CHARACTERIZATION STUDIES ON THE SOLUBLE PROTEINS AND PENTOSANS OF CAKE FLOUR[1]

JOHN HOLME

ABSTRACT

Bleached cake flour (1.32% nitrogen; 14% moisture basis) has been extracted by various aqueous solvent systems to determine the percentage of the total flour protein solubilized under each condition. The effect of factors such as solvent:flour ratio, mixing time, pH, ionic strength, and alcohol concentration upon the amount of soluble protein has been determined. The albumin content of the flour represents about 10% of the total flour protein. The aqueous extracts contain soluble pentosans in addition to the protein. The constituents have been isolated by salt fractionation techniques and characterized by moving boundary electrophoresis and ultracentrifugation. The pentosan fraction (containing about 5% protein) appears to be homogeneous in electrophoresis and sedimentation. The albumin fraction contains at least five electrophoretic components which are also polydisperse in sedimentation characteristics.

One important goal of research on flour is to find an explanation of the functional properties of this material in terms of the composition of the mixture and the properties of the individual components. The fractionation of flour has received new impetus recently with the work of Sollars (16), Yamazaki (18), and Donelson and Wilson (4), who have shown that the baking properties of reconstituted flour can be markedly affected by the relative amounts of such fractions as starch, gluten, water-solubles, and starch tailings incorporated into the reconstituted mixture.

The fractionation of such a complex system as flour is not a simple matter, particularly where definitive fractions are desired. Fractions which are entirely suitable for baking studies are not always amenable to physicochemical characterization. Recent work points to the possible value of new buffer systems for electrophoresis of gluten protein (8) and column chromatography for improved fractionation of this complex protein mixture (17). Electrophoresis of gluten proteins remains a complicated matter, however, because of the profound effect of buffer composition on migration in systems of low ionic strength (2,3,6) and the fact that gluten can only be solubilized in solutions of low electrolyte concentration.

Pence and co-workers (10,11,13) have reported extensively on the amounts of albumins and globulins in various flours and certain physicochemical characterizations of these protein fractions. The pentosans of flour have been characterized chemically by Perlin (14, 15) and Howard (7) and examined in baking studies by Pence *et al.* (12).

[1]Manuscript received June 5, 1961. Contribution from the Procter & Gamble Company, Miami Valley Laboratories, Cincinnati 39, Ohio.

A recent review has summarized this work and that of Gilles and others on the chemical composition of flour pentosans (5).

The present study includes the use of boundary electrophoresis and ultracentrifugation to characterize in part the albumins and pentosans obtained from bleached cake flour by a number of fractionation procedures.

The results show that water extracts of bleached cake flour (2:1 water:flour) contain about 20% of the flour protein, of which one-half is gliadin and one-half albumins. There is also present in this extract a quantity of pentosans about equal to that of albumins. These fractions have been separated by salt-precipitation techniques and examined by moving-boundary electrophoresis. The albumin fraction has been found to contain at least five components. The effect of pH on electrophoretic migration of this mixture has been determined. The pentosan fraction (~95% pure by nitrogen analysis, assuming all nitrogen is protein-nitrogen) is a homogeneous component in terms of electrophoretic and ultracentrifugal sedimentation behavior.

Materials and Methods

A commercial bleached cake flour was used throughout this study. Analytical data include percent nitrogen = 1.3, 14% moisture basis, percent lipid (ethyl ether-extractable) = 0.7.

Electrophoresis was performed in a Perkin-Elmer Model 38A apparatus maintained at 1°C. Ultracentrifugation was performed in a Spinco Model E instrument equipped with a constant-temperature control unit. The initial temperatures varied between 20° and 25°C. During a given run the temperature was constant within ± 0.2°C. Reagent-grade chemicals were used throughout. Unless otherwise stated, all buffers are of 0.1 ionic strength.

Preliminary Extraction with Aqueous Systems. The amount of flour protein which could be extracted by water and other aqueous solvent systems was determined by mixing flour (10 g.) and solvent (20 ml. or more) in a 90-ml. centrifuge tube using a 1/2-in. glass propeller blade rotating at 1,500 r.p.m. All extractions were done at room temperature. The slurries were centrifuged for 10 minutes at 1,800 r.p.m. in an International centrifuge and the supernatants decanted into conical centrifuge tubes for further clarification in a laboratory clinical centrifuge. The amount of protein in these supernatants was determined by semimicro Kjeldahl percent nitrogen × 5.7. The percent of the total flour protein extracted under each condition was then calculated. Variables examined for their effect on extraction of protein included water:flour ratio, mixing time, pH, salt concentration, and aqueous

fraction (which will also contain some globulin, but this was not determined here) represents from 8 to 10% of the total flour protein. The actual yields of solid material from 0.1*M* sodium chloride extracts were about twice this value, and so it is assumed that the pentosans are about equal in weight to the albumin fraction. Increases in solubility of flour protein above the 10% value at the 2:1 solvent:flour ratio represent solubilization of gliadin by the particular solvent; e.g., water, acidic solutions, aqueous dioxane, and ethanol.

TABLE I

EXTRACTION OF FLOUR PROTEIN BY AQUEOUS SOLVENTS

SOLVENT	TOTAL FLOUR PROTEIN EXTRACTED
	%
A. Solvent: flour = 2:1; mixing time = 5 minutes	
Water	19
Sodium chloride, 0.1*M*	9
Formic acid, 0.01*M*	41
Acetate buffer, pH 4, μ = 0.01	28
Phosphate buffer, pH 7, μ = 0.01	10
Dioxane, 25%	38
50%	42
75%	24
100%	1
Ethanol, 50%	50
B. Effect of mixing time — solvent: flour = 2:1	
Water, 15 seconds	20
5 minutes	19
30 minutes	21
Sodium chloride, 0.1*M*, 1 minute	9
5 minutes	9
C. Effect of solvent: flour ratio — mixing time = 5 minutes	
Water, 2:1	19
4:1	42
8:1	55
Formic acid, 0.01*M*, 2:1	41
10:1	66

The data show little effect of mixing time on the amount of protein extracted. The increased solubility of protein at the higher solvent: flour ratios represents solubilization of gliadin as revealed by the fact that addition of sodium chloride (0.1–0.3*M*) effected precipitation of

that protein in excess of the 10% level.

Fractionation of the Water-Soluble Components. The ammonium sulfate fractionation of the water-soluble mixture yielded gliadin, albumin, and pentosan fractions. No work was done with the gliadin in this study. Figure 2 shows typical electrophoretic patterns of the albumin and pentosan fractions at pH 3.8. Since the ascending and descending patterns were essentially enantiographic, only the descending pattern is shown here in accord with the preferred procedure (9). Some variation was found in the yield of the slower-moving albumins (mobilities up to 3 X 10^{-5} cm.^{2}/volt/second). Two examples are shown here; most preparations resembled B rather than A. The effect

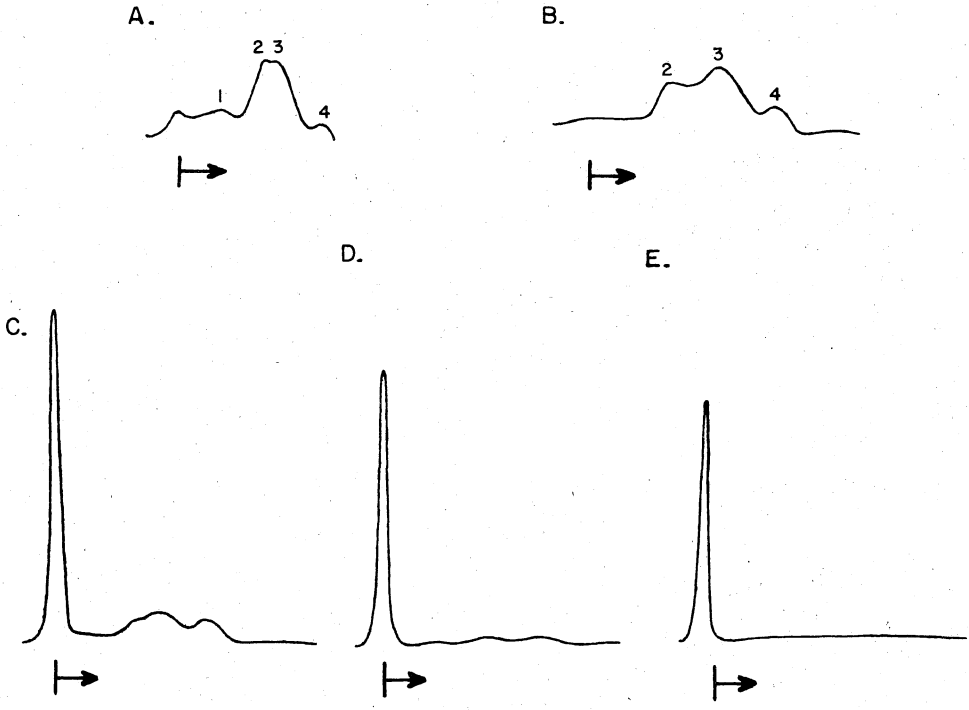

Fig. 2. Descending electrophoretic patterns, at pH 3.8, 0.1 ionic strength, of the fractions from the water-soluble extract of flour. A, albumin fraction, preparation A, not reprecipitated; 6.08 volts/cm., 5,400 seconds. B, albumin fraction, preparation B, not reprecipitated; 6.17 volts/cm., 7,200 seconds. C, pentosan fraction, 1X precipitated; 6.00 volts/cm., 7,200 seconds. D, pentosan, 3X reprecipitated; 6.25 volts/cm., 7,200 seconds. E, pentosan fraction obtained by 2X refractionation of the original 3*M* precipitate at 0.8*M* and 1.74*M* $(NH_4)_2SO_4$; 7.18 volts/cm., 7,200 seconds.

of reprecipitation of the pentosan in removing the protein components is readily seen in C, D, and E. E represents the purest preparation obtained by ammonium sulfate fractionation techniques.

Figure 3 shows the electrophoretic patterns of the albumin fractions in various buffers and Table II gives the mobilities of the peaks. The pentosans were immobile at all pH's in the acid range, although slight negative mobilities were measured at pH 7 and 8.5.

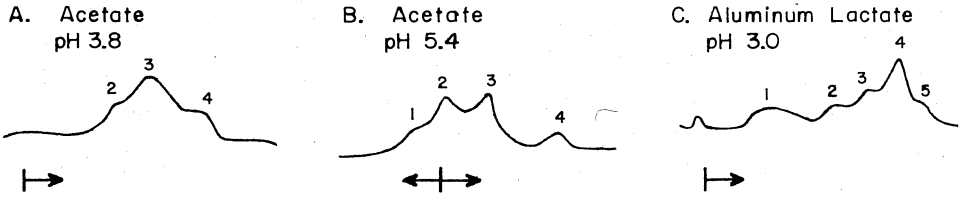

Fig. 3. Electrophoretic patterns of albumin fraction in various buffers. A, acetate buffer, pH 3.8, 0.1 ionic strength; 6.66 volts/cm., 7,200 seconds. B, acetate buffer, pH 5.4, 0.1 ionic strength; 6.24 volts/cm., 6,480 seconds. C, aluminum lactate buffer, pH 3.0, 0.6 ionic strength; 8.49 volts/cm., 5,400 seconds.

TABLE II

ELECTROPHORETIC MOBILITIES OF ALBUMIN COMPONENTS

ALBUMIN PREPARATION	BUFFER[a]	pH	COMPONENT	MOBILITIES
				($cm.^2/volt/sec. \times 10^5$)
Figure 2A Preparation C	Acetate	3.8	1	0
			2	2.2
			3	4.4
			4	7.0
Figure 2B Preparation WG	Acetate	3.8	2	3.7
			3	5.2
			4	6.9
Figure 3A Preparation D	Acetate	3.8	2	3.2
			3	4.5
			4	6.2
Figure 3B Preparation D	Acetate	5.4	1	−1.2
			2	0.3
			3	1.9
			4	4.9
Figure 3C Preparation E	Aluminum lactate	3.0	1	2.7
			2	4.7
			3	5.9
			4	7.1
			5	8.0

[a]All buffers of 0.1 ionic strength.

Sedimentation runs in a synthetic boundary cell indicated quite marked heterogeneity in the albumin fraction. The $S_{20,w}$ value calculated for the broad albumin peak was 2.10 Svedberg units, while that for the very sharp homogeneous peak of pentosans was 1.23. Figure 4 shows the appearance of these peaks in the synthetic boundary cell.

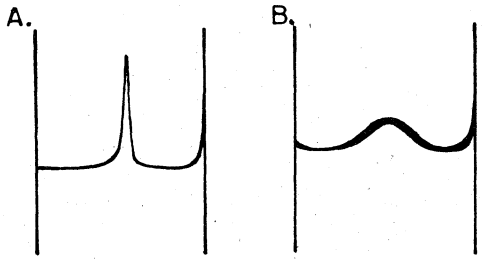

Fig. 4. Synthetic boundary patterns. A, pentosan fraction (3X reprecipitated), 48 minutes at 59,780 r.p.m. after 176 minutes at 21,740 r.p.m. B, albumin fraction (3X reprecipitated), 48 minutes at 59,780 r.p.m. after 128 minutes at 21,740 r.p.m. Both in acetate buffer, pH 3.8, 0.1 ionic strength.

Electrophoresis and Ultracentrifugation of Various Flour Extracts. The extraction of flour by salt and buffer systems gave the yields of soluble protein shown in the table below (all buffers of 0.1 ionic strength).

Solvent	*Flour Protein Extracted*
	%
Sodium chloride, 0.1*M*	8.8
Acetate buffer, pH 3.8	15.3
Acetate buffer, pH 5.4	8.3
Phosphate buffer, pH 7.0	8.2
Veronal buffer, pH 8.5	8.4

The electrophoretic patterns of these buffer extracts are shown in Fig. 5. The sharp pentosan peak is evident in all extracts. Actually, slight migration of this peak is noted at the neutral and basic pH. At least five individual migrating components are indicated in the pH 3.8 and 8.5 buffers. The protein fractions separate readily from the

pentosans during electrophoresis at pH 3.8 and 8.5. Much of the material extracted at pH 5.4 and 7.0 is near isoelectric in the 5.4–7.0 pH range.

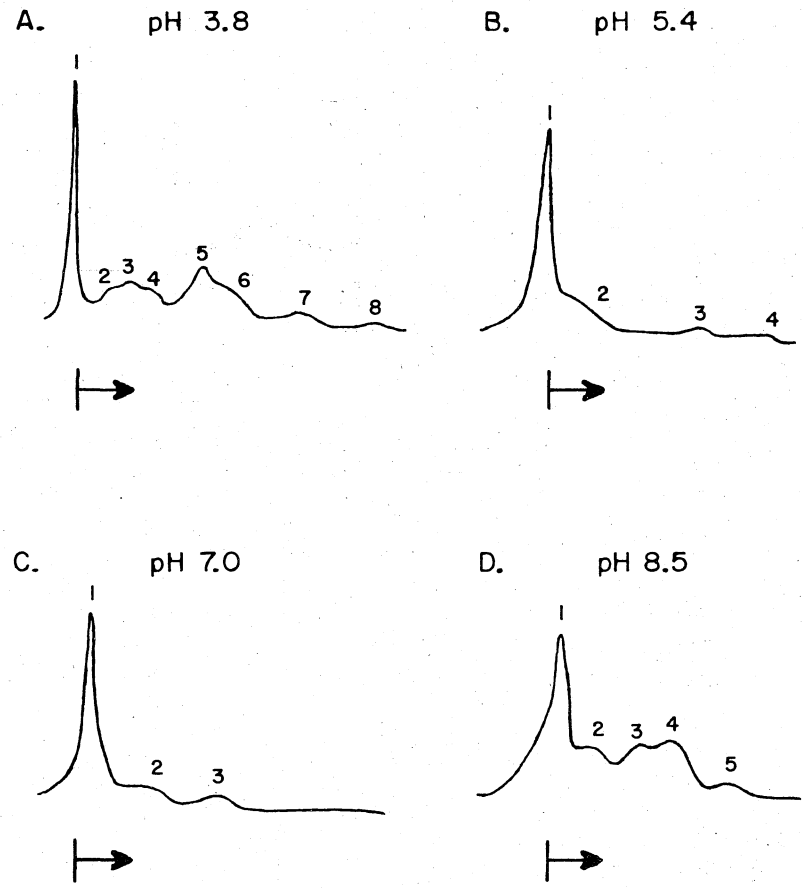

Fig. 5. Descending electrophoretic patterns of buffer extracts of flour. A, acetate buffer, pH 3.8; field strength 6.48 volts/cm., time 7,200 seconds. B, acetate buffer, pH 5.4; 6.11 volts/cm., 7,200 seconds. C, phosphate buffer, pH 7.0; 6.17 volts/cm., 6,300 seconds. D, veronal buffer, pH 8.5; 7.64 volts/cm., 6,120 seconds. All buffers 0.1 ionic strength.

Figure 6 gives the electrophoretic patterns of the 0.1*M* sodium chloride extract following dialysis against the different buffers. These show that the material extracted by the salt solution is essentially

identical to that extracted by the pH 5.4 and 7.0 buffers but contains fewer individual components than that extracted at pH 3.8 and smaller amounts of the faster-moving components extracted at pH 8.5. Table III lists the mobilities of the peaks (or shoulders) indicated in the figures in order of increasing mobility.

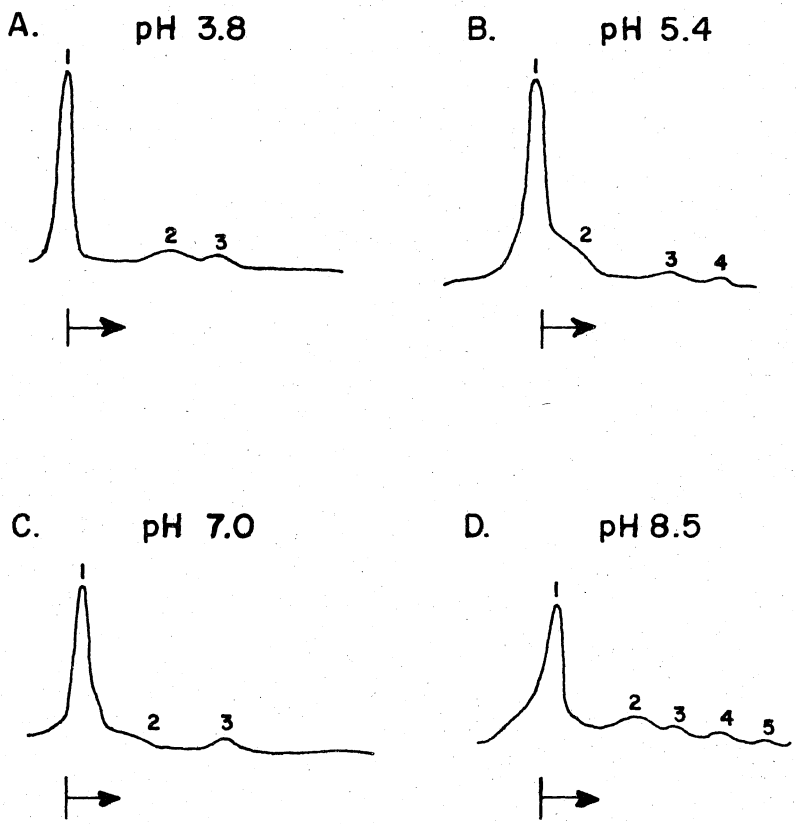

Fig. 6. Descending electrophoretic patterns of the 0.1*M* NaCl extract of flour dialyzed vs. buffers. A, acetate buffer, pH 3.8; field strength 5.27 volts/cm., time 7,200 seconds. B, acetate buffer, pH 5.4; 6.19 volts/cm., 6,480 seconds. C, phosphate buffer, pH 7.0; 6.24 volts/cm., 7,300 seconds. D, veronal buffer, pH 8.5; 7.68 volts/cm., 6,120 seconds. All buffers of 0.1 ionic strength.

TABLE III
ELECTROPHORETIC MOBILITIES OF SOLUBLE FLOUR COMPONENTS

EXTRACTION SOLVENT [a]	ELECTROPHORESIS SOLVENT [a]	MOBILITIES ($cm.^2/volt/sec. \times 10^5$)	
Sodium chloride, 0.1*M*	Acetate buffer, pH 3.8	Component 1	0.1
		2	3.4
		3	5.5
Acetate buffer, pH 3.8	Acetate buffer, pH 3.8	Component 1	0.2
		2	0.9
		3	1.5
		4	2.1
		5	3.6
		6	4.7
		7	6.3
		8	8.5
Sodium chloride, 0.1*M*	Acetate buffer, pH 5.4	Component 1	−0.1
		2	1.0
		3	4.3
		4	6.0
Acetate buffer, pH 5.4	Acetate buffer, pH 5.4	Component 1	−0.1
		2	0.8
		3	4.6
		4	6.6
Sodium chloride, 0.1*M*	Phosphate buffer, pH 7.0	Component 1	−0.5
		2	−1.7
		3	−4.6
Phosphate buffer, pH 7.0	Phosphate buffer, pH 7.0	Component 1	−0.5
		2	−2.1
		3	−4.7
Sodium chloride, 0.1*M*	Veronal buffer, pH 8.5	Component 1	−0.7
		2	−2.7
		3	−4.2
		4	−5.4
		5	−6.5
Veronal buffer, pH 8.5	Veronal buffer, pH 8.5	Component 1	−0.4
		2	−1.4
		3	−2.6
		4	−3.7
		5	−5.2

[a] All buffers of 0.1 ionic strength.

The sedimentation patterns of these solutions, shown in Fig. 7, reveal the similarity in behavior of the pH 5.4 and 7.0 extracts and the marked polydispersity in the pH 3.8 and 8.5 extracts. Table IV lists the sedimentation constants of the components (where measurable). The components having S values in the range of 2.2 and 3.6 compare to the albumin found by Pence and Elder (11) to have

an $S_{20,w}$ = 2.54. The 1.6–1.8 S component is mainly the pentosan fraction, and the patterns show much sharper evidence of homogeneity for this component than for the proteins which give the marked skewness on the leading edge of the sedimenting peak.[2]

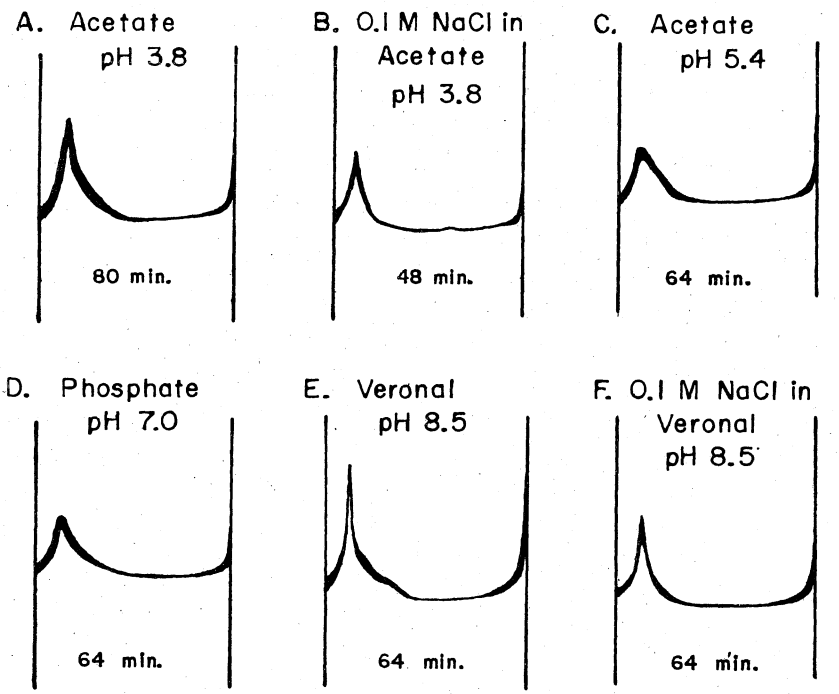

Fig. 7. Sedimentation patterns of 0.1*M* NaCl and buffer extracts of flour. All patterns developed at 59,780 r.p.m.

TABLE IV

SEDIMENTATION CONSTANTS OF SOLUBLE FLOUR COMPONENTS

EXTRACTION SOLVENT [a]	SEDIMENTATION SOLVENT [a]	S [b]
Sodium chloride, 0.1*M*	Acetate buffer, pH 3.8	1.8 (plus heavier components)
Acetate buffer, pH 3.8	Acetate buffer, pH 3.8	1.6 (plus heavier components)
Acetate buffer, pH 5.4	Acetate buffer, pH 5.4	1.6 (plus heavier components)
Phosphate buffer, pH 7.0	Phosphate buffer, pH 7.0	1.7 (plus heavier components)
Sodium chloride, 0.1*M*	Veronal buffer, pH 8.5	1.8 (plus heavier components)
Veronal buffer, pH 8.5	Veronal buffer, pH 8.5	1.6 2.2 3.6

[a] All solvents 0.1 ionic strength.
[b] Not corrected to standard conditions.

[2] The discrepancy between values of 1.7 and 1.2 for the sedimentation constants for the pentosan fraction may arise from a very marked concentration dependence of sedimentation for this material, which has been noted in a purified fraction.

These results, in general, indicate the applicability of moving-boundary electrophoresis to a study of the composition of flour solubles. The numerous individual protein components observed in the albumin fraction confirm the results of Pence and Elder (11). The pentosan is difficult to rid of protein completely, but in this work showed good homogeneity in sedimentation and electrophoretic examination.

Other means of fractionating pentosan from protein and of obtaining the individual albumin components are being studied in this laboratory.

Acknowledgments

The technical assistance of J. P. Smith with the fractionation experiments and W. L. Gagen with the electrophoretic and ultracentrifugal characterization is gratefully acknowledged.

Literature Cited

1. BAKER, J. C., PARKER, H. K., and MIZE, M. D. The pentosans of wheat flour. Cereal Chem. **20:** 267-280 (1943).
2. CANN, J. R. Effect of binding of ions and other small molecules on protein structure. V. On the interpretation of the electrophoretic patterns of proteins in acidic media. J. Phys. Chem. **63:** 210-217 (1959).
3. CHARLWOOD, P. A. Electrophoretic behavior of human serum albumin at pH 4. Biochem. J. **69:** 627-632 (1958).
4. DONELSON, D. H., and WILSON, J. T. Effect of the relative quantity of flour fractions on cake quality. Cereal Chem. **37:** 241-262 (1960).
5. GILLES, K. A. The present status of the role of pentosans in wheat flour quality. Bakers Digest: 47-52 (October, 1960).
6. HOLME, J., and BRIGGS, D. R. Studies on the physical nature of gliadin. Cereal Chem. **36:** 321-340 (1959).
7. HOWARD, B. H. Hydrolysis of the soluble pentosans of wheat flour and *Rhodymenia palmata* by ruminal micro-organisms. Biochem. J. **67:** 643-651 (1957).
8. JONES, R. W., TAYLOR, N. W., and SENTI, F. R. Electrophoresis and fractionation of wheat gluten. Arch. Biochem. Biophys. **84:** 363-376 (1959).
9. LONGSWORTH, L. G. Quantitative interpretation of the electrophoretic patterns of proteins. J. Phys. & Colloid. Chem. **51:** 171-183 (1947).
10. PENCE, J. W. Approximate isoelectric pH's of albumins from wheat flour. Cereal Chem. **30:** 328-333 (1953).
11. PENCE, J. W., and ELDER, A. H. The albumin and globulin proteins of wheat. Cereal Chem. **30:** 275-287 (1953).
12. PENCE, J. W., ELDER, A. H., and MECHAM, D. K. Preparation of wheat flour pentosans for use in reconstituted doughs. Cereal Chem. **27:** 60-66 (1950).
13. PENCE, J. W., WEINSTEIN, N. E., and MECHAM, D. K. Differences in the distribution of components in albumin preparations from durum and common wheat flours. Cereal Chem. **31:** 396-406 (1954).
14. PERLIN, A. S. Isolation and composition of the soluble pentosans of wheat flours. Cereal Chem. **28:** 370-381 (1951).
15. PERLIN, A. S. Structure of the soluble pentosans of wheat flours. Cereal Chem. **28:** 382-393 (1951).
16. SOLLARS, W. F. A new method of fractionating wheat flour. Cereal Chem. **33:** 111-120 (1956).
17. WOYCHIK, J. H., DIMLER, R. J., and SENTI, F. R. Chromatographic fractionation of wheat gluten on carboxymethyl cellulose columns. Arch. Biochem. Biophys. **91:** 235-239 (1960).
18. YAMAZAKI, W. T. The concentration of a factor in soft wheat flours affecting cookie quality. Cereal Chem. **32:** 26-37 (1955).