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A. W. HALVERSON and O. E. OLSON[1]

Introduction

Barley is a well-established cereal crop in North America as well as in other areas of the world where a temperate climate prevails. The grain is used both as a livestock feed and as a raw material of the brewing industry. High protein barley is preferred for feeding purposes and low or medium protein types are needed for satisfactory malting. The large variations which occur in the protein content of South Dakota barley make it difficult to plan an efficient program for production of either feed or malting barley in South Dakota. The present protein composition work may be helpful toward adjustment of feeding and malting practices, making possible the better utilization of barley in the future.

The work discussed here includes protein fractionation studies of three barley varieties.[2] Two of the varieties, Feebar and Plains, were developed from crosses made at the South Dakota Experiment Station in 1936-37 by S. P. Swenson.[3] The third variety, Odessa, has a well-established reputation in this area because of satisfactory malting and yielding properties. Feebar and Plains barleys are high and medium protein types, respectively. Both yield well and are resistant to stem rust. Both of these varieties are used entirely for feeding purposes, since neither has been found suitable for malting.

The South Dakota study is the first barley protein work carried out in this area. The early work of an American, Osborne, in 1895 established methods for barley protein fractionation (2). Osborne found that the solubility behavior of barley protein was similar to that of the other cereals, since the same protein fractions (albumin, globulin, prolamin and glutelin) were obtained from barley as from corn and wheat. Later studies by European and Canadian investigators reported most of the quantitative data that are available at present. The work of the British investigator, Bishop (3), in 1928 showed that the individual protein components of barley vary in a regular pattern which depends directly upon the total nitrogen content of the grain. The more recent reports by Canadian workers, Anderson and Ayre (4), and by European workers, Fink and Kunisch (5) and Urion *et al.* (6), have adequately confirmed the early work of Bishop.

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[2]Part of the data submitted in this report has previously been published (1).

[3]Formerly, Associate Agronomist.

Procedure

Protein fractionation studies were carried out with Feebar, Odessa and Plains barley samples of varying nitrogen content. The samples (1950 and 1951 crops) were grown by the South Dakota State College Experiment Station at five locations in the state. These were the State Experiment Station at Brookings, The Eureka, Highmore and Cottonwood substations and the Redfield Development Farm (irrigated). The three varieties were grown on adjacent plots at each location.

The effect of fineness of grinding upon protein fractionation data was studied during the first year of the research work. The three different grinding methods employed with Feebar and Odessa samples were the Wiley mill (1 mm. sieve), the hammer mill (0.5 mm. sieve) and the ball mill (48 hours of continuous grinding).[4] Plains barley was ground with only the Wiley mill during the first year, so no data on grinding effects are available with that variety.

Fractionation procedures employed with the samples were essentially the same as those described by Bishop (3). The procedure used for Wiley-mill ground samples is as follows. The fractionation included extraction of the ground barley with water to remove albumin, proteoses + peptones, and non-protein nitrogen. Extraction of samples with salt solution (5 percent potassium sulfate) yielded globulin in addition to the constituents extracted with water. Subtraction of the nitrogen of the water extract from that of the salt extract furnished globulin nitrogen data. Finally, the salt-extracted barley samples were extracted with 70 percent aqueous ethanol solution to remove alcohol-soluble protein (hordein). The insoluble residual protein (glutelin) of the extracted samples was calculated by difference.

Albumin was determined on the water extract by adding 20 ml. of acetate buffer[5] and 60 grams of magnesium sulfate to the extract (200 ml.) and then heating the solution at 82° C. for 40 minutes in a water bath. The precipitated albumin was collected by filtration and measured by Kjeldahl analysis for nitrogen. Non-protein nitrogen was analyzed for by the modified method of Blish (7). The proteose + peptone fraction was calculated by subtracting the sum of the nitrogen of the albumin and the non-protein nitrogen fractions from the total nitrogen of the water extract.

Hammer- and ball-mill ground samples were fractionated by essentially the same procedure as that outlined for the Wiley-mill ground samples. However, employment of a trichloroacetic acid precipitation procedure as described by Bishop (8) enabled elimination of the water extraction step and thereby shortened the fractionation procedure considerably. By determining the quantity of albumin + globulin nitrogen in the salt extract by pre-

[4]Ball-milled samples were ground in the Wiley mill (1 mm. sieve) before ball-mill grinding.

[5]The acetate buffer was composed of equal volumes of normal solutions of sodium acetate and acetic acid. The pH of the buffer was 4.6.

cipitation with 2.27 percent trichloroacetic acid, it was possible to calculate proteose + peptone and non-protein nitrogen by difference. The further subtraction of non-protein nitrogen from this difference made possible the calculation of the proteose + peptone values.

Non-protein nitrogen was not determined on hammer-milled samples since the values obtained on Wiley- and ball-milled samples were in good agreement and thus appeared unrelated to the method of grinding. With hammer-mill ground samples, the differential calculation of the proteose + peptone fraction was carried out by employing the average non-protein nitrogen values obtained from comparable Wiley- and ball-milled samples.

Much difficulty was experienced in the fractionation of certain of the ball-mill ground samples. The salt extracts often showed unsatisfactory precipitation with trichloroacetic acid since the usual clarification of the filtrates was not evident. Thus, it was necessary to discard the albumin + globulin and the proteose + peptone data for the ball-milled samples. No difficulty was encountered with the non-protein nitrogen determinations on such samples.

The centrifugation procedure employed in the fractionation studies was carefully controlled. A trunnion-type centrifuge head was employed throughout the studies. In the case of the Wiley-milled samples, the centrifugation force was 1370 x *g* (2550 r.p.m.) and for the hammer- and ball-milled samples was 1570 x *g* (2730 r.p.m.). The customary procedure for removing the water, salt and alcohol extracts from the centrifuged samples was merely to decant the supernatant extract. However, when separation of aqueous extracts from insoluble particles by decantation proved difficult, a siphoning procedure was carried out. Even with the siphoning, it was often difficult to make a clear-cut separation between insoluble and soluble barley constituents with some ball-milled samples. With these samples, centrifugation caused the insoluble barley residue to settle as a loose stratified mass rather than as a compact one suitable for a decanting operation.

Samples which had a fine powdery texture did not centrifuge properly, while those with a coarse granular consistency could be centrifuged without difficulty during the extraction procedures. Obviously the particle size of some of the ball-milled samples was too fine to allow for proper centrifugation with the equipment employed.

All fractionation analyses were performed in duplicate. Suitable blank Kjeldahl nitrogen determinations were carried out to eliminate possible contamination from reagents employed in the protein fractionations. Separate nitrogen and moisture analyses were made on all samples which were ground by different methods. Thousand-kernel weights were determined by counting and weighing 100 kernel samples in triplicate. A high degree of reproducibility was observed in all determinations.

Fractionation with 1951 barley employed only the hammer-mill ground samples. The same experi-

mental locations, with the exception of Cottonwood, were employed as in the previous year. Fractionation included determination of the three major protein fractions, salt-soluble, alcohol-soluble and insoluble nitrogen, and no determinations of the separate salt-soluble nitrogen constituents were made.

Results

Comparison of Methods of Grinding on Extraction of Nitrogen Fractions

Protein fractionation data on 1950 barley samples that were ground by different methods are shown in Table 1. The table shows that Wiley-mill ground samples had lower salt-soluble and higher glutelin (insoluble protein) nitrogen contents than did the hammer- and ball-mill ground samples. Hordein (alcohol-soluble protein) was not noticeably affected by method of grinding. The content of all of the protein fractions increased with increase in the total nitrogen content of the samples.

Figure 1 expresses the data of Table 1 as the percentage of total nitrogen represented by each protein fraction. The pronounced difference in protein composition between the coarsely ground Wiley-mill samples and the more finely ground hammer- and ball- mill samples is clearly evident. The figure also shows that Wiley- and hammer-mill ground samples had regular protein composition patterns while the ball-mill samples showed an erratic protein composition picture.

Table 2 shows data on the salt-soluble nitrogen components of the same samples reported in Table 1. The increased nitrogen obtained in the salt-soluble extract from the more finely ground samples (hammer versus Wiley mill) largely represented the albumin + globulin constituents. However, the extent of the increase in extracted nitrogen contributed by the separate albumin and globulin constituents is not shown by the data. The table further indicates that slightly more proteose + peptone nitrogen was present in the extracts from the more finely ground samples. The non-protein nitrogen contents of extracts from coarse (Wiley-mill ground and fine ball-mill ground) samples were similar.

Changes in Protein Composition and Protein Content

Table 3 summarizes the data on the salt-soluble, alcohol-soluble and insoluble nitrogen fractions of 1950 and 1951 hammer-mill ground samples. The data are graphed in Figures 2, 3 and 4 to facilitate comparison between varieties as well as between crop years.

Figure 2 shows the relation of total nitrogen of samples to the nitrogen represented by each of the different protein fractions. The salt-soluble protein fraction remained relatively constant with variation in nitrogen content, except for increases in some high nitrogen samples. Both hordein and glutelin increased with increase in total ni-

trogen of barley at all nitrogen levels studied. The protein compositions of 1950 and 1951 samples showed close agreement. Feebar and Odessa barley were similar in their protein composition, but Plains barley was slightly higher than the other two varieties in hordein as well as slightly lower in both salt-soluble and glutelin nitrogen.

Figure 3 compares total nitrogen of barley with protein fraction percentages expressed as percent of total nitrogen. The figure shows that with an increase in total nitrogen the proportion of salt soluble nitrogen decreased and the proportion of hordein nitrogen increased. In Feebar barley, the proportion of glutelin nitrogen decreased with increasing total nitrogen content, while for the other two varieties a rather constant glutelin nitrogen to total nitrogen ratio was observed. The barley samples grown in 1950 and 1951 showed a similar composition picture.

Figure 4 presents data on the relation of kernel nitrogen to the amount of each of the protein fractions per kernel. Differences in kernel size among varieties accounted for a different kernel nitrogen range for each variety. (See kernel weight and kernel nitrogen data of Table 3.) With kernel nitrogen as a plotting basis, the relation of each of the protein fractions to total kernel nitrogen appeared linear with each variety. Hordein and glutelin content both increased with increase in kernel nitrogen, but of the two fractions, hordein increased the more rapidly. Change in the total kernel nitrogen did not appear to affect salt-soluble kernel nitrogen in Odessa and Plains barley samples. With Feebar barley, increase in total kernel nitrogen was correlated with a slight increase in salt-soluble nitrogen. The 1950 and 1951 data were comparable with each variety. However, since the Plains data showed no overlapping between the two years, the results are not as definite with this variety as with the other two.

Table 4 summarizes the hammermill sample data of Table 2. Presentation of the salt-soluble component data as percent of the dry matter, as percent of the total nitrogen, and as content per kernel gives a rather complete picture of the composition of the salt-soluble fraction of barley. The content of the various salt-soluble components generally increased slightly with increase in nitrogen content of samples. While Feebar and Odessa samples both showed increases in contents of the salt-soluble constituents in the high nitrogen samples, Feebar showed much the greater increase of the two varieties. Examination of the kernel composition data of the table indicated a general constancy in kernel content of salt-soluble components with each variety.

Table 1. Nitrogen Fractions in Barleys of Different Total Nitrogen Contents When Ground By Different Methods, As Percent of Dry Matter (1950 Crop)

Name of Variety Type of Grinding Mill Location Where Grown	Total Nitrogen %	Barley Protein Fractions Salt-Sol. Nitrogen %	Hordein Nitrogen %	Glutelin Nitrogen %
Feebar				
Wiley mill				
Redfield	1.97	0.54	0.56	0.87
Eureka	2.01	0.53	0.54	0.94
Brookings	2.58	0.60	0.93	1.05
Highmore	2.79	0.65	0.99	1.15
Cottonwood	3.10	0.70	1.09	1.31
Hammer mill				
Redfield	1.97	0.67	0.59	0.71
Eureka	2.01	0.69	0.60	0.71
Brookings	2.58	0.78	0.95	0.85
Highmore	2.79	0.82	1.06	0.91
Cottonwood	3.10	0.91	1.21	0.97
Ball mill				
Redfield	1.97	0.66	0.55	0.76
Eureka	2.01	0.73	0.59	0.69
Brookings	2.58	0.82	0.96	0.80
Highmore	2.79	0.82	1.11	0.87
Cottonwood	3.10	0.99	1.19	0.92
Odessa				
Wiley mill				
Redfield	1.79	0.49	0.51	0.80
Eureka	1.97	0.55	0.55	0.87
Brookings	2.38	0.55	0.82	1.01
Highmore	2.44	0.57	0.82	1.06
Cottonwood	2.79	0.64	1.02	1.13
Hammer mill				
Redfield	1.79	0.71	0.52	0.56
Eureka	1.97	0.73	0.58	0.66
Brookings	2.38	0.73	0.89	0.76
Highmore	2.44	0.77	0.88	0.79
Cottonwood	2.79	0.85	1.05	0.89
Ball mill				
Redfield	1.79	0.64	0.50	0.64
Eureka	1.97	0.75	0.60	0.62
Brookings	2.38	0.87	0.79	0.72
Highmore	2.44	0.81	0.82	0.82
Cottonwood	2.79	0.77	1.02	1.00
Plains				
Wiley mill				
Redfield	2.22	0.54	0.68	1.01
Eureka	2.38	0.56	0.74	1.08
Brookings	2.53	0.56	0.88	1.09
Highmore	2.81	0.64	1.06	1.11
Cottonwood	2.85	0.63	1.00	1.23

Table 2. Salt-Soluble Nitrogen Components in Barley Grown at Different Stations, As Percent of Dry Matter (1950 Crop)

Type of Grinding Mill Salt-Soluble N Fraction	Redfield %	Eureka %	Brookings %	Highmore %	Cottonwood %
Feebar Barley					
Total Nitrogen Content	1.97	2.01	2.58	2.79	3.10
Wiley mill					
Total salt-soluble N	0.54	0.53	0.60	0.65	0.70
Albumin N	0.13	.....	0.10	0.11	0.11
Albumin + globulin N	0.30	0.31*	0.33	0.34	0.37
Proteose + peptone N	0.14	0.14*	0.15	0.15	0.15
Non-protein N	0.10	0.08	0.12	0.17	0.18
Hammer mill					
Total salt-soluble N	0.67	0.69	0.78	0.82	0.91
Albumin + globulin N	0.42*	0.45*	0.49*	0.49*	0.53*
Proteose + peptone N	0.14*	0.15*	0.17*	0.17*	0.19*
Ball mill					
Total salt-soluble N	0.66	0.73	0.82	0.82	0.99
Non-protein N	0.10	0.10	0.13	0.15	0.19
Odessa Barley					
Total Nitrogen Content	1.79	1.97	2.38	2.44	2.79
Wiley mill					
Total salt-soluble N	0.49	0.55	0.55	0.57	0.64
Albumin N	0.13	.....	0.10	0.11	0.10
Albumin + globulin N	0.24	0.31*	0.32	0.31	0.39
Proteose + peptone N	0.14	0.13*	0.13	0.13	0.12
Non-protein N	0.11	0.10	0.10	0.13	0.13
Hammer mill					
Total salt-soluble N	0.71	0.73	0.73	0.77	0.85
Albumin + globulin N	0.46*	0.46*	0.45*	0.47*	0.51*
Proteose + peptone N	0.15*	0.16*	0.17*	0.18*	0.21*
Ball mill					
Total salt-soluble N	0.64	0.75	0.87	0.81	0.77
Non-protein N	0.09	0.11	0.11	0.12	0.13
Plains Barley					
Total Nitrogen Content	2.22	2.38	2.53	2.81	2.85
Wiley mill					
Total salt-soluble N	0.54	0.56	0.56	0.64	0.63
Albumin N	0.11	.....	0.11	0.11	0.10
Albumin + globulin N	0.30	0.33*	0.34	0.37	0.38
Proteose + peptone N	0.14	0.14*	0.13	0.15	0.14
Non-protein N	0.10	0.09	0.09	0.12	0.11

*Samples that were analyzed for albumin + globulin and proteose + peptone nitrogen by the trichloroacetic acid precipitation method.

Table 3. Protein Composition of Hammer-Mill Ground Barley Samples of Different Nitrogen Contents (Moisture-free Basis)

Variety and Description*	Weight per Kernel (gms.)	Total Nitrogen (percent)	Protein Fractions, as Percent of Dry Matter			Protein Fractions, as Percent of Total Nitrogen			Protein Composition of Kernel			
			Salt-Sol. N	Hordein N	Glutelin N	Salt-Sol. N	Hordein N	Glutelin N	Total N (mg.)	Salt-Sol. N (mg.)	Hordein N (mg.)	Glutelin N (mg.)
Feebar												
(H, '51)	0.039	1.75	0.63	0.48	0.64	36.0	27.3	36.7	0.69	0.25	0.19	0.25
(R, '50)	0.039	1.97	0.67	0.59	0.71	33.8	30.0	36.2	0.77	0.26	0.23	0.28
(E, '50)	0.035	2.01	0.69	0.60	0.71	34.6	29.9	35.5	0.69	0.24	0.21	0.25
(E, '51)	0.037	2.18	0.74	0.72	0.73	33.8	32.8	33.5	0.80	0.27	0.26	0.27
(R, '51)	0.037	2.37	0.71	0.85	0.81	29.9	36.0	34.0	0.88	0.26	0.32	0.30
(B, '50)	0.037	2.58	0.78	0.95	0.85	30.3	36.9	32.8	0.97	0.29	0.36	0.32
(B, '51)	0.035	2.63	0.76	1.00	0.87	29.0	37.9	33.2	0.93	0.27	0.35	0.31
(H, '50)	0.033	2.79	0.82	1.06	0.91	29.4	37.9	32.7	0.92	0.27	0.35	0.30
(C, '50)	0.030	3.10	0.91	1.21	0.97	29.4	39.2	31.3	0.92	0.27	0.36	0.29
Odessa												
(R, '50)	0.033	1.79	0.71	0.52	0.56	39.8	29.2	31.0	0.59	0.24	0.17	0.18
(E, '51)	0.033	1.80	0.73	0.53	0.55	40.2	29.2	30.6	0.60	0.24	0.18	0.18
(E, '50)	0.032	1.97	0.73	0.58	0.66	37.1	29.2	33.7	0.62	0.23	0.18	0.21
(H, '51)	0.033	1.97	0.70	0.63	0.64	35.6	32.1	32.3	0.64	0.23	0.21	0.21
(B, '51)	0.031	1.99	0.68	0.63	0.68	34.3	31.7	34.1	0.62	0.21	0.20	0.21
(B, '50)	0.032	2.38	0.73	0.89	0.76	30.6	37.4	32.0	0.75	0.23	0.28	0.24
(H, '50)	0.028	2.44	0.77	0.88	0.79	31.7	36.2	32.1	0.68	0.21	0.25	0.22
(R, '51)	0.031	2.50	0.75	0.88	0.87	30.0	35.2	34.8	0.78	0.24	0.28	0.27
(C, '50)	0.027	2.79	0.85	1.05	0.89	30.4	37.7	31.9	0.77	0.23	0.29	0.24
Plains												
(E, '51)	0.034	1.81	0.71	0.57	0.53	39.0	31.7	29.3	0.62	0.24	0.20	0.18
(H, '51)	0.035	2.11	0.69	0.74	0.68	32.6	35.2	32.2	0.74	0.24	0.26	0.24
(R, '51)	0.035	2.36	0.72	0.86	0.77	30.6	36.6	32.8	0.82	0.25	0.30	0.27
(B, '51)	0.030	2.49	0.72	0.94	0.83	29.1	37.7	33.2	0.75	0.22	0.28	0.25
(B, '50)	0.035	2.53	0.71	1.03	0.79	28.0	40.6	31.3	0.88	0.25	0.36	0.28
(H, '50)	0.035	2.81	0.79	1.18	0.83	28.2	42.1	29.6	0.98	0.28	0.41	0.29

*Description indicates the location and year of sample collection. The locations are abbreviated as follows: B, Brookings; C, Cottonwood; E. Eureka; H, Highmore; and R, Redfield. The figures, '50 and '51 signify the years 1950 and 1951, respectively.

Table 4. Salt-Soluble Nitrogen Components of Hammer-Mill Ground Barley Samples (Moisture-free Basis)

Variety and Description*	Weight per Kernel (gms.)	Total Nitrogen (percent)	Components, as Percent of Dry Matter				Components, as Percent of Total Nitrogen				Kernel Composition Data, Expressed as Content Per Kernel				
			Total Salt-sol. N	Albumin + Globulin N	Proteose + Peptone N	Non-protein N	Total Salt-sol. N	Albumin + Globulin N	Proteose + Peptone N	Non-protein N	Total N (mg.)	Total Salt-sol. N (mg.)	Albumin + Globulin N (mg.)	Proteose + Peptone N (mg.)	Non-protein N (mg.)
Feebar															
(R, '50)	0.039	1.97	0.67	0.42	0.14	0.10	33.8	21.5	7.3	5.1	0.77	0.26	0.17	0.06	0.04
(E, '50)	0.035	2.01	0.69	0.45	0.15	0.09	34.6	22.3	7.6	4.6	0.69	0.24	0.16	0.05	0.03
(B, '50)	0.037	2.58	0.78	0.49	0.17	0.12	30.3	19.1	6.5	4.8	0.97	0.29	0.18	0.06	0.05
(H, '50)	0.033	2.79	0.82	0.49	0.17	0.16	29.4	17.6	6.1	5.7	0.92	0.27	0.16	0.06	0.05
(C, '50)	0.030	3.10	0.91	0.53	0.19	0.18	29.4	17.2	6.3	6.0	0.92	0.27	0.16	0.06	0.05
Odessa															
(R, '50)	0.033	1.79	0.71	0.46	0.15	0.10	39.8	25.5	8.5	5.8	0.59	0.24	0.15	0.05	0.03
(E, '50)	0.032	1.97	0.73	0.46	0.16	0.11	37.1	23.5	8.3	5.3	0.62	0.23	0.15	0.05	0.03
(B, '50)	0.032	2.38	0.73	0.45	0.17	0.10	30.6	19.0	7.2	4.3	0.75	0.23	0.14	0.05	0.03
(H, '50)	0.028	2.44	0.77	0.47	0.18	0.12	31.7	19.2	7.4	5.0	0.68	0.21	0.13	0.05	0.03
(C, '50)	0.027	2.79	0.85	0.51	0.21	0.13	30.4	18.4	7.3	4.7	0.77	0.23	0.14	0.06	0.04

*See footnote for Table 3.

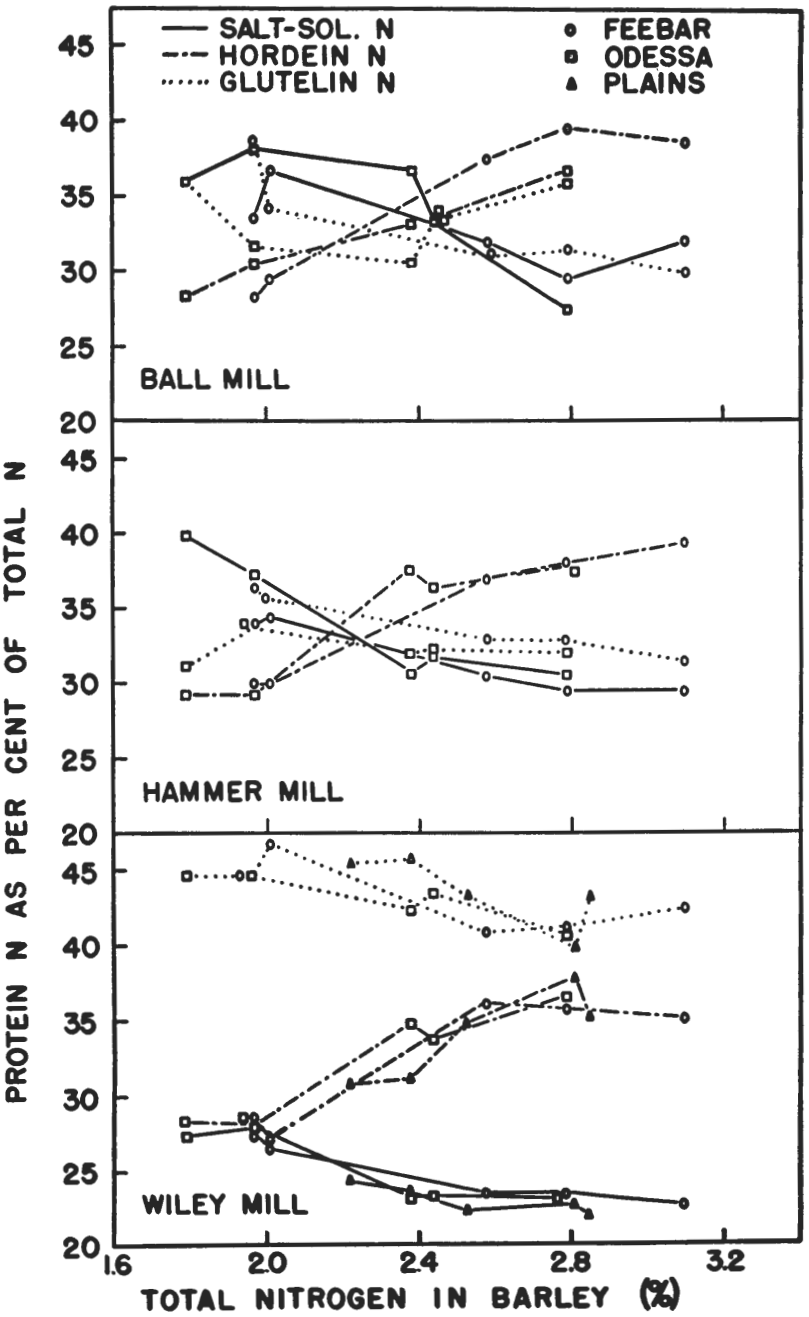

Fig. 1. Effect of the method of grinding and the nitrogen content of barley upon the percentage distribution of nitrogen in the different protein fractions

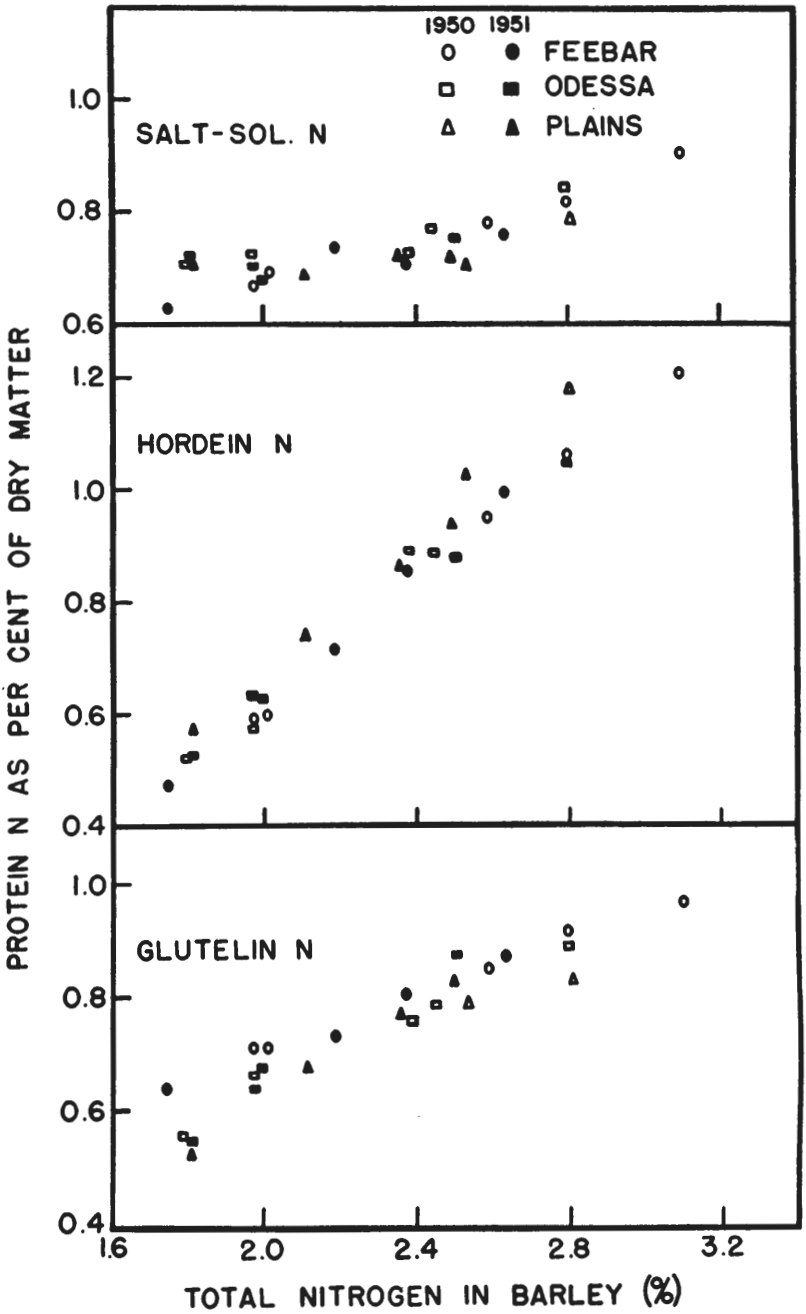

Fig. 2. Effect of total nitrogen content of barley upon dry matter percentages of salt-soluble, hordein and glutelin nitrogen

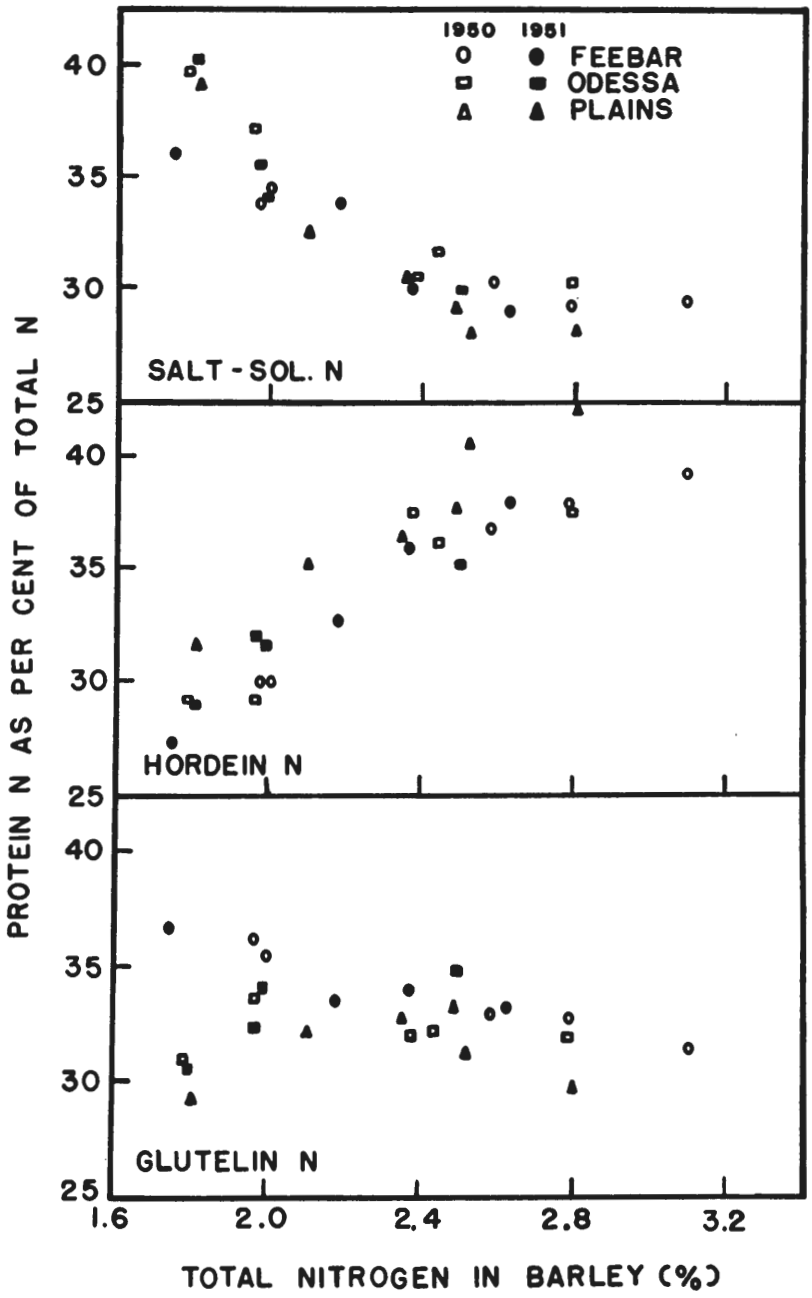

Fig. 3. Effect of total nitrogen content of barley upon the percentage distribution of total nitrogen among the different protein fractions

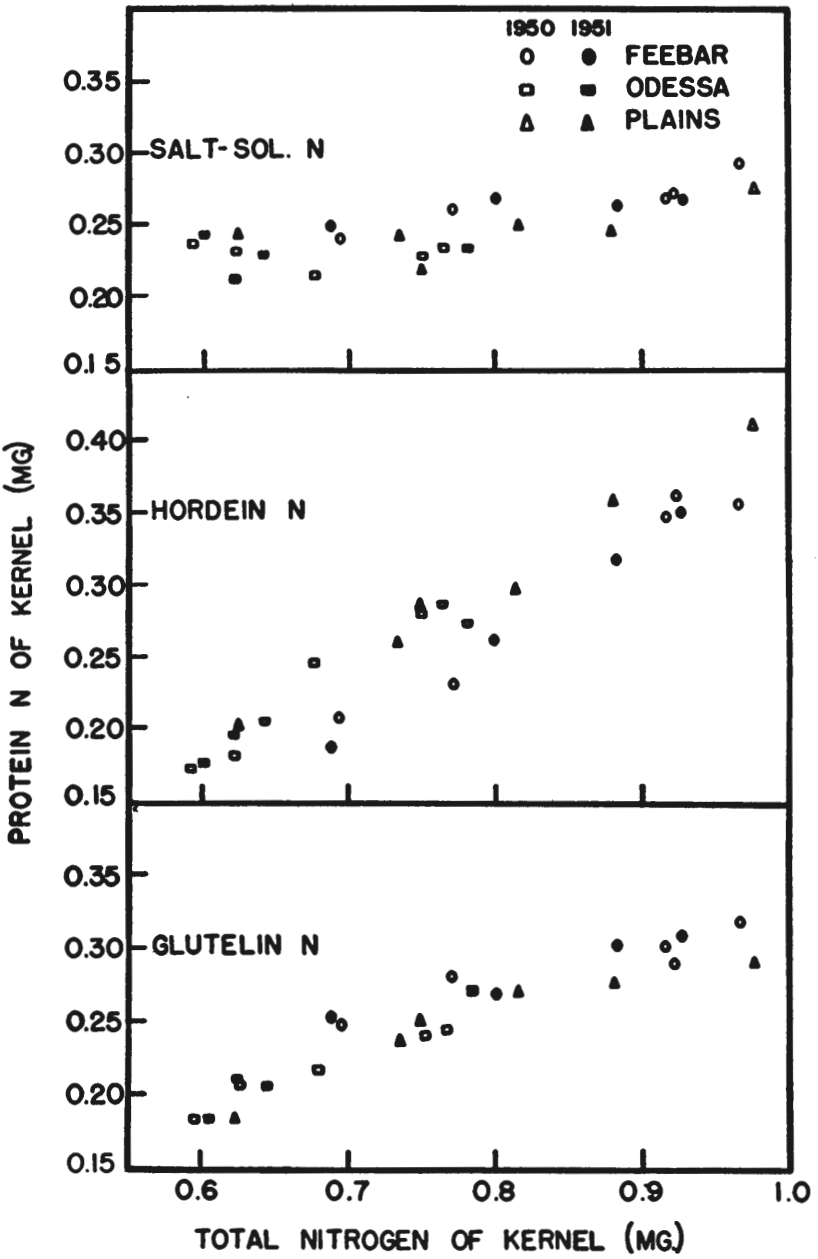

Fig. 4. Effect of kernel nitrogen content of barley upon salt-soluble, hordein and glutelin nitrogen in three varieties

Discussion

The advantages of grinding samples with the hammer mill (0.5 mm. sieve) rather than with the Wiley-mill (1 mm. sieve) or the ball mill are shown by the data presented in Fig. 1. A more efficient extraction of salt-soluble nitrogen resulted with hammer-milled samples than with the less finely ground Wiley-milled samples. Also, the irregular results which were encountered with the salt-soluble fraction of certain ball-milled samples were avoided by hammer-mill grinding. It is obvious that a uniform degree of grinding among different samples is essential for obtaining comparable results in protein fractionation work. Samples ground with the hammer mill should have been comparable, since the sieve would standardize maximum particle size. Grinding with the ball mill does not insure standard particle size.

The methods employed for determination of the salt-soluble components reported in Table 2 are recognized as capable of only approximate separations. The fact that the non-protein nitrogen values were observed to differ between salt and water extracts from identical samples was a good indication that the particular analytical procedure employed may have significantly affected the salt-soluble fraction results. However, the importance of such data as a reference for future work was adequate reason for presentation of the data in Table 4.

Discussion of the protein composition picture is best approached by division of the samples into two categories. Differentiation between the two categories is dependent upon kernel size differences. Samples in the first category consisted of normal-sized (plump) kernels which generally contained less than 2.6 percent of nitrogen. The second category was composed of undersized (shriveled) kernels of varied nitrogen content which most frequently fell in the high nitrogen range (2.4 to 3.1 percent of nitrogen).

With plump kernel samples an increase in nitrogen content was always accompanied by a direct increase in the proportion of hordein and a similar decrease in the proportion of salt-soluble nitrogen. The composition picture with such samples was in close agreement with the previously reported studies of Bishop (3) and of Anderson and Ayre (4) who worked with barley samples of similar or lesser nitrogen content. The actual values reported with the hammer-mill ground samples showed better agreement with data obtained by the Canadian investigators (Anderson and Ayre) than with the data obtained by the English investigator, Bishop.

Shriveled kernel samples displayed an abnormal protein composition picture. The plateauing of the high nitrogen portion of the composition curves (Figs. 1 and 3) was largely due to these samples. The shriveled kernels maintained high nitrogen contents in spite of large reductions in kernel weight (see Table 3). Thus, the kernel weight reductions caused increases in nitro-

gen percentages in samples which showed practically no change in kernel nitrogen contents. This effect contrasted to that observed with plump kernels in which increases in nitrogen percentage were always accompanied by increases in kernel nitrogen content.

The presentation of the kernel data as in Fig. 4 furnished an explanation of the plateau effect observed with the high nitrogen shriveled kernel samples. The graph shows that both shriveled and non-shriveled samples of the same barley variety will have the same protein composition when the kernel nitrogen contents of the samples are the same. When these same samples differ in nitrogen percentage to a significant extent, a plateau in the protein composition curves is the obvious result.

Kernel nitrogen data appear limited in application because of differences in kernel size among different varieties. While such data are valuable for providing an accurate protein composition picture with a single variety, the possibility of applying the same data to other varieties is difficult. The importance of the kernel studies applies chiefly to interpretation of shriveled kernel data and it should be kept in mind that shriveled kernels are in themselves an abnormal product which occurs only in limited instances even in the Great Plains area.

It appears from the data presented that expression of the protein fractions as percent of the dry matter of the samples provides the most practical means for prediction of protein composition (Fig. 2). The uniform relationship between total nitrogen and protein composition obtained with a wide assortment of samples indicated that nitrogen content can be used as a reference standard for prediction of protein composition. Thus, it becomes possible to determine protein composition of barley samples by determining nitrogen content by Kjeldahl analysis and then referring to the graph which shows protein composition throughout the range of nitrogen contents.

Summary

Two years of protein composition studies with Feebar, Odessa and Plains barley samples, grown at points within the state and varying in nitrogen content from 1.75 to 3.10 (10.9 to 19.4 percent protein), are reported.

Studies of different methods of grinding the samples showed the hammer mill (0.5 mm. screen) to be superior to either the Wiley mill (1.0 mm. screen) or the ball mill, this conclusion being based upon the consistency of results and completeness of separation of fractions with the method of analysis used.

In all three varieties, as the total protein content increased, the glutelin N and hordein N contents also increased but the salt-soluble N remained almost constant. The hordein N increase was relatively greater than the glutelin N increase. When the various nitrogen fractions were

expressed as percent of total nitrogen, it was found that the proportion of glutelin N remained about constant, of hordein N increased and of salt-soluble N decreased with increasing total protein content.

In a few cases, samples of barley of high nitrogen content showed an abnormal distribution of the various nitrogen fractions. In these cases, the kernels were smaller than normal (shriveled). On determining kernel weights and calculating the amount of nitrogen in each fraction per kernel, these abnormal variations were explained.

On sub-fractionation of the salt-soluble fraction into albumin and globulin N, proteose + peptone N and non-protein N, it was found that the albumin and globulin N predominated. The proportions of subfractions of the salt-soluble N fraction showed little change with change in total protein content.

Although the Plains variety was found to vary slightly from Feebar and Odessa in its protein composition, reasonably accurate prediction of protein composition of barley, when total protein content is known, seems possible. It appears that climate and soil factors affect protein composition but only as total nitrogen affects the picture.

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