Characterization of Wheat Flour Proteins by Differential Solubility in Conjunction with Disc Electrophoresis[1]

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ABSTRACT

By the Maes extraction procedure (used for removal of proteins from flours and whole meals from different types and species of wheat) up to 97% of proteins present were extractable, and were further characterized by disc electrophoresis. Certain solvents extracted proteins with closely similar electrophoretic mobility. The proteins extracted by 0.10N KOH (about 25% of the total) could not be resolved into different electrophoretic components. Wheats and flours studied included: A, species of different and B, the same chromosome number; C, different varieties of wheat of the same species; and D, HRS flours denatured by various physical and chemical means. No consistent relation appeared between total protein extracted from flours or amount extracted by any one solvent and the baking and rheological behavior of flours of groups A, B, and C, except as protein content itself varied. Proteins of group A flours showed great differences in electrophoretic mobility between wheats of different chromosome number. Group B flours were more similar electrophoretically; group C flours showed relatively little difference between varieties. Group D flours (with baking and rheological properties drastically altered from the control) showed differences in solubilities of proteins in various solvents, but were electrophoretically identical. It was concluded that this procedure was of more value for identifying genetic types than for differentiating between flours as to baking and rheological properties.

Since the classic work of Osborne (1), which demonstrated the existence of five different proteins in wheat flour, a great deal of effort has been directed toward relating the proteins of a flour to its rheological and baking properties. The many methods which have been applied include further fractionation of the proteins, separation and reconstitution of flour components, sulfhydryl studies, ion-exchange chromatography, gel filtration, moving-boundary electrophoresis, starch-gel electrophoresis, immunochemistry, and disc electrophoresis. One of a recent series of publications by Maes (2) described the application to investigations of baking quality of wheat flour of a system for sequential extraction of proteins. A series of solvents was used, selected on a basis of their solubilizing properties on the classic protein groups, albumins, globulins, gliadins, and glutelins. More recently, Mattern and co-workers described an adaptation of this process which they used in conjunction with starch-gel electrophoresis to characterize the proteins extractable from hard red winter (HRW) wheat (3).

In the course of work being carried out in our laboratory on air-classified flour, we wished to investigate the possibility of detecting deterioration of baking properties of flour by means of such a procedure. This communication describes an adaptation of the Maes procedure which, when used in conjunction with the sensitive technique of disc electrophoresis, can be used to characterize the proteins of a small sample of flour or finely ground grain. The experiments described may

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also be interpreted as an appraisal of the value of disc electrophoresis in this field.

EXPERIMENTAL: MATERIALS AND METHODS

Extraction and Disc Electrophoresis of Proteins

The Maes extraction procedure involves continuous extraction of the proteins of a sample of flour suspended in a sand-pumice mixture, with the use of a series of solvents in sequence. For these experiments, columns were fashioned from PVC piping of 1/2-in. internal diameter, cut to sufficient length to contain the extraction mixture, which consisted of 44 g. acid-washed fine sand, 2 g. flour, and 2 g. pumice. These materials were ground and intimately mixed in a mortar before addition to the column. A threaded collar cemented at each end of the column carried a pierced, threaded plug of diameter 3/8 in. The plugs were removable at each end for ease of cleaning, and allowed pressures in excess of 20 p.s.i to be built up in the column if necessary. Effluents were monitored by a nesslerization procedure (4).

Graham (5) drew attention to the fact that after four successive extractions with a solvent, little or no further nitrogen can be removed by extraction with that solvent, although more nitrogen may be removed by the subsequent use of different solvents. By means of the Maes-type continuous process, it was established by analysis of successive 5-ml. aliquots of effluent that no further nitrogen could be extracted from the 2-g. sample of flour by any of the solvents used after 50 ml. of the solvent had passed through the column, although further extraction of nitrogen was possible with subsequent solvents. The procedure adopted involved extraction with 75 ml. of each solvent, followed by careful mixing and determination of total nitrogen in the extract. The volume of each solvent was measured by means of a Beckman Model 746 solution-metering pump, and flow rates varied considerably, depending on the nature of the flour and the solvent used. As a result, the time which elapsed during the passage of 75 ml. through the column ranged from 35 min. to over 2 hr. KOH extractions were invariably the slowest. Earlier attempts to monitor the effluent continuously by spectrophotometry at 280 nm. were abandoned, because KOH extracts were invariably cloudy.

All protein extracts were freeze-dried before disc electrophoresis to facilitate the application of accurate quantities of protein to the disc-electrophoresis gels. A 200-γ sample of protein was applied to the gel in each case. Salt, lactic acid, and KOH extracts were dialyzed 24 hr. against two changes of distilled water before they were freeze-dried. Disc electrophoresis was done at pH 8.9 and 4.5 by modifications of the procedures of Ornstein and Davis (6) and Reisfeld et al. (7), respectively. Disc electrophoresis at pH 4.5 gave sharper resolution of protein components than electrophoresis at pH 8.9. A Canalco electrolytic destainer was used to destain the gels in each case. After the gels were destained, they were photographed in color and, where necessary, the slides were monitored on a Joyce Loebl Chromoscan densitometer adapted to scan 35-mm. slides.

RESULTS

Extractability of Nitrogen

Three experiments were carried out, the object of the first being to establish the

amount of nitrogen extracted by various solvents used singly, and the qualitative nature of the proteins extracted. The second experiment concerned various sequences of solvents, the relative amounts of nitrogen extracted by each solvent in a sequence, and the nature of the proteins extracted. The third experiment was designed to establish whether qualitative and quantitative differences existed between the proteins extracted from flours of different types, as measured by the Maes extraction-disc electrophoresis (MEDE) procedure. A hard red spring (HRS) wheat flour of 13.6% protein was used in all three experiments.

Some of the results are included in Tables I to IV. The amount of protein extracted by the various solvents used singly ranged from 9.9 to 76.5% of the total protein (N X 5.7) present in the original flour, with 0.1N KOH the most effective. Several of the solvents extracted proteins identifiable with the gliadin-type proteins normally extracted by 40% isopropanol or 60% ethanol (Fig. 1). Also, several solvents extracted different proteins when used alone from those extracted when they were employed in various sequences, following other solvents. For example, 2% NaCl and 3.85% lactic acid extracts contained a number of components when used as single solvents, but when used in sequence following water or isopropanol, or both, fewer components were present and those present were more characteristic of the flours.

TABLE I. TOTAL NITROGEN EXTRACTED FROM A COMMERCIAL HARD RED SPRING FLOUR BY VARIOUS SOLVENTS USED SINGLY ON A MAES-TYPE COLUMN

No.	Solvent	N Extracted mg.	Percent of Total N Present %
1	Distd. water	7.9	16.7
2	0.0005M NaCl	4.7	9.9
3	0.001M NaCl	4.8	10.2
4	0.005M NaCl	5.5	11.7
5	0.01M NaCl	5.2	11.1
6	0.05M NaCl	6.0	12.8
7	0.1M NaCl	8.1	17.2
8	2% NaCl	7.9	16.8
9	0.5M NaCl	6.9	14.6
10	1.0M NaCl	5.4	11.4
11	2.0M NaCl	4.4	9.4
12	$Na_2P_2O_7$ buffer	17.7	37.5
13	0.01M Acetic acid	14.9	31.6
14	0.05M Acetic acid	32.3	68.4
15	0.01M Formic acid	17.1	36.2
16	0.05M Formic acid	16.9	35.8
17	Al lactate buffer	31.7	67.2
18	3.85% lactic acid	21.9	46.4
19	0.1N KOH	36.1	76.5
20	40% 2-propanol	29.4	62.3
21	70% ethanol	26.4	55.9
22	0.25N Sodium salicylate	30.4	64.2
23	0.50N Sodium salicylate	32.3	68.4

TABLE II. EFFECT OF VARYING SOLVENT SEQUENCE ON TOTAL NITROGEN EXTRACTED FROM HARD RED SPRING FLOUR WITH THE USE OF MAES-TYPE COLUMNS

No.[a]	Solvent Sequence					Nitrogen Extracted					Total N Extracted	Total Nitrogen Extracted (Percent of Total N Present)
	A	B	C	D	E	A	B	C	D	E		
						mg.	mg.	mg.	mg.	mg.	mg.	%
1	18	20	8	19	...	25.2	2.3	0.1	12.7	...	40.3	85.3
2	8	20	18	19	...	7.9	19.3	3.4	11.7	...	42.3	89.6
3	19	20	8	18	...	39.9	1.4	0.4	0.3	...	42.0	89.0
4	20	8	18	19	...	27.4	1.5	2.2	15.1	...	46.2	97.9
5	20	8	18	19	...	26.5	2.0	2.4	15.1	...	46.0	97.5
6	8	18	20	19	...	7.7	15.5	0.4	9.8	...	33.4	70.8
7	18	8	19	20	...	24.8	0.7	11.7	2.0	...	39.2	83.7
8	1	20	8	18	19	6.2	17.2	2.3	3.9	13.8	43.4	92.0
9	3	20	8	18	19	4.9	13.9	1.7	3.0	10.5	34.0	72.0
10	5	20	8	18	19	5.2	14.1	1.5	0.8	12.2	33.8	71.6
11	6	20	8	18	19	6.0	18.5	1.3	2.9	14.0	42.7	90.5
12	7	20	8	18	19	8.1	17.2	1.3	3.7	14.2	44.5	94.3
13	9	20	8	18	19	6.9	20.4	1.0	2.1	9.7	40.1	85.0
14	11	20	8	18	19	5.4	19.7	0.3	1.8	8.9	36.1	76.5

[a]Solvents used are coded as in Table I; e.g., No. 7 is 0.1M NaCl, etc.

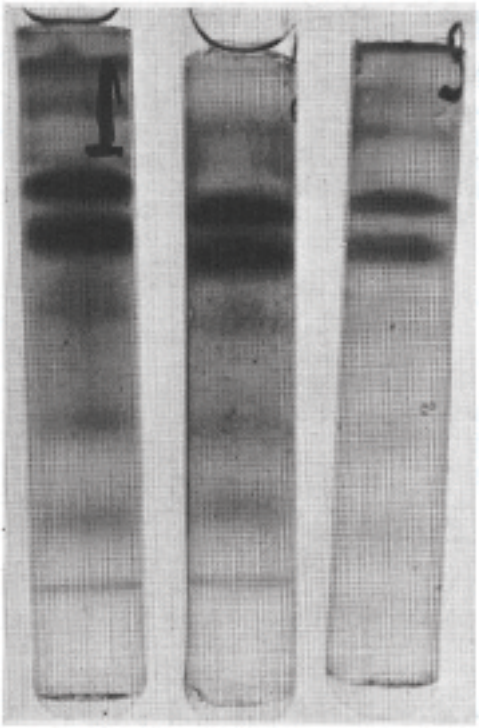

Fig. 1 (left). Electrophoresis of (left to right) water, 2% NaCl, and 40% 2-propanol extracts of the same HRS flour. Throughout the series of figures the direction of migration is from the top to the bottom.

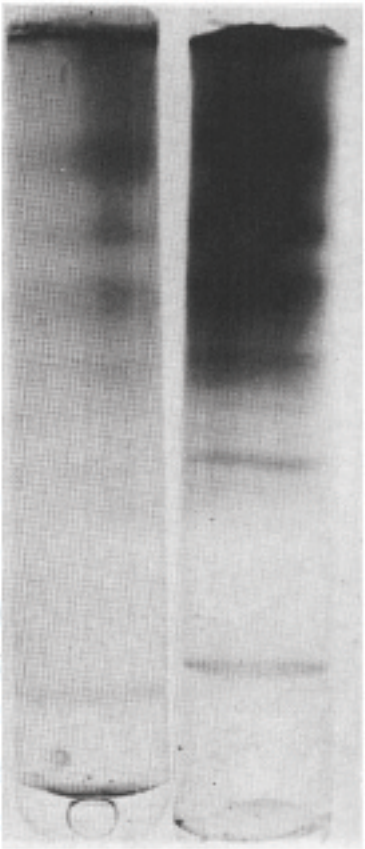

Fig. 2 (right). Electrophoresis of lactic acid-soluble proteins of HRS flour (left) without and (right) with preliminary extraction with water.

No further research was carried out to verify the assumptions of Maes regarding the identity of proteins extracted by the several solvents used. Since, however, no further protein could be extracted by 40% isopropanol or 2% NaCl after exhaustive extraction with these solvents, it is quite likely that the lactic acid-extractable proteins consisted of lower-molecular-weight (MW) fractions of the glutenin complex, or, in terms of the classic definitions of proteins, low-MW glutelins.

Variations of the solvent sequence led to extraction of different over-all amounts of protein from the HRS wheat flour used. The most efficient sequence appeared to be 40% isopropanol as first solvent, followed by 2% NaCl, 3.85% lactic acid, and 0.1N KOH in that order. The resolution of components extracted by 2% NaCl and 3.85% lactic acid was improved, however, if water or 0.001M NaCl was used as a preliminary extractant before the isopropanol, without affecting the

amount of protein extracted by either solvent (Fig. 2). There was evidence that certain solvents used early in the sequence lowered the extractability of proteins by following solvents. For example, when 0.01M NaCl was employed as first solvent, the amount of nitrogen extracted by 40% isopropanol fell considerably, although the components did not appear to be changed electrophoretically.

The precision of the extraction procedure was checked by using the isopropanol-salt-lactic acid-KOH sequence. A commercial baker's flour was analyzed five times. The results (Table III) indicate a satisfactory degree of precision. For examination of flours of different types, however, the extra extractability was forfeited in return for greater resolution of protein components, and distilled water was used as a preliminary solvent in a five-solvent sequence.

In the third experiment, the MEDE procedure was applied to a large number of flours of different types. Typical of the results obtained are those included in Table IV. Protein extractability varied from 22 to 88 mg., corresponding to between 79 and 96% of the total protein present, and did not appear to be related to either the amount of protein present or the flour type. It may be considered that the coarser particle size of the durum flour could have been responsible for the lower

TABLE III. PRECISION OF MAES-TYPE PROCEDURE FOR TOTAL NITROGEN EXTRACTION ON HARD RED SPRING FLOUR[a]

Nitrogen Extracted				Total N Extracted	Total N Extracted (Percent of Total N Present)
A	B	C	D		
mg.	mg.	mg.	mg.	mg.	%
29.6	1.6	2.6	13.1	46.9	97.7
30.6	1.4	2.8	11.5	46.3	96.3
29.5	1.2	2.6	12.8	46.1	96.0
29.2	1.4	2.4	12.4	45.4	94.5
29.4	1.3	2.7	13.2	46.6	97.0

[a]Solvent sequence was A:B:C:D = 20:8:18:19 throughout.

TABLE IV. TOTAL NITROGEN EXTRACTED FROM DIFFERENT TYPES OF FLOUR BY MAES-TYPE PROCEDURE[a]

No.	Flour Type	Flour Protein	Nitrogen Extracted				Total N Extracted	Total N Extracted (Percent of Total N Present)
			A	B	C	D		
		%	mg.	mg.	mg.	mg.	mg.	%
1	Hard red spring	13.5	25.8	2.0	2.6	14.9	45.3	96.0
2	Soft white winter	7.2	11.3	0.9	1.2	8.6	22.0	87.1
3	Hard red winter	11.8	19.8	2.0	3.1	13.3	38.2	92.3
4	Soft white spring	7.9	13.3	1.3	0.3	7.1	22.0	79.4
5	Durum	13.3	24.7	1.3	3.1	11.8	40.9	87.6
6	Club wheat	12.5	21.6	2.6	3.9	13.0	41.1	93.0
7	Australian hard white	11.9	22.8	2.1	2.6	11.1	38.6	92.8
8	Australian soft white	11.3	19.8	1.8	0.7	14.0	36.3	91.7
9	Argentine	9.1	13.5	1.5	2.4	8.4	25.8	81.2
10	English	7.7	12.1	0.9	1.5	7.6	22.1	81.3
11	HRS pin-milled high-protein	27.7	58.2	4.8	4.3	20.8	88.1	90.6

[a]Solvent sequence was A:B:C:D = 20:8:18:19 throughout.

extractability of proteins of this flour compared with, for example, those of HRS and hard winter types, but this is not supported by the fact that the extractability of the proteins of soft winter and spring flours was even lower, although their mean particle size is considerably smaller than either hard spring or durum types. A further point of interest lay in the results obtained with the pin-milled, air-classified flour fraction. Although the recovery of protein (90.6%) was somewhat lower than that achieved for ordinary HRS wheat flours, the large absolute amounts of nitrogen extracted (88.1 mg.) show that high protein alone should not be a limiting factor on the amount of nitrogen extracted. There appeared to be little or no relation between the amount of protein extracted by any solvent from any flour and the baking and rheological behavior of the flour, except insofar as variations existed in the protein content.

Electrophoresis of Proteins of Flours of Different Types

The flours used in this section included both laboratory and experimentally milled material. The isopropanol extracts of the flours of HRS and durum wheats gave distinctly different patterns, corresponding to components with different rates of migration (Fig. 3). The number and mobility of the lactic acid-soluble components, however, appeared to be rather more characteristic of different wheat

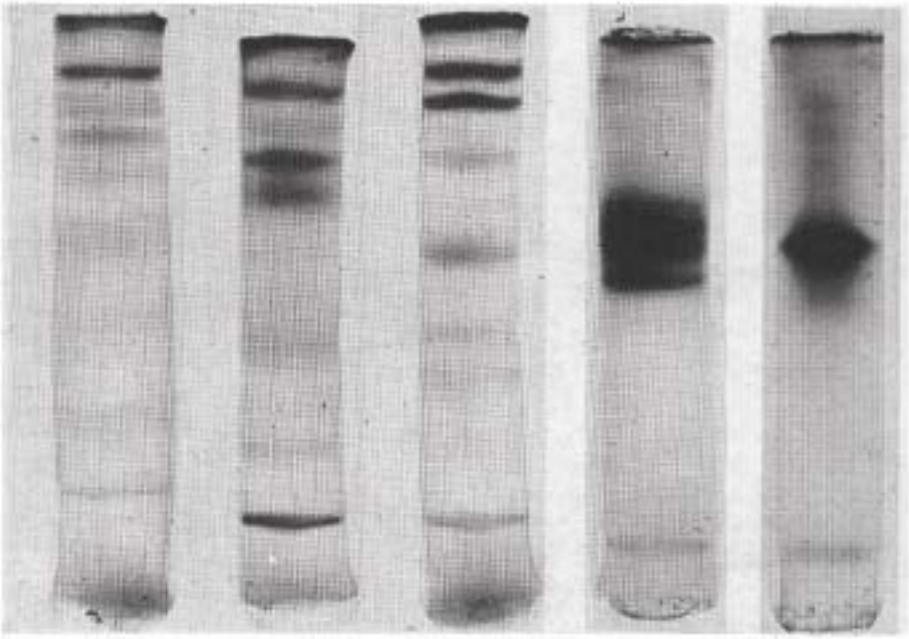

Fig. 3 (left). Electrophoresis of 2-propanol-soluble proteins of (left to right) HRS, soft white winter, and durum flours.

Fig. 4 (middle). Electrophoresis of lactic acid-soluble proteins of HRS flour.

Fig. 5 (right). Electrophoresis of lactic acid-soluble proteins of durum flour.

types than either the salt-soluble or gliadin-type (40% isopropanol-soluble) proteins (Figs. 4 and 5). One major component and four or five minor components were present in durum wheats. Four major components occurred in HRS wheats and also in club wheat flours, although the mobility of the components in the latter was somewhat different. There was, however, very little difference between soft white winter, HRW, soft white spring, HRS, and Garnet flours either in components present or in mobility of components; this suggests that the differences noted between HRS, club wheat flour, and durum flour proteins may have arisen as a result of the different genetic constitution of species, rather than of varieties within a species. Although disc electrophoresis at pH 4.5 gave clearer resolution of components than electrophoresis at pH 8.9, the higher pH showed up more clear-cut differences between flour types.

Electrophoresis of Proteins of Different Species and Types of Wheat

The above experiment was extended with examples of different species of wheat, as well as different wheat types. Some of the species of wheat were obtainable only in small amounts, insufficient for the normal laboratory experimental milling procedures. To obtain material for the study by a uniform procedure, use was made of the particle size index test originally described by Cutler and Brinson (8) as modified by Symes (9). In this test 10 g. of wheat is ground by a standard grinding procedure and the whole meal is sifted for 10 min. through a 200-mesh sieve having an aperture of 37 μ. Preliminary results showed that the flour obtained was quite suitable for analysis by the MEDE procedure, which meant that sufficient material for analysis could be obtained from the yield of a single plant, if necessary. Included among the species studied were *Triticum monococcum, T. dicoccum, T. polonicum, T. sphaerococcum, T. durum, T. compactum, T. aestivum (vulgare), Aegilops squarrosa,* and Tetra-Canthatch. Two F_2 crosses involving Tetra-Canthatch and *Aegilops squarrosa* were also included. The *T. aestivum* samples consisted of Canthatch, Manitou, Thatcher, Winalta, Genesee, and Kenhi; *T. durum* was represented by Stewart 63, Ramsey, and one pure-line variety of *T. durum*.

The results indicated that in some cases considerable differences existed between the types studied; once again, the proteins extracted from different varieties of the same species did not differ greatly from each other (Figs. 6, 7, 8). There were clear-cut differences between wheat types of different chromosome number (Fig. 6) which appeared to be consistent in that the electrophoretic distribution of proteins from the tetraploid *T. dicoccum, T. durum, T. polonicum*, and Stewart 63 (Fig. 7) was very similar, and that of the hexaploid *T. aestivum* varieties and *T. compactum* and *T. sphaerococcum* also had a great deal in common with each other (Fig. 8).

Electrophoresis of Proteins of Different Varieties of the Same Wheat Type

Proteins of four varieties each of HRS, durum, and club wheats were extracted and subjected to electrophoresis at pH 8.9. Although there were considerable differences between the three types with particular respect to gliadin-type proteins and proteins soluble in lactic acid, the different varieties within each type were practically indistinguishable from each other (Fig. 9). There was some evidence that

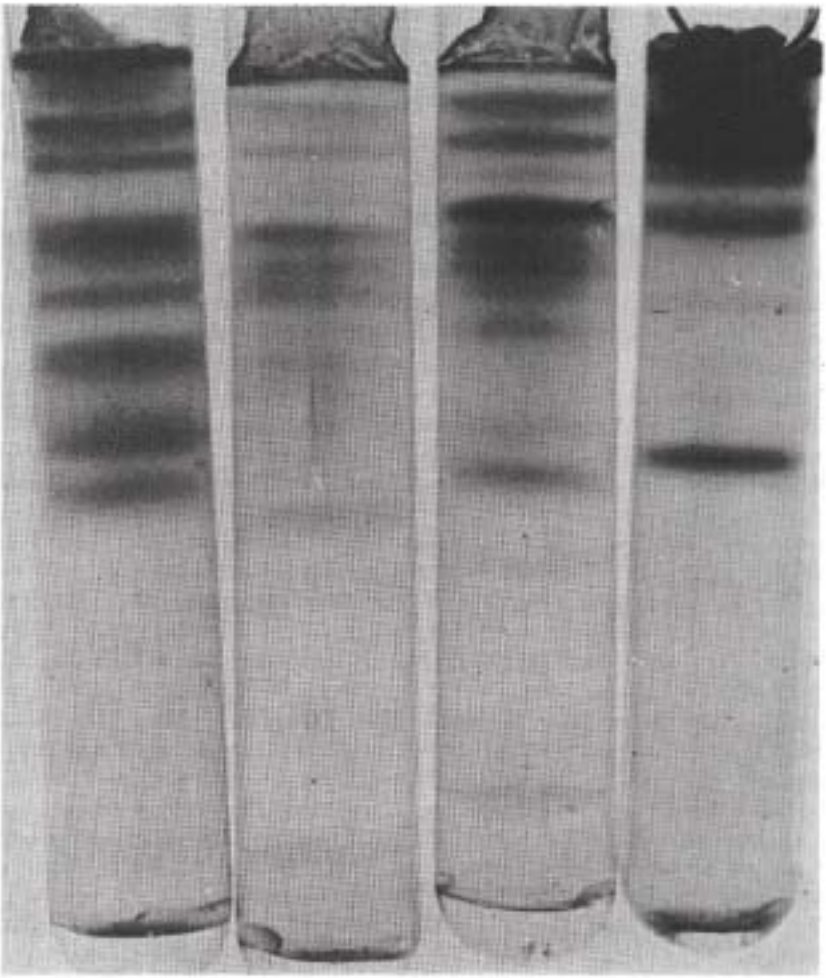

Fig. 6. Electrophoresis of 2-propanol-soluble proteins of (left to right) *Triticum durum*, *T. aestivum* **(Manitou),** *T. sphaerococcum*, **and** *T. monococcum*.

the lactic acid-soluble proteins of Thatcher differed from those of other HRS types, but the differences could not be related to any particular aspect of flour quality, since Manitou, which is similar to Thatcher in baking quality, differed in electrophoretic behavior but was practically identical with Selkirk and Garnet, which differ from both Manitou and Thatcher in quality.

Electrophoresis of Proteins of Denatured Flours

A sample of commercially milled HRS wheat flour was divided into five 1-kg. portions. The first portion was untreated, the second was ball-milled for 2 days, the third was treated with water-saturated n-butanol and the butanol removed by

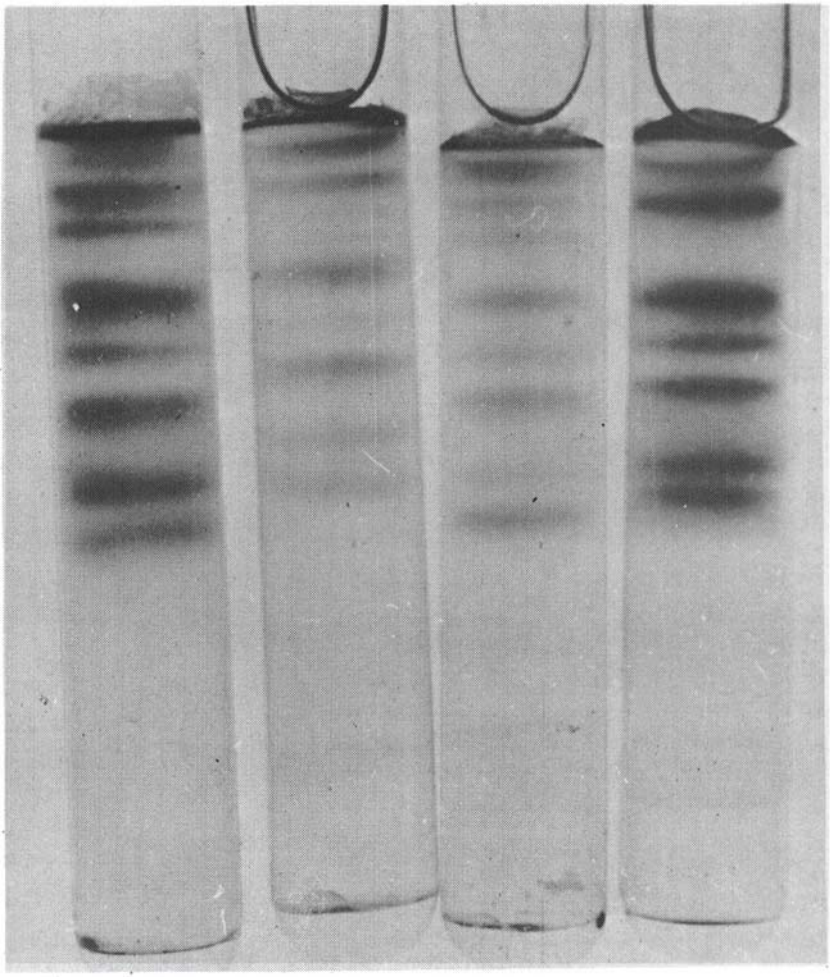

Fig. 7. Electrophoresis of 2-propanol-soluble proteins of (left to right) *Triticum durum, T. dicoccum, T. turgidum,* **and** *T. polonicum.*

humidification and evaporation (10), the fourth was heated overnight at 135°C., and the fifth was treated with excessive amounts of potassium iodate. Farinograph and extensigraph data supported the fact that each of the treated flours was considerably modified as compared with the control. Although Maes extraction revealed that the solubility relations of the proteins had undergone some modification (Table V), disc electrophoresis did not reveal any major differences in the protein components extracted by any of the solvents employed (Fig. 10). This experiment was repeated via disc electrophoresis at pH 4.5 with similar results. These results were construed as evidence that the properties of wheat-flour proteins

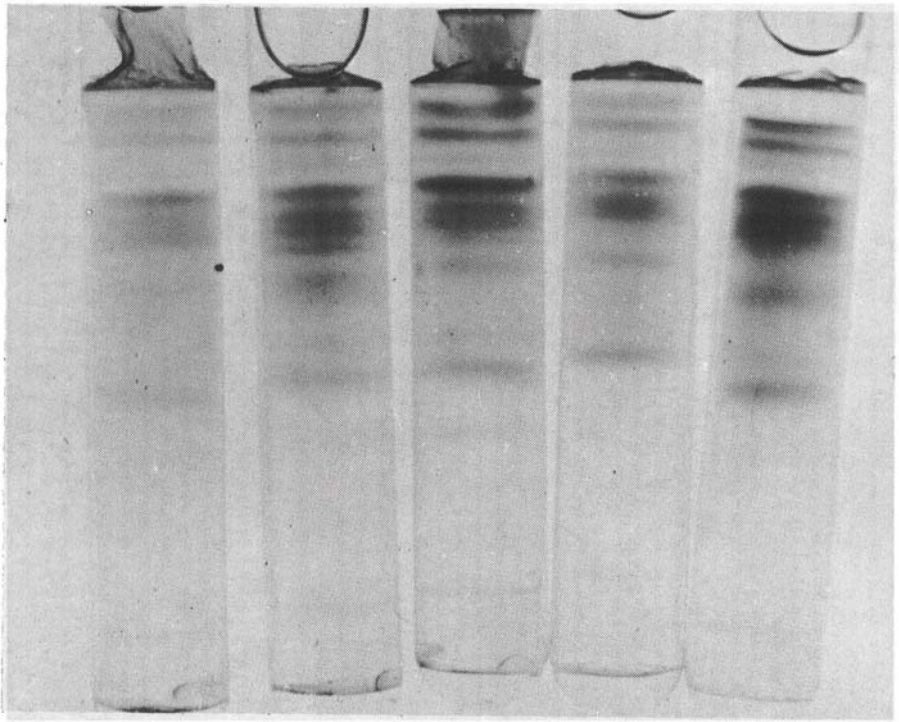

Fig. 8. Electrophoresis of 2-propanol-soluble proteins of (left to right) HRS, SWS, *Triticum sphaerococcum*, SWW, and club wheat (*T. compactum*) flours.

TABLE V. PROPORTION OF PROTEINS EXTRACTED BY DIFFERENT SEQUENTIAL SOLVENTS FROM A HARD RED SPRING WHEAT FLOUR WHICH HAD RECEIVED MODIFYING TREATMENTS

Flour Treatment	Water	40% 2-Propanol	2% NaCl	3.85% Lactic Acid	0.1N KOH
Control	24.1	46.2	1.6	1.8	26.3
Ball-milled 6 hr.	48.2	9.7	0.1	0.1	41.9
Ball-milled 24 hr.	11.4	33.2	2.8	6.5	46.1
Heated at 130°C. 6 hr.	14.4	41.0	5.2	5.5	33.9
Heated at 130°C. 24 hr.	8.9	40.4	5.0	8.3	37.4
Extd. water-satd. n-butanol	14.3	36.7	4.1	7.4	37.4
Oxidized IO_3^-, 10 p.p.m.[a]	19.8	31.6	6.3	11.1	31.2
Oxidized IO_3^-, 40 p.p.m.[a]	41.5	13.2	3.3	5.4	36.6

[a]Extensigraph dough prepared, freeze-dried, and ground to flour for MEDE before and after stretching. No differences were apparent as a result of stretching.

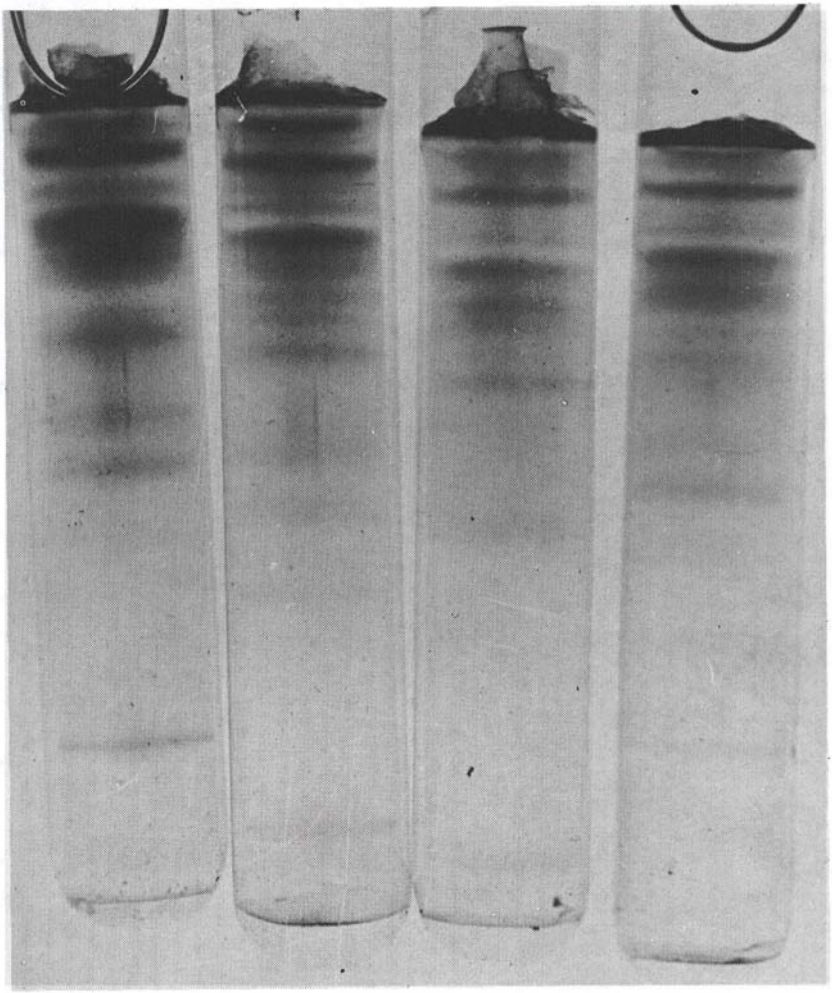

Fig. 9. Electrophoresis of 2-propanol-soluble proteins of (left to right) Garnet, Pembina, Thatcher, and Manitou flours (all HRS varieties of *Triticum aestivum*).

measured by disc electrophoresis do not necessarily bear any relation to the baking or the rheological behavior of the flours.

DISCUSSION

A method for fractionation of wheat and flour proteins based on their solubility in a sequence of solvents is described. The process is inherently simple and is capable of extracting up to 97% of all proteins present with a high degree of precision. Subsequently, the different proteins extracted were characterized by

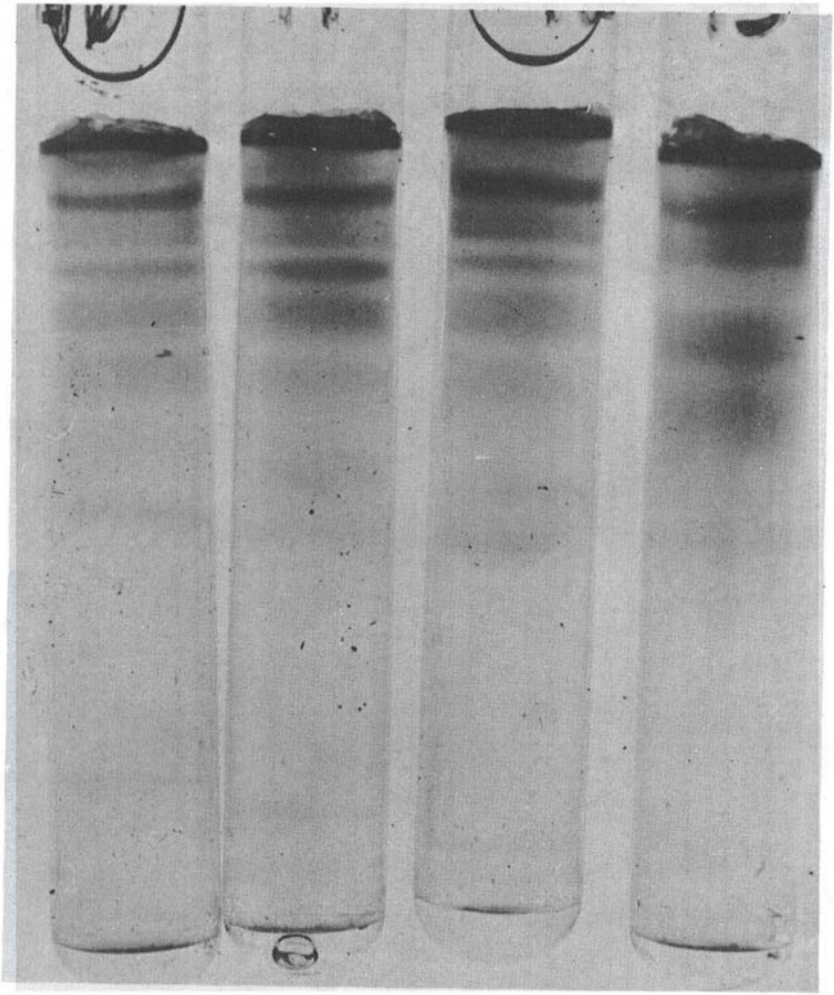

Fig. 10. Electrophoresis of 2-propanol-soluble proteins of (left to right) control HRS flour, and ball-milled, heat-damaged, and overoxidized flours.

means of disc-electrophoresis, which provides a simple, rapid, and sensitive method of separating the components of the different proteins. With suitable equipment a large number of samples could be handled, which would make the process suitable for screening purposes in a wheat-breeding program. The process may be applied to either flour or whole-wheat meal, and for the latter the yield of a single plant would be sufficient for testing purposes.

Differences between wheat varieties in the electrophoretic composition of gliadin-type proteins, albumins and globulins, and the lower-MW glutelins may coincide with differences in the baking and rheological properties of the respective flours, but do not appear to be deeply associated with these differences in behavior.

For example, in one experiment a HRS wheat flour was subdivided into five portions, four of which were damaged by various physical and chemical means. For all five flours upon extraction and electrophoresis, no marked differences were revealed between the electrophoretic properties of the proteins, despite drastic changes in the baking and rheological properties of the flour.

The results have demonstrated that considerable differences exist between wheat varieties belonging to species of different chromosome number. Several species and varieties were tested, and many of the varieties arising from the species with the same chromosome number appeared to possess proteins with similar solubility properties and electrophoretic patterns. Hexaploid wheat varieties of widely different nature gave proteins remarkably alike in electrophoretic properties. Electrophoretic patterns are more likely to be associated with the genetic constitution of the variety and its parents, and should be used to classify varieties on this basis only. There was some evidence that the proteins extractable by 3.85% lactic acid (when used in a solvent sequence) may give more specific differences between wheat types than the other solvents used. Further studies on this aspect of the work are contemplated.

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