# THE INSTITUTE OF BREWING RESEARCH SCHEME.

## BARLEY PROTEIN RESEARCHES.

## PREDICTION OF EXTRACT II.

## THE EFFECT OF VARIETY ON THE RELATION BETWEEN NITROGEN CONTENT AND EXTRACT.

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It has been shown in earlier papers that the quantities of the individual proteins of barley are regularly related to the total protein content of the grain in any one variety, but there are quantitative differences between varieties.[1] Consequently there is no direct means of elucidating the effect of differing quantities of the individual proteins except by comparing barleys of different variety or of different total protein content. Before any effects on brewing value could be separately attributed to these individual proteins it was therefore necessary to determine in what way differences in total nitrogen content or in variety affected the properties of malts.

The present study does this for extract, one of the factors in the brewing value of malt which can be measured quantitatively. One result of this study is that large numbers of varietal analyses are put in a form available for use in practice. The effect of total protein (or total nitrogen content) on extract was first studied chiefly in one variety.[2] There it was shown that both nitrogen content and grain weight affect the extract of malts, made from Plumage-Archer barley, in the manner indicated by the equation :—

(1) $E = 110{\cdot}1 - 11{\cdot}2\ N + 0{\cdot}18\ G.$

This equation was derived from analysis of a limited number of samples (34) and a limited range of nitrogen content (1·3 — 1·7). In the present study the number and nitrogen range have been extended, giving in consequence more accurate results.

It was shown in the previous paper[2] that different varieties require some change in the equation and the question arose as to whether it was necessary to change only the constant of the equation (110·1 in the original) or whether the nitrogen in different varieties behaves differently. This consequence would follow if the individual proteins had specific effects on extract; but none were found in the samples here studied. The importance of the degree of modification of the malt is also shown.

### I.—Constancy of Nitrogen and Thousand Corn Weight Effects on Extract.

#### (a) *Examination of Six Representative Varieties.*

Six varieties were chosen to cover as wide an extract range as possible and six samples of each variety were studied. The extracts of the malts from these samples have been plotted against the nitrogen content of the barleys in Diagram 1. The proof that the effect of thousand corn weight is the same in all varieties has been anticipated—it is known that this effect is in any case small—and the extracts plotted are those calculated for a constant 38 grms. thousand corn weight. That is, 0·2 lb. has been added for every gram that the actual thousand corn weight was below 38 grams and 0·2 lb. subtracted for every gram that the thousand corn weight was above 38 grams.

It is clear from the diagram that in each variety, extract falls off as the nitrogen increases and, since the lines for the different varieties are approximately parallel, the effect of nitrogen content on extract is substantially the same in all the varieties. This implies, for example, that whether compared at 1·0 per cent. of nitrogen, at 2·0 per cent. or at intermediate values Plumage-Archer malts will yield an extract which exceeds by a constant quantity that given by Atlas malts of the corresponding barley nitrogen content.

This leads to the suggestion that a single nitrogen factor can be used for all these varieties and, if the same can be shown for the thousand corn weight effect, then one equation can be used for all varieties except that the constant (A) must be changed for

[1] L. R. Bishop, this *Journ.* 1928, 101; 1930, 336.
[2] L. R. Bishop. *ibid.* 1930, 421.

RELATION BETWEEN NITROGEN CONTENT AND EXTRACT FOR DIFFERENT VARIETIES.

ALL EXTRACTS CORRECTED TO THAT FOR 38 GMS. 1,000 CORN WEIGHT.

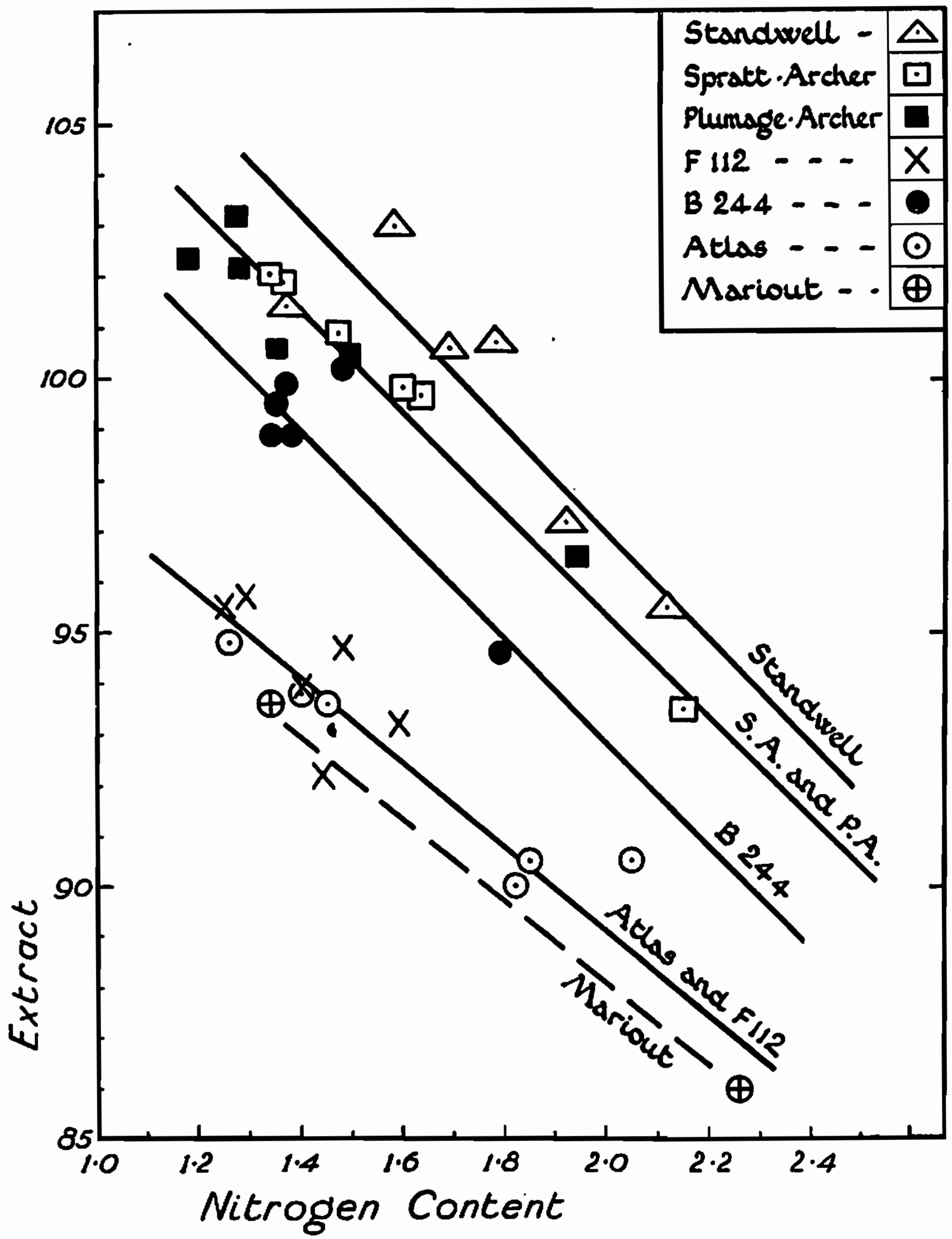


*Diagram* 1. The quantities of extract yielded by barleys of different varieties are seen to decrease regularly as the nitrogen content of the grain inceraces. The points fall very close to straight lines which are almost parallel. The results show that the difference between any two varieties in extract is the same whatever the nitrogen content of the grain. For extract calculation it is therefore necessary to know the variety and its extract constant. The diagram illustrates very clearly the individuality and regularity in the behaviour of varieties.

each variety. This "within varieties" equation was calculated statistically from the analytical results and so were the individual equations for each of the six varieties. It was then shown that the nitrogen factors of the individual equations did not differ from one another more than would be expected on the theory of errors. The same was shown for the individual thousand corn weight factors. (This proof is elaborate and is not given here. Details can be supplied if requested). The results imply that one nitrogen factor and one thousand corn weight factor can be used for the varieties tested. In other words nitrogen appears to have the same effect on extract in all these varieties and so does thousand corn weight.

(*b*) *Examination of* 851 *Samples.*

An alternative method of proving the same point was afforded by examination of all the sets of data available and calculation of the "within varieties" equation. Equation (2) was thus obtained. In it allowance is made for constant differences in extract between varieties compared at equal nitrogen percentages. If nitrogen had different effects in different varieties then the nitrogen factor in the equation would be vague and uncertain, that is, subject to large errors. If, on the other hand, nitrogen had the same effect in all varieties then the factor would be accurate, that is it would have a small standard error. This was actually found to be the case and varieties with abnormal nitrogen extract relationships must have been absent or represented only by small numbers. Similar remarks apply to the thousand corn weight.

The equation obtained was :—

(2) $E = A - 10{\cdot}42\ (\pm\ 0{\cdot}28)\ N + 0{\cdot}217\ (\pm\ 0{\cdot}016)\, G. \pm 1{\cdot}37$

Where $E$ = *Extract in brewers' lb. per* 336 *lb. of dry malt, determined by the Institute of Brewing Standard Method.*

$A$ = *A constant depending on the variety* (*see below*).

$N$ = *Nitrogen per cent. on dry barley.*

$G$ = *Thousand corn weight of dry barley in grams.*

*The standard errors of the factors are given in brackets.*

This equation was derived from analyses of 851 barleys and their malts. They came from a very wide range of soil and seasonal conditions which are too numerous to specify here individually.

The table below shows the sets used :—

The small standard error of the nitrogen factor shows further that experimental stocking and commercial bulk maltings produce very closely similar nitrogen-extract relationships. When the first paper was written the validity of this was questionable, but subsequent results have removed this doubt (see [3] and above).

[3] H. M. Lancaster and H. Lloyd Hind, this *Journ.* 1932, 290.

TABLE I.

SETS OF DATA USED IN CALCULATING EQUATION (2).

| Year. | Number of Samples. | Variety. | Malting Methods. |
|---|---|---|---|
| 1922 | 89 | Plumage-Archer | Stocking. |
| 1923 | 87 | ,, ,, | ,, |
| 1924 | 81 | ,, ,, | ,, |
| 1925 | 116 | ,, ,, | ,, |
| 1926 | 129 | ,, ,, | ,, |
| 1926 | 34 | ,, | Experimental Bulk. |
| 1927 | 78 | Spratt-Archer | Stocking. |
| 1928 | 50 | ,, ,, | ,, |
| 1931 | 104 | Commercial English 2-row | Commercial Bulk. |
| 1931 | 47 | Californian 6-row | ,, ,, |
| Var. | 6 | Standwell | Stocking. |
| ,, | 6 | Plumage-Archer | ,, |
| ,, | 6 | Spratt-Archer | ,, |
| ,, | 6 | Indian | ,, |
| ,, | 6 | F.112 | ,, |
| ,, | 6 | Atlas | ,, |

TABLE 2.

VARIETAL EXTRACT CONSTANTS
*for substitution for A in equation.*
$E = A - 10\cdot5\,N + 0\cdot20\,G.$

| Variety. | Malting Method. | Number of samples. | A. | Standard error of A. | B. Average Extract Yield. |
|---|---|---|---|---|---|
| | | | Naked Barley. | | |
| Indian naked | S | 3 | 116·8 – | — | 104·0 |
| | | | Two-row Barleys. | | |
| Golden Pheasant | S | 10 | 110·5 c | 0·4 | 98·0 |
| Cambridge 59/120 | S | 7 | 110·1 c | 0·4 | 98·0 |
| Standwell | S | 10 | 110·0 c | 0·3 | 100·4 |
| 825 | S | 23 | 110·0 c | 0·1 | 101·0 |
| 824 | S | 23 | 109·4 c | 0·2 | 101·0 |
| Beaven's Archer | S | 11 | 109·2 c | 0·3 | 98·0 |
| Webb's Sunrise | S | 21 | 109·2 c | 0·2 | 98·0 |
| Chevallier | S | 4 | 108·9 c | 0·2 | 100·8 |
| 35/51 | S | 12 | 108·7 c | 0·2 | 101·0 |
| Spratt-Archer | S | 39 | 108·6 c | 0·1 | 101·0 |
| Plumage-Archer | B | 34 | 108·3 – | — | 100·0 |
| Plumage | S | 4 | 108·3 c | 0·3 | 101·7 |
| Archer-Goldthorpe | S | 10 | 108·3 | 0·4 | 99·0 |
| Archer | S | 8 | 108·2 c | 0·6 | 96·0 |
| Goldthorpe | S | 4 | 107·8 c | 0·3 | 101·1 |
| Gartons 1917 | S | 6 | 107·7 c | 0·7 | 95·0 |
| | | | Six-row Barleys. | | |
| B.244 | S | 7 | 106·0 c | 0·4 | 97·0 |
| Garton's Square-head | S | 7 | 105·2 c | 0·3 | 95·0 |
| Indian Six-row | S | 38 | 104·8 – | 0·2 | 97·0 |
| Carter's Six-row | S | 2 | 104·6 c | — | 99·0 |
| F.112 | S and B | 14 | 103·0 c | 0·3 | 94·0 |
| Tennessee Winter | S | 5 | 102·9 – | 0·6 | 94·0 |
| Atlas | S | 12 | 101·5 – | 0·3 | 93·0 |

S=Malted in stocking.
B=malted in bulk.
c=corrected for soil and seasonal errors through a control.

TABLE 3.

APPROXIMATE VARIETAL EXTRACT CONSTANTS.
Where only a few samples were available.
Six-row barleys.

| Variety. | Malting Method. | Number of samples. | A. Extract Constant | B. Average Extract Yield. |
|---|---|---|---|---|
| July | B | 1 | 108·5 | 101·0 |
| O.A.C. 21 | S and B | 2 | 107·3 | 94·0 |
| Coast | S | 2 | 101·9 | 90·0 |
| Trebi | S and B | 3 | 101·3 | 92·0 |
| Mariout | S | 3 | 101·0 | 91·0 |
| Smooth Awn | S | 1 | 99·8 | 89·0 |
| Hero | S | 1 | 99·4 | 86·0 |
| Vaughan | S | 2 | 98·3 | 85·0 |

This general equation may be stated in rounded numbers as :—

(3) . . . . $E = A - 10{\cdot}5N + 0{\cdot}20G$

This equation applies to all the varieties tested, and being based on a much larger number of samples is therefore substituted for the Plumage-Archer equation originally given (1 *loc. cit.*), as it is more accurate and more convenient for general use.

Another result of this more extensive examination was to show that undermodification was probably responsible for the relatively low extracts of malts from barleys with very high thousand corn weight. The limitation put forward in the earlier paper is therefore withdrawn and thousand corn weights above 42 grams should be treated just as those below that figure.

## II.—Extract Constants for Different Varieties.

Having shown that the factors for both nitrogen content and thousand corn weight are similar for all varieties, there remains the constant in the prediction equation and this has been shown to differ considerably in different varieties. In Tables 2 and 3 is given a list of constants calculated for such varieties as it has been possible to test and with the number of samples decided by opportunity.

The constants are for substitution in the general "within varieties" equation (3) given, *i.e.*, in the equation.

$$E = A - 10{\cdot}5N + 0{\cdot}20G$$

Where A = *The appropriate varietal constant.*

In a large number of cases, varieties have been grown by the National Institute of Agricultural Botany alongside a control [Plumage-Archer (1924) or Spratt-Archer (37/6)]. In these cases it has been possible to eliminate the soil and seasonal effects and obtain what is probably an accurate varietal figure from relatively few samples. These cases are marked by c in Table II. They have been adjusted by correcting the extract of the variety by the same amount as the control differs from its own predicted value. The calculation method used has been to predict the extract of both variety and control from the Plumage-Archer equation and subtract the predicted values from the corresponding extracts obtained by analysis. If the differences are represented by $V - v = D$ and $C - c = d$, the correction to be added to the Plumage-Archer constant is given by $D - d$.

Where the control was not Plumage-Archer itself, Spratt-Archer was used and its constant (108·6) substituted for 108·3. With some varieties no control variety for eliminating interfering factors was available and the results are less exact.

It must be emphasized that these varietal constants are those obtained under approximately normal malting conditions for "pale ale" malts. Wide deviations from these conditions will give other constants, particularly with certain varieties. See Section III.

The standard error of A given in Table 2 is the standard error of the mean, *i.e.*, the amount by which the figure given is likely to differ from the true figure. It will be appreciated that where only a few samples are available the figure given is very likely to be inexact. This applies particularly to Table 3.

It will be seen from Tables 2 and 3 that there is an almost continuous series of constants ranging from 110 to 98. This means that varietal effects on extract apart from nitrogen and thousand corn weight may give differences up to 12 brewers' pounds of extract, or as much as 19 lb. when naked barleys are taken into consideration. It is therefore very necessary to know both the variety and its constant when calculating extract from nitrogen and thousand corn weight. Average figures can be given for English and Californian varieties but, since different varieties occur, these are necessarily inexact. (Table 4.)

TABLE 4.

*Average value for constant A for commercial samples.*

| | A. | Range. |
|---|---|---|
| English .. .. | 108 | 107–110 |
| Californian .. | 101·5 | 98–103 |

The constants A are arranged from the point of view of the chemist who wishes to predict the extract. He requires to know the variety, its prediction constant and the nitrogen content and thousand corn weight.

One of the requirements of the buyer is met by the column B in Tables 2 and 3, which gives an approximate estimate of the extract yield of the variety under average soil and seasonal conditions, malted to the degree of modification usual in the samples examined. It will be seen that some of the two-row varieties with high constants do not give the highest extracts, as these varieties tend to be high in the nitrogen content.

## III. Accuracy of Extract Prediction.

Subsequent work has demonstrated the effect on extract of the degree of modification of the barley during malting. This degree of modification is the resultant in the main of two sets of factors, one is the vitality and physical state of the barley at malting (the "missing factor"), the other is the malting conditions employed (times, temperatures, etc.). Studies have shown that, with the normal range of malting conditions and most barleys, the barley factor is as important as malting conditions; each, from a provisional estimate appears responsible for some 5 per cent. of the variation in extract. The ideal aimed at would be to measure the "missing factor" in the barley itself when the extract for given malting conditions could be predicted. In this paper the resultant effect of the two sets of factors has been measured in the malt by the degree of modification. This has been measured, as previously proposed, by the percentage of the total barley nitrogen becoming permanently soluble in the wort.[4]

The importance of the barley factor only becomes prominent with poorer barleys. With "pale ale" grade barleys it is usually not important, and it is estimated that predictions from nitrogen content, thousand corn weight and the appropriate varietal factor (Table 2) should give a standard error of about $\pm 0\cdot 8$ lb., as previously suggested ([2]).

It is among the mild ale grade barleys that the barley factor assumes more importance. These are graded lower than pale ale barleys on external appearance, which is not a reliable guide to the physical state and vitality of the barley (see [5]); nevertheless, it is chiefly here that the unsound barleys occur and predictions from nitrogen and thousand corn weight show larger errors than $\pm 0\cdot 8$ lb.

An equation taking into account the degree of modification eliminates these errors and is useful in checking the determination of extract on malts. It has, therefore, been calculated for as many varieties as possible. The equation obtained from the analyses of 150 barleys and their malts was :—

$$(4) \; .. \; E = a + 0\cdot 12G + 0\cdot 11P - 7\cdot 5N \ldots\ldots \pm 0\cdot 8 \text{ lb.}$$

where P=percentage of permanently soluble nitrogen on barley total nitrogen. The varieties used and their appropriate constants are given in Table 5.

TABLE 5.

*Varietal Constants in Equation* (4).

| Variety | a | Variety | a |
|---|---|---|---|
| Standwell .. .. | 103·8 | Plumage .. | 102·6 |
| Chevallier.. .. | 103·2 | Goldthorpe .. | 102·2 |
| 825 .. .. | 102·9 | Spratt-Archer | 102·0 |
| 824 .. .. | 102·8 | B.244 .. .. | 100·1 |
| 35/51 .. .. | 102·6 | Californian Bay Brewing* .. | 96·3 |
| Plumage-Archer .. | 102·6 | F.112 .. | 95·9 |

* Atlas, Coast and Tennessee Winter.

The remaining errors come chiefly from errors in sampling and analysis and for closer estimates current practice here needs improvement.

### (c) *Analytical and Sampling Errors.*

The importance of avoiding these sources of error is clear. The analysis sample should be made from a mixture of sub-samples taken all over the bulk. Systematic errors in either nitrogen or extract determination do not reveal themselves until the prediction method is used, but here they lead to a large proportion of bad agreements between extract and prediction. It is the extract determination which is liable to the greatest random errors, and occasional determinations may be 1 lb. or more in error if only single determinations are made. Comparison of the results with the prediction will in these cases reveal a discrepancy and repetition of the extract determination will show if this is the source of error. This method of checking has been found useful in several laboratories.

## IV. Physiological Characterization of Varieties.

The barley grain of all varieties is influenced in composition chiefly by soil and season, and the influence of these is similar in all varieties. The characteristics of varieties show themselves as regular small differences in

[4] L. R. Bishop, *this Journ.*, 1931, 345.
[5] E. J. Russell and L. R. Bishop, *ibid* 1933, 287.

composition superimposed on the soil and seasonal effects, *e.g.*, in the samples examined in the Institute series, Golden Pheasant grown beside Plumage-Archer has been regularly 0·16 per cent. higher in nitrogen content [5], and while Plumage-Archer and Spratt-Archer have given closely corresponding nitrogen contents under all conditions, Spratt-Archer has been regularly about 3 grams lower in thousand corn weight.

The extract constants given in Table 2 are another example of constant varietal difference in composition in spite of the large variations produced by soil and season. Varietal characteristics in composition may therefore be given quantitative expression as *plus* or *minus* so much, when compared with a standard variety—say, Plumage-Archer.

It may, therefore, be suggested that the extract prediction constants (A, Table 2) are useful indices in barley breeding, summarising as they do the characteristics of any variety in a single figure; and the suggestion is made that the mode of inheritance of this figure should be worked out with the object of breeding improved varieties.

*Acknowledgements.*

The greater proportion of the barleys on which these results are based were grown by the National Institute of Agricultural Botany, in some cases especially for this investigation. We are very much indebted to this Institute.

The analyses of those grown in the years 1922-26 were made by Mr. Lloyd Hind. For samples and analyses of bulk malts we are indebted to Hugh Baird & Sons, Ltd., and Edward Sutcliffe, Ltd., and to Messrs. G. D. Clarkson, and S. F. Weeden. Altogether the results of analyses of some 1,500 barleys and their malts have been utilised.

## Summary.

Nitrogen and thousand corn weight have been shown to have similar, if not the same, effects on the yield of extract in all the varieties studied. There are, therefore, constant differences between varieties in their extract yields at corresponding nitrogen contents and thousand corn weights. Consequently the extract of barleys of any variety can be predicted if the analysis and the appropriate constant are known. A list of a number of these constants is given.

The remaining sources of error are—another factor in the barley, malting conditions and analytical and sampling errors.

The constancy of the extract prediction "constant" for each variety throws light on the physiology of the barley grain and on possibilities in breeding new varieties.

*Rothamsted Experimental Station,*
*Harpenden,*
*Herts.*