Distribution of Phosphorus Compounds in the Protein Fractions of Various Types of Wheat Flours

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ABSTRACT

Experimental flours were milled from ten U.S. wheat samples, including soft, hard, and durum wheats with protein contents between 9.2 and 17.6% (dry wt.), and their protein and phosphorus composition was studied. Along with glutens, the protein components of flours were quantitatively extracted and classified into four groups according to Osborne's classic solubility criteria. These groups appear to be differentiated by their electrophoretic behavior. Significant differences in protein composition of the various wheat classes were shown. Determinations of total, inorganic, lipid, phytic, total nucleic, DNA and RNA phosphorus, applied comparatively to flours and glutens as to their protein fractions, showed noticeable variations between samples. Albumins, globulins, gliadins, and glutenins appear differentiated by both rate and nature of their P compounds. The particularly high content of globulins in nucleic P invalidates the theory that flour phytates may be involved in the precipitation of this protein fraction after dialysis. Distribution of the main P compounds of flours among their protein fractions is shown, in comparison with gluten.

According to the literature on the phosphorus composition of wheats and flours, phytic and inorganic phosphorus—both depending on extraction rate (1) and on storage conditions (2)—appear, with phospholipids (3), to be the best-known phosphorus compounds.

Because of the importance of phytates in nutrition, data have been reported concerning the phytic P content of flours (2,4,5,6). Studies have been made on the phospholipid composition of both wheats and flours in connection with extraction rate, gluten quality, and baking value (7).

It has been shown that nucleic compounds exist in a high concentration in the germ, and a recent work (8) has specified the total nucleic P content of the wheat grain; also, nucleic compounds appear as normal components in flours and glutens (6,8,9). Nevertheless, not even the most recent data are precise.

Several cereal chemists have attempted to specify the influence of certain phosphorus compounds on the technological behavior of wheat flours. Many observations suggest that phospholipid compounds may be associated with gluten proteins during dough formation and that they may strongly contribute to dough properties (10,11).

Recent studies pointed out that total P in gluten was correlated to flour quality (12), and significant correlations were obtained between total acid-soluble, ester-type P and loaf volume (6). A possible relation between nucleic compounds in flours and water absorption in doughs was also postulated (13).

It is now thoroughly established that gluten, which determines quantitatively and qualitatively the properties of flours, is a very complex protein

material; and it may be assumed that phosphorus compounds, which remain associated as lipid, nucleic, or other forms, play a role in gluten properties.

Little is known concerning the part played by these compounds in the physicochemical characteristics of the so-called soluble proteins in flours, less important in quantity but having evident technological interest (14,15).

The objective of the present comparative study of various types of wheat were:

1) To provide quantitative information about the main phosphorus compounds present in flours;
2) To set up, on the basis of the classic criteria for solubility, the protein composition of flours as types and rates of the P compounds which remain associated with the protein material after isolation; and
3) To specify the distribution of P compounds comparatively among flours, glutens, and their protein fractions.

MATERIALS AND METHODS

The flours were milled on a laboratory Buhler mill to an extraction rate of about 70% from ten U.S. wheats harvested in 1960, including four soft wheats (Omar, Brevor, Lucas, and Trumbull), four hard wheats (Early Triumph, Bison, Selkirk, and Conley), and two durums (Lakota and Wells), having protein contents between 9.4 and 17.6% (dry wt.).

Glutens were hand-extracted with distilled water, and the protein preparations were isolated as described below and freeze-dried. Water content was determined either by 90-min. heating at 130°C. (grains and flours) or by drying at 50°C. under reduced pressure to constant weight (glutens and proteins).

Phosphorus was determined according to the reduced molybdate colorimetric method of Briggs adapted by Macheboeuf and Delsal (16), and nitrogen by the Kjeldahl procedure. Amino acids were determined by the Moore and Stein method, with the Auto-Analyzer Technicon. Starch-gel electrophoresis was done as previously described (17), with the aluminum lactate buffer, pH 3.2, described by Jones *et al.* (18); some experimental conditions reported by Coulson and Sim (19) were observed.

Quantitative Extraction of Proteins and Separation into Four Groups

The extraction was founded on the solubility criteria initially defined in Osborne's work (20).

Salt-Soluble Proteins. Flour (10 g., dry wt.) was gently suspended in 70 ml. 0.5*M* NaCl buffered to pH 6.8 (21,22) and allowed to stand 1 hr. in the cold. The slurry was centrifuged and the flour residue washed twice with 40 ml. of extractant.

The total salt extract (supernatant plus washings) was dialyzed in the cold against water during 4 days in the presence of thymol to prevent contamination. The water-soluble fraction (albumins) was then separated by centrifugation from the insoluble one (globulins) and washed several times with distilled water; both were freeze-dried.

Nonprotein N and total salt-soluble protein N were determined respectively from dialyzable and undialyzable N after dialysis of an aliquot (20 ml.) of 0.5*M* NaCl extracts (assays in triplicate).

The respective rates of albumins and globulins were obtained from N determinations in both soluble and insoluble freeze-dried materials. As unavoidable losses occurred during handling, their concentration in flour was estimated by expressing the analytical values with respect to total salt-soluble protein N. The very low amounts of gliadin made soluble with albumins and globulins in these conditions were disregarded.

Gluten Proteins. After extraction with 0.5*M* NaCl, the flour residue was suspended in 50 ml. 60% ethanol and placed in the cold overnight. The alcohol-soluble prolamine fraction was then extracted 2 hr. at laboratory temperature with mechanical shaking (280 shakes/min.) in the presence of glass balls. A first extract was obtained by centrifuging. The residue was washed twice with 20 ml. 60% ethanol, extracted by shaking 1 hr. with 50 ml. extractant, then finally washed twice (20 ml. each time). When dialyzed 4 days in the cold and freeze-dried, the alcohol-soluble material (extracts plus washings) contained the gliadin fraction of the flour.

The residual glutenin fraction was quantitatively extracted as follows: flour residue was mixed with 50 ml. glycol monochlorohydrin (GMC) (30% water). kept one night in the cold, extracted 2 hr. with shaking, and centrifuged; it was extracted again 1 hr. with 50 ml. GMC, then washed twice. The collected extracts and washings were dialyzed as before against water, then freeze-dried.

Gliadin and glutenin contents were obtained from determinations of total N (N $\times$ 5.7) on aliquots of 60% ethanol and 30% water GMC extracts respectively (assays in duplicate).

Determination of Phosphorus Compounds

Phospholipid P was determined from total lipids extracted at room temperature with water-saturated butanol (23) for flours, at boiling point by the ether-ethanol system (1:3, v./v.) for glutens, and by either condition for protein preparations.

For each material, four consecutive extractions were made and the combined extracts were evaporated to dryness under reduced pressure. The crude lipid residue was extracted with chloroform, then filtered. Aliquots of the chloroformic lipid solutions were then taken for P determinations. Assays for flours and glutens were made in triplicate.

Extraction of other phosphorus compounds was made according to the principle of Ogur and Rosen's method of differential solubility in a perchloric acid medium at two different temperatures and acid concentrations (24).

When used at high temperatures on materials having high starch content, perchloric acid (PCA) has the disadvantage of forming gels which do not allow quantitative extraction. Consequently PCA was used only for glutens and protein preparations; trichloroacetic acid (TCA), which does not form gels with starchy materials, was used for flours.

The amount of sample directly extracted in a centrifuging tube varied according to the type of material: 5 g. for flours, 1 to 2 g. for glutens previously defatted, and between 50 and 500 mg. for protein preparations.

A first cold acid extract was obtained by extracting four times the sample at 0°–2°C. by 0.25*N* PCA or 5% TCA; it contained inorganic and

phytic P and traces of ester and nucleotidic P. According to the nature of the sample, variable amounts of nucleic P can also be extracted.

A second warm acid extract was then made by treating the residue four times at 90°C. with 0.5*N* PCA or 10% TCA. Nucleic and other compounds containing P were made soluble in this way.

Total P determinations were made on both extracts. Inorganic ester, phytic, and nucleic P were similarly determined on the corresponding extracts.

Inorganic P was determined either directly from cold acid extracts or from a 0.5*N* HCl solution of the precipitate obtained after treatment by the magnesium reagent ($MgNO_3 \cdot 6H_2O$, 120 g.; NH_4Cl, 100 g.; distilled water to make 1,000 ml.) in the presence of ammonia.

Ester P was estimated for flours only, by making the cold acid extract 1*N* in HCl and submitting the mixture to mild hydrolysis during 15 min. in a boiling-water bath. The difference between inorganic P checked after and before hydrolysis gives the ester P value.

Phytic P. It was observed that the ferric precipitation method (5) was not useful when noticeable amounts of nucleic P were present with phytic P in cold acid extracts.

The method described by Michel Durand (25), with precipitation by Mg^{++} and Ca^{++} ions and where nucleic P does not interfere, was more specific. It was applied to the determination of micro amounts of phytic P in blood (26) and appeared useful for some protein preparations with low phytic P content. Its principle is as follows: inorganic and phytic P are precipitated together by ammonia-magnesium reagent, when nucleic compounds remain in solution. The precipitate is dissolved in 0.5*N* HCl, and phytic P is specifically precipitated by Ca^{++} ions, inorganic P remaining in solution; phytic P is then determined after ashing of the insoluble Ca phytate.

Determination. A volume of cold acid extract, equivalent to 200–300γ phytic P, is measured in a conical centrifuging tube; 2 ml. of magnesium reagent is added and the mixture is neutralized with 8*N* NH_4OH in the presence of neutral red, then made alkaline with 1 ml. 8*N* NH_4OH. The precipitate is a mixture of crystalline $MgNH_4$ phosphate and amorphous Mg phytate. After standing overnight in the cold, it is separated by centrifugation, dissolved in 5 ml. 0.5*N* HCl, and immersed in a boiling-water bath for 10 min.; 5 ml. 25% Ca acetate is then added and the Ca phytate precipitate is heat-coagulated for 5 min. It is recovered by centrifuging and ashed for P determination.

Another version, which omits Ca precipitation, consists of ashing an aliquot of the 0.5*N* HCl solution, which gives the total of inorganic plus phytic P, and determining inorganic P directly. Phytic P is obtained by the difference between the determinations.

Identification of phytates was made by paper chromatography with the mixture n-butanol, n-propanol, acetone, 80% formic acid, 30% TCA (40:20:25:25:15, v./v.) as solvent, comparatively with check samples. The spots were localized by spraying with Hanes and Isherwood's reagent: 4%

ammonium molybdate, 1*N* HCl, 60% PCA, H_2O (25:10:5:60, v./v.), and heating 30 min. at 90°C.

Total nucleic P. It was noted that a part of the total nucleic compounds is made soluble in cold acid medium; consequently, determinations were made on both cold and warm acid extracts.

The presence of different types of P compounds rendered unspecific the direct estimation of nucleic P by phosphorus determination; indirect estimation from ribose values was no more significant by reason of other pentose-containing components. UV absorption of the extracts at 260–265 mμ appeared more specific for estimating nucleic compounds.

Consequently, total nucleic P from both cold and warm acid extracts was estimated indirectly from absorbance at 260–265 mμ, and converted into nucleic acid values from a standard curve established with a sample of purified ribonucleic acid (RNA); total nucleic P values were then calculated on the basis of 9% P content.

For certain protein preparations, the warm perchloric acid extracts presented a maximum absorption more or less shifted toward higher wave lengths around 270 mμ; this interference of protein impurities was, nevertheless, limited and did not affect the nucleic values significantly.

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) P. DNA P was estimated from DNA values on the basis of 9% P content from both cold and warm acid extracts. DNA colorimetric determinations used Dische's diphenylamine reaction in the presence of acetaldehyde at 600 mμ as described by Burton (27), and experimental values were calculated from a standard curve established with a sample of purified thymus DNA. RNA P was then estimated by difference between total nucleic P and DNA P.

Identification of nucleic compounds used hot 10% NaCl extraction, precipitation with ethanol, partition between RNA and DNA being made after alkaline hydrolysis (28).

The DNA fraction is hydrolyzed in its purine and pyrimidine bases 1 hr. at 100°C. with 70% PCA. Heating 1 hr. at 100°C. in 1*N* HCl converts the RNA fraction in pyrimidine nucleotides and purine bases. In both cases, nucleic derivatives are identified, in comparison with check samples, by their absorption in UV light after paper chromatography in the 3° butanol HCl solvent.

Residual P. A portion of the P compounds made soluble in hot PCA or TCA extracts is not of a nucleic nature. The identity of this phosphorus fraction, which corresponds to the difference between total soluble P and nucleic P, was not elucidated. Assays to characterize a possible form of phytic P, more firmly bound and soluble only in hot acid medium, were unsuccessful.

Morever, treatment by hot PCA or TCA leaves a residue containing variable amounts of insoluble P. Consequently, residual P designates both soluble and insoluble phosphorus compounds which remain undetermined after hot acid treatment. For flours, residual P was calculated by the difference between total P and the sum of inorganic, ester, lipid, phytic, and nucleic phosphorus.

RESULTS AND DISCUSSION

Patterns in Fig. 1 show that the four constitutive protein groups extracted from flours as described previously are clearly differentiated by their electrophoretic mobilities.

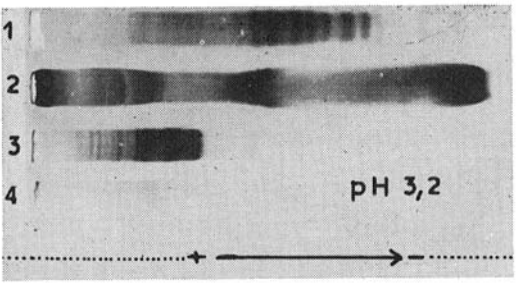

Fig. 1. Electrophoretic behavior of albumins (1), globulins (2), gliadins (3), and glutenins (4) in aluminum lactate buffer, pH 3.2; 0.5*M* urea; 8 v./cm.; 150 min. Starch, 10%; ionic strength, 0.005.

The albumin fraction appears practically free of other protein types, whereas the globulin one, apparently spoiled with some gliadin components, shows a band in the albumin zone which may correspond to the slow globulin fraction reported by Gehrke *et al.* (29). The gliadin fraction contains negligible amounts of glutenin; conversely, the glutenin one is practically free of gliadin.

Data in Table I specify the nonprotein and protein composition of flours as concentrations and rates of the four protein groups. When nonprotein N and salt-soluble proteins appear relatively steady, the gliadin and glutenin contents reflect the variations in total protein.

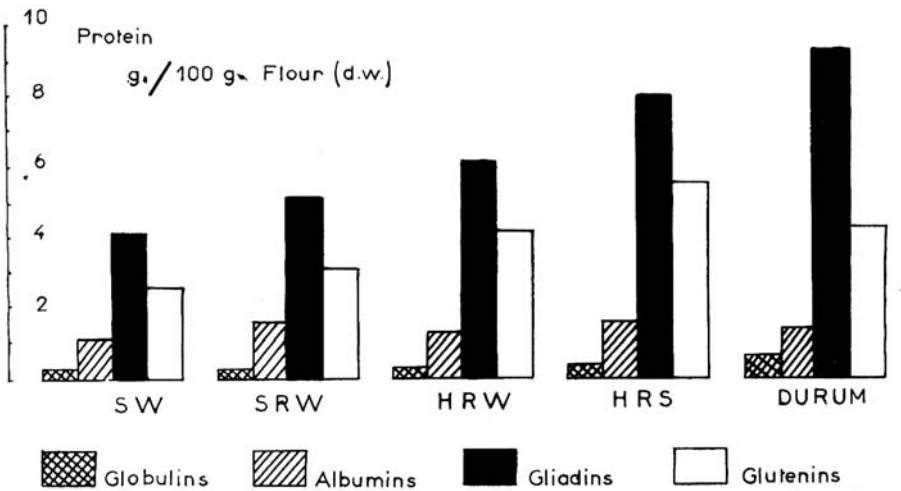

Fig. 2. Composition in globulins, albumins, gliadins, and glutenins of flour from various wheat classes.

The albumin content of soft wheat flours is the lowest, whereas durum flours have the highest globulin content. The proportions of each type of salt-soluble protein appear on the whole to vary inversely to the protein content of flours.

Gliadin rates are similar in soft and hard wheat flours; consequently, the relative increase of the glutenin fraction accounts for the high gluten content in hard wheats (34.1%) compared to the soft ones (29.3%). On the contrary, durum wheats seem characterized by a high gliadin (60.5%) and a low glutenin rate (25.8%).

Figure 2, in which each of the four protein groups is expressed in percent of dry flour, illustrates and summarizes the observed differences between the various wheat classes.

I. Phosphorus Compounds

Flours

In Table II, the main phosphorus characteristics of wheats are shown for comparison.

The total P content of flours varies between 92 (Omar) and 140 (Selkirk): mean, 114 mg./100 g. dry flour. Lower values were obtained from Australian wheat flours: 90 ± 2 mg. (2). In their study on 40 HRW flour samples, Watson *et al.* (6) reported values between 116 and 147 mg. total P/100 g. dry flour (mean, 133 mg.), higher than those on both HRW samples in this study.

When extraction rates are taken into account, the total P content of flours represents an average of 18% total P of wheats for soft and hard types and 33% for durum.

A very low proportion of total P is inorganic: it amounts to between 6.2 and 17 mg./100 g. dry flour (mean 9 mg.) and forms 5 to 15% total P (mean 8%). Lower values (4.4 to 5.5% total P) were reported for Australian flours (2); those concerning HRW flours (6) are higher (28 to 60.5, mean 39.6 mg.) but correspond to a lower mean proportion (3% total P).

Phytic P in flours remains quantitatively the most important type of phosphorus, but, as indicated by the mean values shown in the table below, its concentration and proportion appear rather different between the samples.

Wheat	*Phytic Phosphorus*	
	mg./100 g.	*% of total P*
Soft	30.7	29.3
HRW	30.5	28.6
HRS	57.1	41.7
Durum	41.5	34.9
Mean	38.1	32.8

Similar values were reported by Lee and Underwood (2) from Australian flours: 26 ± 1 mg./100 g. dry flour, i.e. 29.4 ± 0.9% of total P. But those obtained by Watson *et al.* (6) appear considerably lower: the phytic P content of 40 HRW flour samples varies between 2.1 and 14.6 (mean 6.5 mg./100 g. dry flour) and the mean proportion is only 4.9%. These important differences may originate either from milling conditions or from analytical determinations of phytic P.

TABLE I

PROTEIN COMPOSITION OF FLOURS

	Soft Wheats				Hard Wheats				Durum Wheats	
	Omar, SW	Brevor, SW	Lucas, SRW	Trumbull, SRW	Early Triumph, HRW	Bison, HRW	Selkirk, HRS	Conley, HRS	Lakota	Wells
	g. per 100 g. flour (dry wt.)									
Total prot.	7.85	9.55	9.50	11.40	11.75	12.95	16.60	15.75	16.35	15.70
Dry gluten	5.40	9.00	8.15	9.80	11.60	12.70	17.35	15.65	15.75	14.30
	Nitrogen, mg./100 g. flour (dry wt.)									
Total	1,375	1,675	1,670	2,000	2,060	2,270	2,910	2,760	2,870	2,750
Nonprotein	55	78	47	58	76	54	105	102	115	96
Protein	1,320	1,597	1,623	1,942	1,984	2,216	2,805	2,658	2,755	2,654
Albumins	180	185	249	264	211	234	238	290	246	242
Globulins	85	87	74	98	78	62	97	68	105	110
Gliadins	645	825	860	960	1,000	1,160	1,490	1,320	1,570	1,680
Glutenins	410	500	440	620	695	760	980	980	834	622
Gliadins + glutenins	1,055	1,325	1,300	1,580	1,695	1,920	2,470	2,300	2,404	2,302
Dry gluten	718	1,355	1,280	1,570	1,810	1,990	2,590	2,410	2,400	2,260
	Nitrogen, % total N in flour									
Nonprotein	4.0	4.6	2.8	2.9	3.7	2.4	3.6	3.7	4.0	3.5
Albumins	13.1	11.1	14.8	13.2	10.2	10.3	8.2	10.4	8.6	8.8
Globulins	6.1	5.1	4.5	4.9	3.8	2.7	3.3	2.5	3.6	4
Gliadins	46.9	49.3	51.5	48	48.5	51.2	51.2	47.9	54.8	61.1
Glutenins	29.9	29.9	26.4	31	33.8	33.4	33.7	35.5	29	22.6

TABLE II

PHOSPHORUS COMPOUNDS IN WHEATS AND FLOURS

	SOFT WHEATS				HARD WHEATS				DURUM WHEATS	
	Omar, SW	Brevor, SW	Lucas, SRW	Trumbull, SRW	Early Triumph, HRW	Bison, HRW	Selkirk, HRS	Conley, HRS	Lakota	Wells
WHEATS	Phosphorus, mg./100 g. wheat (dry weight)									
Total	341	372	420	451	468	409	432	498	286	227
Inorganic	19.2	15.3	25.2	38.3	15.9	27	45	19.4	14	11.4
Phytic	252	283	343	328	329	321	338	371	192	148
	Phosphorus, % total P in wheats									
Inorganic	5.8	4.1	6	8.5	3.4	6.6	10.4	3.9	4.9	5
Phytic	75.5	76.1	81.6	70.8	72.2	78.5	78.3	74.4	66.8	65.1
FLOURS	Phosphorus, mg./100 g. flour (dry wt.)									
Total	92.1	99.7	107.3	117.5	110.4	103.5	140	133.8	125.1	111.8
Inorganic	5.5	5.5	11.1	17.7	13.9	8.6	7.4	8.4	6.3	6.2
Phytic	24.2	27.4	34.5	36.8	30.3	30.8	59.7	54.5	47.7	35.2
Lipid	11.6	13.1	12.4	14.9	13.3	12.8	16.2	16.8	14.9	14.5
Total nucl.	14.2	17.4	13.4	18	16.6	17	22.8	20.5	22	24.7
DNA	2.4	2	2.6	2.7	2.3	2.5	2.2	2.7	2.4	2.9
	Phosphorus, % total P in flours									
Inorganic	6	5.6	10.3	15.1	12.6	8.3	5.3	6.2	5.1	5.5
Phytic	26.3	27.5	32.2	31.4	27.5	29.7	42.6	40.8	38.1	31.7
Lipid	12.6	13.1	11.6	12.7	12	12.3	11.6	12.6	11.9	12.9
Nucleic	15.4	17.4	12.4	15.3	15	16.4	16.3	15.3	17.6	22.3
Flour phytic P, % wheat phytic P	8.5	8.2	7.7	9.3	8.3	8.2	14.4	11.9	19.1	20.8

The proportion of wheat phytic P released in flours during milling appears very low and confirms that the grain endosperm is poor in phytin compounds. On an average, phytic P in flours accounts for about 8% wheat phytic P in winter soft and hard types, about 13% in hard spring, and 20% in durum wheat flours.

Lipid P. On an average, HRS flours appear relatively richer in lipid P (16.5 mg.) than durum (14.7 mg.) or winter hard and soft types (13 mg.). Nevertheless, the proportion of lipid P remains appreciably constant: durum 12.4, hard 12.1, soft 12.5% total P. Values reported recently on 40 HRW flour samples (6) are notably lower: 4.4 to 6.7 (mean 5.7 mg./100 g.), i.e., 4.3% total P.

Nucleic P appears, after phytic P, to be the most important phosphorus type in wheat flours. Its concentration (mean 18.7 mg./100 g. dry flour) varies between 13.4 (Lucas) and 24.7 (Wells); flours from durum (23.3) and HRS (21.6) contain relatively more nucleic P than those from HRW (16.8) and soft (15.7). Its proportion (% total P) appears higher in durum flours (20%) than in soft (15.1%) and hard (15.7%).

Concentration of DNA P in flours does not vary significantly (2 to 2.9 mg., mean 2.5 mg./100 g.); with regard to total nucleic P, DNA P accounts for 15.7% in soft, 12.7% in hard, and 11.2% in durum flours. To our knowledge, no values have been previously reported for DNA P, and the only work concerning nucleic composition of flours was that of Watson *et al.* (6) from 40 HRW samples; they found a total nucleic P mean content of 77 mg./100 g. dry flour (63 to 87.5), corresponding to 57.4% total P.

In our study, the mean content of the ten flour samples in total nucleic P (18.7 mg.) is equivalent to approximately 200 mg. nucleic compounds. If we consider that this value concerns only the endosperm of wheat grain, it remains in the same order as that of 270 mg. reported by Äyräpää (30) on barley grain. For wheat grain, quantitative determinations concern only the RNA fraction. According to Matsushita (31), the RNA content would be about 118 mg. and the values reported recently by Mihailović *et al.* (8) on eleven Italian and Yugoslav wheat varieties are between 63 and 106 mg.

In the present study, the mean RNA content of flours, estimated by difference between total nucleic P (18.7 mg.) and DNA P (2.5 mg.), lies around 180 mg./100 g. dry flour. Compared with that reported on the whole grain, these values on flours, though in the same order, appear higher but remain very far from the values reported by Watson, representing a mean nucleic acid content of about 850 mg.

For a better understanding, the mean data of the present study for percent total P are compared with those of Watson *et al.* in the table below.

Phosphorus	*Present Study* % of total P	*Watson* et al. (6) % of total P
Inorganic	8	3
Lipid	12.3	4.3
Nucleic	16.3	57.4
Phytic	32.8	4.9
Ester	.....	30.4
Residual	30.6	0

Variations in the minor phosphorus types (inorganic and lipid) are approximately in the ratio 1:3. Ester P values were determined only on some flour samples and are not reported here owing to lack of precision and reproducibility of the experimental values, which range between about 1 and 2% of total P. The most important fluctuations concern the major nucleic and phytic components. It must be noted further that proportions of the various P types determined in the present study account only for 60 (Omar) to 76% (Conley) flour total P; a residual P fraction (mean 30.6%), possibly of a phosphoprotein nature, remains undetermined.

The above data are illustrated in Fig. 3.

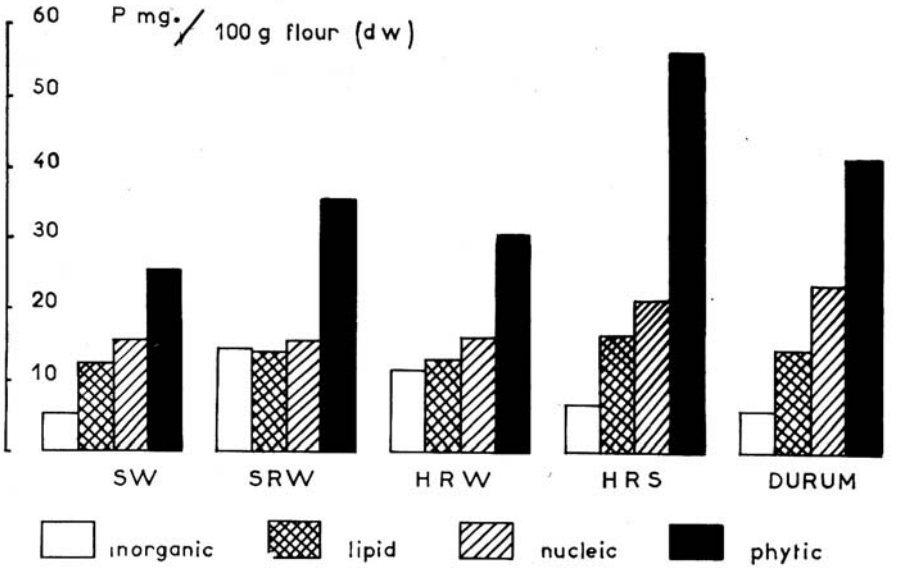

Fig. 3. Phosphorus compounds in various types of wheat flours.

Protein Fractions and Glutens

Total Phosphorus Content. Data in Table III indicate, in addition to their protein content, the total P composition of gluten and protein fractions isolated from flours.

The protein values give evidence that nonprotein materials are made soluble with the extracted proteins; they remain relatively constant inside a similar preparation, and lie around 50% for albumins, 85% for globulins, 92% for gliadins, 75% for glutenins, and 88% for glutens.

More marked differences become apparent concerning total P contents. Expressed in γ/100 mg. dry wt., the mean values are respectively 31 for albumins, 1,100 for globulins, 90 for gliadins, 190 for glutenins, and 250 for glutens.

In salt-soluble proteins, the P content of albumins appears very low,

TABLE III

PROTEIN AND TOTAL PHOSPHORUS CONTENT OF PROTEIN FRACTIONS AND GLUTENS ISOLATED FROM WHEAT FLOURS

	Soft Wheats				Hard Wheats				Durum Wheats		Mean Values
	Omar, SW	Brevor, SW	Lucas, SRW	Trumbull, SRW	Early Triumph, HRW	Bison, HRW	Selkirk, HRS	Conley, HRS	Lakota	Wells	
Albumins											
Protein, % (dry wt.)	46.7	45.8	52.2	55.3	51	45	50.8	59.2	50	50.4	50.6
Total P, γ/100 mg. dry mat.	33	35	30	34	28	29	34	26	32	27	30
Total P, γ/100 mg. protein	71	76.5	57.5	61.5	55	64.5	67	44	64	53.5	61.5
Globulins											
Protein, % (dry wt.)	83.8	83.2	82.6	83.8	82.4	83.2	88.5	90.6	86.2	90.2	85.5
Total P, γ/100 mg. dry mat.	1,220	1,130	1,165	880	1,240	1,270	1,215	1,130	945	910	1,100
Total P, γ/100 mg. protein	1,455	1,360	1,410	1,050	1,510	1,525	1,370	1,250	1,100	1,010	1,304
Gliadins											
Protein, % (dry wt.)	91.2	94.5	80	90	93	95.8	95.3	94.5	94	96	92.4
Total P, γ/100 mg. dry mat.	110	89	147	132	88	83	78	76	57	44	90
Total P, γ/100 mg. protein	120	94	184	146	95	87	82	81	61	46	102
Glutenins											
Protein, % (dry wt.)	73.5	83.3	77	82.2	79.8	69.6	74.8	73	67.2	66	74.6
Total P, γ/100 mg. dry mat.	231	194	184	171	161	149	168	200	223	195	188
Total P, γ/100 mg. protein	314	233	239	208	202	214	225	274	332	295	254
Glutens											
Protein, % (dry wt.)	87.4	85.9	90.2	91	89	89.3	85.5	87.8	86.7	89.8	88.3
Total P, γ/100 mg. dry mat.	255	248	244	250	229	210	283	243	282	280	252
Total P, γ/100 mg. protein	292	289	270	274	257	238	332	277	325	312	287

whereas that of globulins is remarkably high. During assays including extended standing of the bags in cold after dialysis, it was observed that the P content of the albumins so obtained was as low as 16γ when that of globulins was raised up to 1,370 γ/100 mg. dry material. Moreover, when the albumin fraction was purified by treatment with $(NH_4)_2SO_4$ according to the procedure of Pence and Elder (21), the isolated material contained only traces of phosphorus (3 γ/100 mg. dry wt.). It may be admitted that, in the experimental conditions used, phosphorus in albumins would originate from traces of globulins which would not be entirely precipitated after dialysis; only 1.5% of globulin impurities is sufficient to raise the albumin P content up to 20 γ/100 mg. dry wt.

For gluten proteins, the total P mean content of glutenins (188 γ/100 mg. dry wt.) is about double that of gliadins (90 γ), whereas it lies around 250 γ for whole glutens.

When expressed on an equal protein content basis, the mean P content of the four protein groups (γ P/100 mg. protein) is respectively 61 (albu-

TABLE IV

LIPID AND LIPID PHOSPHORUS CONTENT OF "SALT-SOLUBLE" PROTEINS, GLUTENINS, AND GLUTENS

	Soft Wheats				Hard Wheats				Durum Wheats	
	Omar, SW	Brevor, SW	Lucas, SRW	Trumbull, SRW	Early Triumph, HRW	Bison, HRW	Selkirk, HRS	Conley, HRS	Lakota	Wells
Flours										
Lipids										
% dry flour	1.65	1.66	1.59	1.81	1.61	1.54	1.90	1.72	1.80	1.80
% protein	21	17.4	16.7	15.8	13.7	11.9	11.4	10.9	11	11.4
Lipid P, γ/100 mg. protein	148	137.1	130.5	130.5	113.1	98.8	97.6	106.5	91.2	92.3
Salt-soluble proteins										
Lipids										
% dry mtl.	7.90	4.35	4.15	4.15	3.40	3.70	5.50	3.80	3.80	3.40
% protein	15.9	8.4	7.5	7.1	6.5	7.4	9.1	6.5	6.5	6
% flour lipids	14.5	7.8	8.8	8.1	6.6	8.1	9.2	7.7	7.2	6.8
Lipid P, γ/100 mg. protein	28.8	23.3	28.6	25.3	26.9	27.6	20.7	23.8	16.1	20.4
Glutenins										
Lipids										
% dry mtl.	18.3	13.4	15	11.9	13	9.5	14.1	9.3	12.8	15
% protein	24.9	16.1	19.5	14.5	16.3	13.6	18.9	12.8	19	22.5
% flour lipids	35.5	27.6	30.8	28.4	40.2	39	55.8	41.7	50.1	41.9
Lipid P, γ/100 mg. protein	188	123	130	128	160	142	141	163	184	192
Glutens										
Lipids										
% dry gluten	12.5	8.2	7.8	6.3	7.8	6.3	6.6	7.5	7	7
% protein	15.2	9.5	9.1	7.4	9.4	7	7.7	8.3	8.3	8.4
% flour lipids	41	44.5	40	34.5	49	52	60	68.5	61.5	56
Lipid P, γ/100 mg. protein	52.5	46.8	48.8	45.2	48.6	34.2	39.7	47.9	35.7	32.5

mins), 1,300 (globulins), 100 (gliadins), 250 (glutenins), and 290 (glutens).

Lipid and Lipid Phosphorus Composition. Results concerning salt-soluble proteins, glutenins, and glutens are presented in Table IV, in comparison with flours.

1. *Salt-Soluble Proteins.* The lipid content of the whole freeze-dried material after dialysis (percent dry matter) varies between 3.4 (Early Triumph, Wells) and 7.90 (Omar). Expressed in percent proteins, it appears relatively constant: if we except Omar (15.9%), the mean values are respectively 7.7% for soft, 7.4% for hard, and 6.3% for durum.

Then, the crude salt-soluble material of flour containing the albumin and globulin fractions is as rich as 7% lipid which constitutes about 7 to 8% of lipid in flour; for Omar, this value reaches 14.5%. A fraction of this lipid material is of a phospholipid nature and the amount of lipid P (γ/100 mg. protein) appears lower in preparations isolated from durum flours (18.2%) than in those from soft (26.5%) and hard (25%).

Complementary assays specified the distribution of total lipid and lipid P between the albumin and globulin fractions. The following values were obtained from individual protein preparations separated by dialysis as described before.

	For 100 mg. Dry Material				
	Protein	*Lipid*	*Lipid P*	*Lipid*	*Lipid P*
	mg.	*mg.*	γ	*% protein*	*γ/100 mg. protein*
Albumin	54	1.8	2	3.2	3.7
Globulin	83	6.1	89	7.3	107

In percent dry material or protein, the globulin fraction appears significantly richer in lipid than the albumin one. The P content of lipids extracted from both fractions is rather similar (1.10% for albumins, 1.45% for globulins), but the concentration of lipid P in the albumin fraction is considerably lower than in globulins, where it accounts for about 7% of total phosphorus.

2. *Gliadins.* Applied to a limited number of gliadin preparations, the ether-ethanol system, used in place of butanol, allowed one to check that this fraction was practically lipid-free. The amounts of extracted lipid material corresponded to about 0.5% of dry material and contained only traces of phosphorus.

3. *Glutenins.* The lipid content varies between 9 and 18% dry material; that is (in percent protein), 18.7% in glutenins from soft wheats, 15.4% from hard wheats, and 20.7% from durums. Lipids in glutenins account respectively for 31% (soft), 44% (hard), and 46% (durum) of flour lipids. Mean values for lipid P (γ/100 mg. protein) are respectively 142, 151, and 188 for soft, hard, and durum preparations.

4. *Glutens.* Total lipid contents, Omar excepted (12.5%), vary between 6.3 (Bison) and 8.2% (Brevor); on an average, glutens from soft wheats contain more lipids (8.7%) than hard (7.05%) and durums (7.03%). In

percent proteins, the corresponding mean values are respectively 10.30, 8.10, and 8.35%; and lipids in glutens account, in percent flour lipids, for 40% (soft), 57.4% (hard), and 54% (durum).

Lipid P contents (γ/100 mg. protein) are relatively high in soft samples (48.4%) compared with hard (42.6%) and mainly durum (34.2%); these data may be compared with other observations on flours (7), showing that the higher the phospholipid content, the lower is the baking value.

The present data about the lipid and lipid P composition of glutens and the protein fractions of wheat flour lead to the following comments. The presence of lipids in salt-soluble proteins of cereals was previously evidenced by Äyräpää on barley (30). According to the pH value used for salt extraction, the amount of lipids in the isolated proteins varied from 82 to 146 mg./100 g. flour. When lipids in salt-soluble proteins extracted from barley account for 2.8 to 4.6% total lipids, the corresponding values for wheat flour vary between 6.6 and 9.2%. Besides, according to the present study, the lipid and phospholipid contained in the salt-soluble proteins of wheat flour would be mainly associated with the globulin fraction.

For gliadin, it is confirmed, as demonstrated or suggested by previous workers (32,33), that this protein is practically free from lipid and consequently from lipid phosphorus.

The lipoprotein nature of the glutenin fraction, already suggested by Guillemet *et al.* (33), was evidenced first by Olcott and Mecham (34) and confirmed by Lee and Wan (35) in their studies with radioactive tracers. The present data confirm that lipids containing phospholipids remain associated with proteins when glutenin is quantitatively extracted; they show besides that lipid and lipid P contents may vary according to the type of wheat.

Composition in Various Types of Phosphorus. Results in Table V concern the qualitative and quantitative phosphorus composition of albumin, globulin, gliadin, and glutenin fractions isolated from the various wheat flours. They are expressed on a basis of equal protein content.

1. *Albumins.* The total P content of albumins appears particularly low, and, as indicated above, extended dialysis or elaborate purification reduced the total P values. One may suppose that P compounds present in the crude preparations originate from traces of globulins remaining after dialysis. Indeed, nucleic and lipid P are present in very low rates in albumins where, as in globulins, no phytic P is detectable.

As shown above, lipid P accounts for about 4 γ/100 mg. protein, i.e., 5% total P. Insoluble residual P itself accounts for 4 to 8% total P. On an average, total nucleic P amounts to 43% total P, and it was noted that DNA P increases in the same way as total P values—i.e., with rates of globulin impurities. Though analytical determinations on albumins were difficult, they indicated that P compounds which are detectable as traces originate from globulins.

2. *Globulins.* The total P content of the water-insoluble fraction of salt extracts after dialysis is more than 20 times that of albumins (mean value

TABLE V

PHOSPHORUS COMPOUNDS IN THE PROTEIN FRACTIONS EXTRACTED FROM WHEAT FLOURS ACCORDING TO CLASSIC SOLUBILITY CRITERIA

(P, γ/100 mg. protein)

	Soft Wheats				Hard Wheats				Durum Wheats		Mean Values
	Omar, SW	Brevor, SW	Lucas, SRW	Trumbull, SRW	Early Triumph, HRW	Bison, HRW	Selkirk, HRS	Conley, HRS	Lakota	Wells	
Albumins											
Total	71	76.5	57.5	61.5	55	64.5	67	44	64	53.5	61.5
Nucl.	19.1	19.8	24.1	27.5	26	35	18.1	11	19.2	26.4	22.6
Globulins											
Total	1,455	1,360	1,410	1,050	1,510	1,525	1,370	1,250	1,100	1,010	1,304
RNA	708	630	575	508	628	685	770	704	429	369	601
DNA	367	380	475	337	432	505	290	346	476	466	407
Resid.	380	370	360	205	450	330	310	200	195	175	296
Gliadins											
Total	120	94	184	146	95	87	82	81	61	46	102
Phytic	35.5	15.9	27.8	25	25.3	37	7.8	26.4	8.9	9.1	22
Nucl.	12.1	13.8	15.3	13.3	13.6	10.7	15.1	16.2	14	15.3	14
Resid.	72.4	64.3	140.9	107.7	56.1	39.3	59.1	38.4	38.1	21.6	66
Glutenins											
Total	314	233	239	208	245	214	225	274	332	295	258
Phytic	49	61	44	46	45	50	48	83	113	96	63
Nucl.	121	108	105	93	90	106	105	109	115	101	106
Resid.	144	64	90	69	110	58	72	82	104	98	89
Lipid	188	123	130	128	160	142	141	163	184	192	155

1,300 γ/100 mg. protein). Globulins from durum wheats have a total P content (1,055 γ) lower than that of soft (1,320 γ) and hard wheats (1,410 γ).

It was shown above that lipid P is present in globulins with an approximate rate of 110 γ/100 mg. protein, accounting for about 7% total P. But assays to evidence phytic P were negative.

On the other hand, a very high proportion of P is nucleic. Total nucleic P accounts for between 70 (Early Triumph) and 84% total P (Conley); values appear higher in globulins from durum (82.2%) than in hard (77.4%) and soft wheats (75.4%).

Identification of nucleic derivatives from globulins by paper chromatography has confirmed chemical determinations and evidenced that both RNA and DNA compounds are present.

On the other hand, a very high proportion of P is nucleic. Total nucleic nucleic P. But rather important variations in nucleic composition are observed between the samples. Thus, DNA P is only 32% nucleic P in HRS, near 40% in soft and HRW flours, but as high as 54% in durum.

The nucleic acid mean content reaches 9.6% (extreme values 7.4 and 10.6%), distributed between RNA (5.7%) and DNA (3.9%). Nucleic

acid composition of globulins according to wheat type is presented in the table below.

	Total Nucleic Acid % d.m.	*RNA* % d.m.	*DNA* % d.m.	*Ratio: RNA/DNA*
Soft	9.2	5.6	3.6	1.55
Hard	10.4	7	3.4	2.05
Durum	8.5	3.9	4.6	0.85
Mean	9.6	5.7	3.9	1.45

Globulins from durum wheat flours appear to be characterized by the importance of DNA; those from soft and durum, by the preponderance of RNA. Determinations on more samples would specify if these observations have genetical or agronomical meanings.

This high nucleic acid content leads one to assume that the globulin fraction of the wheat endosperm would be, in large part, constituted by nucleoproteins. Study of its solubility characteristics revealed similarities with nucleoprotein which seems to be confirmed by its amino acid composition (see table below).

	g./16 g. N		*g./16 g. N*		*g./16 g. N*
Alanine	5.9	Histidine	2.2	Proline	4.3
Arginine	7.8	Isoleucine	3.8	Serine	4.9
Aspartic acid	8.7	Leucine	7.7	Threonine	4.2
Cystine	...	Lysine	7.5	Tryptophan	...
Glutamic acid	11.6	Methionine	2	Tyrosine	3.6
Glycine	5.1	Phenylalanine	3.8	Valine	5.6

A high content of basic amino acids and a relatively low content of glutamic acid reflect a protein composition comparable with that of histones. On a histone preparation isolated from wheat germ (36), basic amino acids accounted for 30% total N. In the present globulin fraction, the sum of lysine + arginine + histidine accounts for 17.5% total N. The high arginine content of globulin was previously reported by Pence *et al.* (22).

Nevertheless, it must be demonstrated whether nucleoproteins in the globulin fraction are specifically contained in the endosperm, or if they originate from germ particles which probably remain in flour after experimental milling. Residual P accounts for 16 (Conley) to 30% (Early Triumph) total P. The insoluble fraction after perchloric acid treatment (2.5 to 4.2% total P) would be phospholipid, but P compounds made soluble with nucleic P in hot PCA are unknown. Assays to identify a possible form of phytic P more firmly bound to proteins were negative, and it was not possible to characterize this undetermined P as phosphoprotein P.

3. *Gliadins.* The total P mean content of gliadins (102 γ/100 mg. protein) is slightly higher than that of albumins and about 13 times less than that of globulins. It appears higher in preparations from soft (136 γ) than those from hard (86 γ) or durum (53 γ).

Whereas lipid P exists only as traces, both phytic and nucleic P are present.

Phytic P mean content lies around 22 γ and is as low as 9 γ for hard wheats. Calculated on the basis of a molecular weight of 660 for six P

atoms, the amount of phytic acid contained in 100 mg. protein approximates 0.08 mg.

Nucleic P mean content is also very low (14γ) and corresponds to 0.16 mg. nucleic acids/100 mg. protein. RNA is the main component and DNA exists only as traces (DNA P accounts for 0.3 to 1.5% total P). Phytic plus nucleic P accounts only for 35% total P. One part of residual P is soluble in hot PCA (41% total P), the other is insoluble (24% total P). If present, phosphoprotein P would be a major P component of gliadins, although to a very low concentration.

4. *Glutenins.* The total P mean content of glutenins (254γ/100 mg. protein) is about 2.5 times that of gliadins and one-fifth that of globulins. Glutenins from durum contain 314γ total P, from hard 229γ, and from soft 248γ. Along with lipid P, phytic and nucleic P are present and account respectively for 24 and 41% total P; these mean values correspond to 0.23 mg. phytic acid and 1.2 mg. nucleic acid/100 mg. protein. Both RNA and DNA types have been identified (31% and 7% total P respectively). Residual P, determined after PCA treatment on undefatted material, averages 34% total P. Though values concerning lipid P appear notably higher, it may be admitted that the residual P fraction in glutenin PCA extracted is entirely phospholipid.

TABLE VI
PHOSPHORUS COMPOUNDS IN GLUTENS

	SOFT WHEATS				HARD WHEATS				DURUM WHEATS	
	Omar, SW	Brevor, SW	Lucas, SRW	Trumbull, SRW	Early Triumph, HRW	Bison, HRW	Selkirk, HRS	Conley, HRS	Lakota	Wells
	Phosphorus, mg./100 g. dry gluten									
Total	223	256	224	244	256	213	281	215	270	233
Inorganic	47	99	102	109	85.5	58	46.3	39.6	111.3	73.5
Phytic	19.3	14.8	5.4	3.4	44.4	42	86.6	36.9	16.9	20.4
Lipid	45.8	40.2	44	41.1	43.2	30.5	33.9	42	31	29.2
Total nucl.	72.9	72.7	54.1	58.1	67.1	75	83.6	71.4	90.3	97.1
DNA	24.6	27.7	25.3	28.6	24.6	22.3	21.3	21.8	25.3	23.8
Residual	38	29.3	18.5	32.4	15.8	7.5	30.6	25.1	20.5	12.8
	Phosphorus, % total P in gluten									
Inorganic	21.1	38.6	45.6	44.6	33.4	27.2	16.5	18.4	41.2	31.7
Phytic	8.6	5.8	2.4	1.4	17.4	19.7	30.8	17.2	6.3	8.8
Lipid	20.4	15.8	19.7	16.8	16.8	14.4	12.1	19.5	11.5	12.4
Total nucl.	32.8	28.4	24.1	23.9	26.2	35.2	29.7	33.2	33.4	41.6
Residual	17.1	11.4	8.2	13.3	6.2	3.5	10.9	11.7	7.6	5.5
RNA	21.7	17.5	12.8	12.1	16	24.7	22.2	23	24	31.5
DNA	11	10.8	11.6	11.7	9.6	10.5	7.6	10.1	9.4	10.2
RNA/DNA	1.96	1.63	1.14	1.03	1.73	2.36	2.92	2.28	2.56	3.08

5. *Glutens.* Data are presented in Table VI. Total P content of glutens, between 213 (Brevor) and 281 (Selkirk) mg./100 g., does not reflect marked differences among the types of wheats, though it has been reported to be inversely correlated with flour quality (12).

Values reported in several studies concerning glutens extracted from French (9), Italian and Yugoslav (8), and U.S. wheats (6) are compared in the table below. The observed differences may reflect agronomical and genetical features as well as conditions used for gluten extraction.

	Total Phosphorus			
	10 Samples, Present Study	*10 Samples, Bourdet* et al. (9)	*17 Samples, Mihailović* et al. (8)	*40 Samples, Watson* et al. (6)
	mg./100 g. dry gluten	*mg./100 g. dry gluten*	*mg./100 g. dry gluten*	*mg./100 g dry gluten*
Minimum	213	154	240	168.5
Maximum	281	293	560	218
Mean	241.5	236.3	390	191.5

Inorganic P accounts for from 39.6 (Conley) to 111.3 (Lakota) mg./100 g. dry gluten and reflects noticeable variations between the samples. In percent total P, glutens from SRW have the highest values (45.1%), HRS the lowest (17.5%). The table below allows comparison of data reported in various studies.

	Inorganic Phosphorus		
	Present Study	*Bourdet* et al. (9)	*Watson* et al. (6)
	mg./100 g. dry gluten	*mg./100 g. dry gluten*	*mg./100 g. dry gluten*
Minimum	39.6	21.2	20.6
Maximum	111.3	77.7	35
Mean	77.1	46.2	14.5
	% total P	*% total P*	*% total P*
	31.8	19.4	14.5

In most samples of the present study, inorganic P is the major phosphorus compound, and on an average its percentage is about twice those reported elsewhere.

Phytic P present in glutens shows noticeable variations between the types of wheat, as indicated in the following mean values.

Wheat	*Phytic Phosphorus*	
	mg./100 g. dry gluten	*% total P*
SW	17	7.2
SRW	4.4	1.9
HRW	43.2	18.5
HRS	61.7	24
Durum	18.6	7.5

Hard wheat glutens, and particularly hard spring, have phytic P percentages which are three times those of soft and durum samples; values in SRW are peculiarly low.

In the present study, the phytic P mean content is 29 mg./100 g. dry gluten (extreme values 3.4 and 86.6); i.e., 11.8% total P (1.4–30.8%). Corresponding values for French samples (9) are 91 mg. (49.9–124.1) and 38% total P (30–46%); i.e., three times as much.

In the study concerning 40 HRW samples (6), it was reported that the phytic P content of glutens was too low to be determined. The present data show that, among the various samples tested, only glutens extracted

from SRW flours are particularly poor in phytic phosphorus.

In percent total P, the mean proportions of lipid P are respectively 18.2 for soft, 15.7 for hard, and only 12% total P for durum samples. Comparable data concerning lipid P in glutens are given below.

	Lipid Phosphorus		
	Present Study	*Watson* et al. (*6*)	*Bourdet* et al. (*9*)
	mg./100 g. dry gluten	*mg./100 g. dry gluten*	*mg./100 g. dry gluten*
Minimum	30.5	12.3	31.9
Maximum	45.8	19.2	53.2
Mean	38.1	15	46.8
	% total P	*% total P*	*% total P*
	15.9	19.7	7.9

Nucleic P appears quantitatively the most important organic phosphorus compound in glutens. Expressed in mg./100 g. dry gluten, it varies between 54 (Lucas) and 97 (Wells). With regard to total P, its mean proportions are respectively 27.3 (soft), 31.1 (hard), and 37.5% (durum).

Contents of DNA P are relatively constant with the mean values 26.5 (soft), 22.5 (hard), and 24.5 (durum) mg./100 g. dry material. Nevertheless, proportions of DNA P in nucleic P are different according to the type of wheat flour: in glutens from soft, 42% of nucleic compounds are DNA, whereas the rate is only 30% in hard and 26% in durum.

The respective concentrations of both nucleic types in glutens seem to differentiate the classes of wheat as shown by RNA/DNA ratio values: 1.40 (soft), 2.30 (hard), and 2.80 (durum).

In Table VII are compared data of the present study and data recently

TABLE VII

NUCLEIC COMPOUNDS IN GLUTENS EXTRACTED FROM WHEAT FLOURS. VALUES REPORTED IN DIFFERENT STUDIES

	PRESENT STUDY (10 SAMPLES)	BOURDET *et al.* (9) (10 SAMPLES)	MIHAILOVIC *et al.* (8) (17 SAMPLES)	WATSON *et al.* (6) (40 SAMPLES)
	Phosphorus, mg./100 g. dry gluten			
Total	241.5(213–281)	236.3(154–293)	390 (240–560)	191.5(168.5–218)
Total nucleic	74.2(54.1–97.1)	52.2(39.4–73.1)	82.5(53.8–120.6)	114 (104–125)
RNA	49.7(28.8–73.3)	20.7 (7.6–37.4)	40.9(20.9–60.6)	.....
DNA	24.5(21.3–28.6)	31.6(17.9–45.5)	41.6(16.9–65.3)	.....
	Phosphorus, % total P in gluten			
Total nucleic	30.8(23.9–41.6)	22.6(16.7–30)	22 (9.6–39)	59.8(50–67.7)
RNA	20.5 (12–31.5)	9.2 (3–16.5)	10.9 (5.3– 22.4)	.....
DNA	10.2 (9.4–11.7)	13.4(10.6–18.1)	11.1 (3.7– 18.1)	.....
RNA/DNA	2.07(1.03–3.08)	0.73(0.31–1.57)	1.10(0.34)–2.79)	.....

reported elsewhere. Average values reported by Watson *et al.* for total nucleic P appear higher than other published values. As total P content in gluten is low, the proportion of nucleic P rises near 60% total P and is two

or three times that reported in other studies. Total nucleic P in glutens from French wheats (52.2 mg./100 g.) is lower than in Italian and Yugoslav samples (82.5 mg.) but corresponds to a close mean concentration (22.6 and 22% total P). Similarly, proportions for RNA P (9.2 and 10.9% total P) and DNA P (13.4 and 11.1%) are very near.

A large diversification in the U.S. samples may explain that RNA P mean concentration (20.5% total P) is about twice that reported for European wheats, when DNA P (10.2%) is slightly lower. The RNA P/DNA P ratio values reflect the dissimilarities in nucleic composition of glutens; below 1 for French samples, they are between 1 and 2 in other series.

It was previously assumed that the RNA/DNA ratio (or its inverse) may reflect genetical features of wheats (9). Further studies came to the conclusion that the ratio of nucleic acids in the grain could not be specific for a wheat variety, since it is affected by agronomical conditions of growing (8). Complementary studies will be necessary to corroborate this fact, the theoretical and practical importance of which in wheat production is evident.

Residual Phosphorus. Determination of nucleic P by difference in Watson's work allows one to account for 100% gluten total phosphorus. In the present study, the sum of the various P experimental values reaches between 83% (Omar) and 96.5% total P (Bison). The residual unknown P fraction accounts, on an average, for 9.5% total P; it is lower in durum gluten (6.5%) than in hard (8.1%) and soft samples (12.5%). One may suppose it concerns phosphoprotein components.

Figure 4 gives a comprehensive view of the experimental data reported above.

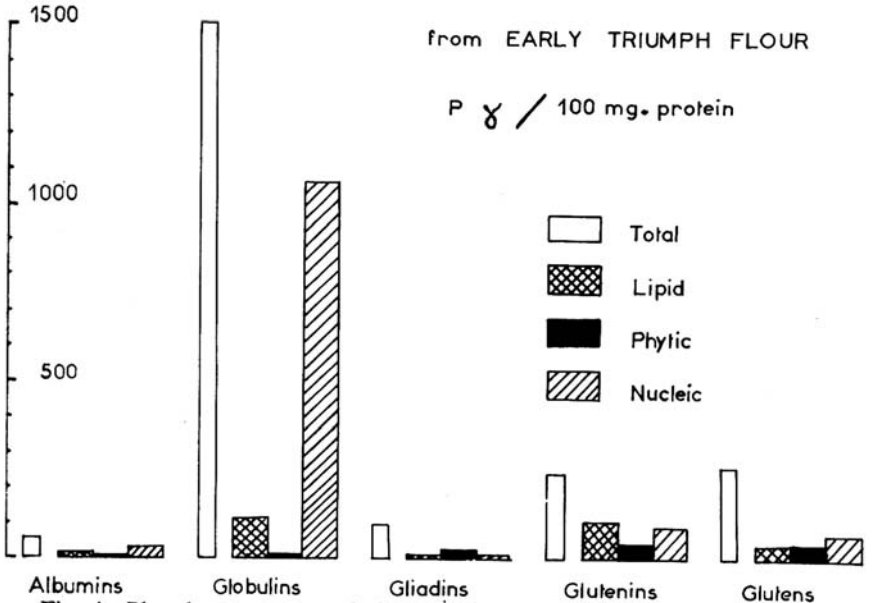

Fig. 4. Phosphorus compounds in protein fractions and glutens isolated from a hard red winter flour.

From the present study, more precise information becomes apparent about phosphorus compounds present in the protein material of wheat flours.

The high P content of the water-insoluble fraction of salt-soluble proteins was reported several times. Neufeld and Mecham (13), after dialysis of a 0.75*M* $MgSO_4$ flour extract, have isolated protein fractions containing 0.5 to 2% P. Direct heating of the salt solution precipitated a protein material, with a 9% total P content and containing phytic and nucleic compounds. The globulin fraction, water-insoluble after dialysis, which was isolated from water extracts of corn by Craine and Farenholtz (37), contained 1% P as such and 0.8% after 0.5% NaCl extraction. Rondelet and Lontie (38), from salt extracts of barley, have stated that the amount of P precipitated with globulins during dialysis depended on speed of protein insolubilization. Considering that the precipitated P was phytic, they made dialysis in a slightly alkaline medium (pH 7.5) against a low-ionic-strength buffer to eliminate phytates. Nevertheless, they did not specify the quantity and type of phosphorus compounds contained in the globulin fraction recovered after a last dialysis against water. In the author's mind, phytates share in insolubilization of proteins.

This conclusion is not apparent from the present data; on the contrary, our data agree favorably with Djurtoft's observations (38). This author has stated indeed that, after dialysis of a salt extract of barley, the water-insoluble fraction contains only 7% of the extracted phytates when the soluble one is entirely free.

Our data show that no detectable amounts of phytates are present in globulins after dialysis, whereas nucleic P accounts for near 80% total P. The presence of nucleic compounds in globulins was indeed previously indicated. Äyräpää (for barley, 30) and Matsushita (for wheat, 31) suggested that the globulin fraction, compared with albumin, was rich in nucleic compounds.

Similarly for gluten proteins, the disproportion in nucleic composition of gliadins and glutenins evidenced in the present study is in good agreement with other previous observations. According to Matsushita (31), the alcohol-soluble fraction of wheat flours has a nucleic acid content lower than that of the alkaline-soluble fraction. Kondo and Morita (39) have stated that rice glutelin contains 2.65% nucleic acids, and subscribed to its nucleoprotein nature. Preliminary observations by the same authors about wheat glutenin have been specified elsewhere (31): the precipitate (F fraction) obtained by neutralizing an alkaline extract of gluten, previously alcohol-extracted, contained 1.77% RNA. Though values obtained in the present study appear lower (1.2%), they confirm that nucleic compounds in gluten are preferentially associated with the glutenin fraction.

II. Distribution of Flour Phosphorus Compounds between Protein Fractions and Glutens

The reported data allow one to specify the qualitative and quantitative distribution of phosphorus in the protein material comparatively between different types of wheat, as expressed in Table VIII.

Total P

On an average, 6.3% total P is recovered in salt-soluble proteins, 14.2%

TABLE VIII

DISTRIBUTION OF FLOUR PHOSPHORUS COMPOUNDS BETWEEN PROTEINS AND GLUTENS IN DIFFERENT TYPES OF WHEAT

(Phosphorus, % corresponding P in flour)

Type of Phosphorus	Protein Fractions	Soft Wheats		Hard Wheats		Durum Wheats		Mean Values	
Total	Albumins	0.80		0.65		0.70		0.70	
	Globulins	6.25	7.05	5.05	5.70	5.50	6.20	5.60	6.30
	Gliadins	6.10		5		4.10		5.30	
	Glutenins	6.60	12.70	10.2	15.20	11	15.10	8.90	14.20
	Glutens		18.80		28.20		31.90		25.20
Lipid	Alb. globul.	3.55		3.10		2.55		3.20	
	Glutenins		30.30		50.10		52.90		42.70
	Glutens		26.40		36.30		30.90		31.30
Phytic	Alb. globul.	traces		traces		traces		....	
	Gliadins	3.7		4.4		2		3.8	
	Glutenins	4.7	8.4	6.5	10.9	11	13	6.6	10.4
	Glutens		2.8		17.5		7		9.5
Nucleic	Globulins	30.6		24.7		23		26.7	
	Gliadins	4.1		5.1		5.8		4.8	
	Glutenins	19.3	23.4	26	31.1	19.7	25.5	22.1	26.9
	Glutens		32.5		54.9		60.5		47

in gliadins and glutenins, and 25.2% in gluten. The higher total P concentration in glutens originates probably from inorganic phosphates which remain adsorbed after washing when they are eliminated from gliadins and

glutenins after dialysis. Furthermore, as reported in previous studies and confirmed here by electrophoretic data, salt-soluble proteins (and mainly globulins), not entirely eliminated during washing, also may be responsible for the high proportion of flour total P recovered in glutens.

Comparatively between wheats, percentages of flour total P recovered in salt-soluble proteins are similar: values for albumins are about 0.7% (0.65 to 0.80), whereas for globulins, which account for only 4% flour proteins, the corresponding values reach 5.6% (5.05 to 6.25). Because they are quantitatively predominant, gluten proteins contain 14.2% flour total P, 8.9% of which belong to glutenins. As the protein content in flours increases, the proportions of flour total P in glutenins increases, whereas that in gliadins decreases.

Lipid P

Only 3.2% flour lipid P is present in salt-soluble proteins, whereas glutenins contain 42.7% and glutens 31.3%. Apparently, values in salt-soluble proteins decrease slowly as flour protein content increases. On the contrary, the proportion of flour lipid P recovered in glutenins increases with flour protein content: 30.3% in soft samples, 50.1 and 52.9% in hard and durums respectively. Proportions of lipid P in glutens evolve in the same order, but are appreciably lower. This may be ascribed to an incomplete extraction of lipids by the solvent system used and to more or less important losses of gluten during washing. In spite of this it can be seen that phospholipids in gluten are associated as a whole with the glutenin fraction.

Phytic P

As indicated before, salt-soluble proteins are practically free of phytates, although very low amounts are detected in gliadins and glutenins. Only 3.8% flour phytic P is recovered in gliadins and 6.6% in glutenins, i.e., 10.4% in whole gluten proteins. The corresponding mean value obtained directly from glutens is very near, 9.5%. Nevertheless, proportions in phytic P, determined for each type of wheat comparatively from gliadin and glutenin fractions and from glutens, differ appreciably. Even if gluten recovery is not quantitative, it seems that flour phytates are either adsorbed (hard wheats) or eliminated during washing (durum and soft wheats). In fact, when specifically and quantitatively extracted, gluten proteins from hard and durum samples appear to retain relatively higher amounts of flour phytates than the soft ones; but in all cases, values remain very low.

Nucleic P

Though globulins account for only 4% flour proteins, by themselves they contain 26.7% flour nucleic P. That proportion varies according to the type of wheat and seems to decrease when the protein content in flour is increased; mean values are 30.6% for soft wheats and only 24.7 and 23% for hard and durum samples. Gluten proteins (81% flour proteins) retain on an average as much flour nucleic P (26.9%) as globulins. By itself, the glutenin fraction (38% gluten proteins) contains 22.1% flour nucleic P, whereas the gliadin one (62% gluten proteins) contains only 4.8%. Per-

centages of flour nucleic P recovered in both gliadins and glutenins are higher for hard (31.1%) than for durum (25.5%) or soft (23.4%). Values obtained from glutens are systematically higher than those from individual gliadin and glutenin fractions. One may observe mainly for hard and durum preparations, that percentages determined from glutens approximate values given by the sum of globulins + gliadins + glutenins. This would be additional evidence that globulins are responsible for that excess of nucleic P and remain associated with gluten proteins after washing.

The above data show that the protein fractions quantitatively extracted from flours, on the basis of criteria for solubility, account more specifically than glutens for the distribution of phosphorus compounds in flour protein material. Not only may losses occur during gluten recovery, but nongluten proteins may be also associated with the classic gliadin and glutenin fractions.

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