Fractioning Barley Proteins by Computer Factor Analysis[1]

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ABSTRACT

The amino acid composition of six related barley varieties was studied as a function of N-fertilizer level. The amino acid spectra of a "storage protein group" and a "nonstorage protein group" were determined for each mutant by factor analyses of the whole grain amino acid data expressed in different units. The amounts of these two "protein groups" at five fertilizer levels were calculated by regression analysis. The results were compared with those for chemically obtained protein solubility fractions. Varietal differences were greater in the storage protein group than in the nonstorage group. Two principally different high-lysine characteristics were observed in the storage protein group: a gradual shift from hordeins towards glutelins and a drastic replacement of hordeins by proteins of the albumin/globulin type of composition.

Amino acid analysis is often used to study the nutritional value of the proteins in cereal varieties. Full interpretation of the 15–20 items of amino acid data from each sample represents a tedious and demanding task; for large sample sets, authors tend to focus on a few amino acids such as lysine and methionine and tend not to use or only briefly mention other amino acids.

These interpretative difficulties are typical for modern chemical analysis. Laboratory techniques such as chromatography, electrophoresis, mass spectrometry, and infrared spectroscopy have recently been greatly improved and produce many measurement variables from a number of samples in a short time. But the data interpretation methods remain more or less as they were many decades ago.

The purpose of this article is to show how computer analysis can simplify and extend the interpretation of multivariate data such as cereal amino acid spectra.

The proteins in six barley varieties (Bach Knudsen 1976) were "fractioned" by numerical computer analysis into groups of "storage proteins" and "nonstorage" proteins. Large tables of amino acid spectra were reduced to simple, quantitative protein data without protein fractionation in the laboratory.

Then these numerically obtained protein groups were compared with more familiar chemically obtained protein solubility fractions such as albumins, globulins, prolamines, and glutelins. (In barley the prolamine fraction is termed "hordein.")

Finally, chemically obtained protein fractions from one grain sample were used to characterize the protein patterns in many other samples by regression computations, a technique that can greatly reduce the laboratory analysis required for large grain sample sets.

These computer methods have been used to analyze amino acid data from a finger millet variety (Martens 1979).

MATERIALS AND METHODS

Amino acid composition and Kjeldahl nitrogen content were obtained from a standard barley variety (Bomi), from three high-lysine Bomi mutants (Mutants 7, 8, and 1508 [Doll 1975, Doll et al 1974]), and from Hily 82:3 (obtained by [Bomi × Hiproly] × Bomi [Doll et al 1974] with the high-lysine gene retained). A separate high-lysine barley variety, KVL 468 (Viuf 1972), was also included.

The six barley varieties were grown in a pot experiment at five different nitrogen fertilizer levels: 0.5, 2.0, 4.0, 8.0, and 12.0 g of N per pot (Bach Knudsen 1976, 1977). Kjeldahl N and amino acid compositions were obtained by standard procedures; methionine and cysteine were determined as methionine sulphone and cysteic acid, respectively. Tryptophan was determined as described by Eggum (1968).

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Samples of Bomi and Mutant 8 were fractionated for protein solubility (Bach Knudsen 1976) by the methods of Ingversen et al (1972). Kjeldahl N was determined in two replicates. Amino acid data were mostly determined in one replicate. Amino acid raw data (Table I) were given as grams of amino acid per 16 g of N. Every spectrum was normalized to yield a sum of amino acids equaling 100% before the numerical analyses (Appendix, A, unit I data).

Factor analyses (weighted principal component analysis) and regression analyses (weighted least-squares analysis) were preformed in FORTRAN on a NORD-10 minicomputer. The mathematical theories of the multivariate computer methods have been described by Martens (1978, 1979) and by Volden and Martens (1978). A summary is given in the Appendix, which also contains the equations referred to hereafter.

RESULTS AND DISCUSSION

Figure 1 shows the relative contents of lysine and cysteine in the protein of two of the six barley varieties, Bomi and the high-lysine Mutant 1508. The amino acid pair lysine-cysteine is used as a two-dimensional illustration of the 19-dimensional space constituted by the 19 amino acids analyzed. In this 19-dimensional space, the amino acid spectra of the five fertilizer levels of each of the six varieties represent $5 \times 6 = 30$ single points.

For each of the six varieties, factor analysis (Appendix, A and B, equations 1–7) of the five samples showed that, within the present analytical accuracy (coefficient of variation $\approx 2\%$), the five spectra points could be fitted to a single straight line (Appendix, B). This factor line, shown for Bomi and for Mutant 1508 in Fig. 1, is defined by the mean vector of each variety (Table II) and the direction or loading vector (Table III), according to equation 14. Summary statistics of this factor analysis of each variety are given in Table IV.

From the simplex theory (Martens 1978, 1979), if a set of whole grain samples such as those for Bomi or Mutant 1508 (Fig. 1), falls along a straight line, then all of the proteins in these samples may be grouped together into as few as two protein groups. Such a rough grouping might be possible if the quantity of proteins synthesized in the barley varieties were influenced by regulatory systems that respond similarly to increased N-fertilizer levels.

The amino acid spectra of the two protein groups were calculated for each of the six varieties. The storage protein group spectrum was found from amino acid data converted to dry matter basis, (Appendix, C, unit II data; Tables V and VI). Earlier studies showed that albumins and globulins remain constant at different N-fertilizer levels on this dry matter basis in many cereal species, including some barley varieties (Michael 1963, Michael et al 1961). All proteins that remain constant are ignored by centralized factor analysis. The first factor loading vector of unit II data therefore has all elements positive and can be normalized to have a sum of 100%, thus yielding the amino acid spectrum of the "storage protein group." Alternatively, the amino acid spectrum of this storage protein group could be found by difference analysis: subtraction of

the amino acid spectrum of a high-N sample from that of a low-N sample. The two methods give quite similar amino acid spectra for the storage protein group; (a comparison is given in Table VI for Bomi.) The simple difference analysis indicated some variation in protein synthesis at different N-fertilizer levels. However, the low number of samples per variety made these variations difficult to distinguish from analytical errors.

The calculated storage protein group corresponds to the sum of the proteins that the plant synthesizes in response to increased fertilizer levels and therefore is also called the "varying" protein group.

The "nonstorage protein group" spectrum could be determined according to two quite different principles (Martens 1979; Appendix, D; Tables V and VII). Figure 1 shows the intersection between the first-factor lines of two varieties (unit I data). This intersect principle could be used in two slightly different ways (Appendix, D.1 and D.2). The other principle relies on analysis of each variety alone and resembles the principle used to find the storage protein group. Using this second principle, the data were converted to a basis where storage proteins remained constant (unit III data). The nonstorage protein group could thus be estimated directly by factor analysis or by simple difference analysis (Appendix, D.3 and D.4) without the interference of the storage proteins. The nonstorage protein group, which is constant on a dry matter basis with increasing N-fertilizer levels, is also termed the "constant" protein group. It probably contains enzymes, structural proteins, and other biologically important proteins that are in the grain even at low N-fertilizer levels. A comparison of the four methods for estimating this protein group is given in Table VII for Bomi. The methods yielded fairly consistent nonstorage amino acid spectrum estimates, characterized by higher lysine and lower glutamic acid contents then were in the spectrum of Bomi's storage

TABLE I

Raw Data Before Normalization, Showing Amino Acids[a] in Barley Varieties at Five Fertilizer N-Levels

	Bomi					Mutant 7					KVL 468				
	0.5[b]	2.0	4.0	8.0	12.0	0.5	2.0	4.0	8.0	12.0	0.5	2.0	4.0	8.0	12.0
Lys	4.13	3.65	3.51	3.01	2.81	4.26	4.17	3.73	3.51	3.47	3.95	3.81	3.71	3.27	3.20
His	2.46	2.22	2.23	2.06	2.01	2.30	2.29	2.38	2.36	2.40	2.29	2.31	2.30	2.12	2.10
Amm	3.14	2.85	3.04	3.38	3.09	2.54	2.48	2.74	2.83	3.00	2.52	2.51	2.69	2.72	2.76
Arg	5.45	4.51	4.83	4.42	4.29	4.55	4.90	5.00	4.61	4.79	5.15	5.06	5.03	4.61	4.65
Asp	6.18	5.34	4.99	4.72	4.53	6.11	6.55	5.73	5.19	4.78	5.75	5.34	5.29	5.01	5.16
Thr	3.67	3.42	3.13	3.06	2.93	3.61	3.82	3.32	3.43	3.60	3.66	3.44	3.46	3.21	3.30
Ser	4.35	4.44	3.97	3.90	3.78	3.97	3.92	4.25	4.37	4.49	4.37	4.00	4.23	4.14	4.16
Glu	21.99	22.68	23.17	26.49	24.28	19.28	19.56	22.39	23.94	24.75	21.15	20.60	22.25	23.30	24.80
Pro	10.24	10.30	10.84	12.34	12.53	8.78	8.74	10.06	11.26	11.38	10.11	9.60	10.68	10.77	11.75
Gly	4.27	3.86	3.60	3.44	3.29	4.53	4.21	4.34	4.12	4.08	4.12	3.91	3.92	3.61	3.65
Ala	4.22	3.75	3.52	3.25	3.06	4.06	3.90	3.73	3.85	3.88	4.36	4.00	4.01	3.67	3.63
Val	5.59	5.23	5.06	5.03	4.77	4.64	4.90	5.07	5.05	5.07	5.12	4.80	4.92	4.67	4.64
Ile	3.74	3.61	3.52	3.59	3.47	3.25	3.15	3.31	3.39	3.39	3.50	3.24	3.43	3.31	3.41
Leu	7.21	7.10	6.98	6.96	6.77	7.02	6.95	7.28	6.91	6.88	6.99	6.44	6.76	6.38	6.61
Tyr	3.59	3.38	3.31	3.35	3.28	3.37	3.30	3.56	3.57	3.46	3.46	3.41	3.56	3.24	3.48
Phe	5.09	4.81	4.86	5.49	5.41	4.52	4.42	4.72	5.26	5.38	4.78	4.94	4.96	5.08	5.46
Cys	2.37	2.17	2.07	1.89	1.83	2.27	2.21	2.16	1.95	1.87	2.46	2.19	2.05	1.98	1.83
Met	1.97	1.77	1.57	1.61	1.56	1.95	1.91	1.95	1.63	1.60	1.95	1.83	1.68	1.73	1.63
Trp	1.43	1.44	1.31	1.14	1.14	1.59	1.68	1.54	1.37	1.37	1.44	1.41	1.49	1.28	1.29
SUM	101.09	96.53	95.51	99.13	94.83	92.60	93.06	97.26	98.60	99.64	97.13	92.84	96.42	94.10	97.51
% N[c]	1.40	1.70	2.02	2.82	3.07	1.53	1.76	2.22	2.87	3.17	1.87	2.21	2.34	3.16	3.33

	Mutant 1508					Hily 82:3					Mutant 8				
	0.5	2.0	4.0	8.0	12.0	0.5	2.0	4.0	8.0	12.0	0.5	2.0	4.0	8.0	12.0
Lys	5.29	5.33	5.26	4.56	4.54	4.08	4.31	3.85	3.90	3.96	4.15	4.01	4.19	4.31	4.19
His	2.64	2.63	2.72	2.46	2.47	2.22	2.34	2.18	2.32	2.35	2.33	2.34	2.41	2.52	2.42
Amm	1.95	2.19	2.24	2.40	2.33	2.61	2.64	2.66	2.92	2.95	2.52	2.51	2.40	2.72	2.76
Arg	6.55	6.27	6.89	6.52	6.62	4.95	5.45	4.84	4.64	5.22	5.36	5.37	6.34	6.82	6.67
Asp	7.39	7.37	7.30	8.25	8.24	6.05	6.10	5.41	5.41	5.46	6.73	6.22	6.22	7.78	7.89
Thr	4.30	4.28	4.01	3.63	3.53	3.46	3.48	3.13	3.20	3.29	3.57	3.41	3.34	3.28	3.09
Ser	4.70	4.85	4.53	4.23	3.97	4.12	4.24	3.88	3.91	4.07	4.24	4.08	4.00	3.91	3.68
Glu	15.23	15.33	15.56	16.58	16.48	19.81	22.01	21.41	23.71	25.00	20.06	20.18	19.91	20.51	20.59
Pro	6.98	6.78	6.94	6.65	6.45	9.32	10.78	10.82	11.20	11.46	9.18	9.71	8.89	9.24	8.67
Gly	5.63	5.46	5.36	5.06	4.77	4.03	4.20	3.02	3.66	3.71	4.20	4.04	4.24	4.13	3.99
Ala	5.66	4.81	4.82	4.21	4.26	4.54	4.40	3.83	3.97	4.05	4.15	4.00	4.38	4.09	3.92
Val	5.51	5.58	5.72	5.15	4.96	5.11	5.44	4.92	5.17	5.31	5.26	5.28	5.18	5.18	4.93
Ile	3.43	3.38	3.53	3.16	3.13	3.59	3.83	3.62	3.78	3.84	3.59	3.61	3.63	3.48	3.29
Leu	6.92	6.90	6.66	6.07	5.69	8.41	7.28	6.77	7.13	7.30	7.35	7.19	6.78	6.77	6.40
Tyr	3.70	3.61	3.62	3.23	3.11	3.31	3.48	3.23	3.35	3.46	3.28	3.40	3.30	3.29	3.06
Phe	4.02	4.11	4.19	4.22	3.85	4.91	5.49	5.21	5.64	5.83	4.63	4.75	4.60	4.89	4.37
Cys	2.33	2.22	2.07	1.71	1.75	2.10	1.86	1.65	1.62	1.52	2.30	2.28	2.10	1.92	1.86
Met	2.14	2.23	2.04	1.66	1.60	2.03	1.93	1.79	1.78	1.76	1.95	1.71	1.72	1.61	1.58
Trp	1.69	1.66	1.55	1.31	1.32	1.53	1.51	1.38	1.36	1.39	1.35	1.42	1.39	1.23	1.38
SUM	96.06	94.99	95.01	91.06	89.07	96.18	100.77	93.60	98.67	101.93	96.20	95.51	95.02	97.68	94.74
% N[c]	1.64	1.88	2.22	3.10	3.06	1.70	1.85	2.50	3.08	3.04	1.58	1.90	2.37	3.20	3.40

[a] Grams of amino acid per 16 grams of Kjeldahl-N.
[b] Grams of nitrogen in fertilizer applied per pot.
[c] % N = Kjeldahl-N in percent of dry matter.

TABLE II

Mean Levels of Amino Acids

	Mean of Each Variety[a]							
	Bomi	Mutant 7	KVL 468	Hily 82:3	Mutant 1508	Mutant 8	Total Mean[b,d]	Total CV[c,d]
Lys	3.50	3.99	3.76	4.09	5.35	4.35	4.00	15.18
His	2.25	2.44	2.33	2.32	2.77	2.51	2.33	7.02
Amm	3.18	2.82	2.76	2.81	2.39	2.69	2.67	11.47
Arg	4.82	4.96	5.13	5.11	7.05	6.37	5.34	15.35
Asp	5.28	5.92	5.56	5.79	8.29	7.27	6.08	17.86
Thr	3.33	3.70	3.57	3.37	4.23	3.48	3.46	9.32
Ser	4.19	4.36	4.37	4.11	4.77	4.16	4.15	6.51
Glu	24.37	22.79	23.45	22.77	17.02	21.15	21.10	14.30
Pro	11.56	10.41	11.06	10.81	7.25	9.54	9.74	17.46
Gly	3.79	4.43	4.02	3.79	5.63	4.30	4.14	14.74
Ala	3.65	4.04	4.12	4.24	5.08	4.29	4.06	12.09
Val	5.27	5.14	5.05	5.28	5.77	5.39	5.10	5.44
Ile	3.68	3.43	3.53	3.80	3.57	3.67	3.47	5.60
Leu	7.19	7.29	6.94	7.52	6.91	7.19	6.89	6.78
Tyr	3.47	3.59	3.59	3.43	3.70	3.41	3.39	4.53
Phe	5.27	5.04	5.28	5.51	4.38	4.85	4.86	10.48
Cys	2.12	2.18	2.20	1.78	2.16	2.18	2.02	11.92
Met	1.73	1.88	1.85	1.89	2.07	1.79	1.79	10.26
Trp	1.33	1.57	1.45	1.46	1.61	1.40	1.41	9.87
SUM	100.0	100.0	100.0	100.0	100.0	100.0	96.09	

[a] Calculated after normalization to percent of recovered amino acids (unit I).
[b] Five nitrogen levels of six varieties.
[c] CV = coefficient of variation.
[d] In grams of amino acid per 16 g of Kjeldahl-N (from Table I).

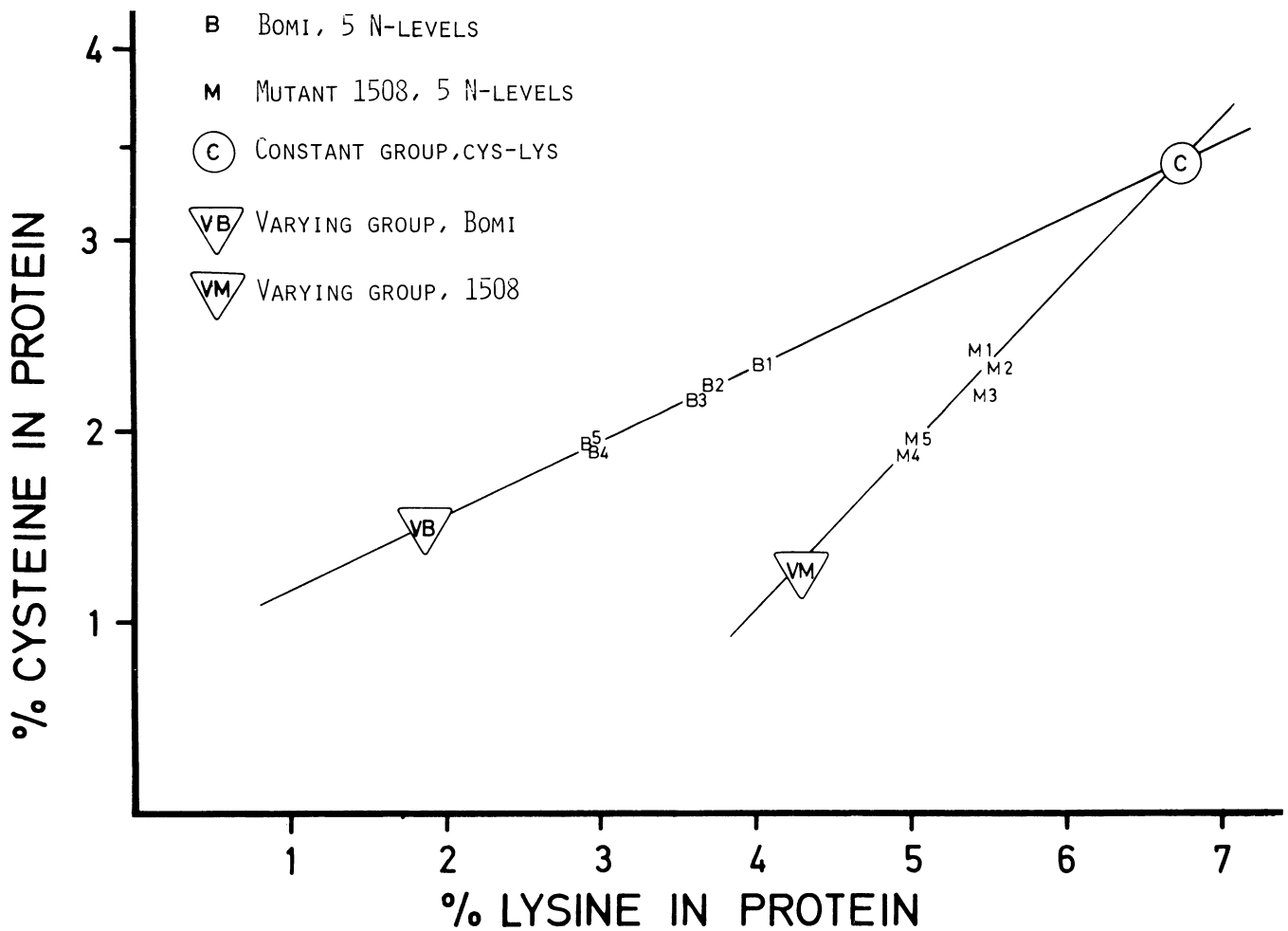

Fig. 1. Cysteine and lysine in barley varieties Bomi and Mutant 1508 and their numerical protein groups. Amino acids in percent of recovered amino acids (unit I). VB and VM, the storage protein group of the two varieties, are found by factor analysis of amino acid data on dry matter basis, (unit II). C is included to illustrate the intersect principle for finding the nonstorage protein group. Analysis of all 19 amino acid dimensions simultaneously showed that the nonstorage protein groups of the two varieties differed slightly.

protein group.

With the exception of Mutant 8, all barley varieties had similar nonstorage protein group spectra (Table V). The storage protein group, on the other hand, showed quite different spectra in the different barley varieties (Table V). A characteristic increase in the lysine content of the storage protein in the high-lysine varieties compared with that in Bomi may be observed. Glutamic acid content decreases for Mutants 1508 and 8 but not for the other varieties. The high lysine and low glutamic acid contents agree with previous findings for the hordein fractions for Mutants 1508 and 8 (Bach Knudsen 1976, Ingversen et al 1973). The low cysteine content in the storage protein of Hily 82:3 may be due to the characteristic "lys" gene of Hiproly (Munch 1972).

The apparent amounts of these two numerical protein groups in the whole grains of the respective barley varieties are given in Fig. 2. The protein amounts, given as percent protein-N vs total Kjeldahl-N in the seed dry matter at the five different N fertilizer levels, were estimated by regression of the whole seed amino acid spectra vs the respective storage and nonstorage protein groups' spectra (Appendix, E).

These numerically obtained protein groups can be compared with more conventional solubility fractions from Bomi. The amino acid spectra of the fractions were fairly similar for Bomi and for Mutant 8 and fairly independent of nitrogen fertilizer level. The fractions from Bomi that will be used in the following discussion are given in Table VIII. The proteins in the sample had been fractioned chemically into three protein solubility fractions (Bach Knudsen 1976): 1) albumins + globulins (salt-solubles), 2) hordein (alcohol-solubles), 3) glutelins + rest (alkaline detergent-solubles and all insolubles).

Regression analysis (Appendix, E) was used to calculate the relative amounts of these three protein fractions in the whole grain samples. Because ammonia and tryptophan values were missing in the solubility fraction data, only the 17 remaining amino acids were used in the calculations, including the initial normalization. The three-phase diagram in Fig. 3 shows the apparent distribution of these protein fractions in the five whole grain samples from Bomi (B1–B5).

At the lowest nitrogen fertilizer level (B1), the protein in Bomi consists of about 10% hordein, 60% glutelins + rest, and 30% albumins + globulins. At the highest fertilizer level (B5), the hordein percentage had increased to about 40%. Glutelins + rest and albumins + globulins had decreased correspondingly to 50 and 10%, respectively.

The two numerically obtained protein groups of each of the six barley varieties were likewise fitted to the solubility-fraction model (Fig. 3). The varying (storage) protein group of Bomi (VB) contains no albumins + globulins, about 60% hordein, and 40% glutelins + rest. Mutant 7, Hily 82:3, and KV 468 show storage proteins (V7, VH, and V4) somewhat similar to that of Bomi, but with a significant shift from the lysine-poor hordein to the lysine-rich glutelins + rest fraction. The other two high-lysine mutants, 1508 and 8, on the other hand, show completely different storage protein groups (V15 and V8), apparently consisting mainly of proteins of the albumins + globulins type.

TABLE III
Loading Vectors of the First Principal Component for Each Barley Variety[a]

	Bomi	Mutant 7	KVL 468	Hily 82:3	Mutant 1508	Mutant 8
Lys	6.23	6.52	6.32	2.46	4.05	−1.40
His	1.85	0.44	1.96	−0.11	0.27	−1.17
Amm	−1.79	−1.57	−1.77	−1.75	−3.69	−2.49
Arg	4.15	2.23	4.76	1.78	−4.11	−12.82
Asp	7.10	11.66	4.62	6.25	−11.73	−14.53
Thr	3.19	2.85	2.97	2.56	3.92	3.24
Ser	2.19	−1.44	0.91	2.11	3.30	3.91
Glu	−23.86	−23.44	−27.93	−26.16	−18.99	−4.30
Pro	−15.88	−12.70	−12.53	−11.61	−0.40	4.94
Gly	4.22	3.65	3.89	4.81	2.73	1.05
Ala	5.16	2.44	5.39	5.16	6.34	1.45
Val	2.77	0.25	3.45	0.84	1.72	2.46
Ile	0.46	0.17	0.44	−0.47	0.75	2.55
Leu	0.91	3.68	2.64	9.93	5.49	6.98
Tyr	0.71	0.35	0.74	0.32	2.51	1.92
Phe	−3.91	−3.58	−3.81	−4.18	−1.74	0.98
Cys	2.49	3.14	4.29	4.25	3.59	4.04
Met	1.66	2.89	2.21	2.31	3.69	2.42
Trp	1.71	2.44	1.43	1.49	2.27	0.78
SUM	100.0	100.0	100.0	100.0	100.0	100.0

[a]The loading vector of each variety is given in the same unit (unit I) as the corresponding mean vector (Table II).

TABLE IV
Summary Statistics of Factor Analysis of Each Barley Variety

	Bomi	Mutant 7	KVL 468	Hily 82:3	Mutant 1508	Mutant 8
% SS[a]						
Total SS	100	100	100	100	100	100
Residual SS						
After one factor	9	9	12	17	13	24
After two factors	4	4	5	8	5	13
% CV[b]						
Total CV	8.2	8.1	6.0	6.5	6.9	6.0
Residual CV						
After one factor	2.2	2.2	1.9	2.5	2.3	2.8
After two factors	1.4	0.8	1.2	1.8	1.4	2.0
%CV for lysine[c]						
Total CV	13.9	13.0	9.7	4.2	5.2	2.2
Residual CV						
After one factor	2.1	3.2	1.9	2.5	2.0	1.6
After two factors	2.4	2.2	0.8	3.1	1.8	1.7

[a]% SS = relative sum of squares, weighted mean of 19 amino acids. Shows how much of original (100%) information in the data is unexplained by one and two factors, respectively. (equation 6).
[b]% CV = Total coefficient of variation, as defined by equation 7.
[c]% CV for lysine is defined by equation 7 with $N_a = 1, i = 1$.

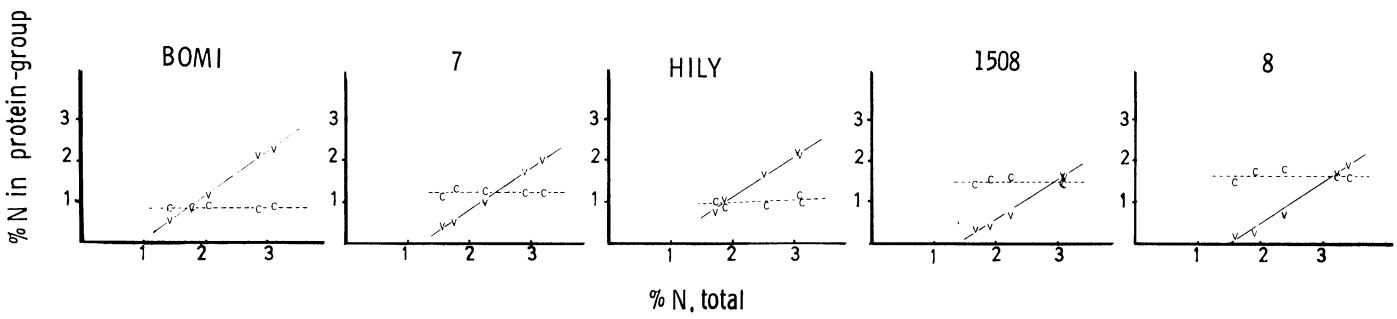

Fig. 2. Regression analysis estimates of amounts of numerical protein groups in Bomi and its mutants given as percent protein-N in dry matter vs percent total Kjeldahl-N in dry matter. C = Constant (nonstorage) protein group, found by graphic intersect analysis in three-factor subspace (Tables V and VII). V = Varying (storage) protein group, found by factor analysis of each variety on a dry matter basis (Tables V and VI).

The numerically obtained nonstorage protein group of all the varieties except Mutant 8 yielded negative amounts of hordein by regression analysis. (Their positions are outside the triangle in Fig. 3). This indicates that the nonstorage protein group of Mutant 8 is somewhat different from that of the other varieties; some hordein is apparently synthesized even at very low N-fertilizer levels in Mutant 8.

In addition, a negative amount of a protein group is obviously meaningless. It is a characteristic artifact that may arise when the protein-fraction "model" does not fit well to the whole grain data. In this case, it probably happens because the extraction procedure we used leaves a significant amount of hordein in the glutelins + rest fraction about 30%.[2] The hordein-free "true" glutelins + rest fraction probably lies further up along the extended hordein-glutelins + rest line (near X), making the "true" triangle include all the constant nonstorage protein groups and "increasing" the apparently negative hordein contents of the nonstorage protein groups to near zero as expected.

The amino acid spectra of the albumins + globulins and the glutelins + rest fractions (Table VIII) are similar to each other so that shifts in positions parallel to this side of the triangle correspond to only minor shifts in the actual amino acid spectra of the nonstorage (constant) protein groups.

Figure 4 shows the calculated contents of the chemical solubility fractions in Bomi, the three Bomi mutants, and Hily 82:3. Fractions isolated chemically from Bomi were used for Bomi, Mutant 7, and Hily 82:3, and corresponding fractions isolated from Mutant 8 (Bach Knudsen 1976) were used for Mutants 1508 and 8. This procedure gave a somewhat better regression fit for these mutants. Calculated amounts of the protein solubility fractions at each of the five fertilizer levels are given as percent protein-N in the seed dry matter vs the total Kjeldahl-N percentage in the dry matter. Figure 4 is analogous to the three-phase diagram (Fig. 3) and is included to illustrate how fractions from a single sample or a few samples may be used to characterize the protein synthesis in other samples, thereby reducing the laboratory work required. Figure 4 shows that the albumins + globulins remain virtually constant in Bomi, Mutant 7, and Hily 82:3, as expected (Michael et al 1961, Michael 1963), but increase drastically with increased N-fertilizer application in Mutants 1508 and 8. Hordein, on the other hand, is synthesized in the three former but not in the two latter varieties. Figure 4 also shows differences in the general level of the various

[2]B. Køie. Personal communication. 1978.

TABLE V
Amino Acid Percentages in Numerically Obtained Storage and Nonstorage Protein Groups of Six Barley Varieties

	Bomi	Mutant 7	KVL 468	Hily 82:3	Mutant 1508	Mutant 8	Mean	CV[a]
Storage protein group[b]								
Lys	1.94	2.34	2.24	3.47	4.36	4.58	3.16	36.32
His	1.81	2.32	1.85	2.35	2.68	2.70	2.29	16.94
Amm	3.61	3.25	3.18	3.29	3.25	3.13	3.29	5.14
Arg	3.84	4.39	3.97	4.41	7.97	8.51	5.52	38.63
Asp	3.64	2.87	4.49	4.40	11.13	9.75	6.05	57.57
Thr	2.57	3.11	2.86	2.84	3.32	2.92	2.94	8.71
Ser	3.49	4.74	4.16	3.62	4.00	3.49	3.92	12.48
Glu	29.60	28.87	30.19	28.41	21.55	21.94	26.76	14.70
Pro	15.78	13.69	13.92	12.92	7.34	8.73	12.06	27.25
Gly	2.79	3.43	3.08	2.93	5.00	4.10	3.56	23.88
Ala	2.41	3.46	2.84	3.23	3.64	4.01	3.27	17.59
Val	4.59	5.07	4.24	5.06	5.32	4.97	4.88	8.01
Ile	3.57	3.38	3.42	3.86	3.36	3.23	3.47	6.32
Leu	6.93	6.25	6.26	6.04	5.62	6.02	6.19	6.98
Tyr	3.33	3.44	3.33	3.33	3.09	3.07	3.27	4.57
Phe	6.33	6.04	6.24	6.32	4.79	4.66	5.73	13.71
Cys	1.51	1.34	1.26	0.96	1.31	1.50	1.31	15.31
Met	1.38	1.08	1.40	1.44	1.19	1.37	1.31	10.84
Trp	0.89	0.94	1.06	1.14	1.08	1.29	1.07	13.45
SUM	100.0	100.0	100.0	100.0	100.0	100.0	100.0	
Nonstorage protein group[c]								
Lys	5.61	5.37	5.89	5.30	5.92	4.26	5.39	11.32
His	2.79	2.68	2.98	2.54	2.82	2.40	2.70	7.72
Amm	2.29	2.37	2.37	2.63	2.42	2.95	2.50	9.83
Arg	6.49	5.57	6.60	5.89	6.43	4.92	5.98	10.96
Asp	7.43	8.09	7.62	7.80	6.92	7.05	7.49	5.97
Thr	4.44	4.38	4.54	4.44	4.72	3.80	4.39	7.10
Ser	5.05	4.43	5.00	5.02	5.28	4.57	4.89	6.59
Glu	15.74	17.69	14.84	14.38	14.43	21.28	16.39	16.44
Pro	6.90	7.38	6.02	6.51	6.76	9.47	7.17	16.87
Gly	5.57	5.39	5.37	5.51	5.87	4.28	5.33	10.22
Ala	5.54	4.81	5.77	5.90	5.78	4.12	5.32	13.30
Val	6.04	5.33	6.25	5.71	6.07	5.55	5.83	6.06
Ile	3.73	3.40	3.77	3.62	3.69	3.63	3.64	3.61
Leu	7.53	7.86	7.60	9.56	7.68	7.54	7.96	9.94
Tyr	3.80	3.73	3.77	3.66	3.96	3.44	3.73	4.64
Phe	4.14	4.25	3.87	3.98	4.01	4.71	4.16	7.19
Cys	2.73	2.77	3.31	3.02	2.74	2.47	2.84	10.18
Met	2.35	2.42	2.46	2.58	2.56	2.08	2.41	7.57
Trp	1.85	2.08	1.94	1.95	1.94	1.49	1.88	10.81
SUM	100.0	100.0	100.0	100.0	100.0	100.0	100.0	

[a]CV = coefficient of variation.
[b]The spectra in this group were found by factor analysis of five samples on a dry matter basis (Appendix, C, unit II data).
[c]The spectra in this group were found by graphic intersect analysis of two and two varieties in three-factor subspace (Appendix, D, unit I data).

protein fractions in the different varieties. Hordein appears to be absent in 1508, but it is present at a low but constant level in Mutant 8. This finding appears to agree with earlier results based on sodium dodecyl sulfate electrophoresis (Køie et al 1975), showing that some proteins in the hordein fraction were present in Bomi but not in Mutant 1508. The same type of studies showed a reduction in the protein of the hordein fraction of Mutant 8 (Bach Knudsen 1976).

The calculations in the present study yield estimates of the major protein groups in the barley varieties. These are, like all estimates, somewhat uncertain. In the first part of the present study, the one-factor straight-line factor analysis model (Table III) adopted for each barley variety is probably an oversimplification. Martens (1979) found a small but highly significant curvature of the "path"

TABLE VI
Two Methods to Calculate the Amino Acid Spectrum of the Storage Protein Group of Barley Variety Bomi[a]

	Difference Analysis[b]					Factor Analysis, One Factor
	Difference Between Amino Acid Values at Two N-Levels[c]					
	3-1	4-2	5-3	Mean	CV[d]	
Lys	2.75	1.91	1.59	2.08	28.67	1.94
His	2.11	1.74	1.71	1.85	12.15	1.81
Amm	3.36	4.10	3.40	3.62	11.56	3.61
Arg	4.30	4.13	3.50	3.98	10.67	3.84
Asp	3.22	3.59	3.92	3.58	9.77	3.64
Thr	2.48	2.39	2.73	2.53	6.86	2.57
Ser	3.83	2.92	3.66	3.47	13.82	3.49
Glu	29.92	31.62	28.19	29.91	5.74	29.60
Pro	14.10	15.15	16.80	15.35	8.85	15.78
Gly	2.74	2.67	2.89	2.77	4.13	2.79
Ala	2.58	2.36	2.34	2.43	5.47	2.41
Val	4.77	4.55	4.51	4.61	3.04	4.59
Ile	3.65	3.44	3.61	3.57	3.12	3.57
Leu	7.71	6.51	6.81	7.01	8.84	6.93
Tyr	3.27	3.19	3.45	3.30	3.90	3.33
Phe	5.21	6.38	6.89	6.16	14.00	6.33
Cys	1.77	1.39	1.47	1.54	12.90	1.51
Met	0.96	1.31	1.65	1.30	26.57	1.38
Trp	1.27	0.63	0.88	0.93	35.01	0.89
SUM	100.0	100.0	100.0	100.0		100.0

[a] From amino acid data (Appendix, C.1 and C.2, unit II data).
[b] Although the factor analysis requires an eigenvalue routine and some matrix manipulations conveniently done with a computer, the simpler difference analysis can be done with a pocket calculator.
[c] Nitrogen levels of fertilizer per pot: 1, 0.5 g; 2, 2.0 g; 3, 4.0 g; 4, 8.0 g; 5, 12.0 g.
[d] CV = coefficient of variation.

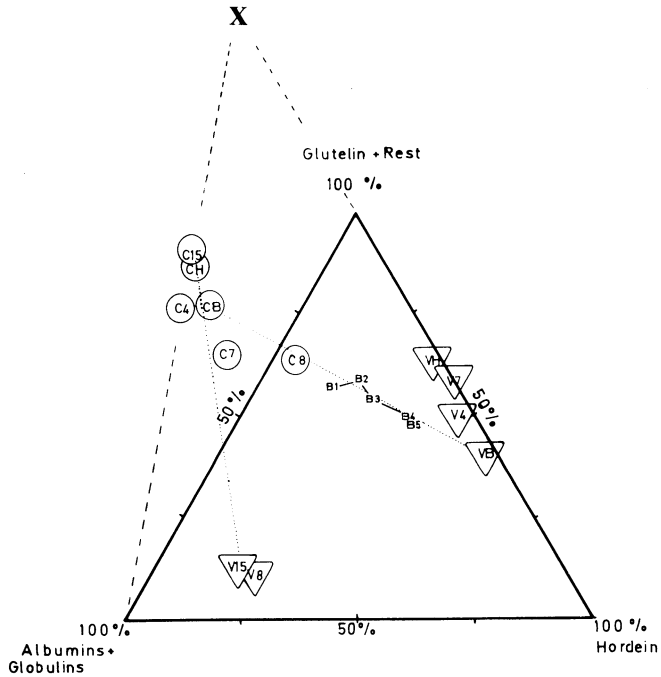

Fig. 3. Three-phase diagram showing percentages of the three chemically obtained solubility fractions from variety Bomi, calculated for: **B1, B2, B3, B4, B5** = Bomi whole grain, five N-fertilizer levels. **V** and **C** = Varying (storage) and constant (nonstorage) protein groups: **VB, CB** = Bomi; **V15, C15** = Mutant 1508; **VH, CH** = Hily 82:3; **V7, C7** = Mutant 7; **V8, C8** = Mutant 8; and **V4, C4** = KVL 4681. **X** = Estimated position of "true" glutelins + rest fraction, completely free of hordein proteins. Based on an assumed hordein contamination level of 30% in the glutelin fraction.

TABLE VII
Four Ways[a] to Calculate the Amino Acid Spectrum of the Nonstorage Protein Group of Barley Variety Bomi

	Graphical Intersect Analysis			Closest Approach Analysis			Difference Analysis				Factor Analysis, One Factor
	vs 1508	vs Hily	Mean	vs 1508	vs Hily	Mean	3-1	4-2	5-3	Mean	
Lys	5.69	5.52	5.61	5.51	3.89	4.71	4.62	5.09	5.80	5.17	4.90
His	2.79	2.78	2.79	2.84	2.36	2.61	2.56	2.69	2.98	2.74	2.67
Amm	2.06	2.52	2.29	2.60	3.07	2.84	3.01	2.15	2.96	2.70	2.89
Arg	6.57	6.41	6.49	6.16	5.07	5.62	5.83	5.05	6.65	5.84	5.96
Asp	7.16	7.70	7.43	7.57	5.71	6.64	7.27	6.89	6.56	6.91	7.13
Thr	4.58	4.30	4.44	4.35	3.52	3.94	4.09	4.35	3.84	4.09	4.09
Ser	5.12	4.98	5.05	4.90	4.33	4.62	4.49	5.77	4.67	4.98	4.64
Glu	15.34	16.13	15.74	16.88	22.96	19.86	18.49	17.81	20.25	18.85	18.46
Pro	7.20	6.62	6.90	6.44	10.59	8.52	8.54	7.54	5.79	7.29	8.03
Gly	5.82	5.31	5.57	5.15	4.05	4.60	4.82	4.93	4.66	4.80	4.81
Ala	5.66	5.41	5.54	5.31	3.97	4.64	4.81	4.95	5.05	4.94	4.87
Val	5.95	6.13	6.04	6.16	5.43	5.80	5.83	6.02	6.10	5.98	5.90
Ile	3.66	3.79	3.73	3.83	3.71	3.77	3.72	3.95	3.76	3.81	3.75
Leu	7.46	7.60	7.53	7.48	7.25	7.37	6.90	7.94	7.81	7.55	7.19
Tyr	3.92	3.68	3.80	3.70	3.52	3.61	3.66	3.72	3.49	3.62	3.65
Phe	4.20	4.07	4.14	4.01	5.03	4.53	4.97	4.00	3.25	4.07	4.57
Cys	2.54	2.92	2.73	2.92	2.27	2.60	2.58	2.85	2.88	2.77	2.68
Met	2.41	2.29	2.35	2.27	1.84	2.06	2.35	2.20	1.64	2.06	2.20
Trp	1.84	1.85	1.85	1.88	1.43	1.66	1.47	2.09	1.88	1.81	1.62
SUM	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[a] Mathematical methods are described in Appendix, D. Graphic intersect analysis and closest approach analysis are two analogue methods, based on amino acid data in unit I. Difference analysis and factor analysis are based on amino acid data in unit III. A constant content in nonstorage proteins of 1% protein-N in dry matter was assumed for Bomi.

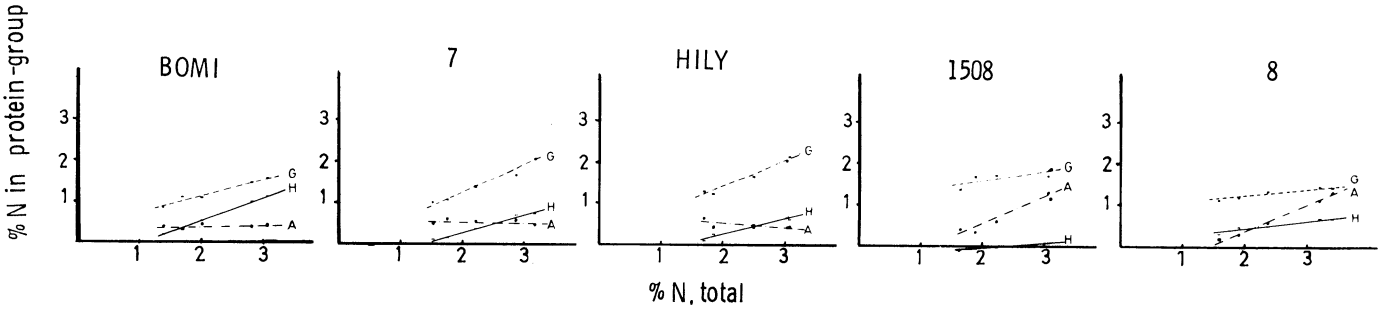

Fig. 4. Regression analysis estimates of amounts of chemical protein solubility fractions in Bomi and its mutants given as percent protein-N in dry matter vs percent total Kjeldahl-N in dry matter. Solubility fractions from Bomi were used for Bomi, Mutant 7, and Hily 82:3; fractions from Mutant 8 were used for Mutants 1508 and 8. **A** = Albumins + globulins (salt-solubles); **H** = hordein (alcohol-solubles); **G** = glutelins + rest (alkaline detergent-solubles + all insolubles).

TABLE VIII
Chemically Obtained Solubility Fractions[a] from Barley Variety Bomi at N-Level Four[b]

	Albumins + Globulins	Hordein	Glutelins + Rest
Lys	6.09	0.90	4.11
His	2.55	1.50	2.70
Amm	...	...	...
Arg	7.37	2.79	4.54
Asp	12.75	1.50	5.95
Thr	4.25	1.80	3.78
Ser	3.97	3.19	4.76
Glu	15.72	41.22	25.41
Pro	6.09	19.86	10.37
Gly	6.52	1.40	4.43
Ala	6.23	1.70	4.32
Val	6.09	3.89	5.95
Ile	3.68	3.59	4.11
Leu	7.08	6.79	8.65
Tyr	3.26	3.29	2.16
Phe	3.97	4.69	5.19
Cys	2.98	1.30	1.84
Met	1.41	0.60	1.73
Trp	...	...	...
SUM	100.0	100.0	100.0

[a]Calculated after normalization to percent of recovered amino acids (unit I).
[b]8.0 g of N-fertilizer per pot.

that the amino acid spectrum of a finger millet cereal variety followed upon N-fertilization. A similar phenomenon may possibly be observed for the amino acid spectrum of Mutant 1508 in the cysteine-lysine graph of Fig. 1, as well as in the amounts-data for all the mutants in Fig. 2. Thus the specific results given here may be subject to further refinement.

In the latter part of this study, uncertainties arise from the regression analysis, which will always give some kind of results, no matter how "wrong" the "protein fractions" in the regression modeel may be. In the present work, the residual terms were model may be. In the present work, the residual terms were inspected visually and noted to be so small that they probably do not affect the main conclusions.

CONCLUSIONS

Multivariate computer analysis was used to simplify amino acid data of barley to simple qualitative and quantitative data of two general protein groups.

One of these numerically obtained groups, the lysine-rich nonstorage protein group, more or less common to all the barley varieties tested, remained at constant amounts in the grain dry matter, although at different levels in the different varieties. The other group, the storage protein group, displayed large amino acid differences between varieties and changed in amounts with N-fertilizer level.

These numerically obtain ein groups were compared with conventionally obtained protein solubility fractions in a three-phase diagram, which showed two main types of high-lysine mutation effects in the storage protein group. Finally, the protein fractions from a single sample were used to study the quantitative protein synthesis in many other samples.

The conclusions obtained from the present numerical calculations apparently agree well with what was known about some of the barley varieties included in the study. Thus the multivariate analyses offer a thorough yet simple interpretation of multivariate data such as that for amino acids.

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APPENDIX

Let N_a = number of amino acids, i (eg, 19)
N_s = number of samples, k (eg, 5)
N_f = number of factors from weighted principal component analysis, j (eg, 1)
N_c = number of protein groups, j (eg, 2)
Let the amino acid (AA) spectra of N_s samples be the $N_a \cdot N_s$ matrix X, with individual spectra x_k and individual AA data x_{ik}.

A. Normalization of Data Before Analysis

To apply the simplex theory (Martens 1979), each spectrum x_k is normalized to give a sum of AA equaling 100%, instead of sums ranging from 89 to 102 (Table I). In other words, x_{ik} is converted from "gAA/16g of N" to "percent of recovered AA." This latter unit is called unit I.

B. Number of Protein Groups in Each Barley Variety

By weighted principal component analysis (or other factor analysis method) of unit I data, the number of factors (underlying tendencies of variation) N_f is found. In simplex theory, for unit I data, the number of independent protein groups is $N_c = N_f + 1$.

Weighted principal component analysis of unit I data:

Centering: $$Y_{ik} = x_{ik} - \bar{x}_i \quad (1)$$

where $$\bar{x}_i = \Sigma_k x_{ik} / N_s \text{ (Table II)} \quad (2)$$

Weighting, assuming the same relative analytical uncertainty (constant coefficient of variation) in all n_a amino acids:

$$z_{ik} = y_{ik} / \bar{x}_i = \frac{x_{ik} - \bar{x}_i}{\bar{x}_i} \quad (3)$$

Singular value decomposition of Z:

$$Z = L \wedge V + E = LS + E \quad (4)$$

where L is the N_f first eigenvectors of ZZ^T.
V is the N_f first eigenvectors of Z^TZ.
Λ is the diagonal matrix of the N_f first eigenvalues$^{1/2}$ of ZZ^T.
E is the matrix of weighted residuals after N_f factors.
S is the obtained score matrix.

The loading vectors in L are scaled to yield a sum of squares equal to N_a and deweighted:

$$b_{ij} = l_{ij} \cdot \sqrt{N_a} \cdot \bar{x}_i \quad (5)$$

The first deweighted loading vector of each barley variety is given in Table III.

Measures of Goodness of Fit
Relative residual sum of squares:

$$\%SS = \frac{100\% \cdot \Sigma_i \Sigma_k e_{ik}^2}{\Sigma_i \Sigma_k z_{ik}^2} \quad (6)$$

Residual coefficient of variation:

$$\%CV = 100\% \sqrt{\Sigma_i \Sigma_k e_{ik}^2 / N_s \cdot (N_a - N_f - 1)} \quad (7)$$

C. "Storage Protein" Group

To find the "storage protein" group (Tables V and VI), unit I data were converted to dry matter basis (unit II) to obtain constant amounts of nonstorage proteins:

$$x''_{ik} = x_{ik} \cdot h_k / 100\% \quad (8)$$

where x''_{ik} is the AA data in percent of dry matter (unit II).
x_{ik} is the AA data in percent of recovered AA (unit I).
where h_k is the percent Kjeldahl-N in dry matter of sample k (Table II).

Nonprotein N is assumed to be very low in the samples and the recovered AA data are assumed to be representative of the total protein in the sample.

C.1 Factor Analysis of Unit II Data (by Analogy to B)

Centering: $$y''_{ik} = x''_{ik} / \bar{x}''_i \quad (9)$$

where $$\bar{x}''_i = \Sigma_k x''_{ik} / N_s \quad (10)$$

Weighting: $$z''_{ik} = y''_{ik} / \bar{x}''_i \quad (11)$$

Singular value decomposition of Z'':

$$Z'' = L'' \Lambda'' V'' + E'' = L''S'' + E'' \quad (12)$$

Deweighting of first loading vector:

$$a_{il} = l''_{ik} \cdot \bar{x}''_i \quad (13)$$

Because all elements in a_l are positive, vector a_l may be normalized to yield a sum of 100%. The storage protein group spectrum has thus been found by factor analysis of N_s (here, five) samples.

C.2 Difference Analysis of Unit II Data
Simple subtraction of two unit II sample vectors k and m:

$$a_i = x''_{ik} - x''_{im} \quad (14)$$

If all elements in a_i are of the same sign, the vector may be normalized to yield a sum of 100%. The two samples k and m should be so different in Kjeldahl-N content in dry matter that analytical uncertainties in x''_{ik} and x''_{im} do not give elements in a_i of opposing signs.

In the present case we have used N-levels 3 minus 1, 4 minus 2, and 5 minus 3.

D. "Nonstorage Protein" Group

To find the nonstorage protein group (Tables V and VII), two principally different approaches may be used: unit I intersect methods and unit III methods.

D.1 Intersect in Three-Factor Subspace, Unit I Data
After weighted principal component analysis (equations 1–5) on *two* barley varieties simultaneously ($N_s = 10$), the two-factor score plot yielded intersects similar to that for cysteine-lysine (Fig. 1). At this intersect, differences in the two varieties along factor 3 were allowed for, because the variety lines do not necessarily intersect completely in the 19-dimensional space. After the three score values of this "intersect" were determined (S, equation 4), the AA spectrum was found:

$$a_i = \bar{x}_i + \sum_{j=1}^{3} b_{ij} s_j \quad (15)$$

D.2 Closest Approach in 19-Dimensional AA Space
The one-factor solution (equation 5) of each variety gives the direction of the AA spectrum "path" in the 19-dimensional space of the variety. The one-factor solutions of two and two barley varieties (m and n) should, based on the results in D.1, nearly intersect somewhere in the 19-dimensional space.

The AA spectra a_m and a_n of the points of closest approach of these two lines were found by iteratively changing the scores s_m and s_n along their factor axes, minimizing F:

$$F = \sum_i \left(a_{im} - a_{in} \right)^2 \quad (16)$$

where $$a_{im} = (\bar{x}_i + b_{il} s_l)_m \quad (17)$$

and $$a_{in} = (\bar{x}_i + b_{il} s_l)_n \quad (18)$$

$\bar{x}_i$ is defined in equation 2 and b_{il} in equation 5.

D.3 Factor Analysis of Unit III Data
Because centered factor analysis detects and describes *variations* in the data from sample to sample, the nonstorage protein group may be found by eliminating the storage protein variations from the data. This can be done by dividing the unit II data by the amount of storage proteins in each sample. This amount may indirectly be estimated by some experimental measurement of the amount of nonstorage proteins c_k. In the present study we used the amount of alcohol nonsoluble proteins in the dry matter of grain samples (Bach-Knudsen 1977) as a rough estimate of c_k. In Bomi, c_k was approximately constant at 1.0% Kjeldahl-N of seed dry matter at all N-fertilizer levels.

Unit III data were then found by:

$$x'''_{ik} = x''_{ik} / (h_k - c_k) = x_{ik} \cdot h_k / (h_k - c_k) \quad (19)$$

where h_k is as in equation 8.

From these unit III data, the nonstorage protein spectrum could be found by factor analysis of x''', by analogy to equations 9–13.

D.4 Difference Analysis of Unit III Data
x''' were analyzed like x'' (equation 14).

E. Estimate Amounts of N_c Protein Fractions in Different Samples (Weighted Regression Analysis)

Let a_{ij} be the AA data of protein fraction j,
x_{ik} be the AA data of sample k, and
p_{jk} be the amounts of protein j in sample k (to be estimated).

Weighting, assuming the same relative analytical uncertainty (constant coefficient of variation) in all N_a amino acids:

$$w_{ik} = x_{ik} / \bar{x}_i \quad (20)$$

$\bar{x}_i$ is defined in equation 2.

Regression:

$$W = GP + E \quad (21)$$

where $$g_{ij} = a_{ij} / \bar{x}_i \quad (22)$$

P may be estimated by the following method:

$$G^T W = G^T G \hat{P} \quad (23)$$

$$\hat{P} = (G^T G)^{-1} \cdot G^T W \quad (24)$$

Residuals:

$$E = W - G\hat{P} \quad (25)$$

$\hat{P}$ represents the estimated "molar" ratios of the protein groups. If weight ratios are desired instead, differences in "molar weights" between the protein groups must be compensated. In the present study the molar weight per 100 AA units were for simplicity assumed to be identical for the storage and the nonstorage protein groups.

LITERATURE CITED

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