Density Fractionation of Wheat Flours in Nonaqueous Solvents. II. Behavior of Fractions in Water[1]

R. L. CLEMENTS[2]

ABSTRACT

Fractions of different densities were separated from soft and hard wheat flours by sequential flotations of the flours in chlorofluorocarbon-hexane mixtures. The fractions were analyzed for soluble protein, soluble solids, hydration capacity, pH, and electrical conductivity. The fractions could be combined into three broad fractions: 1) a low-density to intermediate-density, high-nitrogen, hydrophilic fraction (25% of the flour), pH 5.5, 2) a high-density, high-nitrogen, high-ash fraction (less than 1% of the flour), and 3) an intermediate-density to high-density fraction (75% of the flour), primarily starch, with nitrogen, ash, and pH dependent on degree of inclusion of fractions 1 and 2.

Sedimentation in aqueous media is a classic method for separating wheat flour into four fractions: water-soluble material, starch tailings, gluten, and prime starch. Together with the lipids, these four fractions have constituted the basis of flour chemistry. Water, however, can cause irreversible interactions and relocations of flour components (Kasarda et al 1971, Simmonds and Orth 1973). Hence, aqueous fractionation is unsatisfactory as a prime separation method for studies affected by such changes. The first part of this article (Clements 1979) described a procedure based on density separations in nonaqueous solvents; discussed the distributions of solids, protein, and ash among the density fractions; and also discussed the effects of flour moisture level on these distributions. In the present report, fractions are characterized with respect to their behavior toward water.

MATERIALS AND METHODS

Nonaqueous Fractionation

The fractions were those prepared in the initial study described in the first part of this article (Clements 1979). Two flours, one from a mixture of soft red and white wheats and the other from a mixture of hard red winter wheats, were extracted with hexane and pin milled. They were then fractionated at three moisture levels (low, intermediate, high) by sequential suspensions in a series of chlorofluorocarbon-hexane mixtures of increasing densities (sp gr 1.350–1.470 at 25°C in increments of 0.010). The suspensions were centrifuged at 0°C, floating material was removed, and sediments were resuspended in the next medium in the density series. The process was repeated until less than 2% of the flour remained as sediment, to give 11–13 fractions, including residue, from each flour.

Analytic Methods

Hydration capacity of fractions was determined by a modified AACC method (1962). Samples (2.00 g of flour or fraction) were weighed into 50-ml polyethylene centrifuge tubes. Each sample was mixed with 40 ml of water (double deionized, 1–2 μmho/cm conductivity), added in small increments with stirring so that it first formed a smooth paste, and dispersed uniformly. After 15 min, the suspensions were centrifuged for 15 min at 1,000 $\times g$ at 0°C, and supernatants were collected. The tubes containing the residues were inverted, allowed to drain for 15 min, and weighed to determine hydration. The supernatants were adjusted to 25°C, and pH and electrical conductivity were measured (Clements 1977b). Aliquots (based on 40 ml of total liquid) were evaporated in an airstream, dried for 1 hr at 100°C, and weighed for determination of soluble solids. Soluble protein in aliquots was determined by a micro-Kjeldahl procedure (N × 5.7). Gluten was isolated from the hydrated residues by repeated suspension in dilute NaCl and decanting, kneaded, and freeze-dried. Protein was determined in the dry gluten samples by a micro-Kjeldahl procedure.

Electrophoresis

Disc electrophoresis on polyacrylamide was according to the method of Ornstein and Davis (1962) as modified by Clements (1970). Flours and fractions were extracted at 0°C with 0.1M potassium phosphate buffer, pH 7.0, containing 25% sucrose and 0.001M EDTA. Sample size was adjusted to give about 0.5 mg of protein per milliliter of buffer (based on water-soluble protein). Suspensions were centrifuged for 15 min at 6,500 $\times g$ at 0°C and extracts (0.5–3.0 μl) were applied to gel columns. After electrophoresis at 2–3 mA per tube, proteins were stained with amido black in 7% acetic acid, and gels were washed with 7% acetic acid to remove excess dye.

Aqueous Fractionation of Flours

The hexane-extracted pin-milled flours were also fractionated by a conventional aqueous method (Yamazaki and Donelson 1976) and analyzed for comparative purposes.

RESULTS AND DISCUSSION

The study reported in the first part of this article (Clements 1979) showed that flour moisture level affects density and hence the flotation of solids and the yields in the fractionation sequence. Results indicated moisture level does not affect fraction composition beyond displacing the distribution of solids in the density series. Therefore, this report is confined primarily to data for fractions obtained from the soft and hard wheat flours at intermediate moisture levels only (soft wheat flour, 9.0% moisture; hard wheat flour, 11.1% moisture). All six flour samples (two flours at three moisture levels) were analyzed, however, and some data from fractionations of flours at high and low moisture levels are included.

Concentrations of both total and soluble protein generally declined with increasing density (Tables I and II, Fig. 1 and 2). Fluctuations in total protein appeared to be due primarily to

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[2]Research chemist, North Central Region, Federal Research, Science and Education Administration, USDA.

fluctuations in soluble protein. Soluble protein (based on Kjeldahl nitrogen in aqueous extracts), however, includes free amino acids. Table III, included for comparison, gives protein distributions among fractions from conventional aqueous fractionations of the two flours. The first study showed that the segregation of protein from starch was greater for soft wheat than for hard wheat flour, eg, fractions I–III from soft wheat flour contained 51% of the total flour protein, but corresponding fractions from hard wheat flour contained less than 22% of the flour protein. Similarly, the yields of soluble proteins from these same fractions were 51% from soft wheat flour and 23% from hard wheat flour. This parallel in nitrogen distribution was general, and suggests a close association of soluble and insoluble ("storage") protein in the endosperm. Soft wheat flour fraction XII (Table I), essentially free starch, contained 0.95% protein, 41% of which was soluble. Barlow et al (1973) reported 90% of the protein associated with starch isolated by

TABLE I
Distribution of Solids and Proteins (db) Among Floating Fractions and Residues From Nonaqueous Density Fractionation of Soft Wheat Flour (9.0% Moisture)[a]

Fraction	% of Total Solids	Soluble Solids			Total Protein	Soluble Protein				Gluten			
		Concn. (%)	Wt./100 g Flour (g)	% of Total	Concn. (%)	Concn. (%)	Wt./100 g Flour (g)	% of Total	% of Fraction Protein	Yield (%)	Protein (%)	% of Fraction Protein	Recovery[b] (%)
I	7.1	22.9	1.61	24.6	73.6	18.6	1.31	41.2	25.3	52.7	91.6	65.6	87.8
II	1.1	17.3	0.19	2.9	52.5	10.5	0.11	3.5	20.1	38.3	86.4	63.1	79.0
III	1.2	21.3	0.26	4.0	54.8	15.0	0.18	5.7	27.3	39.1	86.9	62.0	85.3
IV	1.3	15.0	0.20	3.1	35.8	5.3	0.07	2.2	14.8	20.0	81.8	45.7	53.6
V	1.6	13.8	0.23	3.5	36.2	6.2	0.10	3.2	17.2	23.4	72.1	46.5	56.2
VI	1.7	13.3	0.23	3.5	27.9	6.0	0.10	3.1	21.6	22.1	55.4	43.8	55.9
VII	1.7	11.9	0.20	3.0	24.1	5.6	0.09	2.8	23.5	24.4	44.2	44.7	58.4
VIII	6.3	11.9	0.75	11.5	23.9	6.8	0.43	13.5	28.6	19.3	43.4	35.1	49.2
IX	3.2	9.0	0.29	4.4	14.8	4.8	0.15	4.7	32.4	6.2	32.8	13.8	20.4
X	6.5	6.8	0.44	6.7	9.4	2.6	0.17	5.4	28.0	1.6	22.2	3.7	5.1
XI	40.9	3.5	1.41	21.6	3.2	0.9	0.35	11.0	26.8	—[c]			
XII	25.9	2.4	0.64	9.8	0.9	0.4	0.10	3.1	41.1	—[c]			
XIII	1.5	6.2	0.09	1.4	6.8	1.6	0.02	0.6	24.0	—[c]			
Total	100.0		6.54	100.0			3.18	100.0					
Flour		6.21			12.70	2.60							
Recovery		105.31				122.31							

[a]Flotations at 0°C in Freon TF-hexane, sp gr 1.350–1.460 at 25°C in increments of 0.010.

[b]Gluten recovery = $\frac{\%\text{ of fraction protein contributed by isolated gluten}}{100\% - (\%\text{ of fraction protein contributed by soluble protein})} \times 100$

[c]Gluten could not be isolated.

TABLE II
Distribution of Solids and Proteins (db) Among Floating Fractions and Residues From Nonaqueous Density Fractionation of Hard Wheat Flour (11.1% Moisture)[a]

Fraction	% of Total Solids	Soluble Solids			Total Protein	Soluble Protein				Gluten			
		Concn. (%)	Wt./100 g Flour (g)	% of Total	Concn. (%)	Concn. (%)	Wt./100 g Flour (g)	% of Total	% of Fraction Protein	Yield (%)	Protein (%)	% of Fraction Protein	Recovery[b] (%)
I	1.5	15.8	0.24	4.0	55.8	10.9	0.16	6.2	19.5	70.4	60.8	76.8	95.4
II	2.3	19.8	0.45	7.4	64.6	14.9	0.34	13.2	23.0	70.2	68.6	74.5	96.8
III	1.1	13.1	0.14	2.3	41.0	8.7	0.09	3.5	21.1	45.9	67.9	76.1	96.5
IV	1.6	10.8	0.17	2.8	36.3	6.6	0.10	3.9	18.3	34.2	77.2	72.8	89.1
V	3.0	11.3	0.34	5.6	35.8	6.6	0.20	7.8	18.4	31.7	80.3	71.0	87.0
VI	3.5	8.0	0.28	4.6	24.5	3.9	0.14	5.4	15.7	20.0	79.7	65.0	77.1
VII	7.3	7.3	0.53	8.7	19.9	3.4	0.25	9.7	17.1	13.5	84.0	57.3	69.1
VIII	23.3	6.4	1.49	24.5	14.0	3.0	0.69	26.7	21.1	6.7	80.9	38.6	48.9
IX	15.9	5.4	0.86	14.1	9.3	1.9	0.29	11.2	19.8	3.6	74.6	28.5	35.5
X	29.0	4.4	1.29	21.2	4.2	1.0	0.28	10.9	23.6	—[c]			
XI	11.0	2.6	0.29	4.8	1.2	0.4	0.04	1.5	34.8	—[c]			
XII	0.5	—[d]			14.6	—[d]				—[c]			
Total	100.0		6.08	100.0			2.58	100.0					
Flour		5.96			13.30	2.11							
Recovery (%)		102.01				122.27							

[a]Flotations at 0°C in Freon TF-hexane, sp gr 1.350–1.450 at 25°C in increments of 0.010.

[b]Gluten recovery = $\frac{\%\text{ of fraction protein contributed by isolated gluten}}{100\% - (\%\text{ of fraction protein contributed by soluble protein})} \times 100$

[c]Gluten could not be isolated.
[d]Fraction insufficient for analysis.

solvent sedimentation was water-soluble, but presumably this starch was essentially free of adherent storage protein.

Solubilities of flour proteins in aqueous media are affected by nonprotein factors, including electrolytes and lipids (Simmonds and Wrigley 1972). In this study, free lipids were extracted before fractionation. Electrolytes and other components, however, were redistributed during fractionation, providing a new chemical environment with respect to the proteins. Therefore, the proteins may have behaved differently in aqueous suspensions of the fractions than they would have in suspensions of the whole flour. Consequently, soluble protein may be partially a function of nonprotein components of a fraction.

Soluble solids closely paralleled soluble protein; this indicates a relatively uniform distribution of soluble nonnitrogenous material among fractions regardless of density. For example, fraction I from the soft wheat flour (7% of the flour) contained 18.60% soluble protein, 22.96% soluble solids, and 4.36% nonnitrogenous soluble solids (by subtraction); fraction XII (26% of the flour) contained 0.39% soluble protein, 2.48% soluble solids, and 2.09% nonnitrogenous soluble solids. Nonnitrogenous soluble solids in fractions from the hard wheat flour ranged from 3.45 to 4.93% in fractions I–X, falling to 2.19% in fraction XI.

Concentrations of total protein, soluble protein, soluble solids, and ash (Clements 1979) were all higher in the residue (fraction XIII) from the soft wheat flour than in the floating high-density fractions (Table I, Fig. 1). The residue from fractionation of the hard wheat flour at intermediate moisture (Table II, Fig. 2) constituted less than 0.5% of the flour and was insufficient for complete analysis, but residues from both flours fractionated at high and low moisture levels were analyzed and showed elevated levels of these components. Microscopic examination of residues indicated the presence of aleurone material (Stevens 1973), and final floating fractions probably also contained such material.

Disc electrophoresis of soluble proteins showed qualitative differences among fractions from both flours, evident as the disappearance of specific rapidly moving bands, and the

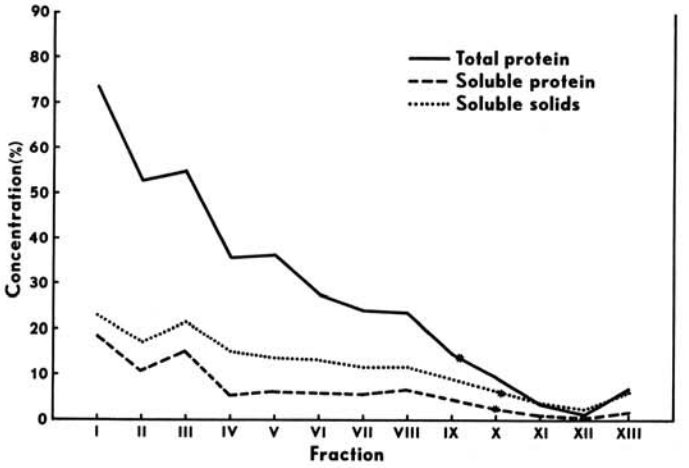

Fig. 1. Total protein, soluble protein, and soluble solids (db) in density fractions from nonaqueous fractionation of soft wheat flour (9.0% moisture). Flotations at 0°C in Freon TF-hexane, sp gr 1.350–1.460 at 25°C in 0.010 increments. Value for whole flour indicated by asterisk.

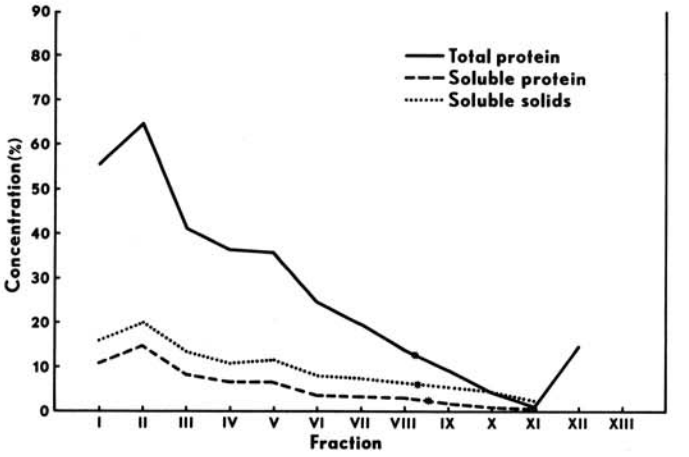

Fig. 2. Total protein, soluble protein, and soluble solids (db) in density fractions from nonaqueous fractionation of hard wheat flour (11.1% moisture). Flotations at 0°C in Freon TF-hexane, sp gr 1.350–1.450 at 25°C in 0.010 increments. Value for whole flour indicated by asterisk.

TABLE III
Distribution of Solids, Ash, and Protein (db) Among Fractions From Aqueous Fractionation of Soft and Hard Wheat Flours[a]

Fraction	% of Flour	Ash			Protein		
		%	g/100 g Flour	% of Total	%	g/100 g Flour	% of Total
Soft wheat flour							
Starch	78.3	0.158	0.124	39.62	0.40	0.31	2.57
Tailings	6.7	0.518	0.035	11.18	13.36	0.90	7.48
Gluten	12.3	0.278	0.034	10.86	82.64	10.16	84.39
Water-soluble	2.7	4.429	0.120	38.34	24.69	0.67	5.56
	Total		0.313			12.04	
Flour			0.449			12.70	
Hard wheat flour							
Starch	73.2	0.181	0.132	39.76	0.52	0.38	3.09
Tailings	12.4	0.465	0.058	17.47	13.49	1.67	13.60
Gluten	11.6	0.318	0.037	11.14	83.24	9.66	78.66
Water-soluble	2.8	3.755	0.105	31.63	20.24	0.57	4.64
	Total		0.332			12.28	
Flour			0.498			13.30	

[a]Hexane-extracted and pinmilled at 18,000 rpm.

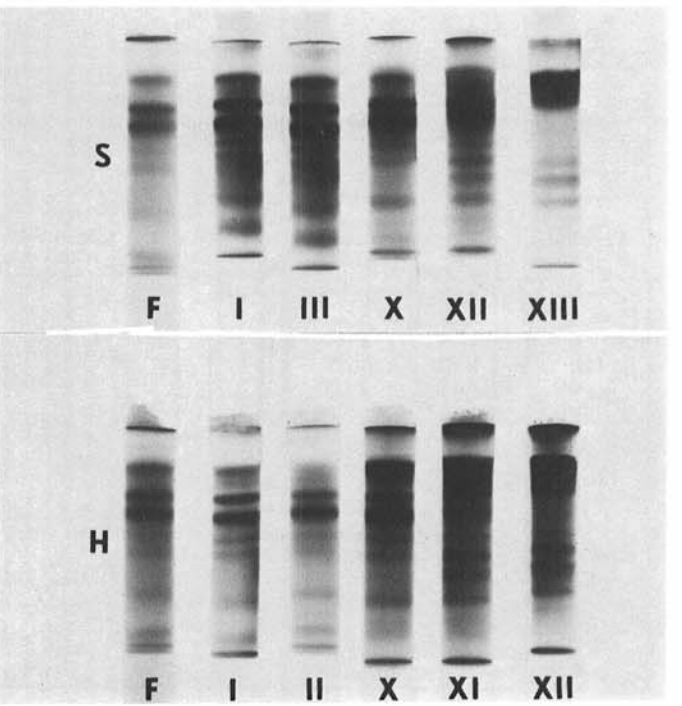

Fig. 3. Disc electrophoretic patterns of extracts of soft wheat flour (S) and hard wheat flour (H) and of selected fractions from nonaqueous fractionations of the flours in Freon TF-hexane mixtures. F = flour; numerals = fractions. Extraction medium: 0.1*M* potassium phosphate, pH 7.0, containing 25% sucrose and 0.001*M* EDTA.

appearance of heavy slowly moving bands with increasing fraction density (Fig. 3). Some bands, however, were common to all fractions. The proteins were buffer soluble, and presumably albumins and globulins.

Glutens were isolated from fractions for determination of the presence and nature of gluten-like material. Under the conditions used, such material could be isolated from all fractions containing more than 9% protein (Tables I and II). Gluten recoveries based on calculated gluten contents (assuming insoluble protein to be gluten) ranged from 5% (fraction X) to 88% (fraction I) for soft wheat flour, and from 36% (fraction IX) to 97% (fraction II) for hard wheat flour. Recoveries from fractions containing less than 20% protein were low, probably because glutens were isolated on a small scale (2-g samples). Normal gluten loss during washing could constitute a substantial proportion of total nitrogen in the low-nitrogen fractions.

Protein in glutens from soft wheat flour fractions ranged from 22 to 92%, compared with 61 to 84% for glutens from the hard wheat flour (Tables I and II). The high protein content (92%) of gluten from soft wheat flour fraction I is significant, since glutens isolated by conventional aqueous methods typically contain considerably less than 90% protein (Table III) (Kasarda et al 1971). Gluten protein from this fraction (7% of the flour) accounted for almost 50% of the flour protein.

Glutens from different fractions of the same flour differed in physical characteristics, ie, glutens from the low-density, high-nitrogen fractions were generally highly extensible and cohesive, whereas glutens from intermediate-nitrogen and low-nitrogen fractions were glutenin-like and relatively tough and brittle. Low values for gluten-nitrogen in low-nitrogen fractions, however, expecially from soft wheat flour, indicate substantial amounts of nonprotein that may have affected physical behavior.

The pH patterns for fractions from the two flours were similar, although pH levels of hard wheat flour fractions were slightly higher than those of the corresponding soft wheat flour fractions (Fig. 4). Generally, pH increased from about 5.5 in fraction I to 6.5 in the residue, but was relatively uniform among the low-density and intermediate-density fractions. The sharp increase in pH in the high-density fractions was not directly associated with starch flotation, eg, pH of soft wheat flour fraction XI (41% of the flour) was about 5.5, and pH of fraction XII (26% of the flour) was 6.2. Protein concentration was low in both of these fractions—3.2 and 0.9%, respectively.

The pH of whole flour was about midway between the pH extremes of the fractions. Also, pH of the hard wheat flour was higher than that of the soft wheat flour by a difference about equal to the displacement between the pH curves for the fractions from the respective flours. Soft wheat flour fractions I–XI constituted 73% of the flour, and the relatively uniform pH (about 5.5) indicated a uniform buffering system among these fractions. The hard wheat flour exhibited a similar uniformity. The sharp rise in pH in the high-density fractions suggests a change in buffering, although dilution by starch could have contributed to the shift toward neutrality. A general relation between low pH and high nitrogen was evident (except in the residues), but pH of individual fractions was not related directly to either soluble or total protein levels in the fractions. The high nitrogen in the residues was associated with high ash, and probably was derived from aleurone. Basic proteins, reported in aleurone cells (Stevens et al 1963), could have affected pH of the heavy fractions.

Hydration was maximum in fraction II from each flour, and declined steadily with fractions of increasing density (Fig. 5). A slight increase in hydration in the last floating fraction (XII) from soft wheat flour may have been related to a decrease in particle size of this fraction (data to be presented in another report). Hydration ranged from 259% (fraction II) to 80% (fraction XII) for the soft wheat flour and 158% (fraction II) to 98% (fractions VIII–X) for the hard wheat flour. The spread in hydration capacity among fractions from the soft wheat flour reflects a high degree of segregation of hydrophilic components. Therefore, although hydration for the unfractionated hard wheat flour (101%) was greater than for the soft wheat flour (96%), the low-density fractions from the soft wheat flour had much higher hydration capacities than did corresponding fractions from the hard wheat flour. Hydration values for high-density fractions from the hard wheat flour (VIII–XI, 79% of the flour), however, were about 100%, whereas values for corresponding fractions from the soft wheat flour (X–XII, 74% of the flour) were lower—80–93%. Hydration capacity generally paralleled total protein, but at a given protein concentration, hydration was greater for soft wheat than for hard wheat fractions, eg, hydration capacities were 200 and 120%, respectively, for fraction IV (36% protein) from the soft and hard wheat flours.

Ash was relatively uniform among fractions from both flours over most of the density range, but rose slightly in the final floating fraction and sharply in the residue (Clements 1979). More than 80% of flour "ash" (ie, mineral) is water soluble (Clements 1977a), and electrical conductivity of flour extracts is a function of ash (Clements 1977b). Conductivity therefore was measured as an indication of variations in mineral extractability among density fractions (Fig. 6 and 7). Conductivity of extracts of low-density fractions from the soft wheat flour was about 300 μmho/cm but fell steadily through the intermediate-density and high-density fractions to about 200 μmho/cm for the last floating fractions, and increased to 400 μmho/cm in the residue. Fractions from the hard wheat flour showed a similar trend, but in the low-density fractions, conductivity was lower and more erratic. Conductivity generally reflected ash levels of fractions, but was lower in high-density fractions (other than residues) than in low-density fractions with comparable ash levels. Apparently, therefore, fractions differed in

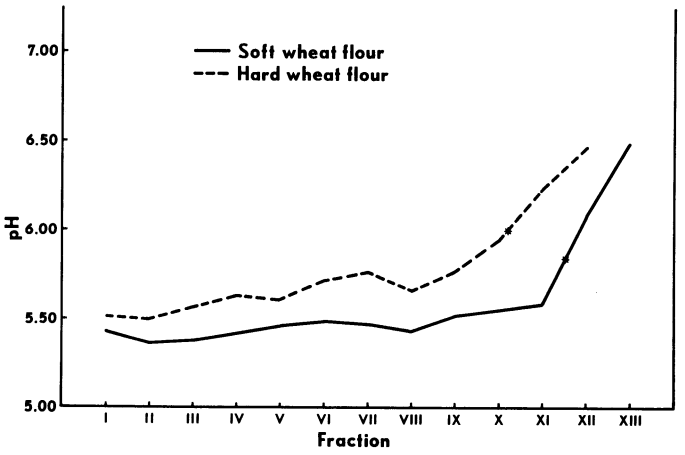

Fig. 4. pH of aqueous extracts of density fractions of soft wheat flour (9.0% moisture) and hard wheat flour (11.1% moisture). Value for whole flour designated by asterisk.

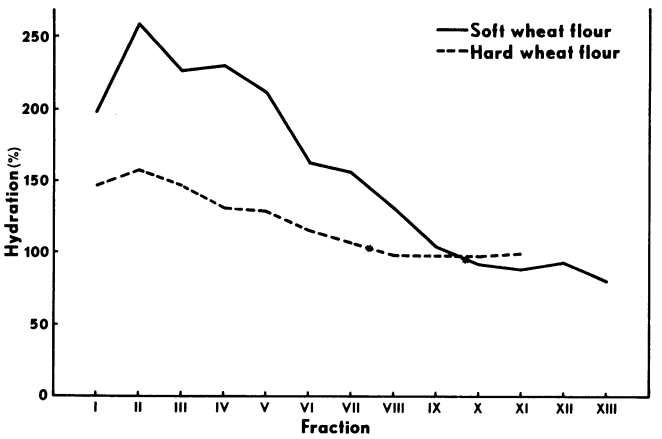

Fig. 5. Hydration capacities (db) of density fractions from nonaqueous fractions of soft wheat flour (9.0% moisture) and hard wheat flour (11.1% moisture). Capacity for whole flour designated by asterisk.

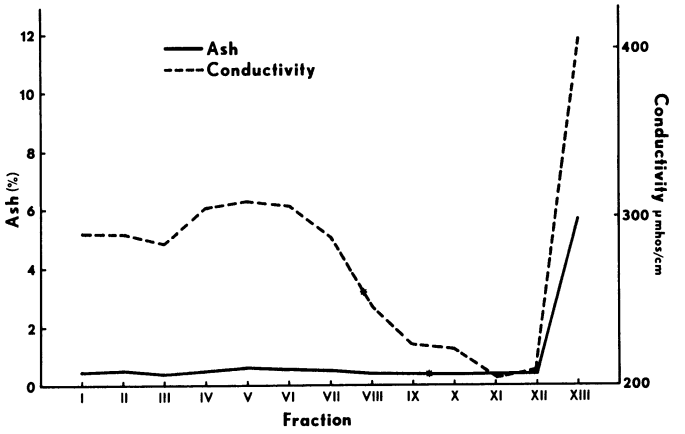

Fig. 6. Relation between ash (db) and electrical conductivity of aqueous extracts of density fractions from nonaqueous fractionation of soft wheat flour (9.0% moisture). Solids/water = 1:20. Values for whole flour designated by asterisk.

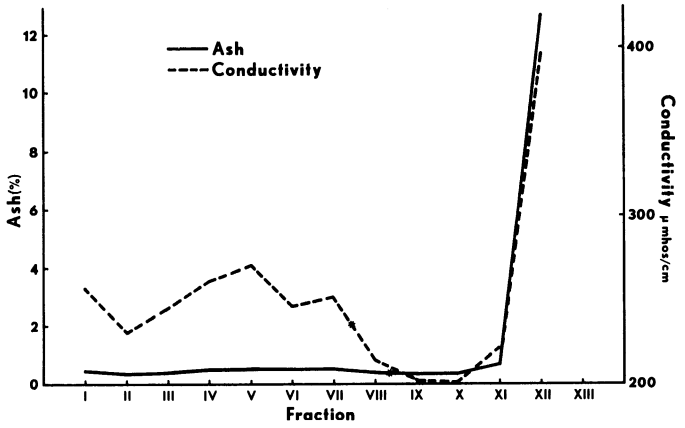

Fig. 7. Relation between ash (db) and electrical conductivity of aqueous extracts of density fractions from nonaqueous fractionation of hard wheat flour (11.1% moisture). Solids/water = 1:20. Values for whole flour designated by asterisk.

mineral species or extractability or both. Although not closely related to soluble solids content, conductivity was generally highest in fractions high in soluble solids, ie, low-density fractions (Tables I and II). Conductivity of residues from all flours was high, indicating substantial levels of soluble minerals, probably from aleurone matter.

The results suggest that the density fractions could be combined into three broad fractions: 1) a low-density to intermediate-density, high-nitrogen, hydrophilic fraction of relatively low pH, constituting about 25% of the flour, 2) a high-density, high-nitrogen, high-ash fraction of relatively high pH, constituting less than 1% of the flour, and 3) an intermediate-density to high-density fraction, essentially starch, constituting about 75% of the flour and with nitrogen, ash, and pH dependent on degree of inclusion of fractions 1 and 2. Degree of separation depends on the degree to which aggregated material is broken down before fractionation. Soft wheat flours are more amenable to fractionation than are hard wheat flours because of the refractory nature of aggregates in the latter (Simmonds and Orth 1973). Selection of appropriate conditions should permit further separation, particularly of the low-density and intermediate-density material.

Acknowledgments

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Literature Cited

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1962. Approved Methods of the AACC. The Association: St. Paul, MN.

BARLOW, K. K., BUTTROSE, M. S., SIMMONDS, D. H., and VESK, M. 1973. The nature of the starch-protein interface in wheat endosperm. Cereal Chem. 50: 443.

CLEMENTS, R. L. 1970. Fruit Proteins. In HULME, A. C. (ed.). The Biochemistry of Fruits and their Products. Vol. 1. Academic Press: London.

CLEMENTS, R. L. 1977a. The distribution of ash among flour extracts and fractions and its relation to electrical conductivity. Cereal Chem. 54: 840.

CLEMENTS, R. L. 1977b. Electrical conductivity of flour suspensions and extracts in relation to flour ash. Cereal Chem. 54: 847.

CLEMENTS, R. L. 1979. Density fractionation of wheat flours in nonaqueous solvents. I. Effects of flour moisture level on distribution of solids, protein, and ash among fractions. Cereal Chem.

KASARDA, D. D., NIMMO, C. C., and KOHLER, G. O. 1971. Proteins and the amino acid composition of wheat fractions. In POMERANZ, Y. (ed.). Wheat: Chemistry and Technology. American Association of Cereal Chemists: St. Paul, MN.

ORNSTEIN, L., and DAVIS, B. J. 1962. Disc Electrophoresis. I and II. Distillation Products Industries: Rochester, NY.

SIMMONDS, D. H., and ORTH, R. A. 1973. Structure and composition of cereal proteins as related to their potential industrial utilization. In POMERANZ, Y. (ed.). Industrial Uses of Cereals. American Association of Cereal Chemists: St. Paul, MN.

SIMMONDS, D. H., and WRIGLEY, C. W. 1972. The effect of lipid on the solubility and molecular-weight range of wheat gluten and storage proteins. Cereal Chem. 49: 317.

STEVENS, D. J. 1973. The aleurone layer of wheat. IV. Effects of extraction by aqueous media. J. Sci. Food Agric. 24: 307.

STEVENS, D. J., McDERMOTT, E. E., and PAGE, J. 1963. Isolation of endosperm protein and aleurone cell contents from wheat, and determination of their amino-acid composition. J. Sci. Food Agric. 14: 284.

YAMAZAKI, W. T., and DONELSON, J. R. 1976. Effect of interactions among flour lipids, other flour fractions, and water on cookie quality. Cereal Chem. 53: 998.

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