# Study of Relationships between Wheat Protein Contents of Two U.K. Varieties and Derived Flour Protein Contents at Varying Extraction Rates. II. Studies by Hand-Dissection of Individual Grains

E. A. FARRAND, Cereal Advisor, RHM Research Limited, The Lord Rank Research Centre, Lincoln Road, High Wycombe, Buckinghamshire, U.K., and J. J. C. HINTON, Senior Scientist, Flour Milling and Baking Research Association, Chorleywood, Rickmansworth, Hertfordshire, U.K.

## ABSTRACT

Some 50 to 70 individual grains representative of milling samples of two U.K. varieties, Maris Widgeon (semihard) and Cappelle-Desprez (semisoft), each at two protein levels, were hand-dissected and protein determinations made on eight weighed arbitrary fractions, classified as embryo, scutellum, pericarp-testa, aleurone, and endosperms 1, 2, 3, and 4. It was concluded that a protein gradient found between the inner and outer endosperm could be expressed logarithmically, and the slope of the gradient was correlated with the protein content of the wheat. An interpretation of the results provided a basis for further studies on the relationship between grain proteins and milling characteristics.

Protein distributions within the endosperms of wheat grains have been reported by Hinton (1). However, to our knowledge such measurements have not previously been applied to particular samples of wheat that have been experimentally and commercially milled. To justifiably relate hand-dissection of a few dozen grains to a 10-ton consignment required careful consideration of sampling problems.

This paper indicates a sampling approach and gives results of protein distributions obtained by hand-dissection of the high- and low-protein Maris Widgeon and Cappelle-Desprez wheats used in section I.

## MATERIALS AND METHODS

### Preliminary Sampling

The samples for Buhler-milling (section I) had already been prepared to give protein contents corresponding with the 10-ton consignments. Samples were further characterized: a) segregation by grain weight into five fractions varying from 15 to 65 mg. per grain, and b) segregation by grain type, vitreous, intermediate, and mealy. Protein determinations were made on each fraction and type. For the type of grain the protein contents were in the order vitreous, intermediate, mealy, and the types selected from both high-protein and low-protein wheats were almost identical in protein content. Therefore, different levels of protein in the original samples were associated with the actual proportions of grain type. In terms of grain weight the larger and smaller grains gave the highest levels and the intermediate sizes the lowest levels of protein. The information was used to build small representative composite samples.

### Sampling for Hand-Dissection

Two factors affecting the protein content of the grains were applied: a) the grain size was arranged so that one-third consisted of grains between 25 and 50 mg. and two-thirds over 50 mg. and b) a high-protein sample was selected comprised of vitreous grains, and a low-protein sample consisting of mealy grains. This facilitated grain selection using the extremes of endosperm texture, but resulted in greater differences between the high and low levels of protein for each variety compared with the milling samples. Since proportions characterizing the original samples were recorded for grain size and endosperm type, the hand-dissection results for the vitreous and mealy components could be appropriately weighted to match the protein content of the milling samples. However, this was considered beyond the scope of this paper.

### Dissection

The method was the same as previously used by Hinton (1). The parts were separated in the natural state (~14% moisture) except for a trace of moisture, applied with a fine brush, to facilitate separation of the pericarp-testa fraction from the aleurone layer. Any endosperm attached to the aleurone layer was removed by gentle scraping, and the endosperm subdivided into four fractions, 1 adjacent to the aleurone, 2, 3, and 4 in a succession inwards to the center. The pericarp-testa, aleurone, and endosperm fractions for analysis were obtained from the median part of the grain. The proportions for the parts were obtained by a separate and complete dissection of six or more grains.

### Determinations

Nitrogen was determined by the micro-Kjeldahl procedure on duplicate samples weighing 0.5 to 1.0 mg. and results expressed at natural moisture content

TABLE I. NITROGEN CONTENT OF DISSECTED FRACTIONS OF MARIS WIDGEON

| | Mealy Grains, N 1.63%, 48 Grains | | | | Vitreous Grains, N 2.27%, 72 Grains | | | |
|---|---|---|---|---|---|---|---|---|
| Fraction | % of Grain | % N Content | N per 100 g. of Grain | N as % of Total N in Grain | % of Grain | %N Content | N per 100 g. of Grain | N as % of Total N in Grain |
| Embryo | 1.2 | 5.83 | 0.070 | 4.2 | 1.2 | 6.07 | 0.073 | 3.3 |
| Scutellum | 1.5 | 4.80 | 0.072 | 4.3 | 1.7 | 4.97 | 0.085 | 3.8 |
| Pericarp-testa | 7.4 | 0.85 | 0.063 | 3.7 | 7.1 | 1.09 | 0.077 | 3.5 |
| Aleurone layer | 5.6 | 3.65 | 0.204 | 12.2 | 5.7 | 3.96 | 0.226 | 10.2 |
| Endosperm 1 | 2.1 | 3.55 | 0.750 | 4.5 | 3.2 | 5.04 | 0.163 | 7.4 |
| Endosperm 2 | 22.0 | 2.15 | 0.474 | 28.2 | 18.7 | 3.15 | 0.590 | 26.6 |
| Endosperm 3 | 29.7 | 1.38 | 0.410 | 24.5 | 28.7 | 1.83 | 0.525 | 23.7 |
| Endosperm 4 | 30.5 | 1.01 | 0.308 | 18.4 | 33.7 | 1.41 | 0.476 | 21.5 |
| Endosperm total | 84.3 | 1.52 | 1.267 | 75.6 | 84.3 | 2.08 | 1.754 | 79.2 |
| Grain total | 100 | | 1.68 | 100 | 100 | | 2.22 | 100 |

TABLE II. NITROGEN CONTENT OF DISSECTED FRACTIONS OF CAPPELLE-DESPREZ

| | Mealy Grains, N 1.45%, 54 Grains | | | | Vitreous Grains, N 2.19%, 72 Grains | | | |
|---|---|---|---|---|---|---|---|---|
| Fraction | % of Grain | % N Content | N per 100 g. of Grain | N as % of Total N in Grain | % of Grain | %N Content | N per 100 g. of Grain | N as % of Total N in Grain |
| Embryo | 1.1 | 6.05 | 0.066 | 4.6 | 1.1 | 6.11 | 0.067 | 3.0 |
| Scutellum | 1.4 | 4.82 | 0.067 | 4.7 | 1.6 | 5.05 | 0.081 | 3.7 |
| Pericarp-testa | 7.4 | 0.83 | 0.061 | 4.2 | 7.3 | 0.93 | 0.068 | 3.1 |
| Aleurone layer | 6.0 | 3.35 | 0.201 | 13.9 | 6.4 | 4.13 | 0.268 | 12.1 |
| Endosperm 1 | 2.3 | 2.70 | 0.062 | 4.3 | 3.3 | 5.10 | 0.168 | 7.6 |
| Endosperm 2 | 9.3 | 1.93 | 0.179 | 12.4 | 19.0 | 3.03 | 0.575 | 26.0 |
| Endosperm 3 | 39.5 | 1.29 | 0.510 | 35.2 | 27.5 | 1.80 | 0.520 | 23.5 |
| Endosperm 4 | 33.0 | 0.91 | 0.300 | 20.7 | 33.8 | 1.37 | 0.464 | 21.0 |
| Endosperm total | 84.1 | 1.25 | 1.051 | 72.6 | 83.6 | 2.04 | 1.723 | 78.1 |
| Grain total | 100 | | 1.45 | 100 | 100 | | 2.21 | 100 |

approximately 14.0%. The dissection results were reported as percent nitrogen and N X 5.7 used for any derived protein values.

## RESULTS

The N content for the dissected parts are given in Table I, Maris Widgeon and Table II, Cappelle-Desprez.

### Maris Widgeon

There was reasonable agreement between the N content for the undissected grains compared with the sum of the dissected parts.

The proportions of anatomical parts showed only slight differences between the vitreous and mealy samples, and the endosperm contents were identical at 84.3%.

All parts of the vitreous grains had a higher N content than those of the mealy grains and this was most marked in the endosperm where the mean N contents were 2.08 and 1.52%, respectively. As a result the endosperm contained 79.2% of the grain N in the vitreous grains compared with 75.6% in the mealy grains. Results are shown in the form of a histogram (Fig. 1), relating percent nitrogen content versus log (endosperm subdivisions). The difference in N gradients within the endosperm shows that the gradient is significantly steeper in the vitreous sample with higher N content. The information indicated that the difference in levels of protein between the samples was mainly due to differences in protein content of the outer endosperm. For example, Fig. 1 was used to estimate the N content of equal proportions of outer endosperms and it was calculated that an arbitrary outer

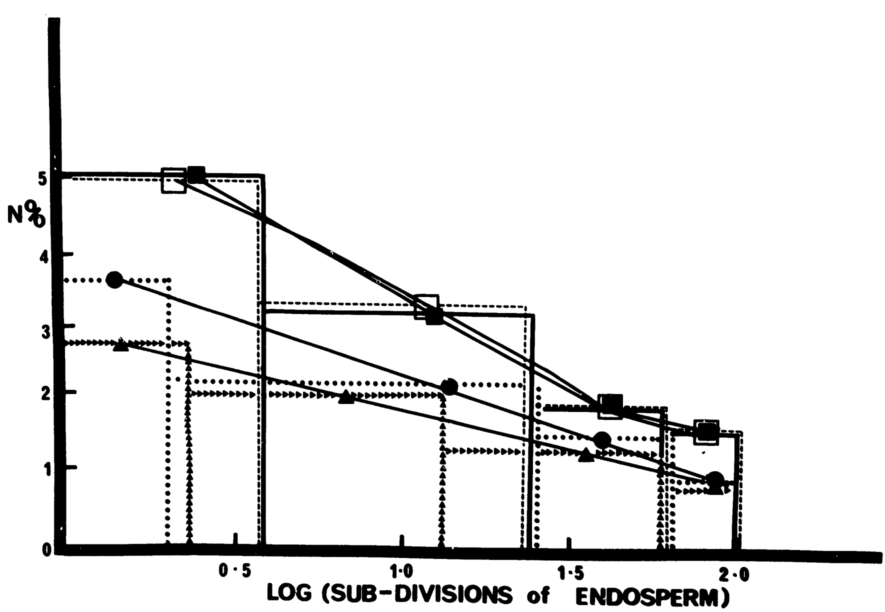


**Fig. 1. Histogram. Percent nitrogen versus logarithm of subdivisions of endosperm. Maris Widgeon: open squares = vitreous, closed circles = mealy; Cappelle-Desprez: closed squares = vitreous, closed triangles = mealy.**

endosperm fraction (2.1% of the grain) accounted for 20% of the difference in N contributions (Table I, column 4) between the vitreous and mealy grains. The difference in N content between the sum of the wheat fractions and the endosperm was 0.16 for the low-protein mealy type and 0.14 for the high-protein vitreous type.

**Cappelle-Desprez**

There was reasonable agreement between the N content of the undissected grains and the sum of the dissected parts. Small differences between the two samples in the proportion of anatomical parts were not considered significant, with the possible exception of the proportion of scutellum, where higher figures were obtained from the vitreous grains for both Cappelle-Desprez and Maris Widgeon. The proportions of endosperm, i.e., 84.1 and 83.6%, were not significantly different.

The difference in N content between the two samples was again reflected in all anatomical parts and was most marked in the endosperm where the mean N contents were 2.04 and 1.25%. Consequently, the endosperm accounted for 78.1% of the grain N for the vitreous grains compared with 72.6% for the mealy grains.

Once again, Fig. 1 shows that the vitreous grains, with higher N content, gave a steeper slope. Estimation of the N content of equal proportions of outer endosperm (2.3% of the grains) showed that the fraction accounted for 27% of the difference in the N contribution made by the endosperm as a whole. The difference in N content between the sum of the wheat fractions and the endosperm was 0.20 for the low-protein mealy type and 0.17 for the high-protein vitreous type.

**Comparison of Maris Widgeon and Cappelle-Desprez**

The smaller proportion of embryo and scutellum found in Cappelle-Desprez compared with Maris Widgeon was considered to be significant for these samples but was not necessarily a varietal characteristic.

*Vitreous Grains.* The N content for the vitreous grains of each variety was similar. The mean N content of the endosperm and the proportions found of the total in the endosperm were also similar. Likewise, the N content of the outer endosperm and the gradient within the endosperm were the same for both varieties.

*Mealy Grains.* The N content for Cappelle grains was lower than for Widgeon grains, and the mean N content of the endosperm and the proportion of the total found in the endosperm were correspondingly lower. Also, the N content of the outer endosperm was lower and the gradient was less steep in the Cappelle.

A broad conclusion drawn from the comparison of the samples was that the greater the N content of the grain, the greater was the proportion of total N found in the endosperm and the steeper the gradient within the endosperm.

Aforegoing points of similarity between the vitreous samples, with similar N contents for the two varieties, and points of dissimilarity between the mealy samples with dissimilar N contents, would be consistent with a conclusion that the distribution of N was more strongly correlated to the N content of the grain than to variety.

The differences in N content between the whole grain, based on the sum of the wheat fractions and the endosperm, indicated a smaller protein loss at the high-protein level, for each variety.

TABLE III. HAND-DISSECTION OF INDIVIDUAL GRAINS

| Wheat | Fraction No. | Flour Fraction % Extraction | Flour Fraction % Protein | Cumulative % Extraction | Cumulative Extraction Function E | Cumulative Flour Protein p | Protein Loss P−p | Protein Ratio P−p/P | $-[\log_e P-p/P]$ |
|---|---|---|---|---|---|---|---|---|---|
| Vitreous Maris Widgeon, protein 14.8% | 1 | 33.7 | 9.3 | 33.7 | 0.9 | 9.3 | 5.5 | 0.372 | 0.99 |
| | 2 | 28.7 | 12.1 | 62.4 | 5.2 | 10.6 | 4.2 | 0.284 | 1.26 |
| | 3 | 18.7 | 20.9 | 81.1 | 73.0 | 13.0 | 1.8 | 0.122 | 2.10 |
| | 4 | 3.2 | 33.4 | 84.3 | 221.0 | 13.7 | 1.1 | 0.074 | 2.60 |
| Mealy Maris Widgeon, protein 10.9% | 1 | 30.5 | 6.7 | 30.5 | 0.7 | 6.7 | 4.2 | 0.384 | 0.96 |
| | 2 | 29.7 | 9.2 | 60.2 | 4.4 | 7.9 | 3.0 | 0.275 | 1.29 |
| | 3 | 22.0 | 14.3 | 82.2 | 101.6 | 9.6 | 1.3 | 0.119 | 2.13 |
| | 4 | 2.1 | 23.5 | 84.3 | 221.0 | 10.0 | 0.9 | 0.083 | 2.49 |
| Vitreous Cappelle-Desprez, protein 14.6% | 1 | 33.8 | 9.1 | 33.8 | 0.9 | 9.1 | 5.5 | 0.377 | 0.98 |
| | 2 | 27.5 | 11.9 | 61.3 | 4.8 | 10.4 | 4.2 | 0.287 | 1.25 |
| | 3 | 19.0 | 20.1 | 80.3 | 59.2 | 12.7 | 1.9 | 0.130 | 2.04 |
| | 4 | 3.3 | 33.8 | 83.6 | 164.0 | 13.5 | 1.1 | 0.075 | 2.59 |
| Mealy Cappelle-Desprez, protein 9.6% | 1 | 33.0 | 6.0 | 33.0 | 0.9 | 6.0 | 3.6 | 0.375 | 0.98 |
| | 2 | 39.5 | 8.6 | 72.5 | 14.0 | 7.4 | 2.2 | 0.229 | 1.47 |
| | 3 | 9.3 | 12.8 | 81.8 | 90.0 | 8.0 | 1.6 | 0.167 | 1.79 |
| | 4 | 2.3 | 17.9 | 84.1 | 198.0 | 8.3 | 1.3 | 0.136 | 2.00 |

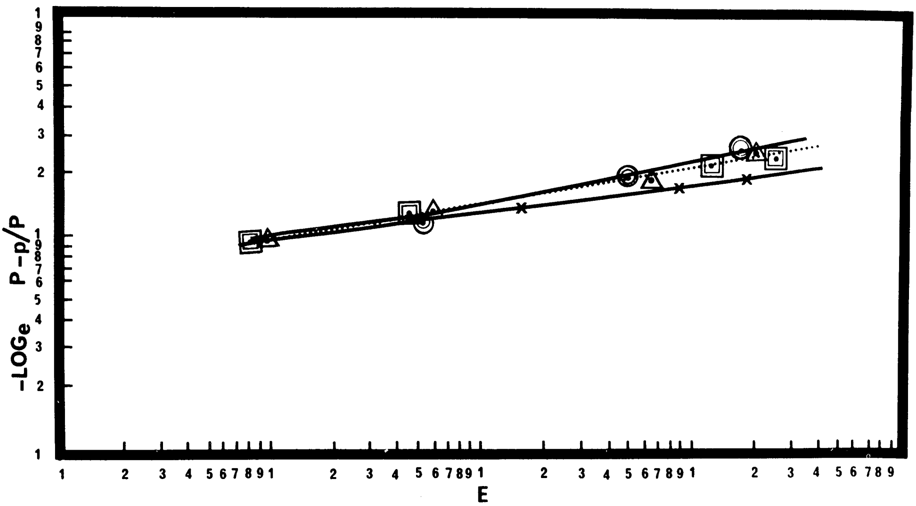


**Fig. 2. Transformed dissection data. Dotted lines = Maris Widgeon; triangles = vitreous, squares = mealy; continuous line = Cappelle-Desprez; circles = vitreous, crosses = mealy.**

**Comparison with Milling Results (Section I)**

The data in Tables I and II were transformed according to the mathematical procedure described in section I. The endosperm subfractions were cumulated to give ideal extraction figures where no feedback occurred between fractions as in the milling procedure. All figures were converted to solids basis; results are shown in Table III, and the sets of four points plotted in Fig. 2.

As found in the milling experiments, the relationships between the protein ratio function and the extraction function are linear. The points for the Maris Widgeon samples are represented by one line, and indicate that in spite of differences in protein content and protein gradient the same protein ratio operated for any given extraction. The vitreous Cappelle gave a similar line with slightly steeper slope. The mealy sample of Cappelle was significantly different and manifested dissimilarities reported in the dissection data. Nevertheless, the fact that a functional relationship

TABLE IV. COMPARISON OF PROTEIN LOSS AND PROTEIN RATIOS AT 74% EXTRACTION

| | Maris Widgeon | | | | Cappelle-Desprez | | | |
|---|---|---|---|---|---|---|---|---|
| | Vitreous high protein | | Mealy low protein | | Vitreous high protein | | Mealy low protein | |
| | Loss | Ratio | Loss | Ratio | Loss | Ratio | Loss | Ratio |
| Hand-dissection | 2.8 | 0.188 | 2.1 | 0.188 | 2.7 | 0.182 | 2.2 | 0.230 |
| Buhler mill | 1.3 | 0.104 | 1.3 | 0.123 | 1.6 | 0.125 | 1.4 | 0.125 |
| Commercial mill | 1.1 | 0.094 | 1.1 | 0.105 | 1.4 | 0.105 | 1.2 | 0.105 |

used to express commercial and Buhler data (section I) also satisfied the hand-dissection results was considered to be a useful starting point for more basic relationships between grain characteristics and milling response. For example, Fig. 2 indicates that, based on protein ratios, there were significant varietal differences for the two types of wheat.

Figure 2 was used to obtain interpolated values for protein ratios at an equivalent of 74% extraction. Table IV gives protein loss and protein ratios for an ideal extraction compared with practical figures that resulted from comminution and sifting procedures according to the milling process (section I). The ideal extraction concept gave substantially higher protein losses and protein ratios, and the dominant role of a fixed protein ratio manifest by correlation of the losses with protein content, except for the atypical mealy Cappelle with a significantly higher protein ratio. The higher losses for the Buhler mill compared with the commercial mill were also associated with higher protein ratios.

It was concluded that the protein ratio was inversely related to the degree of dispersion of the original protein gradient in the grains according to the grinding and sifting operations in the milling process.

## DISCUSSION

The diligence, skill, and persistence required to hand-dissect wheat grains and carry out microanalysis on constituent parts preclude any immediate likelihood of the technique being applied as a routine operation. Consequently, interpretation of milling data in terms of basic varietal characteristics will continue to pose problems, especially when assessing the potential of new varieties.

An induced functional relationship between protein distributions within the endosperms of wheat grains and flour fractions derived from different milling processes could be helpful. Nevertheless, this study showed that at least three factors were involved in a relationship between wheat protein and flour protein for any given extraction.

### Protein Content of the Wheat

Because high-protein levels were mainly due to increased endosperm protein, removal of the epidermal layers resulted in smaller protein loss compared with low-protein levels. This appeared to be a general wheat characteristic.

### Protein Ratio

The lower the protein ratio, the lower the relative protein loss. This phenomenon was considered as a milling effect related to the physical structure of the endosperms and ensuing flour particle size, and mainly accounted for the lower losses for high-protein wheats. The effect was also considered to arise from varietal differences, especially as regards the proportion of mealy grains.

### Constant Protein Ratio

Where mill setting produced the same protein ratio for high- and low-protein wheats, greater relative protein loss was associated with high-protein wheats, and was dependent on both varietal and mill characteristics.

It was considered that the overall quantitative interpretations were compatible with commercial milling experience. Apart from ease of separation of epidermal layers, the main milling characteristic is endosperm texture. Vitreous wheats, irrespective of protein content, give relatively coarse, free-flowing particles, which are easily sifted and dispersed between fractions, thereby minimizing the effects of the endosperm gradient in the grains. Therefore, traditional concepts of good milling characteristics ensure minimum protein loss. During recent years increasing proportions of soft wheats in bread grists have necessitated reappraisal of milling procedures, including the problem of protein loss.

This work has examined only protein, which is the main parameter controlling the breadmaking potential of a flour; nevertheless, other parameters are also involved and subject to similar relative dispersions between flour fractions. Therefore, various aspects of the complex relationship between wheat and flour quality, in terms of the milling process, remain obscure and fruitful for further research.

**Literature Cited**

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