PHYTIC ACID REDUCTION IN WHOLE WHEAT FLOUR DOUGHS BY pH ADJUSTMENT OR WITH SPROUTED WHEAT ADDITION

A Thesis

Presented to the

Department of Food Science and Nutrition

Brigham Young University

In Partial Fullfillment

of the Requirements for the Degree

Master of Science

by

Richard Alan Mayfield

December 1982

This Thesis, by Richard Alan Mayfield, is accepted in its present form by the Department of Food Science and Nutrition of Brigham Young University as satisfying the thesis requirement for the degree of Master of Science.

John Hal Johnson, Committee Chairman

Albert E. Purcell, Committee Member

9 December 1982

Date

Clayton S. Huber, Department Chairman

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

ACKNOWLEDGEMENTS

The use of the laboratory facilities of Deseret Mills and Elevators, Kaysville, Utah was very helpful to the completion of this work. Special thanks is given to Dr. John Hal Johnson and Dr. Albert E. Purcell for their guidance and assistance. Gratitude is expressed to Welles Cannon for his continual encouragement and support. Miss Patty Calder has helped greatly in preparing the final copy of this manuscript. Finally, without the sustaining support and help of my wife, Mava, my daughters Sonya, Kristi, Alicia and my parents this effort would not have reached its completion.

INTRODUCTION

The growing trend in this country of increasing use of whole wheat flour, along with the continued use of whole wheat flour by major segments of the world population, has focused attention on the problem of lowered bioavailability of divalent minerals due to phytic acid. This nutritonal problem has suggested the need for ways to reduce or eliminate phytic acid from whole wheat flour doughs. The purpose of this study was to evaluate proposed methods of phytic acid reduction in whole wheat flour doughs. The first method was based on the addition of acetic or citric acid to obtain the optimum pH of phytase in the doughs. The second method involved the addition of sprouted wheat to increase phytase activity in the doughs.

LITERATURE REVIEW

PHYTIC ACID

Phytic acid has been the subject of study since it was first detected in 1872 by Pfeffer. Even though early chemical studies were done it was not until 1969 that an exact structure (Figure 1) was confirmed (Johnson and Tate, 1969). The chemical designation of phytic acid is myo-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate). The calcium-magnesium salt of phytic acid is termed phytin and phytate indicates the mono to dodeca anion of phytic acid.

Phytic acid is found in a variety of foods. Averill and King (1926) list 57 foodstuffs (grains, flours, soybeans and nuts) with phytin contents ranging from 0.66% to 3.33%. Maga (1982) in his review of phytate lists cereal grains and oilseeds which contain phytate.

Wheat is one of these foods containing phytic acid. Averill and King (1926) list three cultivars of wheat ranging in phytic acid level from 1.16% to 1.36%. Lolas et al. (1976) reported 38 cultivars of wheat ranging from 0.62% to 1.32% phytic acid content. A more complete study by wheat cultivar and area grown shows cultivars grown in midwestern states to be higher in phytic acid content than western wheats (Witmer, 1981). This study reported a range of phytic acid values from 570 mg (0.57%) to 1346 mg (1.35%) per 100 gm of

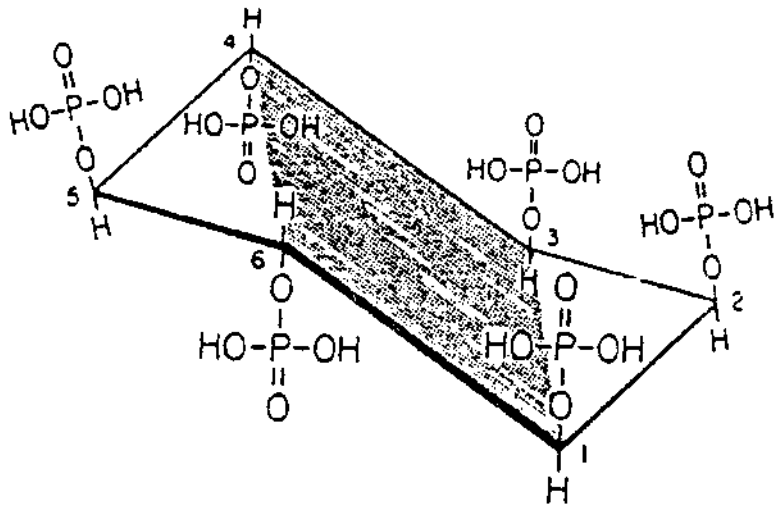

Figure 1. Myo-Inositol 1, 2, 3, 4, 5, 6 Hexakis (Dihydrogen Phosphate) Phytic Acid (Oberleas, 1971).

wheat. The same cultivar (Centurk) grown in three different states also had a wide range of values from 570 mg (0.57%) to 963 mg (0.96%) per 100 gm of wheat.

In the development of the wheat kernel, phytic acid is formed by inositol phosphorylation. Asada et al. (1969) proposed the hypothesis that phytic acid in cereal grains does not occur in a sequential fashion through phosphorylated inositol, but with a hypothetical phosphorylated inositol derivative. This hypothesis has inositol phosphorylated by a phosphate donor to inositol monophosphate which is then complexed by an unknown reagent X to form a phosphorylated inositol derivative. This derivative is then phosphorylated until all six positions on the inositol have phosphates attached. The derivative then releases the phytic acid.

The role of phytic acid in wheat suggested by Williams (1970) is the storage of phosphorus for future plant use and the slowing of metabolism prior to dormancy by the rapid formation of phytic acid near maturity. Phytic acid is shown to contain from about 50% to 80% of the total phosphorus in wheat (Bassiri and Nahapetian, 1977; Lolas et al., 1976; Nahapetian and Bassiri, 1975). This high level of phosphorus suggests a storage role for phytic acid.

BIOAVAILABILITY OF DIVALENT MINERALS

Phytic acid has been suggested as the cause of lowered bioavailability of various divalent minerals. Various investigators have reported lowered bioavailability of calcium due to phytic acid

(Hoff-Jorgensen et al., 1946; Bruce and Callow, 1934). Haghshenas et al. (1972) attributed the occurrence of iron-deficiency anemia of Iranians to high phytate levels from their main dietary staple of unleavened whole wheat bread. This effect occurred despite a high iron content in their diet.

In the 1950's zinc bioavailability for laboratory animals was being investigated due to zinc deficiency problems. O'dell and Savage (1960) reported lowered zinc bioavailability in studies with phytic acid added to various feeds. In studies with rats Oberleas et al. (1966) indicated decreased bioavailability of zinc due to phytate. A further study by O'dell et al. (1972) in evaluation of zinc availability from plant and animal sources indicated that phytic acid appeared to cause a lower availability of zinc from plant sources. More recently Franz et al. (1980) and Witmer (1981) both reported an inverse relationship between phytic acid and bioavailability of zinc.

Finally, Oberleas et al. (1966) also indicated an increased lowering of zinc bioavailability in the presence of excess calcium.

In one study Reinhold (1975) suggested that phytic acid may be a possible cause of human zinc deficiency in Iran. However, Reinhold et al. (1975) reported that fiber and not phytic acid was the primary cause of lowered divalent mineral bioavailability. In contrast to those results, Davis et al. (1977) concluded that phytate and not fiber was the major determinant of zinc bioavailability. Although Reinhold et al. (1976) concluded that dietary fiber was a major factor affecting zinc availability, Davis

et al. (1977) found the correlations between zinc and phosphorus were closer than between zinc and fiber. Since most of the phosphorus was present as phytate it would suggest phytate was more important than fiber.

PHYTASE

Phytase is the enzyme which catalyses the hydrolysis of phytic acid to inositol and phosphate. Peers (1953) reported that phytase purified from whole wheat flours had a pH optimum of 5.15 and an optimum temperature of 55°C. The Michaelis constant for phytase was 0.3×10^{-3}M. The usual heavy metal salts completely inhibit the enzyme. And hard wheats have a higher acitivty than soft wheats.

Nagai and Funahasi (1962) indicated that phytase of wheat bran was not a phytin-specific phosphatase but the same as an enzyme known as plant nonspecific acid phosphomonoesterase. Later studies indicate that phytase from wheat bran can be separated into two fractions F_1 and F_2 by DEAE-cellulose (Lim and Tate, 1971). These fractions yield different substrate degradation patterns and F_1 is activated by lysolecithin. Finally the results of Lim and Tate (1973) suggest that a variety of phytases are present in biological systems.

PHYTIC ACID REDUCTION

During World War II the lowered absorption of calcium due to phytic acid was of great concern because of the higher levels of phytic acid in flour due to higher extraction rates with greater bran content. Pringle and Moran (1942) studied the effects of baking

on phytic acid and concluded that phytase in flour caused the breakdown of some phytic acid during breadmaking. The longer the fermentation time the greater the destruction of phytic acid.

DeLange et al. (1961) studied factors influencing phytic acid hydrolysis in bread baking and reported that longer fermentation times of three, four or five hours increased phytic acid reduction, though their results indicated slight effects after three hours. The addition of acid, acetic and citric acid, increased phytic acid reduction. The addition of calcium acetate, however, inhibited greatly the reduction of phytic acid.

Ter-Sarkissian et al. (1974) in their study of Iranian breads show that modifying baking methods can reduce phytic acid content. They point out that longer fermentation times are successful in reducing phytic acid level but produce an undesirable result in some of the sour dough breads and, therefore, recommend a lower extraction flour in order to reduce phytic acid content. Reinhold (1975) proposed that the nutritional value of Iranian bread could be improved if the traditional unleavened bread were leavened and made from a lower extraction flour. The result of these changes would be lower phytic acid content.

The commercial removal of phytic acid from soy protein uses a different aspect of phytic acid chemistry. Hartman (1979) reported a process of solubilization, pH adjustment to 11.6 to precipitate the phytate and centrifugation to remove it. Finally the high pH of the soy protein dispersion is neutralized. This procedure lowered the phytate content from 2.6% to 0.1% for a typical commercial soy

protein isolate.

Harland and Harland (1980) reduced phytate content in three breads (rye, white and whole wheat) by doubling the yeast in each recipe and extending the fermentation time. Knorr et al. (1981) used commercial phytase or phosphatase in order to reduce phytate levels to 1/8 and 1/12 of initial values. They reported the continued decrease in phytate to a level of only 0.6 mg/100 gm of bread after 96 hours of storage following baking. Finally, Tangkongchitr et al. (1981) followed the loss of phytate through bread-making and concluded that the principal barrier to reduction of phytate in bread-making is the insolubility of magnesium phytate in dough. A later report by Tangkongchitr et al. (1982) indicated that the insolubility of magnesium phytate could be decreased at pH 5 where the limiting factor appeared to be the level of phytase.

PHYTIC ACID DETERMINATION

Heuber and Stadler (1914) developed the method upon which most procedures for phytic acid determination are based. This method uses the fact that phytate in dilute acid forms an insoluble complex with ferric ions. The phytate extract was titrated with standardized ferric chloride solution using ammonium thiocyanate as an indicator. The end point was reached when a flesh-pink color persisted for five minutes. This color was produced by an excess of ferric ion which formed ferric thiocyanate. The major disadvantage with this method is the lack of a sharp end-point due to the ferric phytate percipitate. Young (1936) modified the method by adding a fixed

amount of standard ferric chloride and measured the excess unprecipitated ferric ion colorimetrically. Others have modified this basic method (Earley, 1944; DeLange, 1961).

These methods based on Heuber and Stadler (1914) have been divided into "direct" and "indirect" analysis of phytic acid (Samotus and Schwimmer, 1962). The "direct" methods were based on the determination of phosphorus content of carefully precipitated ferric phytate after hydrolysis. The "indirect" methods measure the unprecipitated iron or the portion of a standardized iron solution precipitated by phytic acid in dilute acid. Generally, the "indirect" methods are more convenient (Oberleas, 1971).

Comparison of these methods have been made by other authors who report wide variation in results. Marrese, et al. (1961) compared three methods of phytin determination and showed wide differences in results. They concluded that investigations about phytin are dependent upon an understanding of what phytin is and with what confidence it can be measured. A comparison of four procedures for phytic acid determination by Makower (1970) also demonstrated a wide variation in results. She concluded that determination of iron in ferric phytate via ferric hydroxide gives results comparable to those obtained in determinations from wet-ashed ferric phytate for phosphorus or iron. The residual iron method is more variable particularly with small amounts of phytic acid and is subject to greater error. Wheeler and Ferrel (1971) in their study of phytic acid determination in wheat and wheat fractions also encountered this variation in results.

A modification of the wet-ashed ferric phytate method was developed by Harland and Oberleas (1977) in which the phytate is concentrated on an anion-exchange resin. The phytate concentrated on the anion-exhange resin is stripped of contaminating inorganic phosphate with a weak sodium chloride solution. The phytate is then eluted with a stronger sodium chloride solution. The final eluant fraction is then digested and the phosphate is measured. A modification of this method proposed by Latta and Eskin (1980) uses the anion-exchange resin to concentrate the phytate and remove inorganic phosphate; however, instead of digesting the final eluant fraction it is mixed with Wade reagent and the amount of phytate is colorimetrically determined. Wade reagent (0.03% ferric chloride - 6 hydrate and 0.3% sulfosalicylic acid in distilled water) has a pink color due to the reaction between ferric ion and sulfosalicylic acid. When Wade reagent is mixed with phytate the ferric ion is bound to the phytate and unable to react with the sulfosalicylic acid, which results in a decrease in the intensity of the pink color. This intensity decrease is measurable and proportional to the amount of phytate present. This method, even though it is simple and rapid, has resulted in low phytate values in comparison to earlier methods. Ellis and Morris (1982) in their comparison of this method and the iron precipitation method concluded that interfering substances in the acid extract resulted in lower phytate levels. They found that this effect is minimized or eliminated by precipitating the phytate and regenerating the purified phytate before adding the sample to the anion-exchange resin.

EXPERIMENTAL PROCEDURE

EXPERIMENTAL DESIGN

The purpose of this study was to evaluate proposed methods of phytic acid reduction in whole wheat flour doughs from two cultivars of Utah wheat. Acetic and citric acids were added to doughs to obtain a flour pH of 5.2. This was done to reach the approximate optimum pH of phytase. Doughs made with 1% sprouted wheat added were also prepared. The sprouted wheat was added to determine if 24-hour or 48-hour sprouting would increase phytase activity. The doughs prepared by these methods were allowed to set for zero, two and four hours. The phytic acid levels of the flours and the doughs were determined and the relative effectiveness of the methods were compared using these results.

The experimental design was reviewed by the Center for Statistical Research at Brigham Young University prior to starting to insure statistical balance. Three replications of each cultivar, method and time were prepared and each replication was analyzed for phytic acid in duplicate.

WHEATS

Two cultivars of wheat from Utah were obtained for this study (Hansel and Manning). Appendix D contains the sources of these wheats and the seed information supplied.

The wheats were graded in accordance with the standards established by the United States Department of Agriculture (1978).

The wheats were also analyzed for protein, using the Kjeldahl method, and moisture using the dielectric method for whole kernel moisture determination.

WHOLE WHEAT FLOUR

The two cultivars of wheat were divided into two 25-pound samples by using a Boerner sample divider. These two samples were then ground into flour using a home flour mill.* The milled flour was then placed in plastic one-gallon jars and frozen at 0°F until the doughs were prepared.

Four 3.000 gm samples of flour from each cultivar were weighed into aluminum cups and dried at 95°C at a vacuum of 24 inches of mercury in an oven for three hours. Samples were allowed to cool in a desiccator, weighed and the moisture calculated from the weight loss.

The pH's of the whole wheat flours were determined using a modification of the AACC method 02-52 (American Association of Cereal Chemists, 1976). Four samples of each flour were placed in beakers and recently boiled distilled water, cooled to 25°C, was added. The water and flour mixture was agitated at 25°C for a period of time and then allowed to settle. The pH of the supernatant liquid above the flour was read using a pH meter with a combination glass electrode (Appendix A).

---

*Magic Mill II, Magic Mill Co., Salt Lake City, Utah. A micronizing mill.

Flour samples were analyzed in quadruplicate for phytic acid by the method of Latta and Eskin (1980) as modified by Ellis and Morris (1982). This procedure measures phytic acid colormetrically using the Wade Reagent (0.03% $FeCl_3$-$6H_2O$ in 0.3% sulfosalicylic acid). The phytic acid was extracted from the flour with 2.4% HCl solution. Excess ferric chloride solution was added to the extract and ferric phytate was precipitated. The precipitate was washed and sodium hydroxide was added to form ferric hydroxide and sodium phytate. The sodium phytate solution was then passed through an anion exchange column. The column was made with 0.5 gm of 200-400 mesh Dowex 1 X 8 resin. The interfering phosphates were removed from the column using 0.1 M sodium chloride. Finally, the phytate was eluted from the column using 0.7 M sodium chloride. This eluant fraction was mixed with Wade Reagent and the absorbance of the solution read at 480 nm. The ug/ml of phytic acid was determined by a least squares linear regression of the standard solution calibration data (Appendix B).

Reagent grade concentrated acetic and crystalline citric acids were diluted to form 1.0 M acetic acid and 0.3 M citric acid. These solutions were used to lower the pH of the whole wheat dough to a pH of 5.2. In order to determine the amount of acid needed to lower the pH of the dough, samples of each flour in quadruplicate were used to determine the pH of the flour. The acids were added, using a buret, to these supernatants until the pH of the liquid had been lowered to 5.2.

The amount of acid added was averaged for the four samples of each wheat and this value was used to determine the total amount of acid needed for each whole wheat flour.

WHEAT SPROUTING

In order to provide the necessary sprouted wheat for this study, each cultivar of wheat was sprouted for 24 and 48 hours. The whole wheat was sprouted by soaking the wheat in two to three cups of warm water for eight to nine hours; then the wheat was rinsed and allowed to sit in a dark location for the remaining time. The sprouts were rinsed every four to six hours during the last 16 to 40 hours. The sprouts were then frozen until the doughs were prepared. Using the beginning and final weight of the sprouts, the amount necessary for 1% sprouted wheat (dry weight) in the doughs was calculated (Appendix C).

WHOLE WHEAT DOUGH

The doughs were prepared by cultivar, method and time as noted in Table 1. Each dough sample was prepared in triplicate in accordance with the schedule in Table 2. This schedule was a random generation by computer in order to provide a statistically balanced experiment.

In Method 1, flour, water and 1.0 M acetic acid were mixed to form a dough consisting of 100 gms of flour (dry matter basis) and 100 ml of liquid. In Method 2, 0.3 M citric acid was used instead of acetic acid. Method 3 was prepared by taking the 1% by weight of 24-hour sprouts and blending them with the water in a household

TABLE 1

DOUGH PREPARATION

Dough Sample Number	Cultivar	Method*	Time (hours)
1	Hansel	1	0
2	"	1	2
3	"	1	4
4	"	2	0
5	"	2	2
6	"	2	4
7	"	3	0
8	"	3	2
9	"	3	4
10	"	4	0
11	"	4	2
12	"	4	4
13	Manning	1	0
14	"	1	2
15	"	1	4
16	"	2	0
17	"	2	2
18	"	2	4
19	"	3	0
20	"	3	2
21	"	3	4
22	"	4	0
23	"	4	2
24	"	4	4

* Method 1 - 1.0 M acetic acid added to lower pH to 5.2 of dough.
* Method 2 - 0.3 M citric acid added to lower pH to 5.2 of dough.
* Method 3 - 1% 24-hour sprouted wheat added to dough.
* Method 4 - 1% 48-hour sprouted wheat added to dough.

TABLE 2

PREPARATION SCHEDULE

Preparation Order	Sample Dough Number	Preparation Order	Sample Dough Number
1	2	37	6
2	12	38	15
3	3	39	15
4	1	40	22
5	4	41	5
6	3	42	14
7	23	43	18
8	3	44	3
9	4	45	19
10	17	46	17
11	11	47	8
12	9	48	7
13	18	49	2
14	8	50	7
15	14	51	23
16	12	52	3
17	2	53	6
18	7	54	4
19	20	55	14
20	18	56	9
21	24	57	13
22	19	58	11
23	21	59	15
24	10	60	24
25	20	61	22
26	1	62	20
27	16	63	24
28	13	64	11
29	23	65	1
30	10	66	22
31	13	67	21
32	8	68	21
33	6	69	19
34	12	70	10
35	9	71	16
36	17	72	16

blender and then mixing with the flour. Method 4 was prepared with 48-hour sprouts in place of the 24-hour sprouts. All four methods were mixed in a household mixer* until a uniform dough was formed (approximately three minutes). The doughs were then placed in plastic freezer bags and either frozen with dry ice for 1 hour time or allowed to react for either two or four hours at room temperature (25° to 27°C). After two or four hours these doughs were also frozen with dry ice. The actual amounts of flour, water, and acid or sprouts used in each method and cultivar are shown in Table 3.

The determination of phytic acid in the dough was done in the same method as the flour except the weighing of the dough was done while it was still frozen for ease of handling (Appendix B).

---

*KitchenAid Mixer Model K5A, Hobert Mfg. Co., Troy Ohio.

TABLE 3

DOUGH INGREDIENTS FOR EACH

	Cultivar	
	Hansel	Manning
Method #1 (Acetic Acid)		
Flour	109.0 gm	109.2 gm
Water	86.6 ml	86.4 ml
Acid (1.0 M Acetic Acid)	4.4 ml	4.4 ml
Method #2 (Citric Acid)		
Flour	109.0 gm	109.2 gm
Water	77.0 ml	77.3 ml
Acid (0.3 M Citric Acid)	14.0 ml	13.5 ml
Method #3 (24-Hour Sprouts)		
Flour	107.9 gm	108.1 gm
Water	91.3 ml	91.0 ml
Sprouts	1.80 gm	1.88 gm
Method #4* (48-Hour Sprouts)		
Flour	107.9 gm	108.1 gm
Water	90.9 ml	90.7 ml
Sprouts	2.18 gm	2.24 gm

* Additional weight of 48-hour sprouts was added to compensate for increased water absorbance by 48-hour sprouts.

RESULTS

WHOLE WHEAT FLOUR

The two cultivars of wheat both graded U.S. No. 1 wheat (Table 4). The two cultivars varied by 1.2% protein and 0.4% moisture (Hansel 13.9% protein and 11.1% moisture; Manning 12.7% protein and 11.5% moisture).

The whole wheat flours were very close in phytic acid level (Hansel 742 mg/100 gm and Manning 745 mg/100 gm) for the cultivars (Table 5). In comparing the standard deviations of the phytic acid determination with previously reported values (Witmer, 1981; Latta and Eskin, 1980; Makower, 1970) these values are within the ranges reported. The pH of both cultivars are a little higher than 6. The moisture content of the flour was approximately 3% lower than the intact wheat kernel. This lowered moisture content is probably due to moisture loss during flour milling.

The determination of the required amount of acid is based on the amount of 1.0 M acetic acid or 0.3 M citric acid added to the flour supernatant to lower the pH to 5.2 for 10 grams of flour. These values were then multiplied by 10 to determine the amount of acid to add to the 100 grams of flour in the dough. the results were 4.4 ml of acetic acid for both cultivars and 14.0 ml of citric acid for Hansel and 13.5 ml of citric acid for Manning.

TABLE 4

GRADING FACOTRS

Factors	Cultivar	
	Hansel	Manning
Class	Hard Red Winter	Hard Red Winter
Dockage	0.0%	0.0%
Protein	13.93% ± 0.66	12.73% ± 0.05
Moisture	11.1%	11.5%
Test Weight (pounds per bushel)	60.8	62.7
Grade	U.S. No 1	U.S. No. 1

TABLE 5

MOISTURE, PHYTIC ACID, AND pH OF WHOLE WHEAT FLOURS

Cultivar	Moisture %	Phytic Acid mg/100 gm	pH
Hansel	8.22% ± 0.24	742 ± 24	6.3 ± 0.1
Manning	8.42% ± 0.13	745 ± 62	6.4 ± 0.1

SPROUTED WHEAT

The 24-hour sprouts had one shoot of approximately 1/8 inch long, while the 48-hour sprouts had three shoots from approximately 1/4 to 5/8 inch long (Table 6). The calculations used to determine the weight of sprouts added to the doughs are shown in Table 6. The end result is the addition of from 1.80 to 2.24 gms of sprouts to the dough to add 1% dry weight of sprouts.

WHOLE WHEAT DOUGH

The phytic acid levels of the whole wheat doughs (method 1 (Acetic Acid)) shows a rapid decrease in phytic acid levels from over 700 mg/100 gm to about 200 mg/100 gm after two hours in both cultivars (Table 8, Figure 2). The reduction rate levels out after two hours and after four hours very little additional reduction in phytic acid level had occurred. Method 2 (Citric Acid) has a similar pattern except the reduction at two hours is slightly greater (Figure 3). Method 3 (24-hour sprouts) had a pattern of very gradual reduction until two hours and it also leveled out to very little additional reduction (Figure 4). Method 4 (48-hour sprouts) for the cultivar Manning showed a similar pattern as Method 3. However, for the cultivar Hansel the pattern appears to rise up to a high of about 630 mg/100 gm at two hours and decrease to a low of about 440 mg/100 gm at four hours.

STATISTICS

The data for the phytic acid levels in the flours and doughs were analyzed for statistical significance using the computer

TABLE 6

WHEAT SPROUTING RESULTS AND CALCULATIONS

		Hansel	Manning
Start Weight	24-hour	75 gm	75 gm
	48-hour	75 gm	75 gm
Finish Weight	24-hour	120 gm	124.6 gm
	48-hour	145.5 gm	149 gm
Initial Moisture Content		11.1%	11.5%
Actual Grams of Wheat (dry matter) Based on 75 gm		66.68 gm	66.38 gm
Grams of Sprouts			
24-hour		1.80 gm	1.88 gm
48-hour		2.18 gm	2.24 gm

The number of grams of sprouts to equal 1 gram dry matter is the total finish weight divided by actual grams of weight (dry matter).

TABLE 7

PHYTIC ACID LEVELS OF WHOLE WHEAT DOUGHS

Dough Sample Number	Cultivar	Method*	Time Hours	Phytic Acid mg/100 gm
1	Hansel	1	0	361 ± 70
2	"	1	2	216 ± 115
3	"	1	4	248 ± 61
4	"	2	0	382 ± 107
5	"	2	2	145 ± 59
6	"	2	4	153 ± 30
7	"	3	0	660 ± 92
8	"	3	2	500 ± 93
9	"	3	4	484 ± 123
10	"	4	0	545 ± 118
11	"	4	2	634 ± 92
12	"	4	4	446 ± 56
13	Manning	1	0	459 ± 143
14	"	1	2	220 ± 23
15	"	1	4	212 ± 73
16	"	2	0	534 ± 125
17	"	2	2	161 ± 49
18	"	2	4	214 ± 52
19	"	3	0	664 ± 113
20	"	3	2	571 ± 108
21	"	3	4	562 ± 71
22	"	4	0	660 ± 105
23	"	4	2	526 ± 182
24	"	4	4	592 ± 53

* Method 1 - 1.0 M acetic acid added to lower pH to 5.2 of dough.
* Method 2 - 0.3 M citric acid added to lower pH to 5.2 of dough.
* Method 3 - 1% 24-hour sprouted wheat added to dough.
* Method 4 - 1% 48-hour sprouted wheat added to dough.

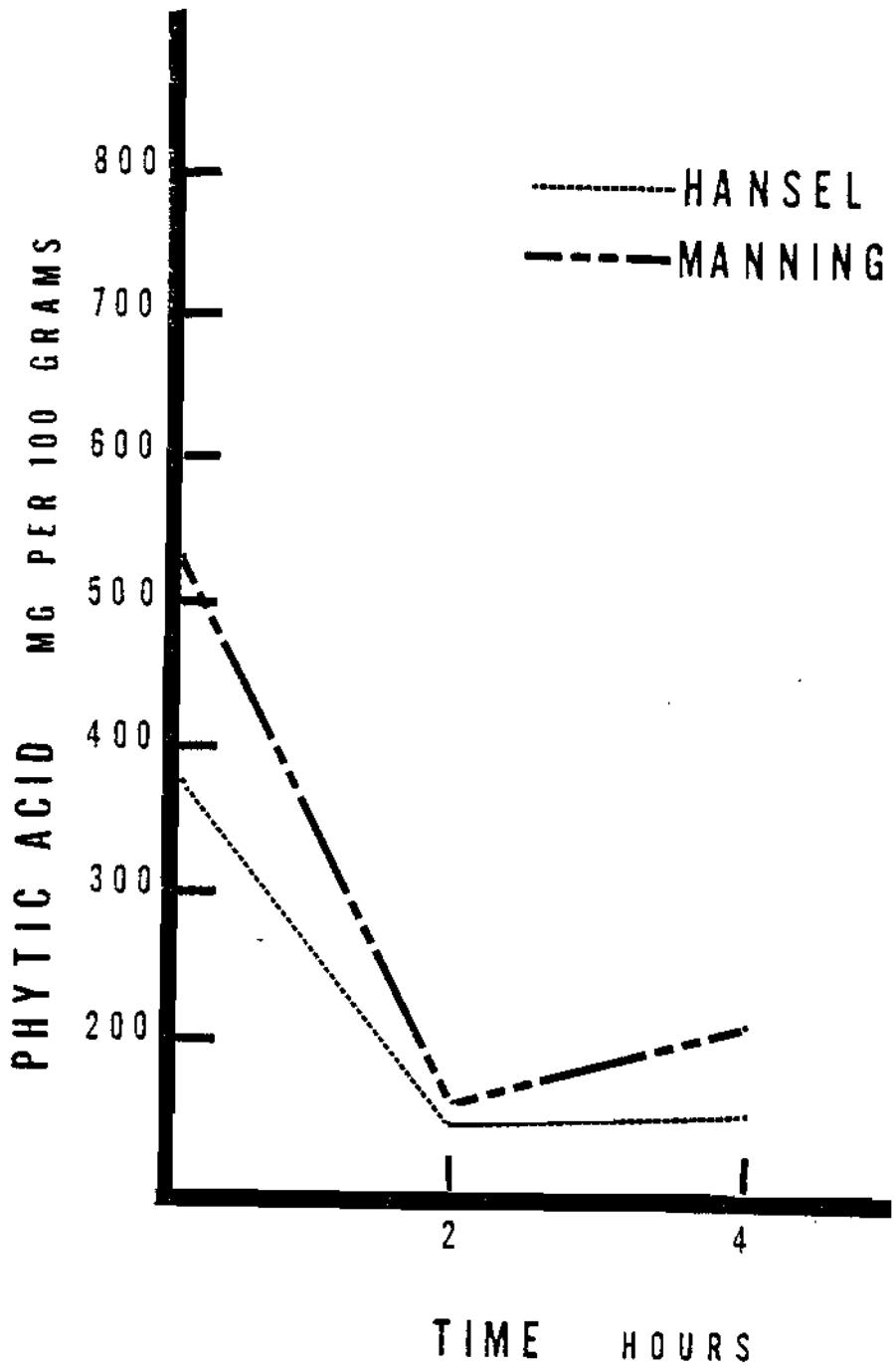

Figure 2. Method 1: Decrease of phytic acid following adjustment to pH 5.2 with 1.0 M acetic acid.

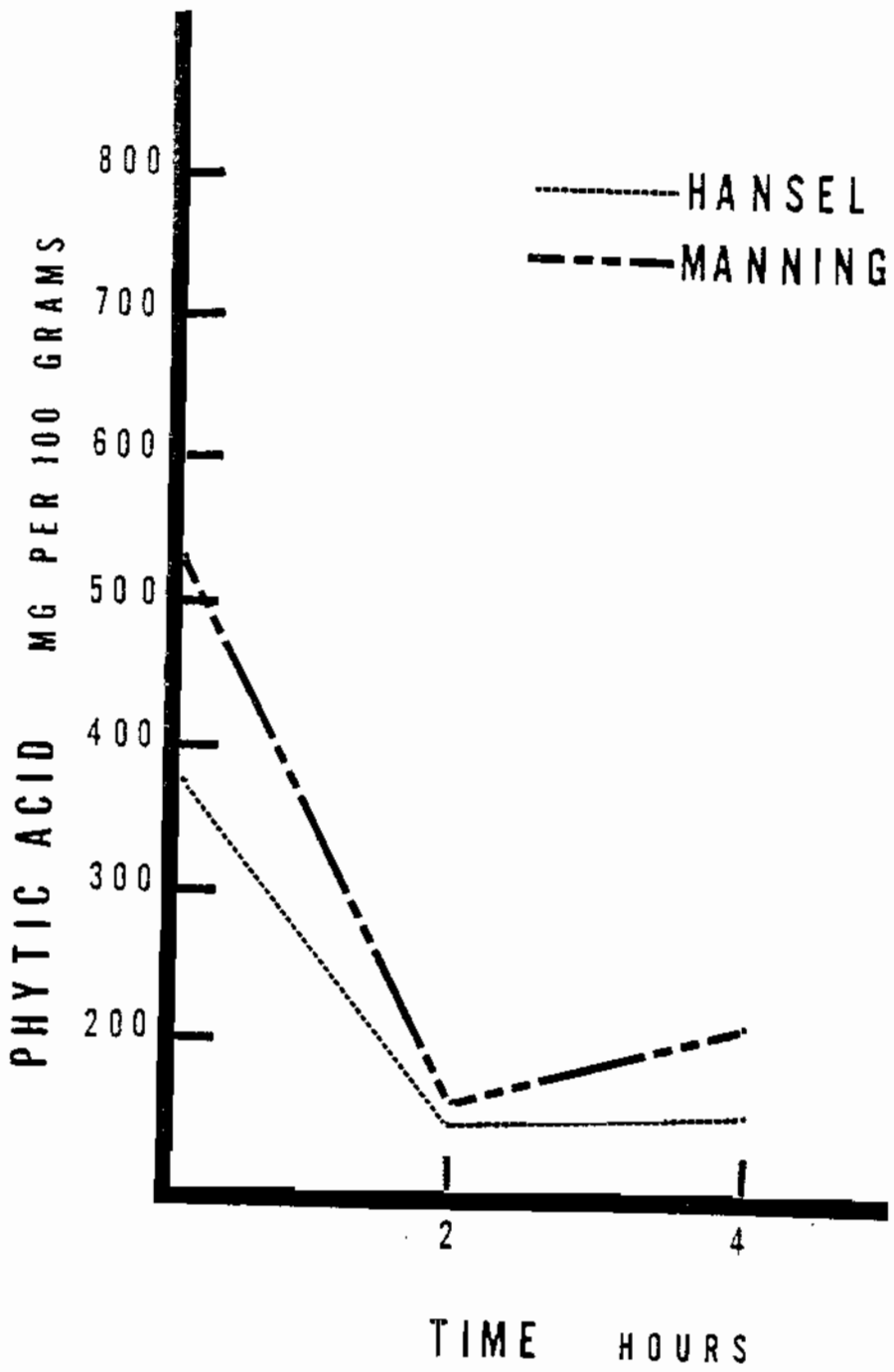

Figure 3. Method 2: Decrease of phytic acid following adjustment to pH 5.2 with 0.3 M citric acid.

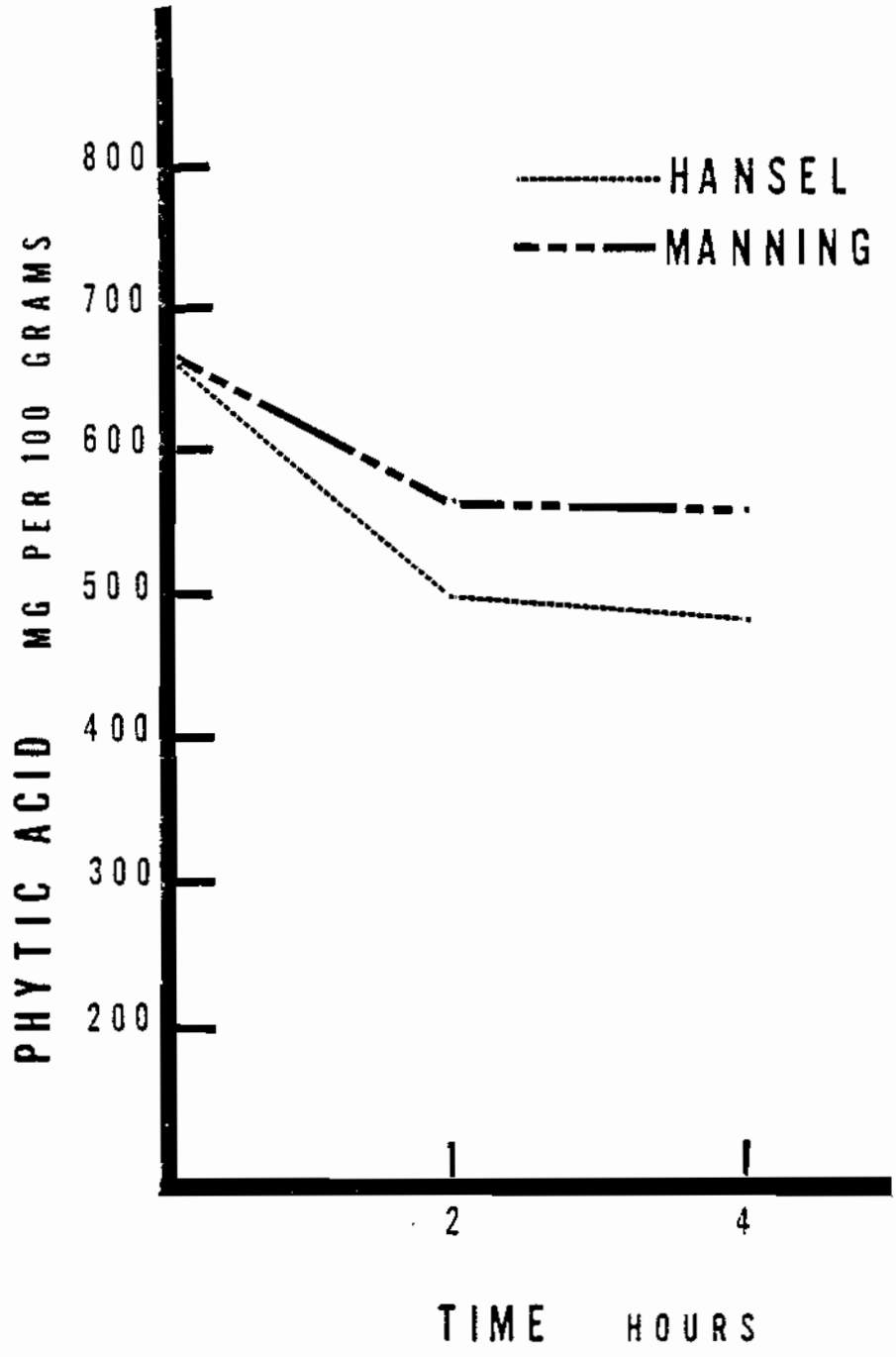

Figure 4. Method 3: Decrease of phytic acid follwoing addition of 1% 24-hour sprouts.

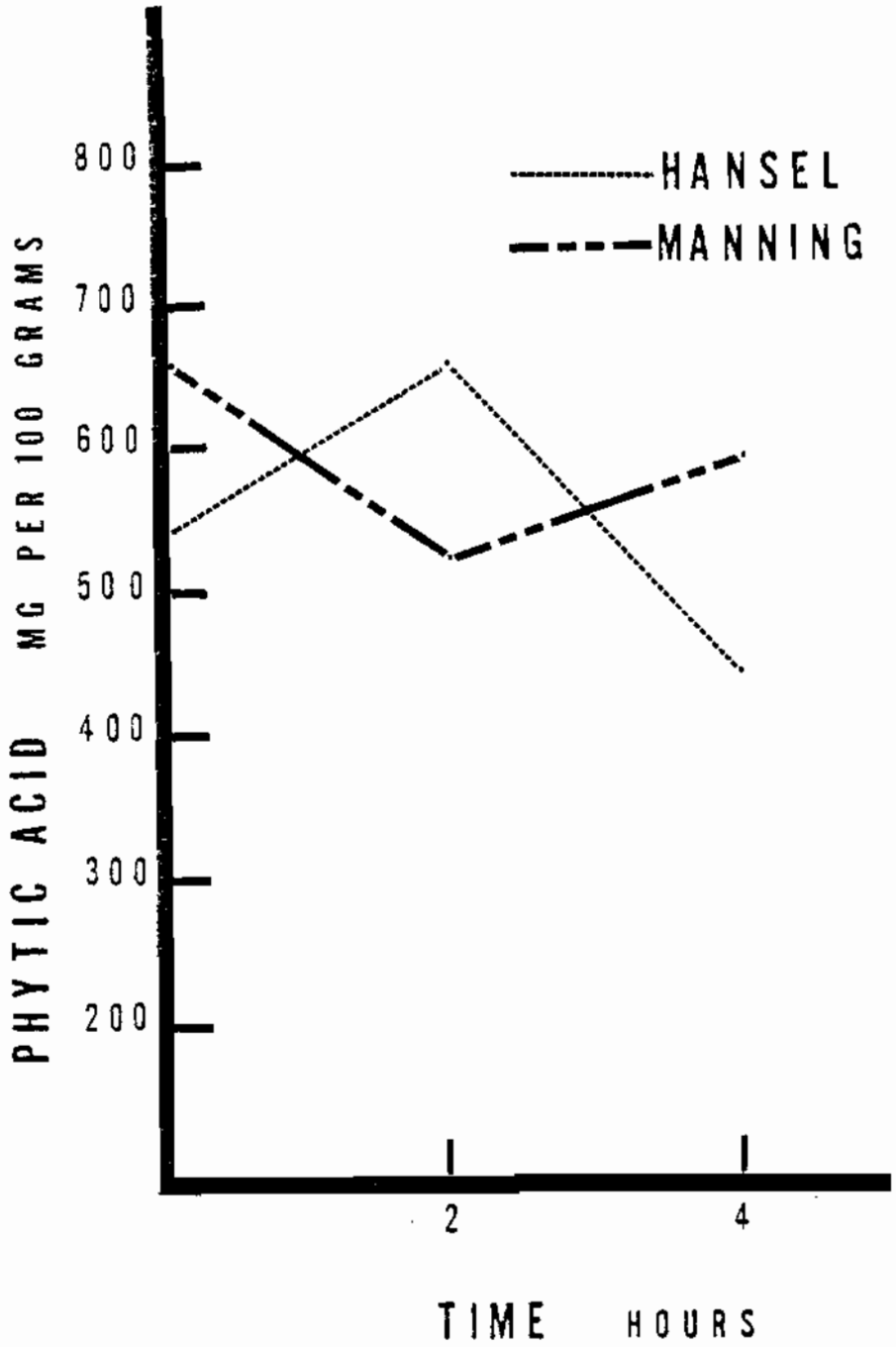

Figure 5. Method 4: Decrease of phytic acid following addition of 1% 48-hour sprouts.

program Rummage II updated April 23, 1982 by Del T. Scott, Melvin W. Carter and Gale Rex Bryce of the Statistics Department, Brigham Young University. This analysis indicated no statistically significant difference between Methods 1 (Acetic acid) and 2 (Citric acid) or Methods 3 (24-hour sprouts) and 4 (48-hour sprouts). However, the analysis indicated statistically significant difference between Method 1 (Acetic acid) and Methods 3 (24-hour sprouts) and 4 (48-hour sprouts) and between Method 2 (Citric acid) and between Methods 3 (24-hour sprouts) and 4 (48-hour sprouts). Finally, the analysis indicated no statistically significant difference between two and four hours of reaction time. The linear statistial model for this analysis and the key to the model are shown in Appendix E. Tables 8 and 9 are an analaysis of variance table for reduction of phytic acid and paired comparisons of the means of the time and methods.

TABLE 8

ANALYSIS OF VARIANCE TABLE
FOR REDUCTION OF PHYTIC ACID

Source of Variation	Error Term	Degree of Freedom	Sum of Squares	Mean Square	F Ratio
V (cultivar)	R	1	80588.8	80588.8	4.433*
M (method)	R	3	3133742.9	1044581.0	57.464*
V X M	R	3	13402.2	4467.4	0.246
T (time)	R	2	876755.3	438377.6	24.116*
V X T	R	2	58339.1	29169.6	1.605
M X T	R	6	262816.7	43802.8	2.410*
V X M X T	R	6	124291.2	20715.2	1.140
R (replication)	E	48	872551.7	18178.2	5.788*
Error		72	226121.5	3140.6	
TOTAL		143	5648609.3		

Simple R-squared .960

R-squared adjusted .920

*Significant at 0.05

TABLE 9

PAIRWISE COMPARISONS OF THE MEANS
FOR TIMES AND METHODS

Pairs	Difference of Means	Standard Error	T-value
T1 - T2	161.458	27.521	5.867*
T1 - T3	169.313	27.521	6.152*
T2 - T3	7.854	27.521	0.285
M1 - M2	20.674	31.779	0.651
M1 - M3	-287.667	31.779	9.052*
M1 - M4	-280.938	31.779	8.840*
M2 - M3	-308.340	31.779	9.703*
M2 - M4	-301.611	31.779	9.491*
M3 - M4	6.729	31.779	0.212

*Significant at 0.05

T_1 = 0 hours
T_2 = 2 hours
T_3 = 4 hours
M_1 = Method 1 - 1.0 M acetic acid added to lower pH to 5.2 of dough.
M_2 = Method 2 - 0.3 M citric acid added to lower pH to 5.2 of dough.
M_3 = Method 3 - 1% 24-hour sprouted wheat added to dough.
M_4 = Method 4 - 1% 48-hour sprouted wheat added to dough.

Refer to Appendix E page 42 for linear statistical model

DISCUSSION

The addition of acid to lower the pH to 5.2 (Methods 1 (Acetic acid) and 2 (Citric acid)) resulted in a similar pattern of reduction in phytic acid. With both acids a sharp reduction in phytic acid occurred during two hours, followed by very little additional reduction for four hours of reaction time (Figures 2 and 3). These results are similar to those reported by Harland and Harland (1980), DeLange et al. (1961) and Tangkongchitir et al. (1981, 1982). This reduction in phytic acid is probably due to enzymatic activity of the phytase present n the doughs. The apparent rate reduction of enzymatic activity after two hours may have at least two possible causes. Tangkongchitir, et al. (1981) concluded that this loss of enzymatic activity was due to the insolubility of magnesiumm phytate in the dough. Tangkongchitir et al. (1982) showed that lowering the pH to 5.0 seems to overcome this insolubility problem. However, Oberleas (1982) suggested that in his studies of precipitation of phytate with divalent minerals, most of the phytate in wheat would be soluble at the pH of 6.2 to 6.6 (the average pH of whole wheat flour). Therefore, the insolubility of magnesium phytate would not explain this rate reduction of enzymatic activity. The second possible cause is that the phytase may be inhibited. The probable reaction is the phytate being hydrolyzed by phytase to either inositol and inorganic phosphate, or a lesser phosphorylated inositol and inorganic phosphate. These products of enzymatic activity, inositol, inorganic phosphate or a lesser phosphorylated

inositol probably inhibit the phytase. This appears to be the most likely mechanism. The addition of acid lowers the pH to 5.2, which optimizes the phytase activity within the dough. This activity continues until a build-up of these inhibiting agents occurs. This build-up is probably localized due to a loss of mobility of the phytate within the dough as the dough structure forms. This localized inhibition and loss of mobility would explain the same pattern of little additional reduction in phytate between two and four hours with the addition of wheat sprouted for 24 and 48 hours. The pH of the sprouted wheat doughs would be that of ordinary whole wheat flour (pH 6.2 to 6.6) which is higher than the optimum pH of 5.2 for the phytase and this would result in a lower level of phytase activity and explains the smaller overall reduction in phytic acid.

DeLange et al. (1961) used 100% extraction flour and added vinegar and citric acid to lower the crumb pH to approximately pH 5.7 to pH 6.1 with fermentation times of three, four and five hours. The results indicated the greatest reduction of phytic acid with the addition of both vinegar and citric acid. The addition of both resulted in a 77% to 80% reduction of phytic acid and a pH of approximately 5.7. The DeLange study also shows little additional reduction after three hours of fermentation. The results match closely the results in this study; however, due to the higher pH's, not as much reduction of phytic acid occurred in the DeLange study.

It is interesting to note that both acid additions caused a significant drop in phytic acid in doughs prepared without reaction time (0 hours). The mixing of the dough and lowering of the pH to

5.2, without a reaction time after mixing, resulted in increased phytase activity which caused considerable reduction of phytic acid before the dough sample was cooled below the inactivation temperature of the enzyme. This same drop is noted in the sprouted wheat doughs at a smaller scale.

The time required to show an apparent rate reduction in enzymatic activity was measured at two hours in this study; howver, it should be noted that no data were collected between zero and two hours, which means the rate reduction could have occurred at any time between zero and two hours after mixing.

Further study in this rate reduction of enzymatic activity could attempt to increase mobility within the doughs possibly by remixing the doughs after two hours of reaction time or determining the inhibiting agents and mixing them in the doughs to observe their effect on phytic acid reduction.

The acid addition reduces phytic acid and in turn may overcome the adverse effect on divalent minerals. This reduction, however, may not be enough to completely overcome the adverse effect of the phytic acid in the doughs.

Addition of wheats sprouted for 24 or 48 hours shows results that would be expected for enzyme action with no additional phytase activity being produced during the first 48 hours of germination. These results are in agreement with the reported decomposition of phytic acid in wheat during germination by Mihailovic et al. (1965). They reported that the majority of phytic acid decomposition occurs from the third to the seventh day of germination. Apparently before the third day of germination little additional phytase activity is

produced. The addition of wheats sprouted for longer than 72 hours (three days) would possibly increase phytic acid reduction; however, this increased germination could cause undesireable results in the dough structure because of increased amylase activity.

The unexpected results of an apparent rise in phytic acid level for the addition of 1% 48-hour sprouts (Method 4, Figure 5) with cultivar Hansel between zero hours to two hours reaction time is not as significant as it appears if it is noted that the standard deviations of the results place both results within a standard deviation of one another. Close examination of the standard deviations will also note that the apparent differences in the zero reaction time phytic acid levels for the cultivar Manning with acid addition are also within a standard deviation of each other. This results in no significant difference between the acid addition methods (Method 1 and 2).

The use of room temperature (25° to 27°C) for reaction time of the doughs instead of the optimum temperature of 55°C for phytase activity was due to the use of room temperature or slightly higher for most breadmaking. The higher temperature (55°C) would possibly begin baking the dough and this could increase even further the loss of mobility within the dough.

The use of both acid addition and sprouted wheat together is a possible method for phytic acid reduction. The effect would probably be reduction of phytic acid; however, the need to increase sprouting time to longer than 72 hours to increase phytase activity would also increase amylase activity and cause possible undesirable effects on the dough structure.

CONCLUSIONS

The addition of acid to whole wheat doughs and allowing the doughs to set for two hours resulted in the greatest significant reduction of phytic acid in whole wheat doughs. The use of 24-hour and 48-hour sprouts did not increase the phytic acid reduction rate above that expected from natural phytase present in the flour. Finally, the greatest reduction in phytic acid appears to occur in the first two hours after the doughs are prepared.

APPENDICES

APPENDIX A

Procedure For Flour pH Determination

Procedure

1. Place 10 gm of flour into a dry 150 ml beaker.

2. Add 100 ml of cool, recently boiled distilled water at a temperature of 25°C.

3. Agitate the flour until an even suspension free from lumps is obtained. Allow suspension to stand at 25°C for 30 minutes, agitating intermittently in such a manner as to keep the flour particles in suspension. Let stand for 10 additional minutes.

4. Using a calibrated pH meter, determine the pH of the supernatant liquid.

Reference
AACC Method 02-51

APPENDIX B

Procedure For Phytic Acid Determination

Reagents

1. 2.4% (w/v) HCl Solution
   Dilute 63.7 ml of concentrated HCl in a 1000 ml volumetric flask with distilled water to the 1000 ml line.

2. $FeCl_3$ solution in 0.6% HCl
   Dissolve 4 gm of $FeCl_3 - 6H_2O$ (Mallinckrodt Reagent lumps) in 1 liter of 0.6% HCl.

3. 1 M NaOH
   Dissolve 40.00 gm of NaOH (Baker Reagent Grade ACS) in a 1000 ml volumetric flask with distilled water to the 1000 ml line.

4. 0.7 M NaCl
   Dissolve 40.91 gm of NaCl (Fisher Scientific ACS) in a 1000 ml volumetric flask with distilled water to the 1000 ml mark.

5. 0.1 M NaCl
   Dissolve 5.85 gm of NaCl (Fisher Scientific ACS) in a 1000 ml volumetric flask with distilled water to the 1000 ml mark.

6. Wade Reagent
   0.03% $FeCl_3 - 6H_2O$ and 0.3% sulfosalicylic acid (Spectrum Crystal Reagent ACS) in distilled water.

7. Standard Solutions
   A series of standard solutions containing 5, 10, 15, 20, 25, 30, 35, 40 mg/ml of phytic acid (Sigma Chemical Company).

8. 5% (w/v) HCl solution
   Dilute 132.6 ml of concentrated HCl in a 1000 ml volumetric flask with distilled water to the 1000 ml mark.

Procedure

1. Determine a calibration curve for phytic acid concentration vs. absorbance. Take 6 ml of a standard solution and add 2 ml of Wade Reagent solution in a 15 ml conical centrifuge tube. Mix for 5 seconds on a vortex mixer. The mixture is centrifuged for 10 minutes. Read the absorbance of the solution at 480 nm with a spectrophotmeter using water as a zero. Using the absorbance values and concentration levels determine the linear Beer's Law plot for the standard solutions.

2. Weigh out 5.000 gm of flour or 10.000 gm of dough and place into a 150 ml or larger beaker. Add 100 ml of 2.4% HCl, extract phytic acid by thoroughly agitating mixture and intermittently agitating solution for at least 1 hour.

3. Take 5 ml of extract and mix with 5 ml of $FeCl_3$ solution. Heat mixture in boiling water for 15 minutes. Cool in ice water. Centrifuge mixture and wash precipitate with distilled water two times. Decant excess liquid from precipitate. Add 5 ml of 1.0 M NaOH solution. Agitate mixture to redisolve phytic acid. Decant liquid into 25 ml volumetric flask. Wash ferric hydroxide precipitate two times with distilled water and add washings to volumetric flask. Add distilled water to the 25 ml mark.

4. Take 10 ml of the redisolved phytic acid solution and add to column. Column is made with 0.5 gm of 200-400 mesh Dowex 1 X 8 resin. The Dowex resin was prepared for use by adding distilled water, agitating the mixture, allowing the resin to settle and pouring off the supernatant to remove the finer particles. This process was done three times.

5. Remove inorganic phosphates or other interfering agents by adding 15 ml of 0.1 N NaCl to the column.

6. Remove the phytic acid by adding 15 ml of 0.7 M NaCl. Catching the elutant as soon as the 15 ml of 0.7 NaCl is added to the column. The elutant is removed from under the column as soon as the level of liquid reaches the top of the resin.

7. The elutant is diluted to a volume of 25 ml with distilled water, and mixed thoroughly. Take 6 ml of mixture and add 2 ml of Wade Reagent in a 15 ml conical centrifuge tube. Mix for 5 seconds on a vortex mixer. The mixture is centrifuged for 10 minutes. Read the absorbance of the solution at 480 nm.

8. Regenerate the columns by adding 15 ml of 5% HCl solution and then add 20 ml of distilled water.

CALCULATION

Ug/ml of phytic acid determined by least-squares linear regression of standard solution calibration data.

$$\frac{\text{ug/ml} \times 25\text{ ml} \times 25\text{ ml} \times 100\text{ ml} \times 100\text{ gm} \times \text{mg}}{10\text{ ml} \times 5\text{ ml} \times \text{dry wt of sample} \times 1000\text{ ug}} = \frac{\text{mg of phytic acid}}{100\text{ gm of dry sample}}$$

Thus:
$$\frac{\text{ug/ml} \times 125}{\text{dry wt of sample}} = \frac{\text{mg of phytic acid}}{100\text{ gm of dry sample}}$$

APPENDIX C

Procedure For Sprouting Wheats

Procedure

1. Weigh out 75 gm of wheat and place it into a mason jar. Attach plastic screening over end of jar with jar ring.

2. Add two to three cups of warm water to the jar at 25°C. Let wheat soak for eight to nine hours in a dark location.

3. Drain wheat and rinse in cool water once and rinse in 25°C water two or three times. Drain and place jar in a tray so that water drains from wheat and air gets to wheat. Store between rinses in dark location.

4. Rinse sprouts every four to six hours with 25°C temperature water two or three times for rest of 24 or 48 hours. Return jar to drain tray and store in dark location.

APPENDIX D

Wheat Cultivars, Their Sources, and Seed Information

Source	Cultivars
Utah Crop Improvement Association Utah Agricultural Experiment Station Logan, Utah 84321	Hansel
Wheatland Seed, Inc. Brigham City, Utah 84302	Mannning

Seed Information	Cultivars	
	Hansel	Manning
Origin	Utah	Utah
Lot Number/Cert. No.	UAES-2/HW-10	NW 3010
Pure Seed	99.71%	99.00%
Inert Matter	0.29%	1.00%
Other Crop	0.00%	0.00%
Weed Seed	0.00%	0.00%
Germination	94.25%	94.00%
Date Tested	9-22-80	8-81

APPENDIX E

LINEAR STATISTICAL MODEL USED FOR ANALYSIS OF VARIANCE OF REDUCTION OF PHYTIC ACID DATA

$$Y_{ijklm} = ug + V_i + VM_{ij} + T_k + VT_{ik} + MT_{jk} + VMT_{ijk} + R_{(ijk)l} + E_{(ijk)m}$$

KEY TO MODEL:

i = 1,2	V(cultivar)	V_1 = Hansel V_2 = Manning
j = 1,2,3,4	M(method)	M_1 = Acetic Acid M_2 = Citric Acid M_3 = 1% 24-hour sprouts M_4 = 1% 48-hour sprouts
k = 1,2,3	T(time)	T_1 = 0 hours T_2 = 2 hours T_3 = 4 hours
l = 1,2,3	R(replication)	if l = 1 1st replication if l = 2 2nd replication if l = 3 3rd replication
m = 1,2	E(error)	if m = 1 1st observation if m = 2 2nd observation

Y_{ijklm} = Phytic acid reduction of the M^{th} observation of the l^{th} replication of the i^{th} cultivar, j^{th} method and k^{th} time

ug = General mean of reduction

V_i = An effect due to cultivar

M_j = An effect due to method

VM_{ij} = Interaction effect between cultivar and method

T_k = An effect due to time

VT_{jk} = Interaction effect between cultivar and time

MT_{jk} = Interaction effect between method and time

VMT_{ijk} = Interaction effect between cultivar, method and time

R = An effect due to replication, also error term for preceeding effects

E = Error term.

LITERATURE CITED

LITERATURE CITED

American Association of Cereal Chemists. Approved Methods of The AACC. Method 02-51. St. Paul, MN. 1976.

Asada, K., Tanaka, K. and Kasai, Z. Formation of Phytic Acid in Cereal Grains. Ann NY Acad Sci 165:801-814, 1969.

Averill, H. P., and King, C. G. The Phytin Content of Foodstuffs. J. Am. Chem. Soc. 48:724-728, 1926.

Bassiri, A. and Nahapetian, A. Differences in Concentration and Interrelationships of Phytate, Phosphorous, Magnesium, Calcium, Zinc, and Iron in Wheat Varieties Grown Under Dryland and Irrigated Conditions. J. Agric. Food Chem. 25:1118-1122, 1977.

Bruce, H. M. and Callow, R. K. Cereals and Rickets. The Role of Inositol-Hexaphosphoric Acid. Biochem J. 28:517-528, 1934.

Davis, N. T., Hristic, V., and Flett, A. A. Phytate Rather Than Fiber in Bran as the Major Determinant of Zinc Availability to Rats. Nutr. Rep. Int. 15:207-214, 1977.

DeLange, D. J., Joubert, C. P., and DuPreez, S. F. M. The Determination of Phytic Acid and Factors Which Influence Its Hydrolysis in Bread. Proc. Nutr. Soc. South. AFR. 2:69-76, 1961.

Earley, E. B. Determining Phytin Phosphorus. Ind. and Eng. Chem. (Anal. Ed.) 16:389-391, 1944.

Ellis, R. and Morris, E. R. Comparison of Ion-Exchange and Iron Precipitation Methods for Analysis of Phytate. Cereal Chem. 59:232-233, 1982.

Franz, K. B., Kennedy, B. M., and Fellers, D. A. Relative Bio-availability of Zinc from Selected Cereals and Legumes Using Rat Growth. J. Nutr. 110:2272-2283, 1980.

Haghshenas, M., Mahloudji, M., Reinhold, J. G. and Mohammadi, N. Iron-Deficiency Anemia in an Irania Population Associated with High Intakes of Iron. Am. J. of Clin. Nutr. 25:1143-1146, 1972.

Harland, B. F. and Oberleas, D. A Modified Method for Phytate Analysis Using an Ion-Exchange Procedure : Textured Vegetable Proteins. Cereal Chem. 54:827-832, 1977.

Harland, B. F. and Harland, J. Fermentative Reduction of Phytate in Rye, White, and Whole Wheat Breads. Cereal Chem. 57:226-229, 1980.

Hartman, G. H. Removal of Phytate from Soy Protein. J. Am. Oil Chem. Soc. 56:731-735, 1979.

Heuber, W. and Stadler, H. Uber Eine Titrationsmethode Zur Bestimmung Des Phytins. Biochem. Z 64:422-437, 1914. As cited in Chemical Abstracts 8:3569.

Hoff-Jorgensen, E., Anderson, O., and Nielsen, G. The Effect of Phytic Acid on the Absorbtion of Calcium and Phosphorus. Biochem. J. 40:555-557, 1946.

Johnson, L. F. and Tate, M. E. Structure of "Phytic Acids". Can J. Chem. 47:63-73, 1969.

Knorr, D., Watkins, T. R., and Carlson, B. L. Enzymatic Reduction of Phytate in Whole Wheat Breads. J. Food Sci. 46:1866-1869, 1981.

Latta, M. and Eskin, M. A Simple and Rapid Colorimetric Method for Phytate Determination. J. Agric. Food Che. 28:1313-1315, 1980.

Lim, P. E. and Tate, M. E. The Phytases. I. Lysolecithin-Activated Phytase from Wheat Bran. Biochim. Biophys. Acta 250:155-164, 1971.

Lim, P. E. and Tate, M. E. The Phytases. II. Properties of Phytase Fractions F_1 and F_2 from Wheat Bran and the Myo-Inositol Phosphates Produced by Fraction F_2. Biochim. Biophys Acta 302:316-328, 1973.

Lolas, G. M., Palamidis, N. and Markakis, P. The Phytic Acid-Total Phosphorus Relationship in Barley, Oats, Soybeans, and Wheat. Cereal Chem. 53:867-871, 1976.

Maga, J. A. Phytate: Its Chemistry, Occurrence, Food Interactions, Nutritional Significance, and Methods of Analysis. J. Agric. Food Chem. 30:1-9, 1982.

Makower, R. U. Extraction and Determination of Phytic Acid in Beans (Phaseolus vulgaris). Cereal Chem 47:288-295, 1970.

Marrese, R. J., Duell, R. W. and Sprague, M. A. A Comparison of Three Current Methods for the Analysis of Phytin Phosphorus. Cro Sci. 1:80-81, 1961.

Mihailovic, M. L., Antic, M. and Hadzijev, D. Chemmical Investigation of Wheat 8. Dynamics of Various Forms of Phosphorus in Wheat During its Ontogenesis. The Extent and Mechanism of Phytic Acid Decomposition in Germinating Wheat Grain. Plant and Soil 23:117-128, 1965.

Nagai, Y. and Funahasi, S. Phytase (Myoinositolhexaphophate phosphohydrolase) from Wheat Bran Part I. Purification and Substrate Specificity. Agr. Biol. Chem. 26:794-803, 1962.

Nahapetian, A. and Bassiri, A. Changes in Concentrations and Inter-Relationships of Phytate, Phosphorus, Magnesium, Calcium, and Zinc in Wheat During Maturation. J. Agric. Food Chem. 23:1179-1182, 1975.

Oberleas, D. The Determination of Phytate and Inositol Phosphates. In Methods of Biochemical Analysis. Edited by D. Glick. Vol 20:87-101. New York: Wiley, 1971.

Oberleas, D. University of Kentucky, Department of Food Science, Lexington, Kentucky. Personal communication, 1982.

Oberleas, D. Muhrer, M. E., and O'Dell, B. L. Dietary Metal-Complexing Agents and Zinc Availability in the Rats. J. Nutr. 90:56-62, 1966.

O'Dell, B. L., Burpo, C. E. and Savage, J. E. Evaluation of Zinc Availability in Foodstuffs of Plant and Animal Origin. J. Nutr. 102:653-660, 1972.

O'Dell, B. L. and Savage, J. E. Effect of Phytic Acid on Zinc Availability. Proc. Soc. Exp. Biol. Med. 103:304-305, 1960.

Peers, F. G. The Phytase of Wheat. Biochem. J. 53:102-110, 1953.

Pringle, W. J. S. and Moran, T. Phytic Acid and its Destruction in Baking. J. Soc. Chem. Ind. 61:108-110, 1942.

Reinhold, J. G. Phytate Destruction by Yeast Fermentation in Whole Wheat Meals. J. Am. Diet. Assoc. 66:38-41, 1975.

Reinhold, J. G., Faradji, B., Abadi, P. and Ismail-Beigi, F. Decreased Absorption of Calcium, Magnesium, Zinc and Phosphorus by Humans Due to Increased Fiber and Phosphorus Consumption as Wheat Bread. J. Nutr. 106:493-503, 1976.

Reinhold, J. G., Ismail-Beigi, F. and Faradji, B. Fibre vs. Phytate as Determinant of the Availability of Calciumm, Zinc, and Iron of Breadstuffs. Nutr Rep. Int. 12:75-85, 1975.

Samotus, B. and Schwimmer, S. Indirect Method for Determination of Phytic Acid in Plant Extracts Containing Reducing Substances. Biochim. Biophys. Acta. 57:389-391, 1962.

Tangkongchitr, U., Seib, P. A. and Hoseney, R. C. Phytic Acid II. Its Fate During Breadmaking. Cereal Chem. 58:229-234, 1981.

Tangkongchitr, U., Seib, P. A. and Hoseney, R. C. Phytic Acid III. Two Barriers to the Loss of Phytate During Breadmaking. Cereal Chem. 59:216-221, 1982.

Ter-Sarkissian, N., Azar, M., Ghavifekr, H., Ferguson, T. and Hedayat, H. High Phytic Acid in Iranian Breads. J. Am. Diet. Assoc. 65:651-653, 1974.

U. S. Department of Agriculture. The Official United States Standards for Grain. Washington, D.C.: Government Printing Office, 1978.

Wheeler, E. L. and Ferrel, R. E. A Method for Phytic Acid Determination in Wheat and Wheat Fractions. Cereal Chem. 48:312-320, 1971.

Williams, S. G. The Role of Phytic Acid in the Wheat Grain. Plant Physiol 45:376-381, 1970.

Witmer, J. O. The Bioavailabity of Zinc in Whole Wheat. Master's Thesis, Brigham Young University, 1981.

Young, L. XL. The Determination of Phytic Acid. Biochem J. 30:252-257, 1936.

PHYTIC ACID REDUCTION IN WHOLE WHEAT FLOUR DOUGHS BY pH ADJUSTMENT OR WITH SPROUTED WHEAT ADDITION

Richard Alan Mayfield

Department of Food Science and Nutrition

M.S. Degree, December 1982

ABSTRACT

The reduction of phytic acid in whole wheat doughs was studied using two cultivars of wheat and two methods at three reaction times. The methods were adjustment of dough pH to 5.2, the optimum pH of phytase, with acetic or citric acids and addition of 1% 24-hour or 48-hour sprouted wheats. The mixed doughs were allowed to react either zero, two or four hours. The addition of the acids and a reaction time of two hours resulted in a reduction of phytic acid by approximately 70%. The addition of 24- or 48-hour sprouts did not increase the reduction of phytic acid above the expected reduction due to natural phytase present in the flour. The rate of reduction slowed significantly after two hours of reaction time for both methods and statistically no difference was noted for two or four hours of reaction times.

COMMITTEE APPROVAL:

John Hal Johnson

John Hal Johnson, Committee Chairman

Albert E. Purcell

Albert E. Purcell, Committee Member

Clayton S. Huber

Clayton S. Huber, Department Chairman