THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Thesis

The undersigned, acting as a Committee
of the Graduate School, have read the accompanying
thesis submitted by Ferdinand A. Collatz
for the degree of Master of Science.
They approve it as a thesis meeting the require-
ments of the Graduate School of the University of
Minnesota, and recommend that it be accepted in
partial fulfillment of the requirements for the
degree of Master of Science.

C. H. Bailey
Chairman

J. F. McClendon

L. S. Knight

April 2   1920

THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Examination

This is to certify that we the
undersigned, as a committee of the Graduate
School, have given Ferdinand A. Collatz
final oral examination for the degree of
Master of Science . We recommend that the
degree of Master of Science be conferred
upon the candidate.

Minneapolis, Minnesota

June 1 1920   *C. H. Bailey*
                          Chairman
              L. S. Knight
              J. F. McClendon
              J. J. Willaman
              R. Adams Dutcher

CERTAIN PHYSICO- CHEMICAL PROPERTIES OF WHEAT FLOURS

1. The relation between flour grades and the specific
conductivity of their water extracts.

II. The optimum temperature for phytase activity mea-
sured by the increase in specific conductivity of a phytin-phytase
solution.

by

Ferdinand A. Collatz

A THESIS

Submitted to the Graduate School of the University of Minnesota
in partial fulfillment of the requirements

For the Degree

of

Master of Science.

St. Paul, Minnesota.

1920

TABLE OF CONTENTS

INTRODUCTION

The grade of flour has always been of great interest to millers and cereal technologists; especially of late years as the modern methods of milling extracts to such a high degree. Naturally these improved methods have led to distinctions as to the fineness of milling and hence the flour grades. Various methods have been devised and practiced by cereal chemists, to determine the grades of flour obtained from the various flour streams, in the milling of wheat. Among the best known and reliable methods employed at the present time are:

1. Percentage of ash.

2. The amount of fibrous debris in the flour.

3. Acidity.

4. Content of pentosans.

5. Color of dry and moistened flour, or Pekar Test.

6. Catalase activity.

Historical. As early as (1893) Vidrodi called attention to the close relation between grade and ash content of various Hungarian wheat flours.

Girard (1895) suggested a method for determining the amount of debris particles in a cubic millimeter of flour.

Snyder (1905) in a report on numerous analysis of patent, second patent and clear flours, finds that first and second patents are invariably lower than .48 per cent ash content.

Wiley (1898) investigated the mineral constituents of wheat and wheat flours and found that the ash content of flour is quite a reliable index to the character of milling to which the wheat has been subjected.

Alway and Clark (1909) while working on the relationship exist-
ing between color and ash content came to practically the same conclusion.

Swanson (1915) agreeing to the previous conclusions, remarks as
to the various factors influencing the ash content: "It may be the kind
of wheat, the kind of soil, the handling of the wheat before milling, and
the method of milling. The last is by far the most important." He finds
that hard, flinty kernels have a higher ash content than soft, yellow ker-
nels, which may influence the ash content of the flour to a considerable
extent. The cleaning of the wheat before milling is also of great im-
portance. The actual milling procedure effects the greatest differences.
"The larger portion of the ash of the wheat kernel is found in the outer
portions which are returned as bran and shorts in the milling process.
Therefore, the cleaner the separation of the outer bran coats from the
inner floury portion, the lower will be the ash content of the flour. It
is for this reason that the lower grades of flour have a higher ash con-
tent than the patent flours." Swanson gives the following limits of ash
content for short patents, long patents and straight flours.

Table I.- Range in percentage of ash, in short patents, long patents
and straight flours.

Flour Grade	Range in Ash	Average Ash Content
	Per cent	Per cent
Short Patents	.368 - .502	.421
Long Patents	.380 - .534	.462
Straight Flours	.413 - .577	.483

Guilio (1915) gives a large amount of analytical data on phos-
phorus content of various grades of flour and the resulting bread. In
general he indicates that the percentage of mineral matter and of organic

phosphorus increases from flour to bran. He concludes that the coarser flours have a greater nutritive value than the white flour, since they contain greater amounts of phosphorus, nitrogenous material, complex mineral substances and crude fat.

Delehage (1917) while realizing the significance of the bran in the flour, did not determine the ash. He determined the amount of bran present by passing his flour through a series of screens with varying fineness of meshes.

Lieberman (1911) determined the amount of bran present colormeterically, by shaking his sample of flour with chloroform and comparing the color obtained with a standard flour.

The acidity parallels the ash content closely as observed by Swanson (1912). He found that the relation between the percentage of acidity and the percentage of ash, amino compounds and water soluble phosphorus was very close. The water soluble phosphorus varied as the acidity, and also with the total phosphorus but not in the same proportion.

. Color tests are used to a great extent in the mills to-day. The principal objection to this is in the inability of the tester to express his results numerically. Notable among these is the Pekar Test, which consists of compressing the flour upon a slab of colorless glass and then comparing it with a standard. This, however, has its disadvantages as flour milled from hard spring wheat has a distinct yellow tinge.

The fact that the enzyme tyrosinase acts upon a dampened flour, to change its color, gave rise to another test worked out by Hein, (1910), who measured quantitatively the extent of color change. This test indicates very closely the grade of flour as the enzyme is found chiefly in the bran and embryo. The greater the enzyme action, the

larger the amount of bran in the flour.

Koning and Mooij (1914) estimated the amounts of bolted and unbolted flour, used in the making of bread, by determining the amount of pentosans present. The unbolted flour had a higher percentage of pentosans than the bolted.

Attention was called to the catalase content of flour by Wender and Lewin (1904). They determined the quantity of oxygen liberated from various flours, under uniform conditions, and found that catalase content and flour grade paralleled each other. In the next year, Wender extended his researches along the same lines and obtained practically the same results as in his earlier investigations.

Liechti (1909) recommends the catalase method as being more exact than color or ash determinations for the grading of flours, as the catalase occurs almost entirely in the bran and the germ.

Bailey (1917) determined the catalase content of a number of American wheat flours, to ascertain the relation between catalase content and grade of flour. After determining the catalase activity of several series of samples, he concludes that while the "parallelism between the percentage of ash and quantity of oxygen liberated by the catalase is not exact, it is remarkably close, however, and shows the test to be of considerable value in indicating the grade of flour."

Of these six methods, the ash content seems to be the most valuable index as to the fineness of milling and the degree of extraction. It is generally agreed among cereal workers and milling chemists, that the higher the extraction, the less bran and germ are present in the flour. Since it is the bran and germ structures which are high in ash, it follows that as the percentage of these structures increases with diminishing refinement, the percentage of ash is higher in the lower grades of flour.

CONDUCTIVITY MEASUREMENTS IN BIOLOGICAL INVESTIGATIONS

Numerous investigators in the field of pathology, physiology, biological chemistry and botany have turned to the conductivity method for a solution of their problems. The method as developed by Kohlrausch, and described in his laboratory guide (1898) consists of the Wheatstone Bridge set-up, which is familiar to all and needs very little description. The method and apparatus has been changed slightly from time to time, notably by Washburn (1913) but the principal remains the same.

Bayliss (1907) used the conductivity method to measure changes taking place in the tryptic digestion of caseinogen, measuring the formation of inorganic phosphorus, peptones and amino acids. He found that the mono-amino, mono-carboxglic acids, such as glycine, alanine and leucine were low in conductivity while the di-carboxylic acids and di-amino acids were relatively high.

Sjogvist (1895) followed the peptic digestion of albumin by means of the conductivity method.

A method was devised by Briggs (1908) for determining the moisture in bulk grains by electrical conductivity, which varies as the logarithm of moisture content.

In 1910, and the years following, Höber devised modifications of the Kohlrausch method for measuring the conductivity of animal cells. He found that the plasma membrane offered too much resistance to an electrical current, when determinations were made by the direct Kohlrausch method. This large resistance, he claims, affects the reading of the actual conductivity of the cell contents, so he devised a method of placing an electrolyte or a conductor of some kind between the plates of an electrical condenser and measuring the resulting change in the capacity

of the condenser. In 1911, he measured the internal conductivity of cells by his damping method. This consists of a secondary coil, in which high frequency oscillations are induced, connected with a coil wound around a beaker or vial, which is to hold the substance to be measured. When a conducting solution is placed in the beaker a damping of the oscillations takes place, which can readily be detected by an instrument for that purpose, usually a telephone receiver.

McClendon (1911) measured the conductivity of striated muscles before and after contraction. He found a greater conductivity in the contracted muscle, and interprets this increase as demonstrating an increase in permeability to anions of some structure within the muscle, since the muscle appears already permeable to kations.

True (1914) in investigating the harmful effects of distilled water used the conductivity method to determine the amounts of salt leached out of the roots of seedlings. He also applied this method to ascertain the amounts of salts absorbed by the plants, when kept in pure culture media.

Stiles and Jorgensen (1914) advocate its use as a simple means of investigating certain classes of problems in plant physiology, the same authors using it to measure the exomose of electrolytes in cell tissues.

Osterhout has perhaps made greater use of the conductivity method than any recent worker in the field of plant physiology, in measuring the permeability of protoplasm to ions. In this work, discs of Laminaria were cut with a cork borer and 100 to 200 of them were packed to-gether so as to form a cylinder. This cylinder was immersed in sea-water, and other liquids, and was kept in place by means of glass rods. At each end of the cylinder was a block of hard rubber containing a platinum electrode. The measurements were made by the Kohlrausch

method. The resistance of the tissue in the sea-water was 1100 ohms and when killed the resistance fell to 300 Ohms.

When the cylinder of tissue was placed in various solutions it was possible to show the rate at which the ions of NaCl or other electrolytic solutions entered into the tissue by the rate at which the resistance fell from 1100 Ohms to 300 Ohms. When solutions of NaCl and $CaCl_2$ were mixed in a certain proportion, it was found that the resistance remained unaltered for twenty-four hours. This was attributed to the antagonistic action of the salts upon one another, both Na and Ca ions being prevented from entering. Sea-water is also such a "balanced solution."

As the intercellular substance has a lower resistance than the living tissue, the question arises, does any current flor through the living protoplasm? Osterhout shows that the resistance decreases when the tissue is killed, demonstrating clearly that the protoplasm does conduct current. The mobility of the various ions can be determined in regard to diffusion. When the protoplasm is in a solution of NaCl and then placed in a solution of RbCl, a sharp fall in conductivity ensues, showing a rapid exosmose of Na ions. The conductivity then slowly rises showing the slow entrance of the heavier Rb ions.

Blackman (1918) while investigating the loss in turger in Pulvinus and in Mimosa pudica, on immersion in warm water found that it was not due to an increase in permeability allowing the exosmose of electrolytes. He measured the conductivity of the solutions, which allowed him to study the exosmose,(if any occured), of the electrolytes. His experiments show that the conductivity method is far superior to indirect plasmolysis.

Green (1918) determined the rates of diffusion of NaCl and $CaCL_2$ in colloidal gels of gelatin, agar agar and gelatin, and a mixture

of flour and gelatin.   By immersing his gel in the liquid between the electrodes, he measured the rates of diffusion in NaCl, $CaCl_2$ or in mixture of the two solutions and finds that there is no antagonism.

Clowes (1918) finds that the conductivity method permits him to measure the permeability of cancer tissues.   He finds that cancer tissue is more permeable than normal tissue.   This permeability bears a direct relation to the rate of growth.

This partial resume of the literature points out some of the uses to which the conductivity method has been put in the biological sciences during the last few years.   In the inorganic field of chemistry a large number of uses have been found for this method, as it has the advantages of accuracy and speed.

EXPERIMENTAL

The Problem. The resistance offered by a regular cube of a conductor having sides one centimeter long, is called the specific resistance or resistivity of the material, and the reciprocal of this is called the specific conductance or conductivity. The latter is usually denoted by K and for convenience, the specific conductivity at 30° will be denoted as K_{30} in the text following. If a solution does not conduct at all it is assumed that there are no ions present; thus the number of ions (or degrees of ionization of any salt) is directly proportional to the specific conductivity. Since a large part of the mineral constituents of flour are extracted as dissociable salts after digestion with water, it was believed that the specific conductivity of the aqueous extract would give one an idea of the ash content of the flour. Accordingly the following experiments were conducted to see what relationship existed between ash content and specific conductivity of the extracts.

Materials Used. Two flours were used in the preliminary experiments to ascertain the best methods of extraction with respect to time and temperature. These flours consisted of a high grade patent containing 0.43 per cent of ash, and a clear flour containing 0.92 per cent of ash. Later on when a method of extraction had been adopted and the technique perfected, the specific conductivities of two series of several flours each were determined.

Apparatus. In making the conductivity determinations a Leeds and Northrup High Frequency Generator was used as a source of current. The number of cycles were approximately sixty thousand per minute.

A ten meter bridge, calibrated in the middle for fifty centimeters and the remainder wound on spools was used in balancing the known

resistance against the unknown resistance of the solution to be measured. This long bridge permitted a great accuracy in reading.

A De Khotinsky water thermostat with a maximum variation of 0.1 degree was used in bringing the cells to constant temperature. The readings were all taken at 30°C. unless otherwise specified.

A tunable telephone was used as a detector, being supplemented by a "Weilaphone", an instrument very similar to a stethoscope, which was attached to the receiver. The receiver was tuned so as to give a tone silence of 2 m.m. on the bridge. Readings were taken by approaching from both sides of the tone silence until a constant reading was taken. The resistance box was of the ordinary type.

The ordinary Freas cell was used in the preliminary experiments. When work upon the two flour series was begun a new set of Freas Cells were used. These were similar to the others with the exception that the bottom of the cells were cut out. This was a distinct advantage as the cell could be dipped into any solution which had come to temperature and its conductivity thus determined. With the closed types the cells had to be rinsed out several times with the desired liquid, and then allowed to come to temperature before the conductivity could be determined. Three portions of the desired solution were put in as many vials and when they had come to temperature, the electrode was immersed in the first vial for a short time, then in the second for a short time, so that when the electrode was placed in the third it had come to temperature and had been rinsed off in the solution. In this way a large number of determinations could be made in a short space of time. The cells were kept in redistilled water when not in use. They were frequently cleaned with a cleaning solution of 50 per cent pure chromic acid. The cell constant was determined every day with a solution of

$\frac{N}{10}$ KCl, the specific conductivity of which is 0.01412 at 30°C. *   The

cells were replatinized as the occasion demanded.

Methods of Extraction. Different ratios of flour to water had

been tried out in this laboratory previous to the present work. It had

been found that when 20 grams of flour were mixed with 100 cc of water,

the resulting mixture was too viscous to work with, and 10 grams of flour

mixed with 100 cc of water was determined upon as the best ratio of water

to flour. In mixing the flour for extraction purposes, a little water

was added at a time and the flask vigorously rotated in order to get all

the flour in contact with the water. Care must be taken that no unwet-

ted material adheres to the bottom of the flask and all lumps must be

broken. This is accomplished by vigorous shaking and giving the flask

a rotating movement. All flasks used in the extraction were of Jena glass.

The conductivity water, which had previously been brought to the tempera-

ture at which the extraction was to take place was added slowly from a

pipette.

It had been observed by Bailey (1918) and Swanson (1919) that

periodic shaking, during the period of extraction, gives practically the

same results as continuous shaking. Periodic shaking was therefore adopt-

ed, the usual procedure being to shake every five minutes for fifteen min-

utes and then at fifteen minute intervals thereafter.

At the end of the extraction period the solutions were centri-

fuged at a high speed for five minutes in order to throw down as many

flour particles as possible. The supernatant liquid was then poured

through a filter paper to remove any light floating particles. In some

unpublished work Bailey noticed that a very slight difference existed in

the conductivity of the filtered extract and that which had not been fil-

tered.

* Landolt, Börnstein and Roth; Tabeln; 4 te auflage p. 1117

The cells were rinsed out several times with the filtered solution, then partially filled with it and allowed to come to temperature. The time necessary for the cell to reach the temperature of the bath varied and was determined for each cell. A constant reading was always obtained in at least fifteen minutes.

TEMPERATURE and TIME as a VARIABLE

From the very first, it was apparent that the temperature and the time at which the extraction was to be made were quite significant. Swanson and Tague (1919) in determining the phosphorus content of wheat extracts employed different temperatures and lengths of time at which to make their extractions. They report:

1. "That the total and inorganic phosphorus increases with time of extraction, when extracted at 5°C.

2. The total and inorganic phosphorus increases with time of extraction when digested at 20 degrees, and total phosphorus shows no increase after 16 hours.

3. The total and inorganic phosphorus increases with time of digestion at 40 degrees C. and reaches a maximum in 4 hours. The proportion of inorganic phosphorus to total phosphorus is much greater at the lower temperatures and at the end of four hours the inorganic phosphorus is almost equal to the total.

4. At 50 degrees the maximum is reached in 2 hours."

With this information at hand it was decided to extract at temperatures of 0, 25, 40, and 60 degrees. The specific conductivities of these extracts are given in Tables II and III and illustrated graphically in Fig. I, showing that the extraction of the electrolyte is more complete at the higher temperature and the maximum conductivity is obtained in a shorter space of time.

Table II.- Specific conductivity x 10^{-4} of the patent flour extracted at different temperatures for different lengths of time.

Temperature of extraction.	0°	25°	40°	60°
Time in minutes	K x 10^{-4}	K x 10^{-4}	K x 10^{-4}	K x 10^{-4}
15		5.478	5.797	6.161
30	4.601	5.590	5.916	6.253
60	4.600	5.668	5.958	6.272
120	5.264	5.798	6.110	6.347
240	5.515	5.830	6.181	6.444
480	5.609	5.950	6.2107	6.443
960	5.780	5.957		

Table III.- Specific conductivity x 10^{-4} of the clear flour extracted at different temperatures for different lengths of time.

Temperature of extraction	0°	25°	40°	60°
Time in minutes	K x 10^{-4}	K x 10^{-4}	K x 10^{-4}	K x 10^{-4}
15		8.789	9.355	9.780
30	6.477	9.167	9.880	9.872
60	6.770	9.367	10.018	9.936
120	7.378	9.999	10.260	10.182
240	8.041	10.160	10.347	10.195
480	8.890	10.401	10.680	10.474
960	9.333	10.593	10.770	10.474

The influence of temperature and time may be summarized as follows:

1. At 0° a steady rise in conductivity was noted with time of digestion in both the patent and the clear flours, which rise continued even after 960 minutes.

2. At 25° a slight increase was noted in the conductivity of patent extract with time of extraction, while in the clear the increase is quite rapid at first, slowing down after eight hours of digestion.

3. At 40° there is the same general increase in the conductivity of patent flour extract, while in the clear the increase is very rapid during the first two hours, then gradually slowing down until at the end of eight hours it had reached its maximum.

4. At 60° an increase is noted in the conductivity of the patent flour extract as at 25° and 40°. The conductivity of the clear flour extract increased during the first fifteen minutes but rises very slowly after this. The curve is very similar to that of the patent flour.

The difference between the 60° and the 40° curve in the clear flour will be discussed later, as it is probably due to an enzyme action.

THE RELATION OF K_{30} TO FLOUR GRADES AS DETERMINED BY ASH CONTENT

As a result of the data presented in Table II and III, it was decided that thirty minute extractions at 25° would give comparative results and would be a sufficient guide to the ash content. This temperature is easy to maintain in the laboratory and in addition enzyme activity at 25° is not accelerated to such a degree that a slight difference in time affects a material difference in the specific conductivity of the extract. The half hour period was selected simply because the time factor is of great importance in the milling industries in making flour analysis, and this was the shortest extraction period which it was believed
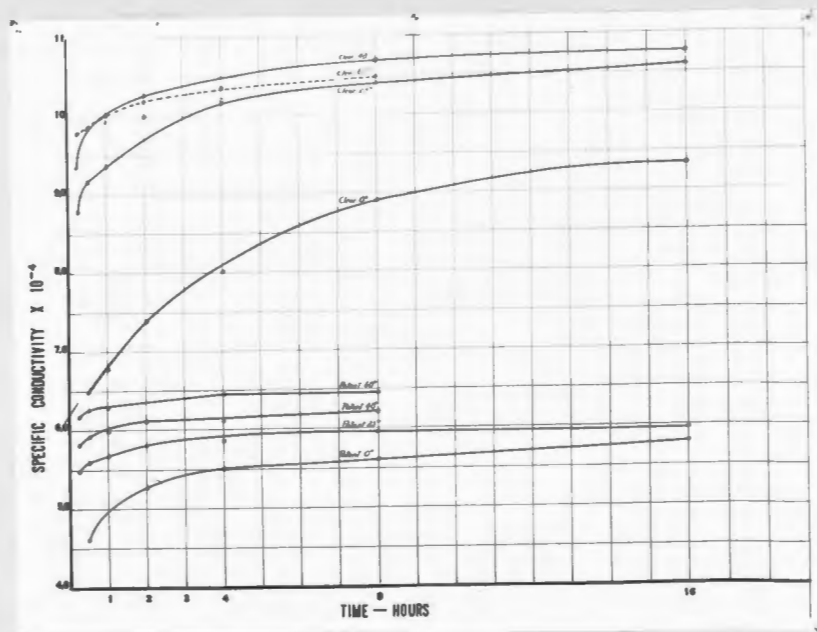
Fig. 1.- Relation of time and temperature of extraction to the specific conductivity of patent and clear flour extracts.
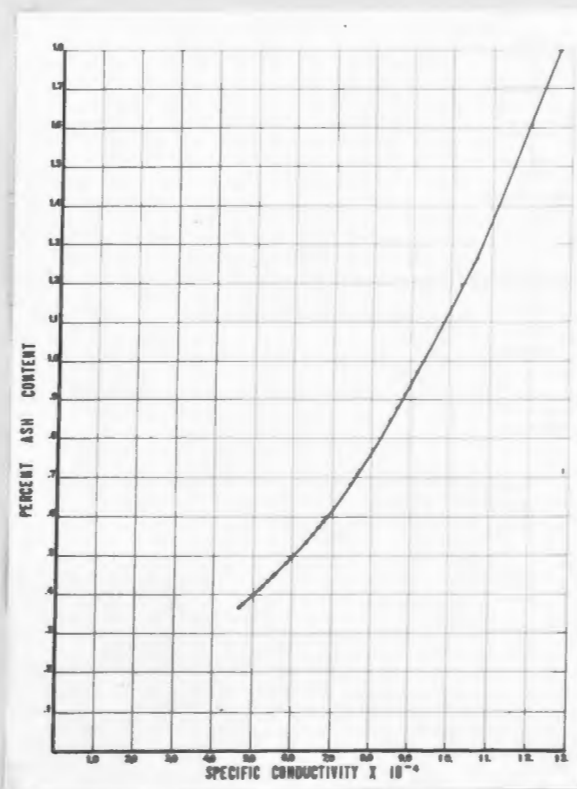

Fig. 2.- Relation of specific conductivity to ash content of Series A and B.

7.19.4m

would give really comparative results.

In comparing K_{30} with the ash content, two series of flour were used. Series A consisted of four break flours and five middling flours given in Table IV obtained from a modern roller mill.

Series B consisted of five break flours, one sizings, seven middlings, one stone stock, three tailings flours and one dust flour as shown in Table V, obtained from a modern roller mill of 500 barrels capacity. Included in this series is a patent (90 per cent) a first clear and a second clear, which were made up of the flours listed above. The patent (90 per cent) was made up of the seven middlings, the stone stock, the first tailings and the sizings flours, together with a small portion of the second, third and fourth break flours and the first coarse tailings.

The first clear was made up of the first and fifth breaks, second fine tailings and the dust flour with the remainder of the second, third and fourth break flours and first coarse middlings. Table V gives the value for the ash content of these various flours and their respective conductivity values.

Table IV.- Relation of K_{30} to ash content of flours in Series A

Grade of Flour	:	Per cent Ash	:	$K_{30} \times 10^{-4}$
First Break	:	1.34	:	10.563
Second Break	:	.59	:	6.647
Third Break	:	.67	:	7.690
Fourth Break	:	1.62	:	1.969
First Middlings	:	.44	:	5.395
Second Middlings	:	.45	:	5.547
Third Middlings	:	.56	:	6.338
Fourth Middlings	:	1.17	:	10.242
Fifth Middlings	:	.61	:	6.777

Table V.- Relation of K_{30} to ash content of flours in series B.

Grade of Flour	Per cent ash	K_{30} x 10^{-4}
First Break	.56	6.503
Second Break	.48	5.971
Third Break	.58	6.838
Fourth Break	.80	8.483
Fifth Break	.96	9.167
Sizings	.45	5.564
First Middlings	.41	5.270
Second Middlings	.38	4.744
Third Middlings	.42	5.002
Fourth Middlings	.46	5.514
Fifth Middlings	.43	5.192
Sixth Middlings	.42	5.075
Seventh Middlings	.47	5.870
Stone Stock	.35	4.643
First Fine Tailings	.73	7.624
Second Fine Tailings	.92	8.650
First Coarse Tailings	.66	7.450
Dust Flour	1.38	10.610
Patent - 90%	.44	5.815
First Clear	.90	8.850
Second Clear	1.73	12.678

The flours in the series A and B were extracted for thirty minutes at 25° and the conductivity of the extract determined at 30° in the convential way. The K_{30} of these extracts when compared with the ash content of their respective flours indicates that K_{30} varies almost directly with the ash content. Fig. 2 illustrates this graphically. Slight individual variations were found which made it necessary to subject the data to mathematical treatment in order to plot the curve accurately. The curve is a simple parabola and represents an equation of the second degree.

Using the formula

$$y = a + bx + cx^2$$

where $y =$ percentage of ash

$x =$ specific conductivity $x\ 10^{-4}$

and   a, b and c are variables.

$$a + bx + cx^2 - y = 0$$

$$a + bx_1 + cx_1^2 - y_1 = V_1$$

Squaring both sides of the equation

$$(a + bx_1 + cx_1^2 - y_1)^2 = V_1^2$$

$V =$ variable.

Then the sum of all the variables are

$$V_1^2 + V_2^2 + V_3^2 + \cdots\cdots V_{31}^2 = \text{minimum}$$

Solving for a, b, and c and integrating

$$\frac{\partial \, \xi (a + bx_1 + cx_1 - y_1)^2}{\partial a} = 2 \quad \xi(a + bx_1 + cx_1^2 - y) = 0$$

$$\frac{\partial}{\partial b} \xi (a + bx_1 + cx_1^2 - y_1)^2 = 2 \, \xi x_1(a + bx_1 + cx_1^2 - y_1) = 0$$

$$\frac{\partial}{\partial c} \xi (a + bx_1 + cx_1^2 - y_1)^2 = 2\xi x_1^2(a + bx_1 + cx_1^2 - y_1) = 0$$

Then the corrections for

A is $\cdot \xi (a + bx_1 + cx_1^2 - y_1) = 0$

B is $\xi x_1 (a + bx_1 + cx_1^2 - y_1) = 0$

C is $\xi x_1^2 (a + bx_1 + cx_1^2 - y_1) = 0$

By substituting the sum of the various values for x and y the corrections for

$$a = .2786$$

$$b = .03902$$

$$c = .01252$$

The data used for these calculations and the calculated values for the percentage ash is given in Table VI.

It will be noticed that the conductivity method is extremely accurate in estimating the higher grades of flour, by inspection of the column showing the difference in percent of ash actually observed and that of the calculated Table IV. It will be observed that up to .662 per cent of ash the conductivity method is very accurate, the difference between the actual and the calculated ash content being small. After .662 per cent of ash is exceeded, greater differences were encountered, due probably to factors which will be discussed later.

Table VI. - Calculated values for ash content and deviations from observed
        values.

Grade of Flour		$K_{50} \times 10^{-4}$	% Ash Content	Calculated % Ash Content	Difference %
Stone Stock		4.6430	.346	.367	.021
Second Middlings		4.7440	.378	.375	.003
First Middlings		5.2700	.409	.421	.002
Sixth Middlings		5.0750	.417	.403	.014
Third Middlings		5.0020	.419	.397	.022
Fifth Middlings		5.1920	.427	.414	.013
Patent	x	5.5900	.435	.452	.017
First Middlings	*	5.3953	.442	.433	.009
Second Middlings	*	5.5473	.446	.438	.008
Sizings Flour		5.5640	.451	.449	.002
Fourth Middlings		5.5140	.460	.444	.016
Seventh Middlings		5.8700	.467	.481	.014
Second Break		5.9710	.479	.492	.013
Third Break	*	6.3378	.555	.534	.021
First Break		6.5030	.564	.554	.010
Third Break		6.8380	.579	.597	.018
Second Break	*	6.6467	.585	.572	.013
Fifth Break	*	6.7774	.613	.588	.025
First Coarse Tailings		7.4495	.662	.683	.021
Third Break	*	7.6897	.668	.719	.051
First Fine Tailings		7.6340	.726	.709	.017
Fourth Break		8.4830	.803	.849	.046
Clear	x	9.1670	.920	.973	.053
Clear		8.8500	.900	.914	.014
Second Fine Tailings		8.6500	.919	.878	.041
Fifth Break		9.1670	.955	.973	.018
Fourth Break	*	10.2423	1.171	1.196	.025
First Break	*	10.9630	1.340	1.263	.077
Dust Flour		10.6100	1.383	1.274	.109
Fourth Break	*	11.9689	1.620	1.606	.014
Second Clear		12.6780	1.731	1.797	.066

* - Series A.

x - Experimental flours used in preliminary experiments.

EFFECT OF BLEACHING ON THE K_{30} OF FLOUR EXTRACTS

After having determined the effects of time and temperature upon the K_{30} of flour, it was thought that it might be of interest to note the effects of bleaching upon the conductivity of the flour extract. Accordingly 200 gram portions of the clear flour used in the preceeding work were bleached with varying amounts of NO_2, namely 2, 5, 10, 25, 50, and 75 cc of NO_2 per 200 grams of flour. The conductivity was determined by extracting 10 grams of the treated flour for thirty minutes at 25^0. The results as given in Table VII show that a very small increase in K_{30} is produced by a small addition of NO_2 to the flour.

Table VII.- Showing effect of bleaching on the K_{30} of flour extracted 30 minutes at 25^0

NO_2 used per 100 gms flour	$K_{30} \times 10^{-4}$
cc	
0	9.1670
1.0	9.2840
2.5	9.3768
5.0	9.5730
12.5	9.5100
25.0	9.8130
37.5	10.1830

INFLUENCE OF ENZYMES

In the conductivities of patent and clear flour extracts shown in Tables II and III a certain irregularity in progression with temperature as the variable is noticed in the K_{30} for the 60° extract, which is lower than that of the 40° extract inthe case of the clear flour.

Numerous investigators have shown that phytase, an enzyme which hydrolyzes organic phosphorus to inorganic phosphorus, is contained entirely in the bran and germ of the wheat kernel. Also the fact that about 50 per cent of the mineral constituents of bran are in the form of phosphates led to the belief that this abnormality noticed in the clear flour was due to the action of the enzyme phytase, as the clear flour has a relatively large proportion of bran present. It was assumed that the inhibition of phytase was the reason for the marked decrease in K_{30} of the 60° extract. A review of the literature gave only one reference dealing with the relations of temperature to phytase activity so that the following study of the activity of phytase was necessary to explain the difference noted between the 40° and 60° extract of the clear flour.

THE OPTIMUM TEMPERATURE OF PHYTASE ACTIVITY

Historical. Suzuki, Yoshimura and Tokaishi (1907) observed that when rice bran was allowed to stand for several days, at ordinary temperature, the major part of the organic phosphorus was changed to phosphoric acid. At first they thought it to be due to bacterial action, but later work showed it to be of an enzyme nature, since boiling stopped the action while toluol did not. They claim to have isolated the enzyme and found that it had no tryptic, diastatic or peptic activity and for this enzyme they proposed the name phytase.

A phytin splitting enzyme was found in the liver and blood of the calf by McCollum and Hart (1908)

Forbes and his co-workers (1915) while estimating organic phosphorus tried to devise a method to prevent enzyme activity by dry heat and boiling. As this interferred with their analytical procedure, it was not adopted.

Tortrodt (1910) found that the quantity of phosphoric acid increased with time, when barley meal was digested with dilute acetic acid; also that when a water extract of barley or wheat bran was boiled the formation of phosphoric acid was stopped. This indicated to him that the increase in phosphoric acid during the digestion of barley or wheat bran with water was due to an enzyme. He found that temperature had a decided influence upon the activity of the enzyme; at 3° - 5° it was practically inactivated, 38° is the temperature of maximum activity, while at 50° - 60° or above, the reaction is very slow.

Plimmer (1913) in a series of experiments on organic-phosphorus splitting enzymes finds that there is a specific enzyme present in bran which hydrolyzes phytic to phosphoric acid and inositol.

Anderson (1915a) showed that the organic phosphorus in wheat bran is very rapidly hydrolysed by the enzyme phytase. He notes that when wheat bran is digested in distilled water hydrolysis sets in at once and proceeds with considerable rapidity. The optimum medium was found to be a 0.1 per cent HCl solution, while a 0.3 per cent HCl solution inhibits and boiling water destroys the action of the enzyme. In a later article (1915b) Anderson finds that the chief products of the hydrolysis of phytin, by phytase, in wheat are inorganic phosphorus and certain intermediate products apparently consisting of inosite tri-di and monophosphates.

Boutwell (1917) tried the effect of dry heat upon the activity of phytase at various temperatures by heating the bran and then extracting with water or dilute HCl. He then determined the total and the inorganic phosphorus extracted and compared his results with samples of unheated bran and finds that dry heat increases the amount of inorganic phosphorus. Thus, when the bran was heated to 125° - 135°C for ten hours and then extracted with 2 per cent HCl, the amount of inorganic phosphorus was practically twice that of the unheated, while temperatures as high as 165°C. had no effect upon the activity of the enzyme phytase.

The Problem. Attention has already been called to Fig. 1, where increase in temperature of extraction is accompanied by a regular increase in the specific conductivity of the patent flour extracts. When the clear flour curves are examined, however, we notice a very sharp increase in K_{30} at the beginning of the extraction period and the conductivity increases with the increase in temperature of digestion up to 40°. At 60° this increase appears at the end of the fifteen minute period, but at the end of the thirty minute period the conductivities of the 40° and 60° extracts are identical. From this point on the 40° extract increases very rapidly while that of the 60° extract increases very little and soon reaches its maximum. The reason that the patent flour extracts did not exhibit the same characteristics may be explained by the fact that there is little bran present in the patent flour and the mineral constituents are rapidly extracted, while in the clear flour there is an appreciable amount of bran. The fact that there is such a small amount of organically combined phosphorus present in the patent flour explains why temperature of extraction overshadows, by far, the temperature effect upon the rate of hydrolysis.

As has been pointed out by numerous investigators, the enzyme phytase exists chiefly in the bran, while Boutwell (1917) has also found it in the embryo. This explanation for the difference in E_{30} between the 40° and 60° extracts in the clear flour is believed to be due entirely to an enzyme reaction. In order to determine the optimum and the inhibiting temperatures and to verify these conclusions, it was necessary to measure the enzymatic activity of the phytase between the temperatures 40° and 60°.

EXPERIMENTAL

Materials Used. The phytase used in the following experiments was prepared by extracting finely ground wheat bran with water at a temperature of $2^{\circ} - 3^{\circ}$ C. After several hours of extraction, the extract was filtered into 95 per cent alcohol. The resulting precipitate was dried, taken up with water, filtered and reprecipitated with alcohol. This was repeated three times and after the third time, the precipitate was dried in a vacuum and then pulverized in a mortar, a grayish-white residue resulting. Pure phytin furnished by Dr. J.B. Rather was used in a water solution as the substrate.

Method. As the result of some preliminary trials, it was decided to use 50 mgms of phytin made up to 50 cc with redistilled water. The same amount of phytase preparation was used, namely, 50 mgms and made up to 50 cc with redistilled water. As only 10 cc of each solution was used in making a determination, the remainder was kept in a refrigerator until needed.

The method of determining the specific conductivity of the phytin-phytase solution was the same as employed in the previous work on the flour extracts. The temperature of hydrolysis was controlled by a De Khotinsky water bath, except at temperatures between $45^{\circ} - 60^{\circ}$

Table VIII.- Specific Conductivity of **phytin-phytase** solutions hydrolyzed at various temperatures.

Temperature of Hydrolysis	25°	30°	35°	40°	45°	50°	55°	60°
Time in Minutes	$K_{29} \times 10^{-4}$	$K_{30} \times 10^{-4}$	$K_{35} \times 10^{-4}$	$K_{40} \times 10^{-4}$	$K_{45} \times 10^{-4}$	$K_{50} \times 10^{-4}$	$K_{55} \times 10^{-4}$	$K_{60} \times 10^{-4}$
0	1.1481	1.3124	1.4416	1.3964	1.4551	1.4854	1.6660	1.7887
15	1.2047	1.4488	1.6109	1.7390	1.7719	1.9556	2.2090	2.5933
30	1.2746	1.5871	1.7825	1.8832	2.0921	2.3270	2.6410	2.7193
45	1.3526	1.6901	1.9177	2.1063	2.2212	2.4010	2.7010	2.7700
60	1.4318	1.7508	1.9688	2.1170	2.2506	2.4140	2.7230	2.7938
75	1.4821	1.8146	1.9872	2.1347	2.2592	2.4250	2.7360	2.8015
90	1.5282	1.8226	1.9872	2.1424	2.2620	2.4330	2.7410	2.8271
105	1.5588	1.8257		2.1424		2.4380	2.7410	2.8384
120	1.5727	1.8257						
135	1.5796							
150	1.5796							

When air thermostats were used.

In order to have a check on the phytin and to ascertain whether any hydrolysis took place when the phytin was dissolved in water, 10 cc of a freshly prepared phytin solution was placed in the conductivity cell and allowed to come to temperature and its specific conductivity determined Readings were taken at fifteen minute intervals and at the end of the sixteen hours the readings were the same as at the outset.

In measuring the rate of hydrolysis 10 cc of the phytin solution was put in a Freas cell and 10 cc of the phytase solution in a separate vessel were allowed to come to temperature, this usually taking twenty minutes. The phytase solution was then poured into the cell, containing the phytin, and the contents shaken to mix the solutions. The specific conductivity readings were taken immediately and additional readings every fifteen minutes thereafter, until no appreciable decrease in resistance was noticed. The reaction was then assumed to be complete or as far as it was likely to proceed. In this manner the conductivities were determined at 25°, 30°, 35°, 40°, $\overset{45°}{50°}$, 55°, and 60°, the results being given in Table VIII and illustrated graphically in fig. 3. These results are not comparative however, as the conductivity measurements of the solutions were made at the temperature of hydrolysis. In order to make the data comparable, the mobility of ions was taken into consideration. This was done in the following manner. A solution which was hydrolyzed at 55° was allowed to go to completion. The conductivity of it was determined at 55°. The cell and contents were then cooled down to 25° and the conductivity of it immediately determined at this temperature. The specific conductivity at 55° was 2.9585 x 10^{-4} and at 25° it was 1.8882 x 10^{-4} giving an increase of 56.684 per cent for 30° rise in temperature. The increase was found to be 1.89 per cent for one degree rise in temperature
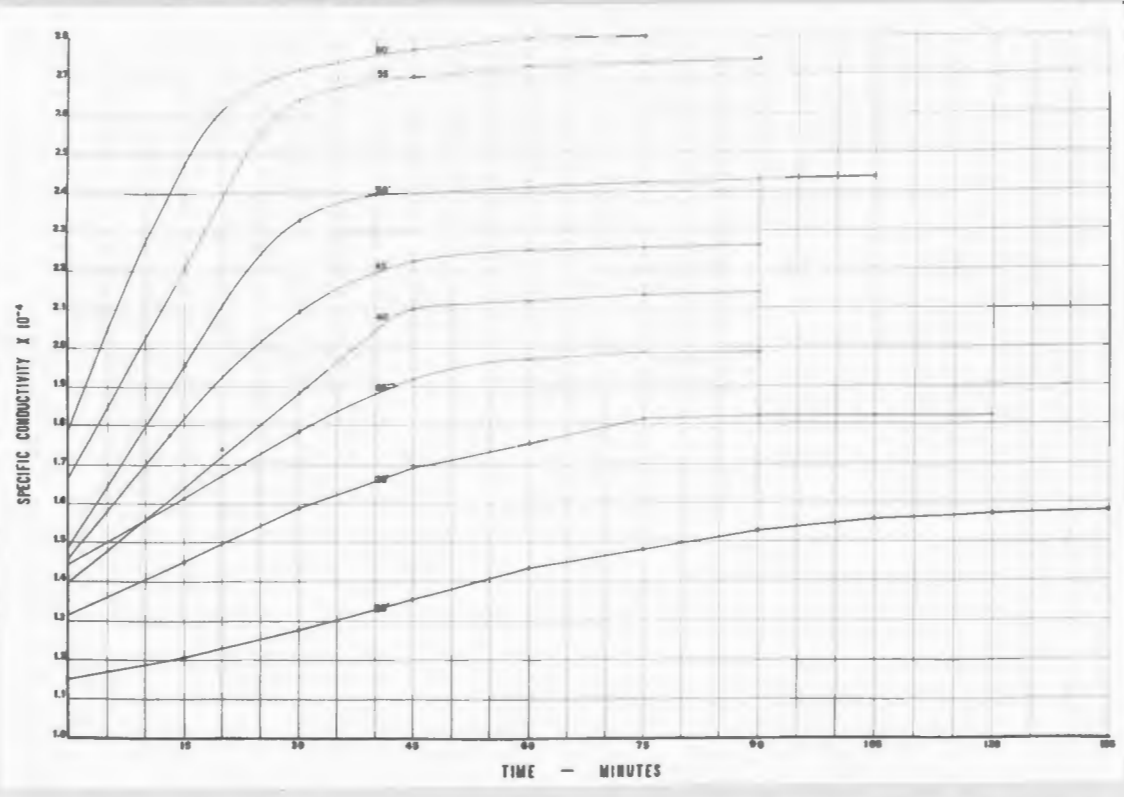
Fig. 3.- Graphs showing the rate of hydrolysis of a phytin-phytase solution at various temperatures expressed as specific conductivity.
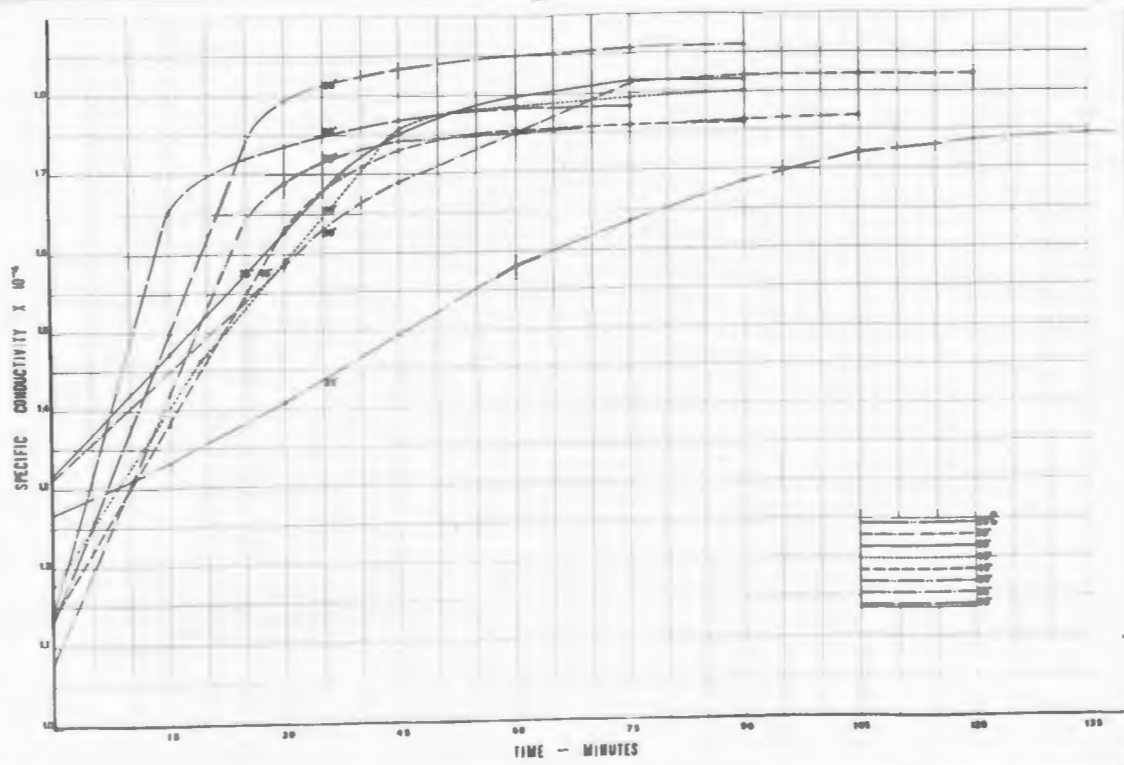

Fig.4.- Graphs showing the rate of hydrolysis of a phytin-phytase solution at various temperatures, expressed as specific conductivity with all values calculated to 30° C.

agreeing quite closely with the mobility of KCl ions as calculated from Landolt, Bornstein and Roth.   The following table gives data taken to calculate the mobility of KCl ions for one degree rise in temperature.

Table IX.- Percentage increase in conductivity for one degree rise in
            temperature (from Làndolt, Börnstein & Roth.)

Solution Used	:	N/10 KCl	:	N/50 KCl	:	N/100 KCl
K_6	:	.00715	:	.001521	:	.000776
K_{30}	:	.01412	:	.003036	:	.001552
$K_{30} - K_o$	:	.00697	:	.001515	:	.000776
Diff per 1° in K	:	.0002323	:	.0000505	:	.00002587
Per cent increase in 1°C	:	1.645	:	1.663	:	1.666

        In this manner it was possible to reduce all of the data in Table VIII to 30°C at which temperature the conductivities of the flour extracts had been made, giving a comparison of the rate of hydrolysis, optimum temperature and inhibiting temperature.   The data calculated to 30° is given in Table X and illustrated graphically in Fig. IV.

Table X.- Specific Conductivity of phytin-phytase hydrolysis calculated to 30°C.

Temperature of Hydrolysis	25°	30°	35°	40°	45°	50°	55°	60°
Time in minutes	$K_{30} \times 10^{-4}$	$K_{30} \times 10^{-4}$	$K_{30} \times 10^{-4}$	$K_{30} \times 10^{-4}$	$K_{30} \times 10^{-4}$	$K_{30} \times 10^{-4}$	$K_{30} \times 10^{-4}$	$K_{30} \times 10^{-4}$
0	1.2679	1.3124	1.3171	1.1745	1.1338	1.0780	1.1316	1.1416
15	1.3304	1.4488	1.4719	1.4626	1.3800	1.4193	1.5004	1.6551
30	1.4076	1.5871	1.6286	1.5839	1.6301	1.6888	1.7939	1.7355
45	1.4937	1.6901	1.7522	1.7610	1.7307	1.7426	1.8346	1.7680
60	1.5811	1.7508	1.7989	1.7806	1.7536	1.7520	1.8496	1.7832
75	1.6367	1.8146	1.8157	1.7994	1.7603	1.7600	1.8584	1.7883
90	1.6876	1.8226	1.8157	1.8019	1.7625	1.7657	1.8618	1.8043
105	1.7214	1.8257		1.8019		1.7694	1.8618	1.8115
120	1.7367	1.8257						
135	1.7444							
150	1.7444							

DISCUSSION

In making water extracts of flours, increase in temperature of digestion within certain limits increases the specific conductivity of the extracts. This generalization holds for the patent flour extracts at all temperatures tried, (Fig.I). In the clear flour extracts, the same general increase is noted in the initial readings, but a decided difference is noticed in the 60° extract after the thirty minute period. Instead of the usual rapid increase, the rise in K_{30} of the 60° extract is very slow after the first 30 minutes, and reaches its maximum very quickly, differing in this respect from the other extraction. The logical conclusion based upon the latter part of the investigation is that this is due to the inhibition of the enzyme phytase.

It was also observed that the K_{30} of a flour extracted at 25° for 30 minutes varies almost directly with the ash content of the flour, (Fig.2). With but few exceptions (Table VI) this method parallels the percentage of ash within the usual experimental error in determining the latter. This method has certain advantages over the ashing method, as it gives results in a shorter space of time, and with practice the operator could run a much larger number of determinations per day, as he is not subjected to
of
limitations, muffle space and expensive platinum dishes.

The ash content of bleached flours can also be determined satisfactorily as the small amounts of NO_2 used to bleach flours make very small difference in their conductivity.

From the work conducted upon the hydrolysis of phytin by Phytase it will be noticed that when pure phytin is hydrolyzed at 25°, Fig.3, a steady increase in conductivity is noted until at the end of 135 minutes the hydrolysis reaches its maximum. At each 5° increase in temperature, the initial rate of reaction is accelerated and reaches a maximum sooner until at 60°

the initial rate is very rapid.   These curves in Fig.3, however represent actual conductivities taken at various temperatures of hydrolysis, and the maximum value recorded does not give a true comparison.   After correcting for the mobility of ions and calculating the data to 30° (Fig.4), it is observed that the rate of hydrolysis at 60° is very rapid, at first, but falls off after the first fifteen minutes and practically comes to a maximum in 45 minutes.

The initial rate of hydrolysis at 55° is not as rapid as that at 60° but continues for a longer period of time.   It does not slow down appreciably until the end of 30 minutes but continues to act and reaches its maximum at the end of 75 minutes.   Hydrolysis at 40° gave a higher conductivity than that conducted at 60°, thus indicating that the discrepancies exhibited in Fig.1 are due to the inhibition of the enzyme through heat.

It is also of interest to note that the optimum temperature of phytase from wheat bran is 55° and not 28° as found by Vorbrodt (1910) with phytase from barley meal.   Vorbrodt further reports the activity of barley phytase to be very slow at 48° whereas in the present work the maximum activity of wheat phytase was observed at 55° and then falling off very rapidly with further elevation of the temperature so that at 60° the extent of hydrolysis was considerably below that of the 40° extract.

S U M M A R Y

1. Preliminary experiments, using temperatures of 0°, 25o, 40° and 60°, periods of extraction of 15,30,60,120,240,480, and 960 minutes showed that within certain limits an increase in time and temperature was accompanied by a corresponding increase in conductivity of the extract. This data shows that a 30 minute extraction at 25° gives comparable results.

2. The specific conductivity of flour extracts is an index of grade, using the ash content of the flour as a criterion. This method has an advantage over that of direct ashing, as more determinations can be made by the operator. The specific conductivity of a flour extract parallels the ash content of the flour and the relation can be expressed by an equation of the second degree.

$$y = a + bx + cx^2$$

3. The rate of hydrolysis of a phytin-phytase solution was studied by the conductivity method. Temperatures of 25°, 30%, 40°, 45°, 50° 55° and 60° were used, readings being taken every fifteen minutes. The optimum temperature for phytase of a wheat bran is 55°. The inhibition of phytase activity starts between 55° and 60°. At 60° the enzymic activity is reduced to such an extent that the conductivity of the digest is below that of the preparation hydrolyzed at 40°.

4. The initial rate of hydrolysis of the phytin-phytase solution increases rapidly with each 5° increase in temperature, reaching its maximum sooner at the higher than at the lower temperature.

LITERATURE CITED

Alway, F. J. and Clark, V. I.

    1909. The color and ash content of different grades of
        Nebraska flour.
        Neb. Sta. 23d Annual Report p.25-30.

Anderson, R.J.

    1915a. The hydrolysis of the organic phosphorus compound of
        wheat bran by the enzyme phytase.
        Jour. Biol. Chem. Vol.20, p. 483

    1915b. Hydrolysis of phytin by the enzyme phytase contained
        in wheat bran.
        Jour. Biol. Chem. Vol 20, p. 475-482.

Bailey, C. H.

    1915. Catalase activity of American wheat flours.
        Jour. Biol. Chem., Vol. 32, p.539-545

    1918. The specific conductivity of water extracts of wheat
        flour.
        Science, N.S., Vol. 47. p. 645-647

Bayliss, W. M.

    1907. On the cause of the rise in electrical conductivity
        under the action of trypsin.
        Jour. Physiol., Vol. 36, p. 221-52.

Blackman, V. H. and Paine, S. G.

    1918. Studies on the permeability of the pulvinus of minosa
        pudica.
        Ann. Bot., Vol.32, p. 69-85

Boutwell, P. W.
    1917. The phytic acid of the wheat kernel and some of its
        salts.
      Jour. Amer. Chem. Soc., Vol. 39, No. 3, p. 491-501.

Delehage, A.

    1917. The examination of 85 per cent flour.
        Ann. Fals., t. 10, p. 554-6 (original not seen.)

Clowes, G. H. A.

    1918. The electrical resistance and permeability of tumor tissues.
        Proc. Soc. Exp. Biol. Med., Vol.15, p. 107-108.

Girard, A.

    1895. Appreciation de la valeur boulangere des farines; dosage
            des debris d'enveloppe et de germe susceptibles de dimineur
            la qualite du pain.
            Compt. rend., t. 121, p. 858

Green, N. B.

    1918. The effect of ions of $NaCl$ and $CaCl_2$ upon the electrical con-
            ductivity of certain colloidal mixtures.
            Plant World, Vol.21, p. 303-316.

Guilio, M.

    1915. Sul valore alimentare delle farine di frumento e del pane
            in rapporto ai composti fosforti, Stazioni sperimentali
            agrarie italiane, Vol. 46, p. 385-456.

Hein, G.

    1910. German patent. 250, 413.

Hober, R.

    1910. Eine methode die elecktrische Leitfähigkeit in Innern von
            Zellen zu Messen.
            Pflügers Arch. f.d. gesamte Physiol., Bd. 133, p. 237-253

    1912. Ein Zweites Verfahren, die Leitfähigkeit in Innern von Zellen
            zu messen.
            Pflügers Arch. f.d. gesamte Physiol. Bd. 148. p. 189-221

    1913. Messungen der innern Leitfähigkeit von Zellen; Dritte Mittel-
            ung.
            Pflügers Arch. f.d. gesamte Physiol. Bd. 150, p. 15-45.

Kohlrausch, F., and Holborn, L.

    1898. Das Leitvermögen der Elektrolyte. Leipsic

Koning, C. J., and Mooij, W. C. Jr.,

    1914. Bijdrage tot het Ouderzoek von Brood.
            Chemisch. Weekblad, Bd. 11. p. 1064-1066

Liebermann, L.v. and Andriska, V.

    1911. Ein neues verfaren zur bestimmung des Feinheitsgrades der
            Weizenmehle.
            Z. Untersuch. Nahr u. genus., Bd. 22, p. 291-294.

Liechti, Paul.

    1909. Die Prüfung von Mehlen auf Grunde ihres Gehaltes an Katalase.
            Chem. Ztg.,Bd. 33. p. 1057

Mc Clendon, J. F.

1911. The increased permeability of striated muscles to ions during contraction.
Amer. Jour. Physiol., Vol. 29, p. 302-305.

Mc Collum, E. V., and Hart, E. B.

1908. On the occurrence of a phytin-splitting enzyme in animal tissues.
Jour. Biol. Chem., Vol.4, p. 497-500.

Osterhout, W. J. V.

1918. Conductivity as a measure of permeability.
Jour. Biol. Chem., Vol. 34, p. 485-87

1918. Note on the effect of diffusion upon the conductivity of living tissues.
Jour. Biol. Chem., Vol.36, p. 489-90.

1918. A method of measuring the electrical conductivity of living tissues.
Jour. Biol.Chem. Vol. 36., p. 557-68

1919. A comparative study of permeability in plants.
Jour. Gen. Physiol. Bol. 1, p. 299-304

Plimmer, R. H. A.

1913. The metabolism of organic compounds; their hydrolysis by action of enzymes.
Biol. Chem. Jour., Vol. 7, p. 43

Snyder, Harry

1905. Testing wheat flour for commercial purposes.
Jour. Amer. Chem. Soc., Vol. 27, p. 1068-74

Suzuki, Yoshimura, and Takaishi.

1907. Uber ein Enzyme "Phytase" das "Anhydro-oxymethylene-di-phosphorsaure" splatet.
Tokyo Imper. Univ. Coll. of Agri., Bul. No 7, p. 503-12.

Stiles, W], and Jorgenson, I.

1914. The measurement of electrical conductivity as a method of investigation in plant physiology.
New Phytol., Vol. 13, p. 226-242.

1918. Quantitative measurement of permeability.
Bot. Gaz., Vol. 65, p. 526-533.

Swanson, C. O.

1912. Acidity in wheat flour; its relation to phosphorus and to other constituents..
Jour. Indus. Engin. Chem., Vol. 4, p 1-7

_____; Willard, J.T. and Fitz, L.A.

1915. Chemical composition and baking qualities of flours from different mill streams. Kansas Flours. Part III.
Kans. Sta. Bul. No. 202

_____; Tague, E.L.

1917. Nitrogen in amino form as determined by formol titration in relation to some other factors measuring quality in wheat flour.
Jour. Amer. Chem. Soc., Vol. 39, p. 482

_____

1919. Determination of acidity and titrable nitrogen in wheat with the hydrogen electrode.
Jour. Agri., Res., Vol. 16, P. 1-13.

True, R. H.

1914. The harmful effects of distilled water.
Amer. Jour. Bot., Vol. 1. p. 255-273

Vidrodi, V.

1893. Untersuchung von Mehlsorten nebst einer neuen Methode zur Bestimmung der Feinheit der Mehle.
Zschr. Angew. Chem. p. 691-696

Vorbrodt, W.

1910. Untersuchungen uber die phosphorverbindungen in den Pflanzensamen, mit besonderer Berucksichtigung des Phytins.
Bul. Int. de l'Acad. Sci. Cracovie, Serie A I, p. 414-511.

Wender, N. and Lewin, D.

1904. Die Katalytischen Eigenschaften des Getreides und der Mehle
Oster. Chem. Z., Bd. 7, p. 173-175

Wenier, N.

1905. Die Feinheitsbestimmung der Mehle.
Z. Untersuch. Nahrungs u. Gemusem. Bd. 10, p. 747-756

Wiley, H. W.

1898. Foods and Food Adulterants; cereals and cereal products.
U. S. Dept. Agri. Bur. Chem. Bul. No. 13, pt. 9