THE QUALITY OF FLOUR FROM WHEAT GROWN IN LIQUID CULTURES CONTAINING VARIOUS CONCENTRATIONS OF PHOSPHORUS

by

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TABLE OF CONTENTS

INTRODUCTION AND REVIEW OF LITERATURE

It has been recognized for many years that various sections of the world produce wheats differing widely in milling and baking qualities. In certain areas, typical soft wheats of low protein content and baking strength are produced which are suitable for making crackers, biscuits, or cakes. In other areas, hard wheats of higher protein content and superior baking strength are grown which are especially adapted for making bread. Of the latter, flour from spring and winter wheat is generally higher in protein and has more desirable mixing characteristics than flour milled from soft wheat, and thus produces a superior loaf of bread. These differences occur not only among wheat classes, but also among varieties in a given class, and even among samples within the same variety grown in different areas. It is generally believed that these variations in baking quality may be due to differences in the chemical and physical properties of the wheats. Because the factors responsible for these differences in quality are numerous and complex, it is difficult to evaluate correctly the role played by any one factor.

The factors which influence quality may be separated into two broad categories, genetical and environmental. Of the two, the latter appears more important. Finney *et al.* (17) observed that the total ash content of wheat is primarily controlled by environment and secondarily by variety. Schrenk and King (44) found that the mineral content of wheat was more greatly influenced by location then by variety. After working with six varieties of wheat grown at fourteen different locations in Nebraska, Sandstedt and Fortmann (42) reported that the baking properties of the flours were markedly influenced by environment. For some varieties, quality of protein appeared to be practically independent of protein content. These varieties responded in a similar manner to environmental changes, with the degree of response determined by variety.

Kraybill (29) indicated that the capacity of a given variety to produce wheat of a certain composition is fixed within certain wide limits by its genetical constitution. Within these limits, wide differences in composition may be obtained, depending upon the environment under which the plant is grown. The effects of long periods of maturity, abundant moisture, and low temperatures during the maturation period produce a wheat of low protein content because these factors favor a relatively greater abundance of carbohydrates than nitrogenous compounds. A wheat of high protein content is produced during short periods of maturity, warm weather, and moderate rainfall because these factors favor the production of nitrogenous rather than carbohydrate material. The application of phosphate fertilizers decreases the protein content of the wheat by stimulating the production of carbohydrates in the developing kernel. Helgeson and Harris (24) discovered that proper manipulation of the concentration of potassium, phosphorus, and nitrogen fertilizers produced wheat varying from 11.1 per cent to 24.9 per cent protein. McCalla and Woodford (33) reflected the generally accepted view that loaf volume is highly correlated with the protein content of the wheat within any one variety, but that the regression of loaf volume on protein content varies from one variety to another.

LeClerc and Yoder (30) demonstrated the influence of climate on the properties of wheat with an experiment involving soils from California, Maryland, and Kansas. Soil from two states was shipped to the third state and the same variety of wheat was grown on each of the three plots for four years. The results indicated that climate was the principal factor influencing the protein content and test weight of the wheat. Beeson (5) concluded that uptake of available nutrients by the wheat plant is affected by variety and soil moisture as well as by the relative ratios of the elements in the soil. Brenchley and Hall (8) observed that for the filling of the endosperm, each plant possesses a special mold. The uniform material, possessing the same ratio of nitrogenous to non-nitrogenous materials and ash, moves continually into the grain. The character of this pattern is determined by such factors as variety, soil, and season. Bequette (6) and Watson (51) discovered that both location and variety were significant in determining the concentration of phosphorus in flour and wheat, although location appeared to be more important.

Many workers, including Kent-Jones and Amos (28), Swanson (47), Bailey (4), and Sullivan (46), agree that phosphorus is one of the elements present in high concentrations in the ash of both wheat and flour. Rogozinski (40) found that total phosphorus content decreased markedly as the milled product became more refined. Masoni (32) reported that the total amount of phosphorus is relatively scarce in patent flour and increases continuously as the grade lowers in the secondary products, until it becomes a maximum in bran. Proskuryakov and Temerin (39), however, reported only a slight correlation between the ash and phosphorus content of wheat and its products.

In a study of three varieties of wheat grown over a three-year period at thirteen different locations in Kansas, Schrenk and King (44) reported that the ash and mineral contents varied appreciably and were correlated with available soil nutrients. Areas producing wheat with high mineral content did so consistently, indicating that differences due to rainfall and other environmental factors during the period did not affect the mineral content significantly. The increased ash content of Western Kansas wheats was the result of combined increases in each of the major ash constituents. Schrenk (43) showed in a later study that non-nitrogenous fertilizer applications caused a decrease in the protein content, but an increase in mineral content. The application of these fertilizers resulted in a limited amount of soil nitrogen, which thus became the limiting factor. Holdefleiss (26) agreed that the application of phosphate fertilizers had a tendency to decrease the protein content. Rosenbluh (41) believed that phosphate fertilizers reduced the protein content by altering the physiological balance between nitrogen and phosphorus. Ames and Boltz (2) found that the addition of phosphate fertilizers increased the size and plumpness of the kernel. The addition of phosphorus without nitrogen increased the yield, but decreased the protein content. They obtained the highest protein content from wheat grown on soil deficient in phosphorus, but well supplied with nitrogen. El Gindy *et al.* (11) noted little effect of soil or fertilizer treatment on milling and flour yield. Total ash was increased by nitrogen, potassium, and phosphorus fertilization. The amount of specific elements in the ashes of wheat and flour fractions varied greatly among varieties, and there was no consistent relationship among elements. This was attributed to a complex heredity background influencing the composition of the ash. Davidson and Shollenberger (9) applied sodium nitrate to wheat plants in the fall and early spring, but the quality or composition of the grain was not greatly affected. Application at heading time, however, increased the protein content and greatly improved the quality. When Borasio (7) measured flour quality with a Pneumodynamometer, he noted no great differences as a result of different fertilizer applications. Similarly, Neuman and Lemmerzahl (34) reported that the baking quality of flour from grain grown with commercial fertilizers was not significantly different from that of unfertilized wheat, and on the average was not inferior. They did not express the concentration of fertilizers used. Gericke (20) found that nutrients absorbed early are used in vegetive development and those absorbed late are not capable of causing new growth, but rather improve the physical character and composition of the kernel.

Thomas (50) discovered that if either nitrogen, potassium, or phosphorus in the soil was decreased below its critical concentration, the uptake of the other two elements would likewise be decreased. This condition resulted in a disturbance of the physiological balance and produced decreased yields. In another study, Thomas (49) found that when only two of the critical fertilizer elements were applied to an infertile soil, the uptake of the third element was depressed, but increased on a more fertile soil. In experiments with maize, Pierre and Parker (37) observed that the plants absorbed all the inorganic phosphorus from either the soil extract or soil solution. When organic phosphate was the only source of phosphorus, plants made no growth. The growth was proportional to the amount of inorganic phosphate added. Margulis (31) reported that the baking strength of flour milled from wheat grown in seven different nutrients was less highly correlated with the dibasic than with the monobasic phosphorus.

Numerous studies have been made in attempting to eliminate the variables due to environment and to determine various fertilizer effects by growing wheat under liquid or semi-liquid culture conditions. Duley and Miller (10) grew plants in sand cultures with variations of optimum and minimum nutrient solutions during various combinations of three, thirty-day growth periods. The second thirty-day period was found to be most important for vegetative growth. Top growth was increased by an optimum supply of nutrients regardless of period, while minimum nutrients led to fibrous root development. In general, the composition of the grain was approximately proportional to the supply of nutrients during the period previous to harvest. After studying growth effects of varying concentrations of phosphorus in liquid cultures, Parker (35) proposed that for solutions of low phosphate concentrations to be favorable for proper growth, the plants must be regularly supplied with large volumes of solution per plant. In another study, Parker and Pierce (36) indicated that some root-soil contact was necessary for proper phosphorus nutrition.

Gericke (21), in growing wheat hydroponically found that nitrogen in the form of nitrates was substantially superior to that in the ammonia form. Accordingly, wheat grown in solutions containing ammonium nitrate produced the poorest loaf of bread, although its volume was the greatest due to the unusually high protein content of the flour. In another hydroponic study, Gericke (22) exposed wheat to various chlorides during the latter period of growth. After adjustments to the same protein content were made, he found that the bread scores were highest from the wheat grown in solutions containing calcium chloride. Less favorable effects were observed from sodium, magnesium, and potassium chlorides.

The literature clearly indicates that flour ash is correlated with wheat ash, and that phosphorus is one of the principal elements present in the ash. Since the quality of low ash flours is generally better than that of high ash flours, one might assume that the better the flour quality, the less the total phosphorus content of the flour. Working (52) discovered that if phosphatide was added to flour, a deleterious effect on baking quality would result, while the removal of the phosphatide restored the original baking quality. Bequette (6) and Watson (51) studied extensively the relationship of total phosphorus to flour quality and found a negative correlation between flour quality and total phosphorus content of the flour. The object of the present study was to further define the effect of phosphorus on flour quality, using wheat grown in hydroponic solutions containing different amounts of phosphorus.

MATERIALS AND METHODS

Variables due to environmental fluctuations were reduced by growing the wheat under near constant conditions afforded by liquid culture in a greenhouse. The equipment was patterned after that described by Grossman <u>et al</u>. (23). The bottom of the seed frame was constructed of 0.25 inch mesh galvanized hail screen to facilitate adequate growing space for the roots. Cheesecloth was placed directly on the screen to hold the seeds and vermiculite. The distilled water used throughout these experiments was periodically analyzed and found to be free of phosphorus.

Two varieties of hard spring wheat were used in the phosphorus study, Thatcher and Pusa 52 x Federation. The latter is a white Indian wheat found to be well adapted to greenhouse conditions. The seeds were soaked overnight in distilled water and, prior to planting, were soaked for one hour in a 0.1 per cent solution of formaldehyde to destroy molds (19). The seeds were than dried between layers of absorbant paper and dusted with Spergon [1] to reduce the incidence of molds and fungi. After the seeds (approximately 375 of Thatcher, or 200 of Pusa 52 x Federation) were uniformly distributed on the cheesecloth on the seed frame, they were covered with one inch of vermiculite to retain

[1]U. S. Rubber Company, New York, N. Y.

moisture and exclude light. The seed frame was placed over the germination tray, which had previously been filled with vermiculite. As much water as possible was added, and after several minutes of soaking, the excess water was drained off.

When the plants were ten days old, the seed frame was removed from the germination tray, the excess vermiculite gently brushed from the roots, and the frame containing the plants was placed on top of the hydroponic tank. The appropriate nutrients were added to the tank, which was then filled with phosphorus-free water.

Three concentrations of phosphorus were used. A modification of Hoagland's Solution I (25) was used to provide the highest level of phosphorus. Results from a preliminary small-scale experiment, as well as those obtained by Seidman (45), indicated that for wheat the highest level of phosphorus should consist of only one-half the amount recommended by Hoagland and Arnon (25). Furthermore, the magnesium sulfate concentration should be reduced by one-half. The low level of phosphorus consisted of one-eighth the amount of phosphorus in the highest level, and the third hydroponic solution contained no phosphorus. Iron, in the form of Versenol iron chelate[2] was added to each tank at the rate of 53 milligrams per liter of nutrient solution. Each week the tanks were drained and fresh solutions added. The solutions were adjusted to pH 5 to 6, since Tarr (48) obtained maximum growth of wheat seedlings at this range. Once each week the plants were dusted with powdered sulfur to prevent the elaboration of mold and fungi. A dilute solution of

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[2] Dow Chemical Company, Midland, Michigan.

nicotine sulfate was sprayed over the plants as necessary to kill aphids.

Twelve tanks were planted with Thatcher wheat on February 7 and the wheat was harvested on June 13, 1961. The temperatures during this period ranged from an average minimum of 62°F. to an average maximum of 76°F. Eight tanks were planted with Pusa 52 x Federation on July 10 and harvested November 17. The average temperatures during the early growth of the plants were considerably warmer than during the same growth period of the Thatcher wheat, despite attempts to lower the temperature of the greenhouse by using whitewash on the glass panes and also by using evaporative water coolers. The temperatures ranged from an average minimum of 70°F. to an average maximum of 84°F.

A small-scale experiment, in which wheat plants were grown in hydroponic solutions in two-quart jars, was conducted simultaneously with the second planting to determine the effects of various concentrations of nitrogen on plant growth and on the protein content of the wheat. The variety used in this study, Pusa 52 x Federation, was grown according to the procedure described by Seidman (45). The previously mentioned modification of Hoagland's Solution I (25) was used to provide the highest nitrogen level. One level contained two-thirds of the highest amount of calcium nitrate, another level contained one-third of the highest amount of calcium nitrate, while the two remaining levels were completely lacking in calcium nitrate. One of the latter levels contained only one-half the regular amount of potassium nitrate. Sufficient monobasic calcium phosphate was added to those levels deficient in calcium nitrate to replenish the calcium. The loss of potassium in the solution with the lowest nitrogen concentration was replenished by an appropriate quantity of potassium sulfate.

The mature Thatcher grain was threshed between two boards covered with corrugated rubber matting and milled on a Hobart grinder by the method of Finney and Yamazaki (18). Since the yield of the Pusa 52 x Federation wheat was so low, the heads were harvested individually and the grain was separated from the chaff by hand. Analyses of ash, moisture, and protein contents were determined according to the methods of the AACC (1). The water-soluble protein in the flours were determined by analysis of an extract prepared essentially as a blank without hemoglobin described in the method for the determination of proteolytic enzyme activity by the AOAC (3). One method used trichloroacetic acid buffer; the other method employed a buffer consisting of a mixture of mono- and dibasic potassium phosphate (see appendix).

The procedure of Johnson _et al._ (27) was used in preparing the mixogram curves for the Thatcher sample.

The total phosphorus determinations of both the wheat and flour samples were made according to the colorimetric method described by Pons _et al._ (38).

Because of the limited amount of sample flours available, it was necessary to blend the samples with a high quality hard winter wheat flour in a 25:75 ratio of sample flour to the blending flour. The blended samples were studied for their baking quality characteristics according to the straight dough procedure of Finney and Barmore (14, 15). The resulting loaves of bread were expected to exhibit properties proportional to the components of the blend. Hypothetically, if the blending flour produced loaves of 980 cc. volume, it would contribute three-fourths of the 980 cc., or 735 cc. of the volume of the loaf baked from the blended flour. If the volume of the loaf from the blended flour was 900 cc., the volume contributed by the sample flour portion of the blend would be 165 cc. Since the sample flour contributes one-fourth of the total volume, the expected volume of the sample flour if baked individually would be 4 times the 165 or 660 cc.

The regression coefficient of loaf volume on protein content may be considered a measure of quality of protein _per se_ (16); the largest values denote the best quality of protein. The values for an individual sample can be established from knowledge of the loaf volume and protein content with reference to an established set of regression lines as published by Finney (13). Thus, if the protein content of a flour was 15.1 per cent and the loaf volume 900 cc., the regression coefficient as determined by the graph would be 49 cc. for each per cent protein. By use of this procedure, the volumes of the experimental loaves were adjusted to a common protein level. Similarly, the quality of the protein may be expressed by adjusting all loaf volumes to a common protein level by use of the regression coefficient. For this work all loaf volumes were adjusted to a common protein level of 20.0 per cent.

RESULTS AND DISCUSSION

Preliminary data using hydroponic solutions were obtained using Pusa 52 x Federation wheat. Although this is an excellent variety to grow in the greenhouse, not enough wheat was available for evaluation of its baking quality characteristics, and no quality data could be found in the literature. Since it was expected, based from the data of Bequette (6), that high concentrations of phosphorus would produce grain with inferior baking quality, experiments were designed in which wheat was grown in solutions of varying phosphorus concentrations. Thatcher wheat was selected for it normally produces good baking quality flour, yielding an excellent farinograph curve. Furthermore, since it is a spring wheat, it does not require vernalization before growing in the greenhouse.

The first noticeable evidence of a phosphorus deficiency in the Thatcher wheat plants occurred after one month of growth. Figure 1 illustrates the growth of the plants after 43 days. In solutions lacking phosphorus, the plants continued to be short with little top or root growth, produced very small heads, and matured at an earlier date than the plants growing in the solutions containing phosphorus. The only visible difference in the plants grown in the two solutions containing phosphorus was the fact that the plants grown in the solutions containing the most phosphorus tillered more. Little difference in the root systems of plants grown in the two solutions containing phosphorus was observed. Even though both sets of plants containing phosphorus began to flower about the same time, the plants grown in the lower concentration of phosphorus began to produce heads one week earlier than the plants grown in the higher concentrations of phosphorus. Although little difference in the physical properties of the heads

Fig. 1. Thatcher wheat plants after 43 days of growth.

The phosphorus concentrations, expressed in mg./l., are from left to right:

Back row - 0; 8.5; 68; 0; 8.5; 68. Front row - 68; 0; 8.5; 0; 8.5; 0.

was noticed, the plants grown in the solutions containing the most phosphorus generally produced a second head, whereas the plants grown in the lower concentrations of phosphorus yielded single heads. However, the second heads produced no seeds. Both sets of plants grown in solutions containing phosphorus began maturing about the same time, but the plants grown in the solutions containing the most phosphorus required a longer time for complete maturation.

Most of the heads on the Thatcher plants grown in the solutions lacking phosphorus contained only one kernel. Without exception, these kernels were abnormally small, shriveled, and light in weight. The heads on the plants grown in solutions containing phosphorus yielded a normal number of kernels, but their physical appearance closely resembled those kernels from the plants grown in the solutions lacking phosphorus. Because of the extremely high temperatures in the greenhouse at the beginning of their growth, the Pusa 52 x Federation plants grew very fast and soon became rank. It was then necessary to provide support to each plant. After one month's growth, the plants lacking phosphorus were shorter and had less foliage than the plants grown in the solutions containing phosphorus. After another month's growth those plants grown in the solutions containing high phosphorus concentrations exhibited good healthy growth compared to those plants grown in solutions containing minimal phosphorus. The phosphorus level in solutions was related to the maturity date. The plants grown in solutions lacking phosphorus matured in 90 days, the plants grown in low phosphorus concentrations required 120 days while 150 days were required for the plants grown in the high phosphorus concentration.

In contrast with the Thatcher wheat grown, the Pusa 52 x Federation wheat was generally plump. Even though the heads looked healthy, many of the spikelets were not filled with grain.

In the small-scale experiment, the plants grown in the solutions lacking calcium nitrate were short and produced no grain. There was no significant difference in the growth of the plants to which nitrates had been added, regardless of concentration. These plants appeared to produce more foliage and heads that were plumper than did the plants growing in the phosphorus study. However, the yield per plant was not any better. Furthermore, there was no difference in the protein content of these samples, each of them being high and in the range of 23.6 to 23.7 per cent.

Since the quantity of the Pusa 52 x Federation was so limited, the wheat was neither milled nor baked as for the Thatcher samples, but was examined for quality characteristics by the micro technique of Elling and Barmore (12).

Table 1 summarizes the physical results of the plot yields from the wheat.

Data in Table 2 shows the ash and protein contents of the wheat and flour from the grain containing varying amounts of phosphorus. As expected, the protein and ash contents of both wheat and flour were very high. Due to insufficient sample, neither the Thatcher wheat grown in the solutions lacking phosphorus nor the Pusa 52 x Federation wheat was milled.

Table 1. Summary of yield data.

Level of Phosphorus	Total Number of Heads	Total Weight	1000 Kernel Weight	Test Weight
		g.	g.	lbs./bu.
Thatcher				
Full	965	261.7	14.7	48.5
Low	1265	353.5	16.5	53.0
Zero	1375	9.3	7.9	48.3
Pusa 52 x Federation				
Full	160	15.0	23.4	——
Low	435	13.7	23.9	——

Table 2. Analysis of wheat and flour for protein and ash content.

Level of Phosphorus	Protein Wheat	Protein Flour	Ash Wheat	Ash Flour	Protein Wheat	Ash Wheat
	%	%	%	%	%	%
	Thatcher				Pusa 52 x Federation	
Full	21.4	23.3	3.00	1.12	23.9	2.63
Low	23.8	24.9	2.28	0.82	25.3	2.02
Zero	26.8	——	2.02	——	——	——

The phosphorus contents of the wheat and flour are presented in Table 3. Both the Thatcher and Pusa 52 x Federation samples possessed comparable phosphorus concentrations which were related to the phosphorus level available to the plants during growth. It is evident that as the protein content increased, the phosphorus content decreased. This was in agreement with the work of Holdefleiss (26), Ames (2), Kraybill (29), Rosenbluh (41), and Schrenk (43). It is also evident that as the concentration of phosphorus increased, the ash in both the wheat and flour also increased. This was to be expected, since phosphorus is generally recognized as the principal inorganic element in ash, as reported by Bailey (4), Bequette (6), Schrenk (43), Swanson (47), Kent-Jones and Amos (28), and Sullivan (46).

It is widely recognized that the baking test is the best single measure of flour quality. Results from baking the flour from the Thatcher samples are presented in Table 4. The regression coefficients of loaf volume on protein content appear to be lower than might be expected from Thatcher flour. Even though all samples appeared to have a low quality of protein there was a definite difference between the regression coefficients of the flours containing varying concentrations of phosphorus. The flour containing 1.9 mg. phosphorus per gram of flour showed a regression coefficient of 40, whereas a regression coefficient of 34 was calculated from the flour containing 2.6 mg. phosphorus per gram of flour. Since a negative relationship existed between total phosphorus content and loaf volume or regression coefficient, the quality of bread

Table 3. Analysis for total phosphorus content.

Level of Phosphorus	Thatcher Wheat	Thatcher Flour	Pusa 52 x Federation Wheat
	mg./g.	mg./g.	mg./g.
Full	6.7	2.6	6.6
Low	4.4	1.9	4.5
Zero	0.8	——	——

Table 4. Summary of baking data (Thatcher Wheat).

Level of Phosphorus	Flour Protein	14% M. B. Absorption	Mixing Time	Volume As Rec'd	Regr. Coef.	Corrected Vol. 20%
	%	ml.	min.	cc.		cc.
Blended						
Full	15.2	64.2	2.5	995	58	1275
Low	15.6	63.3	2.2	1035	60	1300
Non-blended (calculated)						
Full	23.3	72.5	1.6	1020	34	890
Low	24.9	68.7	1.8	1176	40	985

baked from the flour of high phosphorus concentration was slightly inferior to that from the flour of low phosphorus concentration.

The mixograms obtained from the Thatcher flour samples are presented in Figure 2. Although the differences in these two mixograms are slight, certain differences are discernable. The flour from the high phosphorus level appeared to have more plastic-like properties than the flour of lower phosphorus concentration. The latter flour appeared to withstand excessive mixing slightly better than the former.

After being tempered at 40°F. for three weeks, the two Pusa 52 x Federation samples were milled on a micromill. Because of the identical yields of meal, it was concluded that the amount of phosphorus in the wheat had no effect on milling properties.

When the meals were made into dough balls and allowed to expand according to the method of Elling and Barmore (12), the sample containing the higher level of phosphorus exhibited a slightly higher expansion volume than did the sample containing less phosphorus. This test was developed on the concept that dough expansion is highly correlated with gas retention, which, in turn, is recognized as being highly correlated with loaf volume. Since in this research quality was determined entirely on loaf volume, the results using the Pusa 52 x Federation variety suggest that the wheat containing more phosphorus displayed the stronger baking properties. However, the quality differences between the two samples were very slight and may have actually been due to certain uncontrollable factors, rather than differences in the phosphorus concentrations.

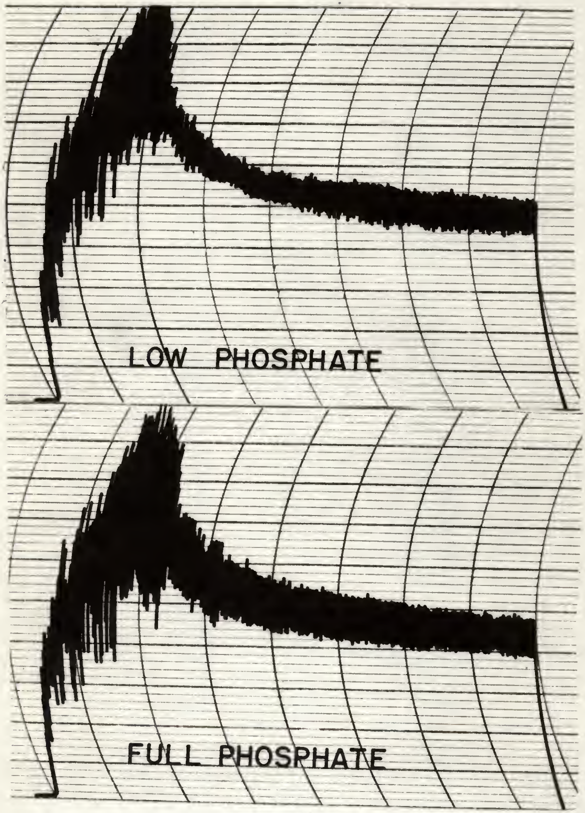

Fig. 2. Mixograms from Thatcher samples.

From the Thatcher loaf volume data, it is evident that the flour protein was not entirely glutenous. The results of analyses for water-soluble protein (Table 5) indicate a high percentage of water-soluble protein in flour of wheat grown in both the high and low concentrations of phosphorus. Considering the results obtained by using the phosphate buffer, the flour with the highest phosphorus content would contain 19.9 per cent water-insoluble protein, while the flour of low phosphorus content would contain 21.3 per cent water-insoluble protein. The loaf volume data suggest that these calculated amounts of water-insoluble flour proteins include an undetermined percentage of insoluble, non-glutenous protein. An insufficient amount of Pusa 52 x Federation sample was available for its determination of water-soluble protein content.

Table 5. Summary of the water soluble protein content of the Thatcher flour samples.

Level of Phosphorus	TCA Method	Phosphate Buffer Method
	%	%
Full	1.1	3.4
Low	1.4	3.6

SUMMARY AND CONCLUSIONS

This research demonstrated the importance of phosphorus to the growth of wheat plants. When the amount of phosphorus in the culture medium was limited, the plant growth was so stunted and hindered that the heads produced shriveled, if any, grain. The maturation time was found to be dependent upon the level of the phosphorus in the medium.

Growth appeared to be more abundant when the plants were grown in glass containers than when grown in galvanized containers coated with asphalt. In many instances, the asphalt cracked and peeled off, exposing the bare metal surfaces. This allowed an interaction between the zinc of the container and certain chemicals in the solution. Further harmful effects may have been due to certain toxic substances in the asphalt paint.

The amounts of ash and protein in all the samples were much higher than would be expected from the same wheat samples grown in soil plots. Conditions apparently favored the production of nitrogenous rather than carbohydrate material. Increasing the concentrations of phosphorus resulted in decreasing the protein content and the amount of water-soluble protein, but increasing the amount of ash.

The total amount of phosphorus found was highest in the wheat grown in the solutions containing the most phosphorus.

A negative relationship existed between the total phosphorus content and loaf volume of the Thatcher samples, indicating that the flour containing less phosphorus displayed the better baking characteristics. On the other hand, the dough expansion was greater for the Pusa 52 x Federation wheat with the higher phosphorus concentration, indicating that its flour was slightly stronger. However, since the differences observed in each case were so small, it is concluded that the phosphorus content of the wheat did not significantly affect the quality characteristics of the flour.

SUGGESTIONS FOR FUTURE WORK

This investigation was designed to further define the effect of phosphorus on flour quality. The results have suggested the following possibilities for future research:

A study of the effects of environment on the baking quality of flours from wheat grown in complete hydroponic solutions in the greenhouse, in soilpots in the greenhouse, and in a field plot.

A study of the effects on flour baking quality of a varying concentration of phosphorus applied at different stages of growth to the wheat plant.

A study of the concentration of different types of phosphorus in the wheat kernel and its flour as related to different concentrations of phosphorus fertilizers.

A study to determine the minimum concentrations of phosphorus required to provide good plant and root growth, and which will yield good quality flour.

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APPENDIX

Method of Growing Plants

Hydroponic Tank Assembly.(23). The assembly consisted of a germination tray, a hydroponic tank and a seed frame. The germination tray measured 1 x 14 x 30 inches. The seed frame was one inch longer and one inch wider. The entire assembly was constructed from 22 gauge galvanized iron, except the bottom of the seed frame which was 0.25 inch mesh galvanized hail screen. This screen was covered with cheesecloth to hold the seeds and vermiculite. All joints were either riveted or spot soldered. The exposed surfaces were painted with two coats of asphalt paint to prevent interaction between the zinc-coated iron and the nutrient solutions. A 0.625 inch soft copper drain tube and a 0.25 inch soft copper aeration tube having 0.033 inch orifices at eight inch intervals were installed in the tank. The copper pieces were not painted. Aeration was provided by continuously supplying air at 20 pounds pressure to the nutrient solutions. A manifold consisted of a one inch electrical conduit in which holes were drilled and 0.25 inch copper tubes soldered at suitable intervals. Air delivered to the manifold was uniformly distributed to the various tanks by means of rubber tubing connecting the copper tubes with the aeration tubes.

Procedure. The seeds were soaked overnight in distilled water, then prior to planting were soaked one hour in a 0.1% solution of formaldehyde (19). The seeds were dried between layers of absorbant paper and dusted with Spergon[1] to reduce decay due

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[1]U. S. Rubber Company, New York, N. Y.

to fungi and mold. Approximately 375 dusted seeds were uniformly distributed on the cheesecloth on the seed frame for the experiment with the Thatcher wheat; about 200 dusted seeds were planted of the Pusa 52 x Federation variety. The seeds were covered with one inch of coarse vermiculite to retain moisture and exclude light. The seed frame was placed on top of the germination tray, which had been previously filled with vermiculite. As much distilled water as possible was added, then the excess water was drained off after about fifteen minutes of soaking.

When the plants were ten days old, the seed frame was removed from the germination tray, the excess vermiculite was gently brushed from the roots, and the frame containing the plants was set on top of the hydroponic tank. The appropriate nutrients were added to the tank, which was then filled with the phosphorus-free water obtained by condensing steam piped into the greenhouse. Iron in the form of Versenol[2] iron chelate and at the rate of 53 mg. per liter of nutrient solution was added each week to prevent yellowing of the plants.

For the Thatcher experiment, twelve tanks were installed, five containing no phosphorus, three containing the full amount of phosphorus, and four containing one-eighth the full amount of phosphorus. Eight tanks were used for the experiment using the Pusa 52 x Federation wheat, two containing the full amount of phosphorus, and three tanks each for the other two concentrations of phosphorus. The water level in the tanks was maintained by adding phosphorus-

[2] Dow Chemical Company, Midland, Michigan.

free water as necessary. Each week the tanks were drained and fresh nutrient solution added.

Hydroponic Jar Assembly. Regular two-quart Mason jars were fitted with 2-3/8 x 5/8 inch corks containing three holes for the plants and one hole for the aeration tube. The corks had been covered previously with several coats of paraffin to reduce fungus and mold growth. The jars were painted with aluminum paint to exclude light from the roots. The aeration tubes were fitted to capillary tubes extending into the nutrient solutions to protect the roots from large, uneven bubbles of air. The aeration tubes were connected to the same manifold used by the hydroponic tanks by a system of glass T-tubes and rubber tubing.

Procedure. The Pusa 52 x Federation variety was used in the small scale experiment controlling the concentrations of available nitrogen. The seeds were treated in the same way as for the phosphorus study except that the dusted seeds were distributed in a shallow glass pan, covered with vermiculite, and thoroughly watered. After ten days of growing, they were transferred to the jars. A small plug of glass wool was placed in the cork holes and around the plant stems for support. The appropriate nutrient solutions were added to the jars, which were then filled with water. Five concentrations of nitrogen were used; besides the full concentration of nitrogen were 5/6, 2/3, 1/2, and 1/4 the amount of nitrogen in the highest concentration of nitrogen. Each week the jars were emptied and fresh nutrient solution added.

Determination of Water Soluble Protein (3)

Preparation of Solutions. The concentrated acetate buffer was prepared by diluting 120 ml. of glacial acetic acid and 164 g. of anhydrous sodium acetate to one liter with distilled water. The solution used was prepared by diluting one volume of the concentrated acetate buffer with 20 volumes of distilled water.

The trichloroacetic acid solution was prepared by dissolving 180 g. of trichloroacetic acid in 320 ml. of distilled water.

For the phosphate buffer, 15.22 g. of dibasic potassium phosphate were mixed with 4.54 g. of monobasic potassium phosphate and diluted to one liter.

Phosphate Buffer Method. 5.0 g. of flour (14% M.B.) and 3 g. of fine pumice were thoroughly mixed into a 125 ml. erlenmeyer flask. 30.0 ml. of phosphate buffer were mixed in, and the sample digested one hour in a 40°C. water bath. The solution was mixed well each 15 minutes. The solutions were then centrifuged 5 minutes at 1800 rpm. and filtered through Whatman No. 5 filter paper. Aliquots of 5.0 ml. were pipeted into Kjeldahl flasks and the per cent water-soluble protein determined.

TCA Method. To the dry ingredients above was added 25 ml. acetate buffer solution that had previously been warmed to 40°C. in the water bath. After the stoppered flasks had been in the bath 15 minutes, 5.0 ml. of the TCA solution was added. The solutions were digested in the bath exactly 30 min. then centrifuged at 1800 rpm. for 5 minutes. Before filtering, the solutions were placed in a boiling water bath for 5 minutes. Stoppers containing 12 inch glass tubing lengths were placed in the flasks during the heating to serve as air condensers. The flasks were cooled 10 minutes in a cold water bath, then the solutions were filtered through Whatman No. 5 filter paper. Aliquots of 5.0 ml. were pipeted into Kjeldahl flasks for the determination of water-soluble protein.

Kheldahl determination of water-soluble protein. The regular Kjeldahl method for the determination of protein was followed with the following exceptions: 1. 25 minutes digest time; 2. 350 ml. cooling water; 3. 1.5 times the normal amount of concentrated alkali; 4. titrated with 0.0714 N NaOH; and 5. enough receiver acid to titrate 24.00 ml. of standard 0.0714 N NaOH was used.

Reduced Molybdate Colorimetric Determination of Total Phosphorus (38)

Preparation of Solutions. The concentrated reduced molybdate reagent was prepared by adding 39.12 g. of reagent grade molybdic anhydride (MoO_3) into a two liter, round-bottom pyrex flask with two necks and adding 800 ml. of concentrated sulfuric acid. A mechanical driven glass stirring rod was introduced into one neck of the flask and a thermometer through the other neck. The solution was heated with continuous stirring on an electrically heated mantle at 150°C. until solution was complete (1.5 to 2.0 hours) as indicated by a clear, greenish color. After the quantitative addition of 2.20 g. of powdered molybdenum metal (99.5% Mo), heating and stirring was resumed until solution was again complete (2 hours). The deep blue solution was cooled, transferred quantitatively to a one-liter volumetric flask and diluted to volume with concentrated sulfuric acid.

The dilute reduced molybdate solution was prepared by pipeting 10.0 ml. of the concentrated reduced molybdate reagent into a 100 ml. volumetric flask containing about 50 ml. of distilled water. Because of the viscosity of the reagent, the inside of the pipet was washed into the flask with distilled water. The dilute reagent was cooled to room temperature and diluted to volume with distilled water. A fresh solution of this reagent was prepared before each use.

The concentrated stock phosphate solution was prepared by dissolving 4.3929 g. of ACS grade, dried monobasic potassium phosphate in 300 ml. of distilled water and 200 ml. of N. sulfuric acid contained in a one liter volumetric flask. Several drops of 0.1 N potassium permanganate were added and the solution diluted to volume with distilled water. This stable solution contained 1.0 mg. of phosphorus per ml.

The dilute stock phosphate solution containing 0.01 mg. of phosphorus per ml. was prepared by diluting one ml. of the concentrated stock solution to 100 ml. This was freshly prepared before each use.

Digestion of Sample. 0.400 g. ground wheat or flour was weighed into a 100 ml. Kjeldahl flask. Three ml. of concentrated sulfuric acid and two six-mm. glass beads were added. The sample was heated until all organic material was charred and a homogeneous solution obtained. After cooling, four drops of 30% hydrogen peroxide were added and the solution was heated until colorless. Usually up to ten drops were necessary to obtain colorless solutions. The solution was heated for 10 minutes after the last peroxide addition. When cool, 20 ml. of distilled water was added and the solution boiled for five minutes to remove any remaining peroxide and to insure conversion of phosphorus to the ortho form. After cooling, the solution was quantitatively transferred to a 100 ml. volumetric flask and diluted to volume. This sample was used for the colorimetric determination of total phosphorus.

Colorimetric Determination of Phosphorus. An aliquot of 5.0 ml. of the sample solution was transferred to a 100 ml. volumetric flask. Sufficient 3.60 N NaOH was added to neutralize the acid. Two drops of indicator, 0.2% aqueous solution of sodium alizarin sulfonate, were added and the acidity adjusted with N sulfuric acid and N NaOH until one drop of the acid turned the solution yellow. The solution was diluted to about 70 ml. with distilled water. A reagent blank was prepared using the same amount of 3.60 N NaOH as for the samples and the acidity adjusted in the same manner. A 10.0 ml. aliquot of the diluted reduced molybdate reagent was added to the blank and each sample solution. All flasks were swirled and placed in a boiling water bath for 30 minutes. After cooling in a cold water bath, the reaction solutions were diluted to volume with distilled water. Color intensity was read from a Bausch and Lomb "Spectronic 20" colorimeter at 720 millimicrons. The instrument was set at 100% transmission with the reagent blank. Milligrams of phosphorus in the sample aliquot were determined by reference to the calibration curve.

Calibration Curve. Aliquots of 0.0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, and 12.0 ml. of diluted phosphate solution (0.01 mg. of phosphorus per ml.) were pipeted into 100 ml.

volumetric flasks. Two drops of indicator and one drop of N sulfuric acid were added to each flask, and the solutions diluted to about 70 ml. with distilled water. After adding 10.0 ml. of reduced molybdate solution, the procedure outlined above for treatment of the sample aliquot was followed. The standard containing 0.0 ml. of the standard solution was used to set the instrument at 100% transmission. The logarithms of the transmittance values obtained for the standards were plotted against the known phosphorus concentrations to obtain the standard calibration curve.

$$\text{Mg. total P/g.} = \frac{\text{mg. P in aliquot}}{\text{sample wt. in g.} \times \text{aliquot factor}}$$

where: sample wt. = 0.4000 g.

$$\text{aliquot factor} = \frac{5}{100}$$

THE QUALITY OF FLOUR FROM WHEAT GROWN IN LIQUID CULTURES CONTAINING VARIOUS CONCENTRATIONS OF PHOSPHORUS

by

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The effects of phosphorus on the quality characteristics of flour was investigated by growing wheat in hydroponic solutions containing different amounts of phosphorus. The varieties studied were Thatcher and Pusa 52 x Federation, both of which are spring wheat varieties. Thatcher wheat is well known for its excellent bread-making quality, but no information on quality was found in the literature concerning the Pusa 52 x Federation variety, a white wheat developed in India. However, the latter is known to grow well in greenhouses.

Three levels of phosphorus were employed to produce the wheat with varying phosphorus content. A solution regarded as a complete nutrient containing 68 mg. phosphorus per liter provided the highest level of phosphorus. A second solution containing 8.5 mg. phosphorus per liter was prepared by an eight-fold dilution. The third level contained no phosphorus. The solutions were replaced weekly to provide a constant concentration of phosphorus available to the plants.

Differences in the external appearances were observed after the plants had grown one month. As the plants became older, the foliage growth and vigor depended upon the amount of phosphorus present in the culture media. Even though plump heads were produced by the plants growing in the solutions containing phosphorus, much of the grain was shriveled and light in weight. Furthermore, the number of kernels obtained from these heads was few. Those plants growing in phosphorus-free solutions produced short heads which occasionally contained one kernel, but were more often without kernels. The amount of phosphorus uptake, as found in the grain, was a function of the amount of available phosphorus in the solutions.

The ash and protein contents of the wheat and flour were higher than would be expected from the same varieties grown on soil plots. As the phosphorus content of the samples increased, the ash content also became greater. However, the protein content, as well as the amount of water-soluble protein, decreased slightly with an increase in the phosphorus content.

Due to the limited supply of Thatcher wheat, its flour was blended in a 25:75 ratio with a standard flour for the baking test. Upon calculation of the data obtained from the blended flour into data which theoretically would have been obtained by baking the experimental flour individually, the sample containing the lower amount of phosphorus yielded loaves with slightly larger volumes than the sample with the highest level of phosphorus. The amount of wheat grown in the solutions lacking phosphorus was insufficient for milling and baking studies.

The yield from the Pusa 52 x Federation plants was even less than for the Thatcher wheat. Consequently, a micro test was employed to determine the quality. No significant differences in quality as determined by the expansion of the dough was observed.