STUDIES ON SHORT- AND LONG-MIXING FLOURS

III. Mixing Properties, Protein and Lipid Composition of Various Fractions[1]

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ABSTRACT

Short-mixing Willet and long-mixing Rodco wheat flours of similar protein contents were separated into fractions of various particle sizes. Farinograph mixing properties, solubility and electrophoretic composition of the proteins, and free and bound lipid content of these fractions were determined. Mixing requirements of the fractions varied considerably and increased as their protein contents increased. All of the Willet series fractions, however, were shorter-mixing than the Rodco series fractions of comparable protein content. Major differences in protein solubility characteristics were: between the series, the higher acid solubility of the Willet gluten proteins; within each series, the greater salt solubility of the proteins in the lower protein fractions. In both series, moving-boundary electrophoretic analyses of fractions showed small differences in the acid-soluble proteins and large differences in the salt-soluble proteins. Ether-soluble lipid increased as the protein content of the fractions increased. Bound lipid, as measured by the difference between the acid hydrolysate and the ether extract, was essentially constant for all of the flour fractions, regardless of their protein content.

Various properties of the constituents of flours of different particle size have been studied. Hess (1) has separated the "wedge" proteins associated with the fine particles of flour from the "adhering" proteins associated with the larger particles and starch. These proteins with different mixing properties (2) are both said to be required for normal dough characteristics. Differences in the electrophoretic properties and amino acid composition of wedge and adhering protein have been shown by Hess and Hille (3). In an effort to study these differences further, Wrigley (4) extracted parent flours and their fine, medium, and coarse fractions successively with sodium pyrophosphate, acetic acid, and sodium hydroxide solutions. The distribution of nitrogen among the solvents was determined. The author states that only minor differences were shown, although the lowest-protein fraction (medium fraction) contained considerably (47–65%) more nitrogen soluble in pyrophosphate buffer than the highest-protein fraction (fine fraction). The low-protein fraction also contained less acetic acid-soluble gluten protein than the other fractions. Studies of Jones and Dimler (5) on the electrophoretic composition of glutens washed from flours of different particle size indicated that the glutens were similar.

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Their results, however, showed that the low-protein fractions contained a greater percentage of water-soluble protein than the higher-protein fractions. They suggest that differences between wedge and adhering protein may be in the water-soluble fraction rather than in the gluten obtained by water washing.

The Willet and Rodco flours of similar protein contents used in our earlier studies (6) had markedly different rheological properties and showed differences in their protein characteristics. The possibility that a certain particle-sized fraction might contain proteins determining mixing differences was investigated. The flours were separated into fractions of various particle sizes. Solubility and electrophoretic characteristics of the proteins in these fractions were determined, and the results were related to the mixing properties of the flours. Free and bound lipid contents were also determined.

Materials and Methods

The flours used were the Multimat-milled Willet and Rodco patent flours described in the first paper of this series (6). These flours were pin-milled at 19,000 r.p.m. in a 160 Z Alpine Kolloplex. Each flour was then separated into fractions with a laboratory Walther classifier. Four fractions were separated from Willet flour and three fractions from Rodco flour by this method. Willet fraction 5 and Rodco fraction 4 are residual coarse material.

Protein contents were determined by the Kjeldahl method and average particle-size values by the Fisher method. Farinograms were obtained with the 80-g. stainless-steel bowl at the second speed (62 r.p.m.), with constant dough weight and constant consistency of 500 ± 25 B.U.

The separated flours were analyzed for the quantity of salt-soluble (albumin and globulin) proteins and acid-soluble (gluten) proteins and prepared for electrophoretic analyses as follows: A 10-g. sample was dispersed in 30 ml. of 0.1*M* NaCl in a 360-ml. Waring Blendor bowl at top speed for 3 min. After centrifugation at 38,000 × *g* at 1°C., the supernatant was retained and the residue redispersed in 20 ml. of water in a similar manner and recentrifuged. Both supernatants, containing predominantly salt-soluble proteins, were combined and diluted to 50 ml., and aliquots were taken for Kjeldahl and electrophoretic analyses.

The residue was then redispersed and recentrifuged; one 50-ml. portion and three successive 40-ml. portions of 0.02*M* formic acid were used. These extracts, containing predominantly acid-soluble gluten proteins, were combined and diluted to 200 ml., and aliquots

were taken for Kjeldahl nitrogen and electrophoretic analysis. All samples were analyzed in duplicate. Quantities of insoluble protein remaining in the residues were determined by difference. Electrophoretic analyses of salt-soluble proteins and acid-soluble gluten proteins were made as given in our earlier report (6). Lipid analyses were obtained by the AOAC ether extraction method 13.018 (7) and the AOAC acid hydrolysis method 13.019 (7). The amount of bound lipids was calculated as the difference between these values. Protein and lipid values and farinograph absorptions have all been calculated on 14% moisture basis.

Results and Discussion

The distribution of the material and protein among the separated flours is given in Table I. Protein contents and particle size values of the fractions are also given.

TABLE I

YIELD AND ANALYSES OF SEPARATED WILLET AND RODCO FRACTIONS

FRACTION	PERCENT OF TOTAL MATERIAL	PROTEIN	PARTICLE SIZE	PERCENT OF TOTAL PROTEIN
	%	%	μ	%
Willet, P-M[a]		14.2	8.2	100
Willet, fraction 1	9.7	34.1	4.7	24.0
2	11.3	27.7	6.3	22.4
3	19.4	9.2	10.3	12.7
4	20.6	6.4	13.1	9.3
5	39.0	11.4	18.9	31.6
Rodco, P-M		13.5	11.1	100
Rodco, fraction 1	8.5	24.6	4.9	16.3
2	16.4	11.9	7.7	15.1
3	17.5	7.1	13.2	9.5
4	57.6	13.4	25.0	59.1

[a] P-M, pin-milled.

Farinogram results on the fractions are given in Table II. Mixing characteristics vary markedly, depending upon the protein content of the fraction and the type of parent flour. The Willet fractions are all shorter-mixing than Rodco fractions of comparable protein contents. Comparison of fractions of comparable protein content show that the fractions reflect the mixing characteristics of their parent flours. There is no evidence that any one fraction is primarily responsible for the mixing differences between the original flours.

The distribution of the proteins in the fractions according to their solubility is given in Table III. With both flours, the percentage of salt-soluble protein in fractions of comparable protein content is similar, but the lower-protein fractions have higher percentages of

TABLE II

FARINOGRAPH MIXING PROPERTIES OF SEPARATED FRACTIONS

SAMPLE	PROTEIN	ABSORPTION	MAXIMUM	TIME TO MAXIMUM	STABILITY
	%	%	*B.U.*	*min.*	*min.*
Willet, P-M[a]	14.2	60.6	500	4	5
Willet, fraction 1	34.1	94.2	490	24	21
2	27.7	86.5	510	22	15
3	9.2	57.2	495	1	2
4	6.4	57.2	500	0.5	0.5
5	11.4	53.8	520	2	3
Rodco, P-M	13.5	65.3	485	20	19
Rodco, fraction 1	24.6	94.2	495	40	18
2	11.9	69.5	495	22	20
3	7.1	57.2	525	2	2
4	13.4	60.0	490	12	14

[a] P-M, pin-milled.

salt-soluble proteins than the higher-protein fractions. The low-protein, adhering protein fraction associated with the starch is thus high in proteins of the salt-soluble, albumin, and globulin types. Data of Wrigley (4) and Jones and Dimler (5) actually show greater solubility of the proteins in the low-protein fraction.

Acid-soluble gluten proteins were highest in the Willet series fractions. Greater solubility of Willet gluten proteins was also shown in our previous studies (6). Within either the Willet or Rodco series, the greatest difference in the percentage of the acid-soluble gluten proteins was between the lowest-protein fraction and the other fractions. Some of this difference was accounted for by the greater proportion of proteins removed with the salt extraction of the lowest-protein fraction.

The quantity of acid-insoluble proteins was consistently higher for the Rodco fractions. This also agrees with previous results with Rodco flour (6).

TABLE III

PROTEIN DISTRIBUTION IN SEPARATED FRACTIONS

FRACTION	PROTEIN	PERCENT OF TOTAL PROTEIN		
		Salt-Soluble	Acid-Soluble	Insoluble
	%			
Willet, P-M[a]	14.2	10	82	8
Willet, fraction 1	34.1	7	84	9
2	27.7	7	85	8
3	9.2	11	79	10
4	6.4	13	73	14
Rodco, P-M	13.5	10	75	15
Rodco, fraction 1	24.6	7	77	16
2	11.9	9	74	17
3	7.1	13	70	17

[a] P-M, pin-milled.

The percentages of acid-insoluble proteins in the fractions within each series were similar except for the lowest-protein Willet fraction. This fraction had a high percentage of acid-insoluble protein. The adhering protein fraction of Willet flour appears to contain a higher percentage of proteins of high molecular weight than the other Willet fractions. Early results of McCalla (8) showed that both the salt-soluble protein and glutenin fraction increased uniformly throughout grain development, whereas gliadin increased more rapidly in the later stages. Thus, the percentage of salt-soluble protein and glutenin protein (0.1*N* NaOH-soluble) was greater at early stages of maturation. Recently, Jennings and Morton (9) and Graham *et al.* (10) have extended these findings. They also found that a larger proportion of the total protein was soluble in 0.01*M* sodium pyrophosphate at early stages of development.

The present results, showing an association of the salt-soluble proteins with the adhering protein fractions, when considered with the maturation studies, suggest that this fraction is laid down early during maturation. The percentage of insoluble (glutenin) proteins is high in the Willet adhering protein fraction. As McCalla (8) found a higher proportion of glutenin-type protein in early stages of maturation, this is a further indication of the early development of adhering protein.

The electrophoretic compositions of the gluten proteins are given in Table IV. Results are averages of duplicates which in general agreed within less than 5%. The major difference between the series is in the greater quantity of the beta component in the Willet fractions. Within each series analyses were quite similar, except for the lowest-protein fractions which had a lower percentage of many of the components.

TABLE IV

ELECTROPHORETIC COMPOSITION OF GLUTEN PROTEINS IN SEPARATED FRACTIONS

FRACTION	PROTEIN CONTENT	ACID-SOLUBLE PROTEIN[a]	PERCENT OF TOTAL PROTEIN IN COMPONENT				
			Alpha	Beta	Gamma	Omega	Fast
	%	%					
Willet, P-M[b]	14.2	82	44	16	10	3	9
Willet, fraction 1	34.1	84	43	18	10	4	9
2	27.7	85	46	15	12	4	8
3	9.2	79	42	15	11	4	7
4	6.4	73	40	13	9	4	7
Rodco, P-M	13.5	75	41	12	11	4	7
Rodco, fraction 1	24.6	77	44	11	12	4	6
2	11.9	74	42	12	10	4	6
3	7.1	71	41	10	9	3	8

[a] Percent of the total protein in fraction.
[b] P-M, pin-milled.

TABLE V

ELECTROPHORETIC COMPOSITION OF SALT-SOLUBLE PROTEINS IN SEPARATED FRACTIONS

SAMPLE	PROTEIN	SALT-SOLUBLE PROTEIN[a]	TOTAL PROTEIN IN COMPONENT							
			1	2	3	4	5	6	7	8
			4.4–5.8[b]	3.8–3.9	3.2–3.4	2.5–2.7	1.0–2.1	0.7–0.9	0.4–0.6	(0)
	%	%	%	%	%	%	%	%	%	%
Willet										
P-M[c]	14.2	11.2	0.5	0.8	0.7	1.4	2.5	0.8	1.6	2.9
Fr. 1	33.8	6.6	0.4	0.6	0.5	0.7	1.2	0.7	0.9	1.6
Fr. 2	25.3	7.6	0.4	0.6	0.5	0.7	1.3	0.9	1.2	2.0
Fr. 3	10.6	12.6	0.6	0.6	0.9	1.3	2.8	1.1	2.1	3.3
Fr. 4	4.9	15.9	0.3	0.9	0.7	1.3	4.7	1.7	2.5	3.8
Rodco										
P-M	13.4	10.5	0.6	0.7	0.6	0.9	1.5	0.7	2.2	3.2
Fr. 1	24.6	8.4	0.4	0.5	0.5	0.6	1.0	0.5	2.0	2.9
Fr. 2	12.5	10.5	0.3	0.6	0.5	0.9	1.6	0.8	3.1	2.9
Fr. 3	7.2	15.2	0.6	0.9	0.8	1.1	2.7	1.2	3.2	4.7

[a] Percent of total protein.
[b] Mobility range, μ (10^{-5} cm.^{2}/volt/sec.).
[c] P-M, pin-milled; Fr., fraction.

Electrophoretic analyses of the salt-soluble proteins are given in Table V. The results were obtained on a different series of samples which were prepared similarly to those of Table I. The percentage of the total protein present in each of the components tended to increase as the particle size of the fraction increased, except for component 1. Component 1 is a composite of minor components. Component 7 contains pentosans (11) and component 2 has been isolated and characterized by Kelley (12). The identity and importance of the other components are, however, largely unknown. Components 5 and 6 may contain some gluten components (present authors' un-

TABLE VI

LIPID DISTRIBUTION IN SEPARATED FRACTIONS

SAMPLE	PROTEIN	ETHER EXTRACT	ACID HYDROLYSATE	BOUND LIPID	PERCENT OF TOTAL LIPID BOUND
	%	%	%	%	%
Willet, P-M[a]	14.2	0.80	1.35	0.55	40.7
Willet, fraction 1	34.1	2.10	2.65	0.55	20.8
2	27.7	1.40	2.00	0.60	30.0
3	9.2	0.45	1.00	0.55	55.1
4	6.4	0.35	0.90	0.55	61.1
5	11.4	0.40	0.90	0.50	55.6
Rodco, P-M	13.5	0.80	1.30	0.50	39.2
Rodco, fraction 1	24.6	2.00	2.50	0.50	21.2
2	11.9	0.88	1.45	0.57	39.3
3	7.1	0.50	1.10	0.60	54.5
4	13.4	0.66	1.20	0.54	45.0

[a] P-M, pin-milled.

published results). However, these represent only part of the increase in salt-soluble protein in the low-protein fraction.

The lipid analyses of these flours are given in Table VI. Values for ether extract, as is known, increase as the protein contents increase. The quantity of bound lipids is, however, essentially constant and the percentage of the total lipid that is bound is maximum for the lowest-protein fractions. This suggests that the lipid might be bound by a nonprotein component.

Conclusion

Flour fractions of various particle sizes separated from Willet and Rodco flours vary in mixing properties, depending upon their protein content and the flour from which they are derived. In general, the fractions of similar protein contents reflect the mixing properties of their parent flour.

The major difference between the protein characteristics of the two series of samples is the difference in the solubility of the gluten proteins. Within each series one major difference is in the greater quantity of salt-soluble proteins from the lower-protein fractions.

This may have a relationship to changes that occur during wheat maturation and, when considered in conjunction with maturation studies, suggests that adhering protein is laid down early in the maturation period. Another difference within a series is the greater percentage of insoluble protein in the lowest-protein Willet fraction.

Adhering protein appears to be unusual in several ways, containing greater percentages of salt-soluble protein, insoluble protein, and bound lipid. Further studies should be made to determine the reason for these differences and the nature of the bound lipid.

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