A STUDY OF THE ROLE OF PHOSPHATIDES IN RELATION TO BAKING BEHAVIOUR OF WHEAT FLOUR

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1. INTRODUCTION

In recent years several investigators have demonstrated that the bromate baking test is a better criterion of the commercial value of experimentally milled Canadian hard red spring wheat flour than the basic method. Larmour and MacLeod (1929) concluded that the bromate formula by itself gave a better measure of baking value than the basic formula. Geddes and Larmour (1933) in an extensive paper on the bromate baking test state that the application of the basic formula of the A. A. C. C. to the evaluation of strength of Canadian hard red spring wheat has been found to give results, which considered by themselves would lead to preposterous deductions. The following comparison cited by Geddes and Larmour shows that the basic method is completely at variance with the accepted valuation of hard red spring wheat flours. Two flours containing 13.9 percent and 13.7 percent of protein gave loaf volumes of 650 c.c. and 640 c.c. respectively, by the basic method. When these flours were baked with bromate the values increased to 730 c.c. and 890 c.c. respectively. They conclude that the results obtained with 0.001 percent potassium bromate place the results at least for any given crop year into relative positions which are more in line with their commercial value.

Papers by Larmour (1931), Geddes, Malloch and Larmour (1932), Geddes and Larmour (1933), Harris (1930, 1931), indicate, that protein content and loaf volume are more highly correlated, when the bromate differential is employed, than in the case when the basic procedure is used. The results of baking tests conducted on flours experimentally milled from Western Canadian hard red spring wheats lead to the conclusion that the bromate

formula gives a much better measure of the relative strength of such flours than the basic formula.

From this it should be expected that an understanding of the constituents affected by the addition of potassium bromate to dough would materially extend our knowledge as to the fundamental nature of the term "strength". Geddes (1930) and Rich (1934) found that the addition of potassium bromate does not affect the gas producing capacity, but that it results in a modification of the gas retaining capacity of bread doughs. This leads to the conclusion that the potassium bromate exerts its effect either direct or indirectly on the gluten proteins. A great deal of evidence has accumulated indicating that the action is an indirect one, and that it acts primarily on the phosphatides; substances which appear to have a detrimental effect on gluten quality.

It has been found convenient to subdivide the relation of phosphatides to baking quality according to the following scheme:

1) Chemical Composition of Phosphatides in Wheat.
2) The Determination of Phosphatides in Wheat, and Their Distribution in the Wheat Kernel.
3) The Effect of Adding Lecithin and Lipoid Products to Flour, and its Relation to Gluten Quality.
4) The Effect of Ether Extraction on the Bread Making Properties of Flour.
5) The Effect of Aging on the Phosphatides of Flour and its Relation to the Bread Making.

The Chemical Composition of the Phosphatides of Wheat.

Winterstein and Hiestand (1907) isolated two phosphatides, one containing 2.23 percent and the other containing 2.21 percent phosphorous from wheat flour. In both cases they found that carbohydrates were combined to the phosphatide complex. They were able to isolate glycero-phosphoric acid, choline and galactose. They concluded that besides choline some other nitrogenous bases must be present. Winterstein and Smolenski (1908) state that several phosphatides exist in wheat. One of these is insoluble in acetone and soluble in alcohol. The phosphatide insoluble in acetone was hydrolyzed. Choline, ammonia, and trigonelline were identified. Smolenski (1908) isolated several solid and liquid phosphatides from wheat germ. The acetone extract gave a crystalline substance, m.p. 60°-61° containing 6.88 percent phosphorous, 2.09 percent nitrogen and 2.10 percent sugar calculated as glucose. A second crystalline phosphatide which is believed to be a pure substance softens at 63°-64°, m.p. 82°-83° and contains 5.48 percent phosphorous. Notthahn and Mayer (1934) isolated a phosphatide from wheat containing 26 percent carbohydrates calculated as glucose. They state that trigonellin is not present.

It becomes apparent that accurate information is lacking on every detail of the structure of wheat phosphatides. Neither the saturated nor the unsaturated acids have been identified. Pure lecithin has not been isolated and the presence of cephalin in the phosphatides has not been proven.

The Determination of Phosphatides In Wheat and their Distribution in the Wheat Kernel.

The first work recorded is that of Kopler (1861) who found the phosphorous content of the ether-extract of wheat oil to be 0.25-0.28 percent. Hask and Phelps (1925) showed that ether extracts less than one-half of the lipoids present in wheat flour. By extracting flour with ammoniacal alcohol they were able to get much higher results. Sullivan and Near (1927a) found that alcohol-extraction followed by ether-extraction gave results that agreed very closely with those obtained by Hask and Phelps. They referred to the alcohol-ether extract of plant tissue which has been evaporated to dryness, and then taken up in chloroform as "lipoids". This extract includes not only phosphatides but also neutral fats, small amount of fatty acids, chlorophyll and sterols.

Hend and Amos (1930) made a critical study of the estimation of fat in wheaton products. They calculated the percentage of phosphorous in fatty bodies extracted from wheaton products, by different methods. Their results are given in the following table.

Percentage of Phosphorous in Fatty Bodies Extracted from Wheaten Products by Different Methods.

(Results obtained on dry material.)

Sample	Soxhlet		Hydrolysis		
	Petroleum Ether	Ethyl Ether	Alkaline	Acid	Alcohol (Lipoid content)
Patent flour	0.30	0.27	1.14	0.21	1.07
Bran		0.11	0.48	0.13	0.34
Germ		0.28	0.53	0.15	0.48

The above results show that both the alkaline hydrolysis, and the alcohol hydrolysis method gave approximately the same values, and that their phosphorous content is much higher than those obtained by the other methods. In the case of the alcohol hydrolysis, it should be mentioned here that Hend and Amos are referring to the method specified by the Association of Official Agricultural Chemists (page 233) 1925 Edition. This method is essentially the same as that used by Sullivan and Near (1927a).

Nottbohn and Mayer (1934) determined the phosphatides of flour by hydrolysing the material with magnesium oxide and quantitatively determining the percentage choline. They state that all the choline present in flour is in the form of lecithin-choline. They obtained results which are three to four times higher than those previously reported;

the percentage of phosphatide in flour and semolina being 0.9 and 1.2 percent respectively. Sullivan and Near (1928) (1933) reported results ranging from 0.18-0.50 percent phosphorous in wheat flour. Klein and Linseer (1933) state that a preponderance of free choline over lecithin choline has been found in plants, fruits, and seeds. They also state that free choline is subject to much greater variation than lecithin choline.

Sullivan and Near (1927b) found that the glutens from clear flours contained more lipoid material than gluten from a patent flour. They also stated (1927c) that the lipoid content of wheats of widely ranging quality varied within a very narrow range, and that the ratio of lipoid to protein content gives a valuable index of the quality of flour. Nottbohn and Mayer (1934) found that gluten contains only 4 percent of the total phosphatides, the wash water contains 15 percent and the greatest bulk is in the starch.

Sullivan and Near (1928) studied the lipoid phosphorous of wheat and its distribution. It has been convenient to rearrange Table I and Table II of Sullivan and Near's data and the percentage of phosphatides in wheat and its milled products have been calculated. The ratio of phosphatide to (lipoids minus phosphatides) for different mill streams has also been calculated.

Sullivan and Near (1928) state that the phosphorous in lipoids decreases with decrease of refinement. By calculating the percentage of phosphatides present the reason for the above results becomes obvious. Apparently

the ratio of phosphatides to (lipoids minus phosphatides) is much higher in a refined flour than in the low grade streams. The ratio for patent flour is 22.75 percent, whereas the ratio for germ is only 10.15 percent.

The Effect of Adding Lecithin, Wheat Germ and Lipoids to Flour and Its Relation To Gluten Quality

Working (1924) found more phosphatides present in the glutens from lower grade flours. In his work the term "lipoids" is used to denote the substituted fats containing nitrogen and nitrogen and phosphorous and the term "phosphatides" includes only the phosphorous containing lipoids. Crude gluten prepared from low grade flour using distilled water was very soft and weak. Upon long continued washing the gluten gradually became more tenacious, finally becoming practically equivalent to that washed from patent flour. Since the washing of gluten with water improved the quality of the gluten from low grade flour, Working concluded that the converse should be true, i.e. the addition of wheat phosphatide to high grade flour should impair the quality of the gluten. 0.5 percent of wheat phosphatide was added to the crude gluten from patent flour with the result that it became fully as soft as gluten from low grade flour. The addition of other colloids such as pentosans and soaps were found to have an effect very similar to that caused by the addition of phosphatides; that is, the gluten became very soft and lacked tenacity.

TABLE I

DISTRIBUTION OF PHOSPHATIDES IN WHEAT AND ITS MILLED PRODUCTS

Type	Yield %	Ash %	Nitrogen %	Lipoids %	Phosphorous in Lipoids %	Lipoid Phosphorous %	Phosphatides 809/31 %	Lipoids Minus Phosphatides %	Ratio Phosphatides / Lipoid-Phosphatides
Patent	58.0	0.464	2.49	1.79	0.7079	0.0127	0.330	1.45	22.75
Clear	12.0	0.773	3.16	2.44	0.5550	0.0136	0.355	2.08	17.10
Wheat	-	1.855	2.90	3.08	0.5666	0.0171	0.446	2.57	18.13
Low Grade	2.6	1.647	3.41	4.39	0.4444	0.0195	0.509	3.88	13.12
Total Mill Run Middlings	14.4	4.637	3.43	7.29	0.4331	0.0316	0.825	6.46	12.77
Bran	13.0	6.497	3.25	5.74	0.4346	0.0249	0.650	5.09	12.77
Germ	-	4.628	5.13	12.04	0.3593	0.0433	1.110	10.93	10.15

Working (1928) attempted to develop doughs artificially; thereby dispensing with the fermentation period. The quality of bread produced becomes a measure of the accuracy of the imitation. The addition of one gram egg yolk, plus 0.75 c.c. of 90 percent lactic acid to a dough with no fermentation period, gave a loaf superior in volume and texture to a dough fermented normally for three hours at 32°C. The addition of 0.1 grams lecithin, 3 c.c. alcohol, .001 milligrams potassium bromate, all gave results similar to that of the egg yolk. The amount of lecithin added was 0.03 percent of the flour which is equivalent to 0.001 percent of lipoid phosphorous. An anomaly appears to exist in Working's results. In 1924 he found that the addition of 0.5 percent wheat phosphatide seriously impaired the gluten quality and in 1928 he states that the addition of 0.5 percent phosphatide to an artificially matured dough results in a loaf superior in volume and texture to a dough fermented normally. Working believes that these differences may be attributed to the degree of dispersion of the phosphatides. He states, that of all the constituents of flour, the phosphatides are probably the most likely to be affected by minute amounts of oxidizing agents.

Working (1929) found a significant increase in the amounts of phosphatides dispersed in water in a dough during fermentation.

His theory of the action is as follows:

"Ordinary flour contains much more phosphatide than is necessary to properly develop the dough when acid is added. Apparently this phosphatide is so constituted that it cannot

spread over the interfaces between the gluten fibrils. It seems probable that the action of oxidizing agents consists in breaking up these combinations so the phosphatides can be dispersed in the water present. Fermentation must have this action as well. Alcohol, glycerine, and saponin probably increase the dispersion of phosphatide and possibly also affect the surface forces directly."

Geddes (1930) found that the addition of 5 percent of raw germ to a highly purified middling flour has a decidedly deleterious effect on baking quality. The addition of bromate largely reduced or counteracted the deleterious effect of the germ on the baking quality of the middlings flour. Geddes extracted the flour-germ mixture with ether and found a marked improvement in baking quality. The extracted flour gave only a slight response to bromate. These observations led Geddes to suggest that improvements in baking quality induced by heat, chemical improvers and ageing, are due to some action on the germ constituents, which are present in straight grade flours.

Geddes (1930) added pure lecithin to flour and obtained a slight improvement by the basic method. It is interesting in this regard to note the relative effect of raw germ as opposed to lecithin.

Values are listed below for loaf volume

Control	608 c.c.
Addition 5% raw germ	562 c.c.
Addition 1% lecithin	627 c.c.

If the decrease in volume were due to the quantity of phosphatides in the germ the 1% lecithin should produce a

decrease in baking quality greater than that produced by the germ. 5 percent of germ contains approximately 0.25 percent lecithin. It would appear that the loaf volume depends on the degree of dispersion or some other property of phosphatides, rather than the total quantity of phosphatides.

Saunderson (1930) determined the decrease in lipoid phosphorous content with increasing heat treatment. His data are not very conclusive but the tendency indicates a progressive decrease in lipoid phosphorous paralleled by an increase in the baking quality of the flour-germ admixture.

Rich (1934) added raw germ, ether-extract of germ, and the ether residue of germ to fifth middlings flour. The results obtained for raw germ are in accord with those obtained by Geddes (1930), Johnson (1928). Johnson and Whitcomb (1931), and other workers have found that ether-extraction improved the baking quality of flour. From this it would be expected that the addition of the ether-extract or alcohol-ether extract of germ should have a detrimental effect on baking quality. Rich (1934) added the above extracts, and in both cases obtained a beneficial effect. The addition of the ether residue markedly impaired the baking quality of the flour.

Rich claims that it is not the extract, but the germ residue that impairs the baking quality of the flour. Rich concludes that the reason for the poorer baking quality of the lower grade flours is due to their contamination with germ particles, and that improvement due to artificial maturation is caused by some reaction which apparently in-

volves oxidation of some constituent of the germ content of the flour. His work does not agree with the hypothesis that the phosphatides are the constituents responsible for the reaction, but indicates that some other constituent is involved.

The Effect of Ether-Extraction on the Bread-Making Properties of Flour

Salamon (1908) found that a flour free from fatty matter gave a much larger loaf volume and better texture than the same flour containing fatty matter.

Johnson (1928) states that ether-extraction of flour improves its baking quality. No differences could be detected in quality and quantity of gluten, absorption, and viscosity. The addition of even larger quantities of lard to the extracted flour did not reduce the color, and texture of the bread baked from it. Johnson concludes, that the improvement brought about by ether-extraction is caused by materials other than true fatty acids.

Johnson and Whitcomb (1931) state that the most significant effect observed on extracting flour with ether is the marked change, on the gas retaining power of the doughs. The ether-extracted flours were markedly superior to natural flour doughs in their ability to retain carbon dioxide produced during fermentation. The addition of fats to the doughs prepared from ether-extracted flours reduced their gas retaining power.

Geddes (1930) found that a marked improvement in gluten quality resulted from ether-extraction of flour. When the natural flour, and the ether-extracted flour were baked

with bromate, the response was not nearly as great in the case of the ether-extracted flour. Geddes concludes that ether-extraction markedly reduces the response to bromate, suggesting that the ether has induced in the flour changes similar to those of bromate; in that, the flour constituent on which the potassium bromate chiefly acts has been removed by ether.

Geddes (1933) determined the relative effect of extracting flour-germ mixtures with ether and acetone. He found that ether-extraction produced a more beneficial effect than acetone-extraction. The acetone was found to extract less phosphatide than ether. This fact is considered to be additional proof to the effect that phosphatides are an important factor in gluten quality. However Sullivan and Near (1933) state that acetone extracts almost twice as much phosphatides from the different mill-streams as does ether-extraction.

Martin and Whitcomb (1932) extracted flours known to differ greatly in quality. Flours from Marquis, Kubanka and flour milled from Federation, a soft wheat, were studied. They found that ether-extraction improved the baking quality of the flour from Marquis, but had a deleterious effect on that from both Kubanka and Federation wheats. Ether-extraction improved the gas-retaining power of the dough from Marquis, but impaired the gas producing power of the dough from Kubanka wheat flour; the gas retaining power was not influenced. They conclude that ether-extraction does not affect, in the same way the baking quality of flours from widely different wheats, and

that quality in gluten is affected by a number of factors.

Saunderson (1930) studied the effect of wetting flour in ether and then allowing the ether to evaporate and found a beneficial effect similar to ether-extraction.

The Effect of Ageing on the Phosphatides of Flour

Bailey and Johnson (1924) noted that a freshly milled flour extracted with ether showed practically no change in H+ conc. during storage for three years. Johnson and Green (1931) and Schulerud (1933) studied the march of acidity in stored flours and concluded that the fatty acids alone are responsible for the increase in acidity.

Winkler (1931) studied the effect of ageing germ at two different moisture levels. The lipoid phosphorous decreased from 0.100 percent to 0.071 percent in flour stored at the 7 percent moisture level, whereas it decreased from 0.100 percent to 0.026 percent at the 13 percent moisture level.

The effect of ageing on the phosphatides of the different mill-streams was studied by Sullivan and Near (1933). Their results, in the case of germ, were essentially the same as those obtained by Winkler. However they found that patent flours showed very little change in phosphatide content in three months. The original lipoid phosphorous was 0.0074 percent, which changed at the 13.6 percent moisture level to 0.0070 percent, and at the 8.2 percent moisture level to 0.0078 percent. Though the lipoid phosphorous remained practically unchanged, the acidity of the stored flour at 13.6 percent moisture level markedly increased. This fact is interesting in so

far that is shows that the increase of acidity may be independent of any change in phosphatide content.

Solomon and Binnington (1932) found that in the ageing of germ the oxidative changes were of small magnitude in comparison with the extent of hydrolysis.

Geddes and Larmour (1933) rebaked a series of six flours from each of the crop years 1928 and 1929. The flour had been stored in air-tight containers for 35 months and 23 months respectively since they were first baked. They were rebaked by the basic and bromate formulas, and Geddes and Larmour conclude that in general loaf volumes by both the basic and bromate formulas decreased, but the decline was greater in the case of the bromate formula with the result that the response of the aged flour was in most cases very much less than the response of the newly milled flour.

They further state that this may be interpreted to mean, either that the character of the protein had changed or that some intermediate on which the bromate acts, had altered with storage. Saunders, Nichols, and Cowan (1922) state that they found an improvement in loaf volume after three years storage.

2. PROBLEM

In the specific investigations which have been carried out an effort has been made: (1) to determine whether the phosphatide content of experimentally milled Canadian hard red spring wheat flours of different protein content changes during a three hour fermentation period;

(2) to compare the phosphatide content of basic and bromate doughs, at the initial, and final stage of fermentation in order to determine whether potassium bromate has a specific effect on the phosphatide content; (3) to make a similar comparative study of the water-soluble phosphorous of doughs. This study enables us to have a check on the results obtained in (2) i.e. if the phosphatides are broken down during fermentation, or if the amount of phosphatide dispersed in water increases a corresponding increase should be obtained in the water-soluble phosphorous.

This work was later extended to include comparative studies on the phosphatide and water-soluble phosphorous content of bread obtained by the basic and bromate methods.

3. EXPERIMENTAL

All the work was done on patent flours milled from Canadian hard red spring wheats ranging in protein content from 16.5 to 9.7 percent. The procedure adhered to in mixing the dough, time of fermentation, punching and panning, was that of the basic formula of the A. A. C. C. In the case of the bromate baking method .002 percent potassium bromate was added.

The procedure used in preparing the dough was as follows:-

The doughs were mixed in a Hobart mixer for three minutes. An aliquot was immediately removed and approximately three gram samples were weighed out in duplicate for the determination of lipoid phosphorous, water-

soluble phosphorous and moisture determination. The remaining portion was placed in a proofing cabinet maintained at 30°C. The dough was punched after one hour and forty-five minutes, two hours and thirty-five minutes and panned after three hours of fermentation. At the end of fifty-five minutes proofing period, the dough was removed and treated in the manner indicated above.

The author first attempted to do all the phosphorous determinations on the same sample, i.e. water-soluble, alcohol-ether soluble and insoluble phosphorous respectively. The accuracy of the results could be determined by the checks obtained on the duplicates of the three determinations and the checking of the total of three determinations. The method used is outlined below:-

A 5 gram sample of dough was weighed, placed in a beaker containing approximately 50 c.c. of water and disintegrated into small particles by means of two pointed glass rods. A Buchner funnel of 15 centimetres diameter was prepared by placing one filter paper cut to the proper size on the bottom and another filter paper of larger diameter which fitted over the first snugly against the sides of the Buchner to a height of one inch. Filter paper Whatman No. 1 was used. The author found that 500 c.c. of water was sufficient to completely wash out the water-soluble phosphorous. The filtrate was made up to 500 c.c.'s in a volumetric flask, a portion was centrifuged and a one-fifth aliquot was pipetted into a Kjeldahl flask and the water evaporated to a small volume.

The dough on the filter paper was allowed to dry at

room temperature, pulverized and then the inner filter paper containing all the dough was transferred to a soxhlet thimble. Extraction was carried on with 95 percent ethyl alcohol over night and then with ether for three hours.

Initially the author felt that since all the water soluble phosphorous had been removed it should be unnecessary to re-extract the alcohol-ether extract with chloroform. The alcohol-ether fraction was directly transferred to a Kjeldahl flask, precaution being taken to insure complete transfer of all the material in the soxhlet flask.

The remaining dough in the soxhlet thimble was allowed to dry, and directly transferred to a Kjeldahl flask.

The author first tried to destroy the organic matter by ashing rather than by Kjeldahling. In the case of the lipoid extract it was impossible to get complete ashing at temperatures as high as 850°C. In view of the above results it became necessary to resort to the digestion of the material in a Kjeldahl flask.

Two to three grams of magnesium nitrate were added to each flask, sufficient concentrated sulphuric acid (10 - 20 c.c.) was added and the contents digested for half an hour. After cooling 2 cc. of concentrated nitric acid were added and digestion continued until the contents of the flask were almost dry. The contents were allowed to cool for a few minutes and water was then added. It is important to add the water when the residual acid is still slightly warm so that complete solution of the salts sticking to the sides of the flask may be

effected.

It is essential in this work to keep the sample weight as low as possible because of the difficulty of handling the dough, and yet have a sample weight containing sufficient phosphorous to give a blue colour intense enough to be read accurately. The author found that a sample weight between 3 and 4 grams was satisfactory. Phosphorous was determined by a modification of Fiske and Subarrow's method (1925).

In order to get a rapid development of the blue colour it is necessary to neutralize the residual acid in the Kjeldahl flask. The neutralization with sodium hydroxide produces sodium sulphate. Since the percentage of lipoid phosphorous in patent flours is very small, accuracy of the colorimetric method is essential. The author thought it best to investigate,

1. Development of the blue colour on aging.
2. The effect of various concentration of sodium sulphate on the development of the blue colour.

The development of the blue colour was studied by using the spectro-photometer. It was found that the intensity of the colour increased at a rapid rate for twenty hours and then slowly continued to develop; for at least four days. On plotting transmittancy against time it was found that a curve typical of a unimolecular reaction was obtained. In view of the fact that the deeper the colour the lower the error on reading with a colorimeter the solutions were all allowed to stand for twenty-four hours before they were read.

The author has found no literature bearing on the question as to the effect of a concentration of salts on the development of the blue colour. To four 100 c.c. volumetric flasks, 2 c.c. of a solution equivalent to 0.16 milligrams of phosphorous were added. One flask was kept as a check and the other three flasks were made up to a concentration of 3, 5, and 10 percent of sodium sulphate. The results obtained are tabulated in the following:-

Concentration of Sodium Sulphate - %	Reading on Colorimeter
0	(set at 20)
3	20.8
5	21.5
10	24.0

It appears that the more concentrated the solution the lower the intensity of colour as noted by the naked eye. It became obvious that if the concentration of sodium sulphate in the different solutions to be tested for phosphorous were allowed to fluctuate to any great extent a serious error would result. Furthermore the results obtained would be too low when read against the standard unless sufficient sodium sulphate were added to the standard to balance the amount present in the unknown solution.

As stated previously digestion of the material in the Kjeldahl flask was continued until the contents were practically dry. It was estimated that approximately 1 c.c. of residual acid was left, which on neutralization with sodium hydroxide is equivalent to 2 - 3 grams of sodium sulphate.

In the cases of total and insoluble phosphorous the contents of the Kjeldahl flask were transferred to 250 c.c. volumetric flasks and a one-tenth aliquot was used in determining the phosphorous content. The error involved in this case is negligible as the concentration of sodium sulphate in the solutions in which development of the blue colour takes place is only 0.2 to 0.4 percent. Therefore no sodium sulphate was added to the standard to balance the small amount present in the unknown.

The amount of phosphorous present in the lipoid phosphorous and water-soluble phosphorous fraction is so small that a much larger aliquot must be taken in order to get a blue colour sufficiently intense to be read accurately. The quantity of organic material to be destroyed is only a small fraction of the total weight of the sample. In destroying the organic matter by Kjeldahl digestion 3 - 5 c.c. of sulphuric acid, one-half gram of magnesium nitrate and 1 c.c. of nitric acid were used. The contents were taken practically to dryness, and transferred to 100 c.c. volumetric flasks. In the case of the lipoid phosphorous the total contents of the Kjeldahl flask were used for the phosphorous determination, whereas a one-half aliquot was taken for the water-soluble phosphorous determination. The standard was made up to a 5 percent concentration of sodium sulphate in order to compensate for the salts present in the lipoid phosphorous extract. It was felt that the above precaution, with regard to the standard has largely obviated the error introduced by neutralizing the residual acid with sodium hydroxide.

The following experiment was attempted to check the accuracy of the method:

The procedure outlined above was used in the first two cases. In one case extraction of the material was made with hot water and in the second case extraction was made with cold water.

In the third case anhydrous sodium sulphate and sand were added to a three gram sample of dough. The dough was dried, pulverized, pumice and more sand added and transferred to a soxhlet thimble. Extraction was effected in the usual manner with alcohol and ether. The contents of the soxhlet flask were transferred to a beaker and evaporated on a steam bath, the evaporation being facilitated by passing a stream of air over the mouth of the beaker. The residue was taken up with chloroform, and then filtered into a Kjeldahl flask. The phosphorous of the residue on the filter paper was also determined.

In the fourth case a sample of dough was directly transferred to a Kjeldahl and the phosphorous determined. The results obtained are tabulated in Table II.

These results indicate that the method is quite accurate. The phosphorous of the dough determined directly was 0.109 percent whereas the totals obtained for hot water extraction, cold water extraction, and anhydrous sodium sulphate were 0.111 percent, 0.109 percent and 0.111 percent respectively.

The data indicate the importance of re-extracting the alcohol-ether extract with chloroform. The chloroform insoluble phosphorous amounted to .005 percent. It, therefore,

TABLE II

EXPERIMENT TO DETERMINE THE ACCURACY OF THE METHODS INVOLVED

(DRY BASIS)

Method	Water Soluble Phosphorous %	Alcohol-Ether Soluble Phosphorous %	Insoluble Phosphorous %	Total Phosphorous (by addition) %
Hot Water Extraction	.0242	.0129	.0739	0.111
Cold Water Extraction	.0295	.0138	.0659	0.109
Anhydrous Sodium Sulphate		Chloroform Extract .0082 / Residue .0052	.0979	0.111
Total Direct				0.109

became evident that the best way to determine lipoid phosphorous, using the term lipoid to indicate the alcohol-ether extract re-extracted with chloroform, was to first dehydrate the dough with sodium sulphate.

It is interesting to note that cold water extracts more phosphorous than hot water. This can be explained by noting that whereas hot water forms a colloidal paste with starch, the cold water merely suspends the starch. The extraction with cold water was also much more satisfactory from the standpoint of ease and speed of filtering.

The Effect of Fermentation on the Phosphatides of Flour by the Basic and Bromate Method

Patent flours ranging in protein content from 15.7 to 11.2 percent were used. The results obtained are shown in Table III, (page 35).

Referring to this table - In the three higher protein flours the lipoid phosphorous by the basic procedure is actually higher at the end of three hours fermentation than at the beginning of fermentation. Bormann (1951) reported on the determination of lipoids in alimentary pastes and in baked products. He states that lipoids are not completely extracted by the present tentative method using alcohol and ether. The author feels that the results obtained substantiate this conclusion. Apparently a three hour fermentation period changes the physical state in which the phosphatides are held and renders them more soluble in alcohol-ether extraction. In the case of the 15.7 and 14.7 percent protein flours, the difference between the basic and

TABLE III

THE EFFECT OF THE BASIC AND BROMATE BAKING METHOD ON THE LIPOID PHOSPHOROUS DURING A THREE HOUR FERMENTATION PERIOD

(DRY BASIS)

Protein Content	Lipoid Phosphorous of Dough (0 Hours Fermentation)	Lipoid Phosphorous (3 Hours Fermentation)	
		Basic	Bromate
%	%	%	%
15.7	.0083	.0094	.0078
14.7	.0082	.0094	.0083
13.6	.0088	.0098	.0104
11.2	.0082	.0082	.0081

bromate at the end of three hour fermentation is 0.0012 percent phosphorous. This value is small but nevertheless appears to be significant because Working (1934) found that the addition of wheat phosphatide corresponding to .001 percent phosphorous had a tremendous effect on the quality of the gluten.

It should be mentioned that the results recorded in Table III were obtained by using anhydrous sodium sulphate to dry the dough. Previous to this, five attempts had been made to get accurate results by first extracting the dough with water, and then with alcohol and ether. This method was discarded, but it should be pointed out that almost in every case the phosphorous content of the alcohol-ether extract at the end of the fermentation period was greater for the basic than for the bromate dough. Furthermore in almost every case the phosphorous content of the alcohol-ether extract of the basic dough was greater at the end of the fermentation period than at the beginning.

It is interesting to note that Sullivan and Near (1933) found that the lipoid phosphorous of patent flour stored at 7 percent moisture level increased on ageing from .0074 to .0078 percent.

A Comparison of the Changes in Water-Soluble and Lipoid Phosphorous of the Dough During the Fermentation Period and the Baked Bread.

Certain investigators have noted profound changes occurring in the dough during the baking process. Natalie Kazmin (1933) found that bread production gives an impetus to the splitting

of starch. It was found that in a normal flour the water-soluble substances in dough were 9.1 percent, whereas in the bread the percentage of soluble materials was 15.2 per-cent. Fischer and Halton (1929) compared the pH of doughs and the corresponding bread. In every case the H+ concen-tration of the loaf was greater than that of the correspond-ing dough by about pH = 0.20 to 0.30. That is, the actual baking raises the pH or diminishes the H+ concentration considerably.

Since the final criterion of the quality of flour is the baked loaf, the author thought that analyses should be made at the beginning of fermentation, the end of fermen-tation and on the baked bread. The bread was prepared for analysis by using all the material except the crust. The bread was torn into small parts and allowed to dry at room temperature.

The sample was then ground in the Wiley Mill, thus ensuring a finely ground product. The analysis from this stage was carried on in a manner similar to the pulverized dough. A flour of 14.7 percent protein content was used, which gave a response of 139 c.c. to .002 percent of potassium bromate. The results are shown in Table IV. (page 88)

The results obtained were of great interest. It appears that the baking process breaks down the phosphatides. The break-down is, however, differential. The lipoid phosphorous of the bread baked by the basic method was .0075 percent, whereas the bromated loaf had a value of .0052 percent. A difference of .0023 percent. Harrel (1927) found that H+

TABLE IV

A COMPARISON OF THE CHANGES IN WATER-SOLUBLE AND LIPOID PHOSPHORUS DURING THE FERMENTATION PERIOD AND THE BAKED BREAD - USING THE BASIC AND BROMATE METHOD

(CALCULATED TO A DRY BASIS)

Method	Basic			Bromate		
	Water Soluble Phosphorous %	Lipoid Phosphorous %	Total (By addition) %	Water Soluble Phosphorous %	Lipoid Phosphorous %	Total (By addition) %
0 Hours Fermentation (Dead Yeast Added to Dough)	.031	.0082	.0392	.031	.0082	.0392
3 Hours Fermentation	.032	.0094	.0414	.032	.0083	.0403
Bread	.033	.0075	.0405	.036	.0052	.0412
50 Hours Fermentation	.047			.045	.0045	.0475

concentration of bread baked by the bromate method was slightly higher than bread baked by the basic method. The data obtained in this work may partially explain Harrel's observation. The decomposition of phosphatides would produce a small amount of phosphoric acid; but whether this small amount of acid could appreciably change the H. ion concentration in view of the large quantity of buffer substances is very questionable.

Yashitaka Hasitani and Tsunokaku Suko (1932) have found that a temperature of 98.5° develops in the centre of the dough for approximately ten to fifteen minutes during the time of baking. Paal (1929) states that lecithin shows great thermolability, undergoing complete hydrolysis when warm. It is possible that the action of bromate renders the phosphatides more susceptible to heat.

A portion of the dough was allowed to ferment for fifty hours. By that time the phosphatide content of the bromated dough was .0045 percent. That is, fifty hours of fermentation had decreased the phosphatide phosphorous content from .0082 to .0045 percent.

The water-soluble phosphorous showed no appreciable change during the three hour fermentation period. On allowing the doughs to ferment for fifty hours, the water-soluble phosphorous increased from .031 to .047 percent by the basic method and from .051 to .043 percent by the bromate method. It is interesting to note that during the baking of the bromated dough, the water-soluble phosphorous increased .004 percent at the same time that the lipoid phosphorous decreased .0031 percent.

A Comparison of the Lipoid Phosphorous Contents of Flours of Different Protein Content and the Breads Baked from the Above Flours.

Six patent flours, varying in protein content from 16.5 percent to 9.7 percent were used. Their baking behaviour is shown in Table V, (page 31).

The lipoid phosphorous was determined for the flours shown in Table V, and also for the breads baked from each flour.

With reference to Table VI, (page 32), we find that the total lipoid phosphorous varied within narrow limits ranging from 0.0069 - 0.0083 percent. Herd and Amos (1930) calculated the lipoid phosphorous to phosphatides, assuming that the fatty acid radicals had a molecular weight of 256. Stearic acid has a molecular weight of 239 and oleic acid radical 247. On this assumption the molecular weight of a phosphatide will be 329 + (2 x 238) = 805. Levene and Rolf (1925) studied the plant phosphatides of soybean. They found saturated fatty acids, stearic and palmitic. Representatives of the three different orders of unsaturation were found, namely of oleic, linolic, and linolinic types. They conclude that the theoretical molecular weight for a monophosphatide is 809. Assuming that the weight of a typical wheat phosphatide to be 809, the percentage of phosphatide in patent flours varies within .180 and .216 percent (dry basis.) The author finds that there is no relationship between the amount of phosphatide present in flour and the

TABLE V

LOAF VOLUME GIVEN BY SOUND SAMPLES OF WHEAT OF DIFFERENT PROTEIN CONTENT BAKED BY THE BASIC AND BROMATE METHOD

Flour Protein	Loaf Volume		
	Basic	Bromate	Response
9.7	560	535	- 25
11.4	540	660	+ 120
12.3	570	680	+ 110
14.7	640	770	+ 130
15.7	680	795	+ 115
16.5	700	690	+ 170

@ 13.5 % moisture basis

TABLE VI

A COMPARISON OF THE LIPOID PHOSPHORUS CONTENT OF FLOURS OF DIFFERENT PROTEIN CONTENT; AND THE BREADS BAKED FROM THE ABOVE FLOURS

(CALCULATED TO DRY BASIS)

Protein Content of Flour	Total P. of Flour %	Phosphatide of Flour %	Phosphatide of Bread Basic %	Phosphatide of Bread Bromate %	Phosphatide of Flour / Protein of Flour	Phosphatide of Basic Bread / Protein of Flour	Phosphatide of Bromate Bread / Protein of Flour
16.5	0.104	.216	.234	.216	1.3	1.7	1.3
13.7	0.113	.213	.219	.198	1.38	1.39	1.26
14.7	0.115	.213	.196	.136	1.45	1.33	.93
12.3	0.109	.180	.120	.120	1.46	.98	.98
11.4	0.115	.196	.227	.164	1.72	1.99	1.44
9.7	0.108	.188	.149	.117	1.93	1.54	1.21

/ By assuming that the average molecular weight of a mono-phosphatide present in wheat flour is 809 the percentage phosphatide in a sample is equal to

$\frac{809}{31.04}$ x percent lipoid phosphorous in the sample.

protein content of flour.

In all cases except that of the flour of 12.3 percent protein, the phosphatides of the "bromate bread" were from .02 to .06 percent lower than the loaves baked without bromate, while the lipoid phosphorous was from .0008 to .0024 percent lower. The duplicates were in all cases extracted on the succeeding day, six extractions being done daily, and the differences obtained by the two baking methods are beyond the range of experimental error. The results suggest that potassium bromate has a specific effect on the phosphatide content. The chemical nature of this effect is not known, but its action appears to be facilitated during the baking process.

It is again interesting to note that in three cases the percentage phosphatides of the bread was higher than the percentage in the dough at zero hours fermentation. Newald (1930) determined phosphatides for five different brands and found a range from 0.174 to 0.302 percent. He considered that the phosphatide content of bread is considerably higher than is found in flour analysis. Shulze and Steiger (1934) using the method recommended by Nottbohm and Mayer (1934) found the phosphatide content of flour to be 0.65 percent (dry basis).

Sullivan and Near (1928) state that the ratio of lipoid phosphorous to total nitrogen is lower in the flour than in the total mill-run middlings, bran and germ, i.e. the ratio increases with decreasing quality. It is questionable, however, whether this ratio is of any value. Since the percentage phosphatides in patent flours falls within a very narrow range, it is obvious that as the protein content decreases the ratio of phosphatide to protein will increase.

Bread baked from 15.5 percent protein flour using the bromate procedure gave a value of 1.3 for the above ratio, (Table VI). On using the 9.7 percent protein flour the value for the ratio was 1.2. The response to bromate was 170 c.c. and minus 20 c.c. respectively. It appears that this ratio is of no significance in bread.

Sullivan and Near (1927c) reported that the total phosphorous of wheat was not correlated with protein content. Total phonphorous determinations were made on ten patent flours and it was found that the phosphorous content varied within a very narrow range, 0.108 to 0.115 percent. These results confirm Sullivan and Near's conclusion.

The Effect of Fermentation on Water-Soluble Phosphorous

Water-soluble phosphorous was determined on four patent flours. The determinations were made at zero hours, and three hours of fermentation and in one case on the baked bread. The results are given in Table VII, (page 36).

The results obtained indicate that the changes in water-soluble phosphorous during a three hour fermentation period are of little magnitude.

It is however extremely interesting to note that in all the flours except the one of 14.7 percent protein content, the bromated doughs contain slightly more water-soluble phosphorous than the basic doughs. The difference ranges from .003 to .007 percent. Working (1934) presented data showing that the bromate renders the phosphatides water-soluble. These results tend in some measure to lend support to Working's conclusion. In the case of the bromated dough, the increase

TABLE VII

COMPARISON OF THE CHANGES IN WATER-SOLUBLE PHOSPHORUS DURING THE FERMENTATION PERIOD USING THE BASIC AND BROMATE METHOD

(CALCULATED TO A DRY BASIS)

Protein Content of Flour %	0 Hours Fermentation %	3 Hours Fermentation (Basic) %	3 Hours Fermentation (Bromate) %	Bread (Basic) %	Bread (Bromate) %
16.3	.038	.041	.044		
14.7	.031	.033	.032	.033	.036
13.0	.037	.040	.035		
11.0	.024	.021	.025		

of water-soluble phosphorous may be due to conversion of phosphatides to water-soluble phosphatides.

An attempt was made to directly verify Working's results. An aliquot of the water-soluble phosphorous extract was evaporated on a steam bath, taken up with alcohol and ether, evaporated, taken up with chloroform, and the phosphorous determined in the extract. The results obtained in a preliminary experiment were in the order of .0002 percent of phosphorous for basic and .0004 percent for bromate. The colours were too weak to be read accurately and the results are inconclusive. The above method is not very satisfactory because of the necessity of evaporating a large volume of liquid. Additional evidence as to the inadequacy of this method would appear from the results obtained by Cranner (1932). He established that phosphatides of the lecithin type are dialyzed by water at room temperature from undamaged plant tissues, and that these phosphatides are insoluble in organic solvents. It is therefore not possible to extract them from the aqueous dialyzates by shaking with ether, but since ether absorbs about 2 percent of water, the small amount of water-soluble phosphatides included therein can be isolated by careful evaporation of the ether. The residue obtained is no longer water-soluble, but possesses all the typical properties of lecithin, including its ready solubility in alcohol and ether. He concludes by defining lecithin as the denatured product of an original water-soluble phosphatide complex.

Schmierud (1932) has analyzed the relation of phosphorous compounds to acidity in flours. He has shown that when flour suspensions are allowed to rest with antiseptics added

the amount of soluble phosphorous increases proportionally with the acidity and that this development of soluble phosphates is a regular chemical process appearing in all flours independent of acidity by fermentation. This may be explained as a real chemical reaction between phytin and water. It then becomes obvious that the method used in this work to determine water-soluble phosphorous is subject to a serious error. The water-soluble phosphorous at zero hours fermentation will not be the same as the amount at the end of the time required to wash 500 c.c. of water through the dough. It therefore becomes apparent that a method must be obtained which can give an accurate measure of the changes occurring specifically in the water-soluble phosphatides during the fermentation period. The changes occurring in the water-soluble phosphorous are not necessarily a criterion of the changes in the water-soluble phosphatides.

Grafe (1929) states that the phosphatides are combined to the proteins in yeast. His conclusion is based on the fact that phosphatides are not dialyzable from unautolyzed yeast but when autolysis has taken place by enzyme action the phosphatide becomes dialyzable. The above research may give a new line of attack for determining the relation of phosphatides to the gluten proteins, and possibly to baking quality.

It may be possible to observe the changes in solubility of phosphatides by taking samples of dough at different stages of fermentation, dehydrating the dough by the addition of sodium sulphate, placing the dried pulverized substance in a cellophane or colloidin bag and allowing it to dialyze for twenty-four hours or until equilibrium is reached. The

extract obtained should be free of starch and quite clear. The inorganic phosphorous could be directly determined on an aliquot of the extract. The total phosphorous content could be determined by taking an aliquot of the extract, evaporating the aliquot to a small volume, adding 1 c.c. of normal magnesium nitrate, taking the contents to dryness over a bunsen burner, and igniting in a muffle at 600°C. The ignited substance could then be washed into a volumetric flask and the phosphorous determined. Total phosphorous minus inorganic phosphorous would be equal to the organic phosphorous. Parker and Fudge (1927) have worked out a colorimetric method for determining organic and inorganic phosphorous in soil extracts. They find that the Denige's colorimetric method is more suitable than the Fiske-Subarrow method and is five times as sensitive. Due to its greater sensitivity the Denige method would undoubtedly be more valuable, as it would be more appropriate to the very low concentrations of dialyzable phosphatides.

It appears to the author that the above method would give important data concerning the changes in water-soluble organic phosphorous. Since phytin makes up by far the largest percentage of organic phosphorous, and since it is only dialyzable to a very slight extent it might readily be assumed that the changes in water-soluble organic phosphorous can be attributed to water-soluble phosphatides. This point would require further investigation. Furthermore, there is the possibility that the dehydration of the dough by sodium sulphate may influence the amount of dialyzable organic phosphorous. Magistris and Shafer (1930) studied

the influence of salts, acids and alkalies on the occurrence of water-soluble phosphatides in plants. The elimination of phosphatides is increased by the presence of the alkali metal and magnesium ions and is arrested by the alkaline earths. In their effect on phosphatide exosmosis the cations are arranged in the series K. (greatest), Na; NH_4; ---------- Sr. A similar series has been obtained for the anions.

The above work appears in some measure to offer an explanation for the results obtained by Saunderson (1930). His object was to determine whether the principle effect of chemical improvers was the oxidation of some constituent of the bread doughs. He conducted a series of comparative baking tests on flour-germ mixtures using sodium bromate, potassium bromate, potassium chlorate, potassium iodate, calcium peroxide, and potassium persulphate. These improvers were added at rates which were calculated to yield the same quantities of oxygen in all cases. If their action were due entirely to oxidation the effects should be similar, or at least proportional to their ease of decomposition, but the results obtained were quite different for the various improvers. Geddes (1933) states that further studies on the above problem have indicated a lyotropic series, and this may be interpreted as a direct and specific ionic effect on the gluten proteins. It would be of great interest and of theoretical importance to repeat Saunderson's work, making a corresponding study on the effect of the different chemical improvers on the amount of dialyzable phosphatides.

No literature bearing on the question of the effect of temperature on dialyzable phosphatides has come to the author's

attention. Geddes (1930) conducted extensive experiments on the effect of heat treatment on flour-germ mixtures. He found that, generally, heat-treatment tended to reduce the deleterious effect of the added germ and improved the baking quality of the flour. The results obtained by heat treatment largely parallel the results obtained by the addition of potassium bromate. Furthermore, data has been presented (Table IV) to show that the baking process breaks down the phosphatides. The relation of heat treatment to dialyzable phosphatides of wheat flour should be a fruitful field of investigation.

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4. SUMMARY

1. A method has been outlined for the determination of water-soluble and lipoid phosphorous in doughs obtained from patent flours of hard red spring wheat.

2. In reference to the colorimetric determination a study was made of, (1) the effect of concentration of sodium sulphate, and, (2) the length of time allowed for the development of the blue color. The intensity of the blue color decreased with increasing concentration of sodium sulphate. The intensity of the blue colour increased rapidly for twenty hours and then continued to develop very slowly during at least four days.

3. Cold water extracts more water-soluble phosphorous from doughs than hot water.

4. The quantity of lipoid phosphorous of doughs shows no decrease with a three hour fermentation period. On the other hand a slight increase in lipoid phosphorous was obtained in three out of the four basic doughs. The bromate doughs showed an increase in lipoid phosphorous in only one case. In the other three flours the lipoid content of the dough at the beginning and the end of fermentation was approximately the same.

5. The actual baking has a definite effect on the phosphatides. On baking the bread by the basic and bromate methods this effect is differential. The values obtained (Table IV) for the basic and bromate bread are 0.0075 and 0.0052 percent lipoid phosphorous respectively. A comparison of six flours of varying protein content baked by the two methods showed that in five

out of the six flours the phosphatide content of the bromate bread was from 0.21 to 0.68 percent lower than the phosphatide content of the basic bread. The results indicate that bromate has a specific effect on the phosphatides.

6. A bromated dough fermented for fifty hours decreased in lipoid phosphorous from 0.0082 to 0.0045 percent.

7. The percentage of lipoid phosphorous in the flours studied ranged between 0.0069 - 0.0085 percent, or the percentage in terms of phosphatides ranged between 0.160 - 0.216 percent. No correlation appeared between the quantity of phosphatides and protein content.

8. The total phosphorous of the flours ranged from 0.108 to 0.115 percent. No correlation appeared between the percentage of phosphorous and protein content.

9. In general the water-soluble phosphorous increased slightly during a three hour fermentation period. The increase was appreciably greater for the bromate doughs.

10. It has been shown that the bromate has a specific effect on the phosphatides, rendering them either more insoluble to alcohol and ether or more susceptible to decomposition. It is also known that the addition of bromate to a dough results in a modification of the gas retaining capacity, through some action, direct or indirect, on the gluten proteins. The question then arises as to whether the modification of the gluten proteins is due to the action of the bromate on the phosphatides, or, failing such a direct causal relationship, what is the association between these two concurrent phenomena. In the results obtained in this study the

larger modifications of the phosphatide content occurred during the baking process. Such evidence will not support the view stated above with regard to the action of bromate on the gas retaining properties of the dough. The important modifications of the phosphatides may have occurred during the fermentation period without having been completely measured by the methods used in this study i.e. it is known that the water-soluble phosphatides may be changed to an alcohol-ether soluble condition. Recommendations have been made for a continuance of this work using methods calculated to directly study the transformation of phosphatides to water-soluble phosphatides.

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