alcohol-soluble protein. In this paper the rounded figure of 6·0 is preferred until the barley proteins have all been isolated in unquestionably pure condition, and the proper factor determined.

converted to give extract as a percentage E_1), by the factor 0·763, is :—

(33) . . . $E_1 = 102{\cdot}7 - 6{\cdot}90N - 2{\cdot}12I$ . . $\pm 0{\cdot}76$.

The validity of the comparison is improved further by giving the "true" extract, *i.e.*, the extract corrected for the interfering effect of grains volume. The correcting equation used is :—

(34) . . . $E_2 = 1{\cdot}053E_1 - 3{\cdot}54$ . . . .

(Calculated from results kindly supplied by Mr. F. E. Day.)

Applying this to equation (33), the following equation is obtained :—

(35) . . . $E_2 = 104{\cdot}6 - 7{\cdot}27N - 2{\cdot}23I$

Comparison of equations (32) and (35) shows the extent of agreement between theory and actuality. The nitrogen factor (7·27) is significantly higher than the expected, and so is the insoluble carbohydrate factor (2·23). This was shown by "t" tests (Fisher, §29).

"Sealing Up" of Extract in the Standard Grind.

Calculation from the figures for 23 of the 1922 barleys and their malts (from data of H. Lloyd Hind, this *Journ.*, 1924, 971) demonstrated the different relation between nitrogen and extract in the standard and fine grinds.

(36) Standard Grind.

$E_1 = 81{\cdot}2 - 6{\cdot}46N + 0{\cdot}09G$ . . . $\pm 0{\cdot}76\%$.

(37) Fine Grind.

$E_3 = 80{\cdot}5 - 4{\cdot}87N + 0{\cdot}08G$ . . . $\pm 0{\cdot}70\%$.

Where E_3 is the fine grind extract as a percentage on dry malt.

Part played by the Different Factors.

By means of the analysis of variance (Fisher, §40) it was shown that nitrogen matters only within varieties, and insoluble carbohydrate matters chiefly between, but also to a smaller extent within varieties.

These and other relations discussed in the text are illustrated more simply by means of a table of correlations given below.

Table 3.

Table of Correlations.

	F.	Simple Correlations.						Multiple Correlations.			
		EI.	EN.	EG.	IN.	IG.	NG.	E. NI.	E. NG.	E. IG.	E. NIG.
Six Variety Set.											
Total	35	—·78	—·36	+·46	*—·22*	—·61	*+·25*	·95	·63	·77	·95
Within varieties	30	—·37	—·90	*+·26*	*+·10*	—·43	*—·02*	·92	·91	*·34*	·94
Between ,, ...	5	—·95	*+·39*	*+·54*	*—·62*	*—·68*	+·81	·98	*·55*	·96	·98
Two-row plus Six-row Results.											
Total	150	—·92	—·61	—·44	+·37	+·56	*0*	·96	·75	·92	·96

F = number of degrees of freedom in simple correlations.

Correlations in heavy type very significant ($p < \cdot 01$)

,, ,, ordinary type significant (p between ·05 and ·01).

,, ,, italic type not significant ($p > \cdot 05$)

APPENDIX II.

Table 4.

Analyses of Barleys and Malts Studied.

Analyses are on dry weight and are the mean of duplicates except with the extract.

	Barley.				Extract of Malt		
Number.	Dry Matter %	Nitrogen %	1000 Corn weight grms.	Insoluble Carbo-hydrate %	Analysis	Pre-diction. (1)	Differ-ence.
The results for Six Varieties are given in Appendix II of a previous paper (this Journ. 1934, p. 74)							
	Commercial English two-row.						
1	88·38	1·500	36·9	7·42	100·0	100·5	—0.5
2	88·22	1·517	35·9	7·56	100·5	99·9	+0·6
4	87·90	1·42[illegible]	37·2	7·28	101·1	101·5	—0·4
5	87·72	1·432	36·5	7·33	101·2	101·3	—0·1
6	87·46	1·381	34·9	7·44	101·6	101·5	+0·1
7	87·36	1·405	35·7	7·52	100·7	101·0	—0·3
8	87·08	1·623	34·8	7·82	97·5	98·2	—0·7
9	88·10	1·341	37·1	7·02	102·8	103·0	—0·2
10	88·28	1·532	31·6	7·72	97·9	99·3	—1·4
13	87·08	1·484	36·6	7·02	101·0	101·7	—0·7
14	87·36	1·482	37·0	7·20	100·1	101·2	—1·1
17	87·26	1·400	33·6	7·78	100·6	100·4	+0·2
22	87·90	1·574	31·9	7·89	97·3	98·4	—1·1
23	88·10	1·686	35·0	7·62	98·1	98·2	—0·1
24	88·97	1·511	31·9	8·03	97·5	98·6	—1·1
28	88·22	1·816	37·3	7·64	97·9	97·0	+0·9
29	87·88	1·554	36·7	7·99	97·9	98·3	—0·4
30	87·72	1·275	36·4	7·54	102·3	102·1	+0·2
31	87·47	1·316	36·4	7·50	102·2	101·9	+0·3
32	87·88	1·332	37·8	7·48	102·8	101·8	+1·0
33	87·92	1·347	35·3	7·40	101·7	101·9	—0·2
38	88·26	1·573	41·2	7·21	100·4	100·4	0
11	87·50	1·422	34·7	7·72	99·2	100·3	—1·1
18	88·06	1·504	38·4	7·60	99·6	99·9	—0·3
19	88·88	1·578	34·7	7·08	100·9	100·7	+0·2
20	88·16	1·636	33·1	7·96	95·9	97·7	—1·8
21	88·10	1·475	36·1	8·24	98·3	98·3	0
26	88·76	1·701	33·6	8·28	95·5	96·2	—0·7
43	88·34	1·586	35·1	7·90	97·7	98·3	—0·6
44	88·48	1·562	38·4	7·02	101·1	101·0	+0·1
45	88·82	1·568	36·7	7·33	101·7	100·1	+1·6
46	88·26	1·566	37·6	7·09	100·6	100·8	—0·2
47	87·94	1·604	33·2	7·69	99·4	98·8	+0·6
48	88·24	1·466	36·2	7·34	101·0	101·0	0
34	87·86	1·442	36·0	7·18	101·6	101·6	0
49	88·50	1·477	39·3	7·22	101·0	101·2	—0·2
50	89·04	1·586	34·9	8·08	97·4	97·8	—0·4
54	88·72	1·479	35·65	7·64	101·5	100·0	+1·5
56	88·86	1·486	37·4	7·41	101·0	100·6	+0·4
59	88·76	1·494	34·55	7·44	100·6	100·4	+0·2
60	88·98	1·488	35·6	7·41	100·4	100·6	—0·2
66	88·34	1·268	37·7	7·62	101·6	102·0	—0·4
67	88·84	1·382	41·4	7·78	102·6	100·5	+2·1
35	88·32	1·343	37·6	7·35	102·8	102·0	+0·8
41	87·90	1·422	36·2	7·34	101·8	101·4	+0·4
42	87·70	1·410	37·0	7·16	101·7	102·0	—0·3
55	87·84	1·414	37·4	7·06	101·7	102·2	—0·5
57	87·36	1·452	34·7	7·37	101·8	101·0	+0·8
58	87·72	1·421	35·8	7·32	102·0	101·4	+0·6
89	87·24	1·450	35·4	7·43	100·8	100·9	—0·1
90	87·64	1·411	33·0	7·66	99·7	100·6	—0·9
91	87·26	1·476	37·4	7·31	101·4	101·0	+0·4
120	87·00	1·512	33·8	7·39	101·2	100·4	+0·8
71	88·04	1·627	34·85	7·82	96·7	98·2	—1·5
103	87·86	1·468	35·7	7·52	101·4	100·4	+1·0

Barley.					Extract of Malt.		
Number.	Dry Matter %	Nitrogen %	1000 Corn weigh grms.	Insoluble Carbo-hydrate %	Analysis	Pre-diction. (1)	Differ-ence.
104	87·90	1·458	36·0	7·40	100·4	100·9	—0·5
105	88·30	1·458	35·3	7·66	101·2	100·2	+1·0
121	87·72	1·507	34·6	7·38	101·7	100·5	+1·2
129	87·06	1·438	34·4	7·49	101·1	100·8	+0·3
134	87·10	1·426	37·4	7·34	102·3	101·3	+1·0
135	87·82	1·423	38·2	7·28	101·7	101·5	+0·2
141	87·80	1·338	36·8	7·46	101·3	101·7	—0·4
153	87·84	1·413	35·0	7·65	100 9	100·6	+0·3
65	86·98	1·627	39·2	7·45	100·0	99·3	+0·7
87	87· 2	1·402	35·0	7·56	102·2	100·9	+1·3
97	86·86	1·488	36·5	7·54	99·8	100·2	—0·4
98	88·14	1·605	38·2	7·41	99·1	99·5	—0·4
99	87·58	1·617	37·4	7·53	99·2	99·1	+0·1
122	87·62	1·634	41·1	7·12	98·3	100·1	—1·8
131	87·96	1·496	34·8	7·50	100·1	100·2	—0·1
133	87·32	1·351	38·9	7·32	102·2	102·0	+0·2
137	86·94	1·454	36·1	7·50	99·6	100·6	—1·0
142	87·18	1·452	34·3	7·(0	100·8	100·4	+0·4
158	87·36	1·6.3	37·5	7·32	99·7	99·7	0
172	87·44	1·540	41·1	7·31	97·9	100·4	—2·5
51	87·16	1·579	35·1	7·92	98·6	98·4	+0·2
52	87·28	1·624	34·5	7·!2	97·4	97·9	—0·5
61	86·96	1·434	36·5	7·32	101·6	101·3	+0·3
62	86·60	1·535	36·4	7·44	101·2	100·1	+1·1
82	88·48	1·526	38·0	7·36	99·0	100·4	—1·4
100	87·72	1·592	36·0	7·44	100·0	99·6	+0·4
111	87·82	1·547	34·7	7·39	102·1	100·4	+1·7
112	87·80	1·407	35·5	7·21	101·5	101·8	—0·3
130	86·70	1·370	34·4	7·44	100·8	101·6	—0·8
151	87·20	1·478	37·8	7·16	101·2	101·3	—0·1
152	87·58	1·494	38·3	6·78	101·4	102·3	—0·9
155	87·44	1·548	39·1	7·22	101·0	100·6	+0·4
159	87·44	1·647	37·6	8·02	99·3	97·5	+1·8
85	87·04	1·448	35·1	7·49	100·2	100·7	—0·5
86	86·84	1·436	36·4	7·32	100·7	101·3	—0·6
106	86·96	1·558	35·5	7·61	99·3	99·4	—0·1
113	87·00	1·484	39·3	7·62	101·6	100·0	+1·6
116	87·18	1·617	38·7	7·47	100·2	99·3	+0·9
118	87·26	1·592	38·6	7·66	99·2	99·0	+0·2
119	87·38	1·602	37·5	7·50	99·5	99·3	+0·2
123	87·52	1·629	38·2	7·54	98·5	99·0	—0·5
136	87·44	1·484	36·2	7·44	100·0	100·5	—0·5
145	87·32	1·665	40·9	7·14	100·4	99·7	+0·7
156	87·12	1·500	37·1	7·54	100·2	100·1	+0·1
157	87·12	1·476	36·3	7·37	98·9	100·8	—1·9
160	87·50	1·492	38·4	7·52	100·1	100·2	—0·1
173	87·10	1·638	35·0	7·74	95·0	98·3	—3·3
179	86·86	1·447	37·1	7·28	100·4	101·3	—0·9
181	87·20	1·536	34·5	7·77	100·9	99·2	+1·7

APPENDIX III.

Table 4.

Analyses of Barleys and Malts Studied.

Analyses are on dry weight and are the mean of duplicates except with the extract.

Barley.						Extract of Malt.		
Letter.	Number.	Dry Matter %	Nitrogen %	1000 Corn weight grms.	Insoluble Carbo-hydrate %	Analysis	Pre-diction. (1)	Differ-ence.
		Commercial	Californian	Six-row.				
PR	4/3	87·56	1·529	40·1	9·40	95·7	94·7	+1·0
	5/3	88·08	1·563	40·1	9·34	95·6	94·6	+1·0
	10/3	87·66	1·520	39·4	9·21	95·2	95·3	—0·1
	11/3	87·66	1·547	39·5	9·53	95·2	94·1	+1·1
	12/3	87·20	1·540	40·0	9·68	95·6	93·8	+1·8
IN	3D	87·66	1·420	43·0	9·09	96·6	96·5	+0·1
	4D	87·32	1·394	44·25	9·22	96·0	96·3	—0·3
	5D	87·66	1·393	44·2	9·18	96·7	96·5	+0·2
	6D	87·90	1·450	43·7	8·98	96·4	96·5	—0·1
	7D	88·12	1·421	44·3	8·97	96·8	96·8	0
Buc.	3AB	87·48	1·535	45·0	9·39	93·1	94·6	—1·5
	4AB	87·66	1·520	43·7	9·61	93·9	94·1	—0·2
	18/3	87·00	1·496	43·5	9·52	92·9	94·6	—1·7
	19/C, D, E.	87·55	1·488	44·5	9·54	93·1	94·6	—1·5
	35/C, D, E.	88·11	1·529	44·8	9·75	93·2	93·7	—0·5
PN	8D	87·40	1·691	37·6	9·84	91·9	92·0	—0·1
	9D	87·60	1·655	39·1	9·74	92·6	92·6	''
	10D	87·86	1·635	39·6	9·82	91·6	92·5	—0·9
	11D	87·74	1·654	38·2	9·78	91·8	92·4	—0·6
	12D	88·26	1·695	40·1	9·68	92·2	92·3	—0·1
	13D	87·82	1·659	38·6	9·73	91·7	92·6	—0·9
	21D	88·20	1·654	39·6	9·80	92·4	92·4	0
	22D	88·20	1·649	40·5	9·67	93·4	92·8	+0.6
	23D	88·40	1·650	38·6	9·58	92·9	93·1	—0·2
	24D	89·33	1·621	39·7	9·57	93·1	93·4	—0·3
Cor.	13/3	87·02	1·618	40·4	9·67	93·7	93·1	+0·6
	14/3	87·02	1·569	41·4	9·54	94·6	93·9	+0·7
	15/3	87·68	1·591	40·7	9·46	94·6	93·9	+0·7
	16/3	87·40	1·665	40·0	9·62	93·5	92·8	+0·7
IN/1	25D	89·12	1·688	36·1	9·98	92·1	91·6	+0·5
	26D	89·40	1·672	35·9	9·92	92·8	91·9	+0·9
Buc/Cor	17/3	87·30	1·570	42·6	9·52	93·5	94·0	—0·5
AJ	26/3	88·48	1·397	46·6	8·96	97·6	97·1	+0·5
	29/3	88·95	1·421	45·0	8·99	97·4	96·7	+0·7
	30/3	88·22	1·397	46·1	8·78	97·3	97·5	—0·2
	32/3	88·20	1·396	46·3	8·97	97·6	97·1	+0·5
	35/3	88·70	1·444	44·5	8·98	97·0	96·6	+0·4
	38/3	88·16	1·419	45·0	9·22	97·6	96·1	+1·5
	36/3	89·00	1·430	44·2	9·12	96·8	96·3	+0·5
TC	30/1	88·70	1·650	38·6	9·80	91·0	92·5	—1·5
	31/1	88·21	1·631	37·2	10·08	92·8	91·8	+1·0
	32/1	88·20	1·626	38·6	10·12	93·0	91·8	+1·2
	33/1	88·22	1·630	38·6	10·18	93·1	91·6	+1·5
AM	14D	87·94	1·610	43·8	8·68	97·1	96·0	+1·1
	15D	87·14	1·559	43·7	8·76	96·6	96·2	+0·4
	19D	88·60	1·585	44·0	8·74	97·2	96·0	+1·2
	20D	88·30	1·560	43·6	8·77	97·3	96·2	+1·1

Barley.						Extract of Malt.		
Letter.	Number.	Dry Matter %	Nitrogen %	1000 Corn weight grms.	Insoluble Carbo-hydrate %	Analysis	Pre-diction. (1)	Differ-ence.
			Other Varieties.					
July		87·56	1·240	29·0	8·11	101·6	100·9	+0·7
O.A.C. 21	'28	86·52	2·09	32·1	9·05	92·1	90·6	+1·5
Trebi	'28	86·72	1·77	45·2	8·40	94·3	95·3	—1·0
	'30	85·08	1·946	42·6	8·42	87·9	93·6	—5·7
	'32	87·54	1·436	46·6	8·94	95·8	96·8	—1·0
Commercial Californian	9 & 10, '31	87·72	1·454	41·7	9·13	96·5	96·0	+0·5
Hero	'26	87·14	1·931	45·4	10·14	86·2	89·0	—2·8
Vaughn	7, '31	87·94	2·290	36·1	9·41	85·9	87·8	—1·9
Tennessee Winter ..	'26	87·66	2·262	30·8	10·25	87·2	85·7	+1·5
	2, '31	87·76	1·181	37·4	9·68	97·3	97·0	+0·3
	11, '31	88·00	1·510	38·9	9·46	94·0	94·6	—0·6
Mariout	'26	87·96	2·259	46·5	9·68	87·5	87·3	+0·2
	24, '31	88·22	1·377	44·4	9·36	94·8	96·1	—1·3
Coast	'26	87·74	2·139	32·7	11·25	86·8	84·0	+2·8
Smooth Awn	'26	87·42	1·812	42·8	9·42	89·4	92·0	—2·6
B 244	199, '27	88·48	1·513	32·8	8·04	94·6	98·6	—4·0
	200c, '28	88·30	1·477	32·5	7·41	99·2	100·7	—1·5
	131, '29	88·90	1·788	34·7	7·70	94·0	97·1	—3·1
	110c, '30	88·18	1·370	31·6	7·18	98·7	102·3	—3·6
	111c, '30	87·90	1·354	30·4	7·47	98·1	101·6	—3·5
	115c, '30	89·22	1·342	30·2	7·64	97·4	101·2	—3·8
	116c, '30	88·82	1·377	30·4	7·64	97·5	100·9	—3·4

Rothamsted Experimental Station,
Harpenden, Herts.
22nd December, 1933.