Barley-starch, its production, and some of its non-carbohydrate constituents
by John David Imsande

A THESIS Submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Master of Science in Chemistry
Montana State University

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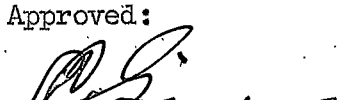

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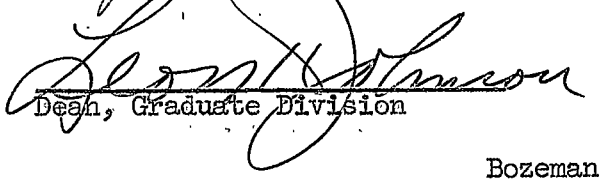

Dean, Graduate Division

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I. ABSTRACT

An investigation was made to determine the practicability of utilizing barley for the production of starch. The separation of starch from protein was attempted in six varieties of barley by Dimler's alkali process and the batter process; however, only Dimler's process gave a satisfactory separation. The yields of starch obtained in the separation of 100 gram samples of the various barley flours varied from 43 to 63 grams, while the protein recovery varied from 81 to 86%. Some non-carbohydrate constituents of these various starches were determined.

II. INTRODUCTION

The cereal grain most common in the Pacific Northwest is wheat. This is due to the relatively short growing season and also the high net returns, per acre, obtained by the farmer.

The increasing wheat prices of a decade ago, which were brought about by the enormous cereal demands of World War II, inspired farmers to bring additional acreage into production. Now, however, our nation has accrued a surplus of wheat, and it has become necessary to reduce the acreage in production. Recently our government initiated a "Soil Bank" plan as a means of bolstering wheat prices. This means many fields must be removed from wheat production.

Barley is a cereal grain which grows well under the climatic condition of the Pacific Northwest. In the past few years, much of the acreage removed from wheat production has been planted in barley as the farmers are permitted to grow barley on their fields even though they are drawing the "Soil Bank" allotments. As a result, a surplus of barley has accumulated. It appears this surplus will continue to increase as more and more acreage is removed from the production of wheat. In view of these facts this study has been undertaken to exploit further the commercial possibilities of barley.

At the present time approximately 95% of the starch in the United States is produced from corn and milo maize. A small amount is produced from cull potatoes. Also, a small amount is produced from damaged and low grade wheat flour (1); however, wheat still could not

compete with corn in the production of starch if it were not for the fact that its protein is used for the manufacture of sodium glutamate.

Several procedures have been developed in an effort to produce starch from wheat economically but because of the high cost of wheat this has not been too successful. However, barley, in certain areas, markets at a much lower price, per pound, than wheat or corn. Also, the process for the production of starch from wheat, which was recently developed by Dimler et al. (2) had been tested successfully on a few strains of barley. In this process, the protein can be recovered in the denatured state and sold for feed or feed supplement.

The batter process (3) is an excellent and inexpensive procedure for the separation of starch and gluten from wheat, but so far as known, it has never been attempted on barley. In this procedure the gluten, or protein, is recovered in its native state. Undenatured gluten could possibly be added to low protein flours and thereby increase their baking qualities.

Most of the starch plants are located in the Corn Belt. This means the consumers of the Pacific Northwest must pay the shipping costs from the Mid-West. The starch production process developed by Dimler et al. (2) could be carried out in any sugar beet plant of the Northwest with only a small addition of equipment. This would appear to be ideal as these plants are now operating only during the sugar beet campaign. These plants could devote the larger portion of each year to starch production and with a little additional equipment could produce dextrose syrup from the raw starch.

The low cost of barley per bushel, its availability, the accessibility of existing production facilities, and an increasing demand for starch and starch products in the Northwest would place barley starch in strong competition with corn starch in this area.

Recently much interest has been extended in the study of the qualities and properties of the various types of starch. It has been reported that a large granule starch is highly desirable. Since barley starch contains large granules it might bring a premium over corn starch for certain uses.

Another possibility resulting from the production of starch from barley would be the commercial production of yeast. Yeast is presently produced for a variety of commercial outlets. They include: Bakers yeast, feed supplement yeast, and yeast for production of vitamins. Yeast is grown best on a media which is low in carbohydrates (1 to 2%). This is approximately the concentration of the starch wash solutions. The recovered protein could be added to the wash solution to give it the protein concentration required for maximum yeast growth. Yeast production, however, would require considerable additional equipment.

In summation, a satisfactory method must be established for the separation of starch from the various varieties of barley, and a thorough investigation of barley starch is necessary to determine its chemical properties. Identification of outstanding starch characteristics would do much to support the production of starch from barley.

III. EXPERIMENTAL METHODS AND MATERIALS

The process developed by Dimler et al. (2) for the production of starch essentially involved the dispersion of the flour protein in a dilute aqueous alkaline solution and then the removal of the starch fraction from the alkaline solution by centrifugation. The alkaline protein solution was then acidified and the alkali soluble protein was precipitated. The starch and protein can then be processed as desired.

Dimler's process was developed for the production of starch from wheat flour, using sodium hydroxide as a protein dispersing agent, and sulfuric acid for the acidification and precipitation of the protein. In order for this procedure to be applied effectively to barley flour the solubility of the protein of various strains of barley and the pH for the maximum precipitation of the barley protein had to be determined.

The following strains of barley: Betzes, Compana, Carlsberg, Hannchen, Vantage and Ymer, were milled to flour in an experimental Buhler mill equipped with 10XX bolting silk. A sample of Compana barley was pearled to determine if pearling would facilitate the production of starch from barley flour. The samples of barley investigated were of known history and selected by Mr. Robert Eslick of the Montana State College Agronomy Department. Each flour prepared was analyzed for its content of starch, protein, pentosan, crude fiber and ash to determine if any of these qualities were correlated with starch production. Starch was determined polarimetrically by the procedure

outlined by Clendenning (4). A Kraav full circle polarimeter was used for these determinations. In calculations, the value 203.5°, which was determined by Clendenning and Wright (5), was used as the optical rotation of purified barley starch. The per cent starch was calculated by employing the following formula: $\% = \frac{\text{Obs. Reading} \times 10^4}{203.5 \times 2 \times 2}$. Nitrogen was determined by the Kjeldahl method. The per cent protein was calculated by multiplying the per cent nitrogen by the factor 5.7 (2). Pentosans were determined by the official A.O.A.C. (1940) procedure, the distillate being redistilled and the furfural precipitated by thiobarbituric acid. Crude fiber was determined by the standard A.O.A.C. method (6) and ash content by ignition for three hours at 700°C.

In applying Dimler's process to barley, various normalities of sodium hydroxide were used to find the apparent maximum solubility. Ten gram samples of the various air dried barley flours were mixed with 100 ml. portions of sodium hydroxide varying from 0.015 to 0.45N until a homogenous mixture was obtained. Mixing time was reduced by first making a paste with 20 ml. portions of the alkaline solutions and then the remaining 80 mls. added. This procedure was carried out between 20°C and 30°C to prevent gelatinization of the starch. The samples were allowed to stand with occasional stirring for 30 minutes to insure protein solubility and then the pH of the solutions was determined. A model H2 Beckman glass electrode pH meter was used for the pH determination. They were then centrifuged for 7 minutes and 10 ml. aliquots of the supernatant were removed for Kjeldahl analysis. The milligrams

of nitrogen were divided by the weight of the flour samples, which were corrected for moisture content, to find milligrams of nitrogen dissolved per gram of dry flour. Five gram samples of the various flours were placed in a vacuum oven at 100°C for 16 hours and the moisture content was calculated as the weight loss.

After the normality of the aqueous sodium hydroxide solution required for maximum protein dispersion had been determined, 100 gram samples of the air dried flours were mixed with 1200 ml. portions of an alkaline solution of the desired strength. Again, the mixing time was shortened if a smooth paste was made and then diluted with the remainder of the 1200 mls. of alkali. Thirty minutes from the initial time of mixing a 50 ml. aliquot was removed and the pH was determined. The suspensions were then screened through a 140 mesh sieve to remove hull particles. After screening, the suspensions were centrifuged for 10 minutes in a size 2, RV 110 International Centrifuge equipped with 250 ml. jars. In all cases, three distinct layers were obtained. On top was the tawny alkaline supernatant, next was a tan viscous layer described by Sandstedt et al. as the "amylodextrin layer" (7), MacMasters, however, designated it "tailings fraction", a more appropriate name (8), and on the bottom was a white cake of starch. The supernatant was decanted and saved for protein recovery analysis. The tailings fractions were removed by means of a spatula, washed with an equal volume of water, and centrifuged. The starch cakes were removed and re-suspended in a small amount of water and re-centrifuged to facilitate a more quantitative

removal of the tailings fraction. The tailings fraction was placed on a large watch glass and the starch was placed on a large weighed filter paper and both were allowed to air dry.

The pH for maximum protein recovery was then determined by taking 7, 100 ml. aliquots from the supernatant and acidifying them with 0.30N sulfuric acid to pH's of 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0 respectively. The sulfuric acid was added from a buret and the volume added to each was noted. The solutions were then centrifuged for 10 minutes and a 10 ml. aliquot was taken from the supernatant for Kjeldahl analysis. The difference between the per cent protein per ml. in each of the acidified filtrates, and the per cent protein per ml. in the original alkaline solutions was taken as precipitated protein. Corrections were made for the volume of acid added to produce the various pH's desired. Undoubtedly some of the precipitated protein is not recovered. Recycling of the alkaline solution would probably produce a greater protein recovery.

The batter process (3), designed for the separation of starch and gluten from wheat flour, was investigated in addition to Dimler's alkali process (2). This process involved forming a homogenous batter of flour and water and then disintegrating the batter in an excess of water by means of a high speed mixer. The batter produces milk starch and small, apparently undenatured, gluten curds upon disintegration.

One hundred gram samples of the air dried flours were added to portions of distilled water varying from 80 to 170 mls. The temperature

of the water used was varied from 20°C to 45°C. The flour and water were mixed with a small hand paddle for 1 to 5 minutes and allowed to stand. The mixing time was inversely related to the temperature of the water used. The duration of standing, which was inversely related to the mixing time and the temperature of the water used, varied from 1 to 60 minutes. When a smaller amount, or cooler, water was used more time was required for gluten hydration.

After the batter was formed 300 mls. of water were added. The combined batter and water was immediately subjected to a high speed mixer in an attempt to shred the batter and wash the starch free, leaving gluten curds and an aqueous starch suspension. The resulting mixture was poured on a 140 mesh sieve in order to separate the gluten curds or mass from the starch milk. The recovery of pure starch from the barley strains investigated, by the above procedure, was unsatisfactory because the barley did not form strong elastic curds.

The raw starches from the various strains of barley, which were obtained by Dimler's method of separation, were analyzed for per cent starch and some non-carbohydrate constituents. Starch was determined polarimetrically as outlined by Clendenning (4) on a Kraav full circle polarimeter.

The non-carbohydrate analysis included: phosphorus, silica, sulfur, calcium, magnesium, potassium, sodium, nitrogen and fat. Nitrogen was determined by Kjeldahl analysis of 5 gram samples of starch. For the other constituents 7 gram samples of the various room dried starches

were washed 3 times in 100 ml. portions of water at room temperature. Each time the starch was recovered from the aqueous suspension by centrifugation. The samples were washed before analysis in order to remove any mechanically held particles.

Five hundred milligram samples of the various washed starches were weighed into 50 ml. Florence flasks. They were then ashed by the following procedure: 2 mls. of concentrated nitric acid were added and after a few minutes 3 mls. of 70% perchloric acid. After addition of the acid the samples were placed under a hood on a hot plate and heated just to dryness. They were removed from the hot plate, allowed to cool, dissolved in a small amount of water and transferred quantitatively into 25 ml. volumetric flasks.

Five ml. aliquots were pipetted into new 25 ml. volumetric flasks and each was diluted to volume. The original solutions were set aside for flame photometrical analyses. From the dilution, 10 ml. aliquots were taken for the photometric determination of phosphorus. A modification of the procedure outlined by Allen (9) was used. A model B Beckman Spectrophotometer was used for this analysis.

Two mls. of dilute phosphoric acid were added to the remaining 20 mls. of the original solutions, and they were then used for the flame photometrical determination of calcium, magnesium, potassium and sodium.

Three standard solutions consisting of all the ions reported in starch, at ratios of their approximate concentrations, were prepared.

These solutions were analyzed for calcium, magnesium, potassium and sodium, and the observed readings for each of the ions were plotted. The concentration of each of the elements investigated was obtained by interpolation from these standard curves. A model D.U. Beckman Flame Spectrophotometer with oxygen-hydrogen flame was used for these determinations.

The procedure used for the flame photometrical determination of calcium was a modification of that which was suggested by Cooley (10).

The procedure employed for the determination of magnesium, potassium and sodium was a modification of the method of Pro and Mathers (11), and current investigations at Montana State College, Bozeman, Montana (11a).

Silica was determined photometrically by the procedure used by Kerr and Trubell (12).

The determination of sulfur was attempted by the photometric procedure of Snell and Snell (13); however, the percentage of sulfur was somewhat questionable due to small samples and the apparent lack of sensitivity of the procedure.

The fat content was determined by acid hydrolysis and extraction from the hydrolysate as suggested by Taylor and Nelson (14). Ten gram samples were used for this analysis.

The "tailings fractions"(7) or "amylodextrins" (8) were pulverized in a type V.A. Braun Pulverizer and analyzed for their nitrogen and starch contents. Nitrogen was determined by Kjeldahl analysis on 2 gram samples of the pulverized "tailings". Clendenning's

procedure for starch analysis, which was employed very effectively for the determination of starch in flour and purity of raw starch, would not work satisfactorily for the determination of starch in the "tailings fractions"; however, a modified method of the wheat procedure outlined by Earle and Milner (15) was used satisfactorily. Instead of using the 3, 10 ml. portions of 0.05N sodium hydroxide as suggested by Earle and Milner, one 20 ml. portion of 0.05N sodium hydroxide was used. Only one sodium hydroxide extraction was necessary because most of the protein had been removed during separation.

IV. RESULTS

The milling yields of the various flours were noticeably different (Table I). Several varieties milled very readily while others were more difficult to mill. In milling Ymer, the flour had a tendency to "ball up". Carlsberg, on the other hand, milled quite readily; however, a much greater percentage of the hull was retained in the flour. In general, the large, heavy kernels milled more readily and gave higher milling yields. Tempering was found to be disadvantageous in the milling of barley flour. In fact, dryness appeared to be a prerequisite for good milling.

Of the two methods investigated for the separation of starch, only Dimler's alkali process (2) was satisfactory. A batter possessing the elasticity and rigidity required for the batter process could not be prepared from barley flour. With low water ratios only a crumbly ball could be formed. Upon disintegration most of the batter was transformed into a flour suspension. A few "curds" resulted; however, they existed as a "slime coated" flour ball.

The dispersing action of aqueous sodium hydroxide on barley protein is strikingly different as shown in figure 1. Only 95.6% of the protein was extracted from Carlsberg and 97.2% from Hannchen in a 0.045N solution. The protein of the other flour strains was 100% soluble in the same strength solution and also in less concentrated solutions. The observed apparent protein solubilities and pH variations given in Table II indicate that the protein or proteins, of the

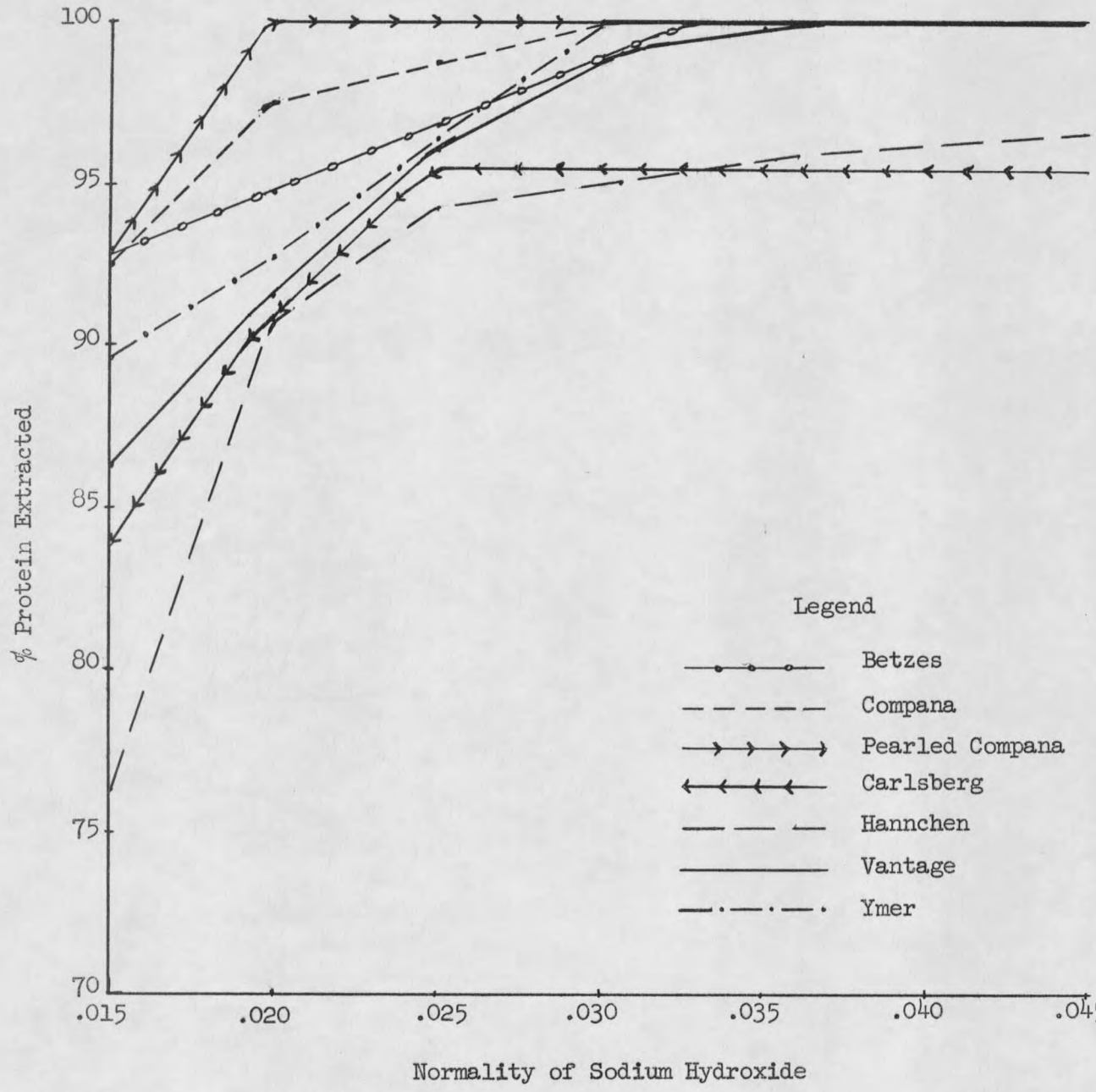

Figure 1. Relationship of the extent of barley protein extraction to the normality of aqueous sodium hydroxide.

various varieties are somewhat different, or that their mechanical "tie-up" is somewhat different. However, if in milling, an abnormal amount of hull and/or germ is thrown into the flour stream, a measurable amount of a foreign protein may be introduced. This situation was apparent in Carlsberg. Carlsberg flour contained an excessive amount of hull particles. This fiber which was obtained by sieving the alkaline flour solution, after it had been allowed to set for 30 minutes, revealed 1.3% nitrogen or 7.5% protein by Kjeldahl analysis. The nitrogen contained in the fiber was approximately equal to the total non-soluble nitrogen or protein of the flour.

In general, about 82% of the dispersed barley protein was very easily recovered (Table III). The maximum recovery (85.7%) was obtained from Vantage. In all cases, the optimum pH for protein precipitation was approximately 5 as is indicated in figure 2; however, there was a slight variation. If the ionic strength is low, the pH where the protein is least soluble would be the isoelectric point of that protein. This evidence substantiates further the hypothesis that the proteins of the various barley varieties are somewhat different.

Of the varieties investigated, Compana appears to be the best suited for the production of starch. The starch yield appears more closely correlated to the starch content of the flour than any other factor. The yields of high quality starch varied from 43 to 63 grams per 100 grams of flour while the "tailings fraction's" varied from 16 to 30 grams (Table IV).

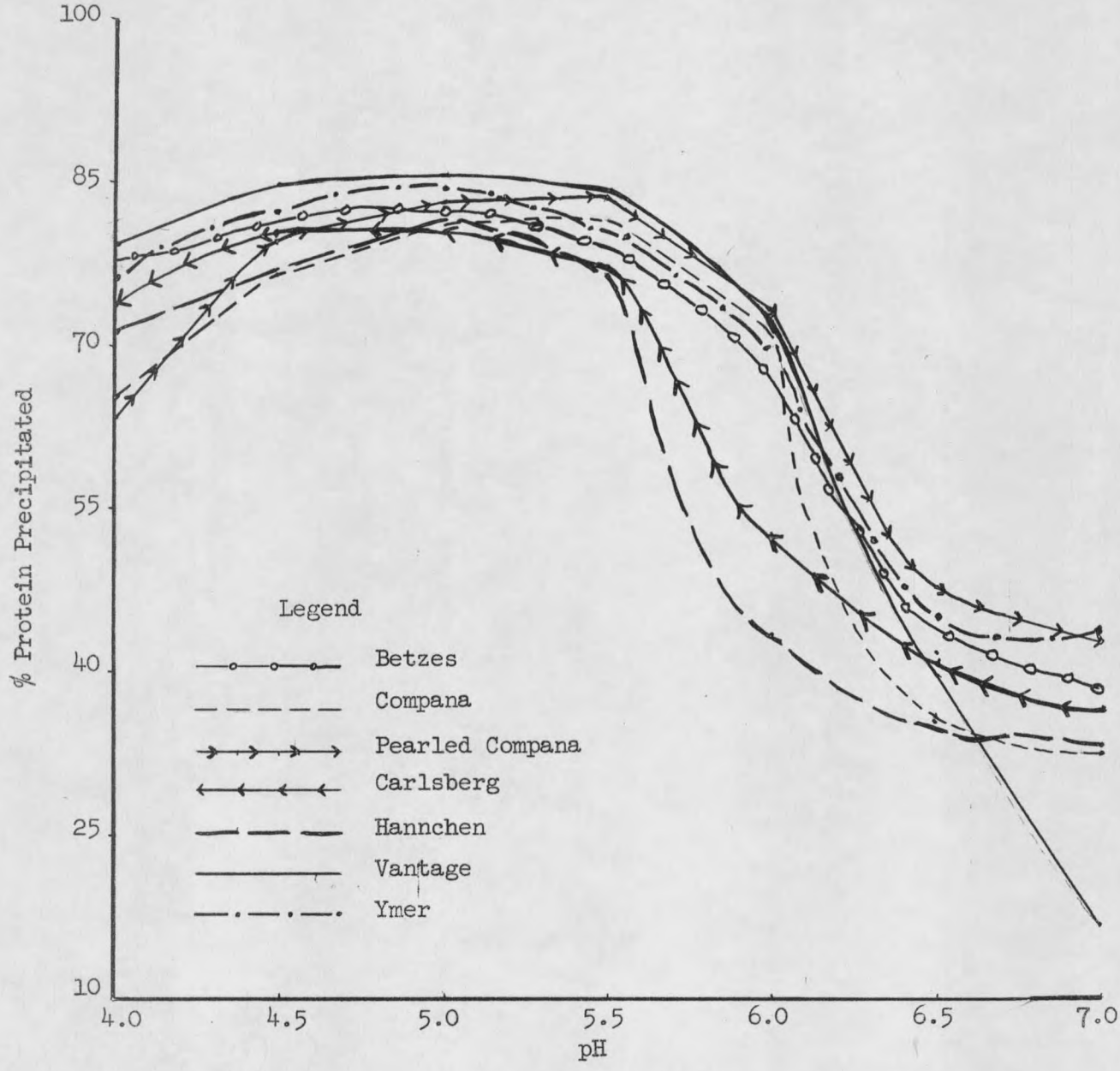

Figure 2. Effect of pH on the extent of precipitation of barley protein by acidification of the alkaline dispersion with sulfuric acid.

The purity of the raw starches varied from 97.3 to 99.7% while the starch content of the "tailings" varied from 57 to 82% (Table IV). These values were obtained polarimetrically and the error involved is reported to be less than 1% (15).

The non-carbohydrate analyses of the raw starches revealed a low nitrogen content (Tables IV and V). The analyses of phosphorus, calcium, magnesium, potassium, sodium, and silica, as listed in Table V, reveals barley starch compares more closely to rice starch than any other cereal grain (16). The sulfur content was not determined accurately, but it appeared to be at approximately the same concentration as found in other starches.

The percentage of "bound" fat found in barley starch was slightly higher than that amount reported in corn starch (14)(Table V).

TABLE I

ANALYSES OF FLOUR SAMPLE
(All data on moisture-free basis)

Strain of Barley	Lbs/bu.	Milling Yield %	Starch Content %	Protein Content %	Pentosan Content %	Ash Content %	Crude-Fiber Content %
Betzes	50.6	45	73	10.5	1.14	1.57	1.25
Compana (No.1)[1]	51.4	58	80	7.7	1.06	1.23	1.03
Pearled Compana[2]	--	57	79	9.0	--	--	--
Carlsberg	47.3	51	67	10.0	1.57	1.98	1.92
Hannchen	50.5	66	74	11.2	1.16	1.54	1.51
Vantage	48.8	43	79	11.8	1.11	1.27	1.23
Ymer	48.6	48	74	10.3	1.07	1.43	1.54

1. Two different Compana samples were used.

2. 57.2 represents hulled berries, pearled berries gave 65%.

TABLE II

PH DETERMINATION FOR MAXIMUM PROTEIN EXTRACTION

	Normality													
	.015		.020		.025		.030		.035		.040		.045	
Strain of Barley	pH	%Ext	pH	%Ext	pH	%Ext	pH	%Ext	pH	%Ext	pH	%Ext	pH	%Ext
Betzes	10.0	93.3	10.5	94.5	10.8	96.6	11.0	99.1	11.1	100.2	11.2	100.0	11.3	100.2
Compana	10.2	92.6	10.6	97.8	10.8	98.3	11.0	100.1	11.1	100.1	11.2	100.3	11.3	100.1
Pearled Compana	10.1	93.2	10.6	100.0	10.9	100.0	11.1	100.0	11.2	100.0	11.3	100.0	11.4	100.0
Carlsberg	9.8	83.8	10.4	90.4	10.7	96.0	10.9	95.3	11.1	95.6	11.3	95.6	11.4	95.6
Hannchen	9.6	76.0	10.3	90.7	10.6	94.6	10.8	94.9	11.1	95.7	11.3	96.9	11.4	97.2
Vantage	10.1	86.6	10.6	91.4	11.0	96.3	11.1	98.9	11.2	99.6	11.3	100.0	11.3	100.0
Ymer	9.8	89.5	10.3	92.4	10.6	96.6	10.8	100.3	11.1	100.0	11.2	99.6	11.3	100.0

Note: pH measured with glass electrode, uncorrected for alkali salt errors.

TABLE III

PH DETERMINATION FOR MAXIMUM PROTEIN PRECIPITATION
(Nitrogen calculated as N x 5.70)

Strain of Barley	Per Cent Protein Precipitated at pH						
	4.0	4.5	5.0	5.5	6.0	6.5	7.0
Betzes	77.4	80.0	82.5	80.2	69.6	44.4	38.0
Compana	65.5	75.8	80.9	80.2	73.9	35.2	31.4
Pearled Compana	63.3	79.7	83.3	83.4	74.3	47.6	42.4
Carlsberg	74.1	80.9	80.3	77.5	53.8	38.9	36.2
Hannchen	71.6	76.2	81.6	76.7	43.4	35.0	31.5
Vantage	79.3	84.4	85.7	83.9	72.3	38.3	14.8
Ymer	75.2	81.0	83.3	80.6	71.0	45.1	42.5