A study of the starch and protein in barley
by Clarence A Ryan

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

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I. ABSTRACT

An investigation was made to determine the per cent amylose in the starch fraction of ninety-seven barley samples. Two barley flours, prepared from Compana and Vantage varieties, were selected for a study on the preparation of starch. The starch was separated from the protein fraction by Dimler's alkali process to see if possible commercial application could be made. The protein fractions were analyzed chromatographically for their amino acid content to see if there was a correlation between amino acid content and malting characteristics.

II. INTRODUCTION

There has been considerable interest recently (1, 2) in finding cereals with a high amylose content. The work of Wolf et. al. (3) has shown that pure amylose can be used to make self supporting films with most of the desirable qualities of other plastic films. They have also shown that good film characteristics are retained with additions of small amounts of amylopectin.

Starch is composed of two fractions, amylose and amylopectin. Amylose is the linear fraction, and usually constitutes about twenty per cent of the starch of most common cereals. The remaining starch is the branched fraction, called amylopectin.

Considerable progress has been made in the selective crossbreeding of corn varieties (4) with some resulting cross-breeds showing a marked increase in amylose percentage. Therefore, if a cereal could be found, which is above average in amylose, cross-breeding could be attempted to increase the percentage of amylose. If this could be accomplished, the resulting starch could be used to replace a considerable amount of alpha cellulose in the production of films. This would be desirable with the present shortage of wood pulp and excess of cereal grains. The present price of alpha cellulose would make starch a competitive product.

In searching for a cereal of this type, barley seemed an obvious choice in the Northwest. This is due mainly to the fact that barley is the only cereal that can be grown on much of the land taken out of wheat production. As a result large surpluses of barley have been built up and this condition will probably become much worse. Therefore, a search has

been undertaken to find varieties of barley suitable for possible cross-breeding.

Only four barley varieties have been reported for their percentage of amylose previous to this paper (5). Of these four, only one, Trebi, has been included in this survey.

Barley starch has several other potentialities. Ordinarily over ninety-five per cent of this country's starch is produced from corn and milo maize. A small amount is produced from wheat, but this is difficult to obtain and therefore expensive. It probably would not be produced if it were not for the fact that the wheat protein is used for the manufacture of sodium glutamate. A small amount of potato starch is also produced from cull potatoes.

In the corn belt, where most starch plants are located, barley has not been seriously considered as a source for starch production. In the first place, barley and corn are about equal on a cost per pound, and starch per pound basis. This makes corn the better choice because of availability and ease of processing.

An ever increasing demand for starch and starch products in the Northwest, the low cost of barley per bushel, and its availability, would tend to make barley starch production more feasible here than in the corn belt. Furthermore, good irrigated barley should yield about as much starch as corn on a pound per pound basis. Starch production in the Northwest would also cut shipping costs now paid both ways on these products. A commercial outlet for barley would be highly desirable in this area.

Because of the high cost of actually building a plant for the pro-

duction of barley starch, it would be necessary to develop a process which could be utilized by using equipment already in the area.

A process for the production of starch from wheat has been developed recently by Dimler, et al. (6). This process could be applied to barley in a sugar beet plant with a small addition of equipment. This situation would be ideal because these plants do not operate twelve months a year and could produce starch during the lay off months. With a few minor additions they could make dextrose syrup from the raw starch.

The protein from the barley could be sold as feed or feed supplement, thus furthuring the economics of such an operation.

The second experimental part of this thesis concerns applications of Dimler's process to the preparation of barley starch.

Barley is used almost universally as malt for the production of beer. The malting process, during which the barley grain is induced to sprout under ideal temperature and moisture conditions, produces high alpha amylase activity. This activity breaks down the starch of the barley grain and other carbohydrate substrates present to fermentable sugars and low molecular weight dextrins.

Not all barley varieties are good malting barleys. This seems to be because of the inability of some barley grains to produce enough alpha amylase for complete conversion.

During the malting stages, no protein or amino acid substrates are added to the incubating barley. This would indicate that 1.) an amino acid pool was present to synthesize alpha amylase, 2.) that synthesis occurred from transfer of amino acids from other proteins, or 3.) that another

enzyme system may be the precursor of alpha amylase.

If one of these possibilities were present, it would seem possible that good malting qualities were tied up in amino acid content of the barley. If so, it could possibly furnish a clue to the composition of alpha amylase, or at least to the amino acids necessary for its synthesis. Since some varieties are definitely known to be good malting varieties while others are not this problem is of some interest.

As far as is known nothing has been done in this direction, although some common amino acids have been added to malt with no improvement shown in malting qualities of the barley.

Apart from the malting aspect, it would be desirable to know the amino acid composition of some barley varieties which could be grown in the Northwest. This is because of the commercial possibilities of barley protein as a by-product of barley starch production. As a feed or feed supplement the amino acid content would be valuable knowledge when considering the metobolic demands of animals being fed.

The glutamic acid in barley could be a possible source for the production of sodium glutamate, which is used widely as a flavor extendor in meat. The amount present in barley protein would be of considerable importance.

With these ideas in mind, a chromatographic determination was made of the amino acid content of Compana barley, which is considered a good malting barley, and Vantage, which has proven to possess very poor malting characteristics. Compana is not the best barley variety which could have been chosen, but it was selected because of its ability to grow well in the Gallatin Valley.

III. EXPERIMENTAL PART I

METHODS

During the study of amylose in barley, the first considerations were the effects of environmental factors on the percent amylose in a single variety. The effects of the following factors were considered using the Compana variety in all cases, 1) the years when planted, 2) the different dates of one year when planted, 3) effects of fertilizers added, 4) stage of growth when harvested, and 5) locations where grown. All samples except 5) locations, were grown in the Gallatin Valley.

This knowledge is necessary if a survey of different varieties is to be made; because if the environment did make a difference in amylose content, then it would not only be a matter of cross-breeding to raise this content, but of having ideal growing conditions as well.

A further study followed in which twenty-two barley varieties from eight states were analyzed for percent amylose. Twenty-eight varieties from twelve foreign countries were similarly analyzed.

Fifteen barley samples of Compana Shrunken Endosperm and five Compana freaks were also analyzed.

The barley samples were selected by Mr. Robert Eslick of The Montana State College Agronomy Department.

Approximately ten grams of each barley variety were pearled for one minute to remove the hulls. A pearling machine consists basically of a rotating emory wheel, which strips the hulls and most of the pericarp from the grain, but this does not affect the ratio of amylose to amylopectin in the barley samples.

Three to five grams of pearled barley were ground in a Wiley mill to pass a forty mesh screen, and the fat extracted for twenty-four hours in a Soxhlet extractor with 85% methanol. The residue was dried at room temperature for at least twenty-four hours before analysis. All percentages are reported on a fat free basis.

The barley samples were analyzed for percentages of fibre, ash, protein and moisture. The percentages of these four were then used as total non-starch in each barley sample. Six random samples of barley were chosen as an average value for the percent ash and fibre. Moisture determinations were made at random on twenty-four samples and since they all fell in a narrow range the average was used to calculate the balance. A protein determination was made on the individual barley samples. The extracted ground barley samples were used in every analysis. The average value for fibre, ash and moisture were used as overall corrections for non-starch material, while individual corrections were made for protein.

The fibre was analyzed by the standard A.O.A.C. method (7). The results varied between 1.33% and 1.65%. The average being 1.49%.

The ash was also analyzed by the standard A.O.A.C. (8) specifications. The ash content varied between 1.98% and 1.69%. The average was 1.88%.

For the determination of moisture, approximately two gram samples were dried in a vacuum oven for twenty-four hours at 100° C. The loss in weight was reported as percent moisture. The values varied between 6.61% and 6.81%. The average was 6.71% moisture.

The total average of these three analyses was 10.08%. This figure was used as a blanket correction on all of the samples as the non-protein, non-

starch component. It is to be observed that an error as great as 1% on this total would only make a difference of 0.20 to 0.25 on the percent amylose and this is within the experimental error of the method.

Protein determinations were carried out by a modified Kjeldahl method (9). The percent protein used in the calculations was obtained by multiplying the percent nitrogen by the factor 5.7 (10).

The amylose was determined according to the method described by Bates, Rundle and French (11) as modified by Lanski (12). To approximately 0.04 grams of the extracted ground barley was added 10 ml of boiling distilled water to gelatinize the starch. Then 5 ml of 1.0 N potassium hydroxide was added to disperse the starch in the samples. The solution was left to stand for one hour, neutralized with 0.5 N hydrochloric acid, using methyl orange as the indicator. A 10 ml quantity of 0.5 N potassium iodide was pipetted into the solution and the volume was brought to 100 ml with distilled water. This solution was titrated potentiometrically with a standard iodine in potassium iodide, potassium chloride solution containing .0001970 grams of iodine per ml in a solution 0.05 N in both potassium iodide and potassium chloride. The instrument used for following the titrations was a Beckman Model H-2 pH meter, set to read in millivolts, and using a calomel reference electrode and a platinum indicator electrode.

The titration is dependent upon the activity of the iodine in solution. Amylose forms a complex with iodine in which 0.215 grams of iodine combine with 1.0 gram of amylose. A complex is not formed between amylopectin and iodine (11). At the end point there is a sharp increase in the iodine activity which can be followed by the increase in potential. The

upper and more horizontal portion of this curve is extrapolated to give the ml of standard iodine used, since this point gives a more accurate result, as shown by experimentation (Figure 1).

The iodine solution was standardized with arsenous oxide. The solution used for analysis of amylose is prepared by tenfold dilution of the standardized stock solution.

A standard curve is plotted by titrating 830 mg of potassium iodide and 373 mg of potassium chloride in 100 ml of distilled water with the standardized iodine solution. From this standard curve, the milligrams of iodine in solution are subtracted form the total iodine used at the end point when titrating a sample of starch. This gives the milligrams of iodine bound in the complex with amylose. Knowing that .215 grams of iodine is bound per gram of amylose, the weight of amylose was calculated. This weight of amylose, divided by the weight of starch in the sample, times one hudred, will give the percent amylose in the starch. The experimental error was rather high plus or minus 1.0%. This was due largely to the instrument which could be read to only two significant figures.

RESULTS

There was no striking difference found in the percentages of amylose in the starch due to the environmental factors which were observed. Compana, fertilized with 11-48-0, appeared slightly higher in amylose. These samples were rechecked and the results were reproducable (Table I).

No barley varieties were found with abnormally high amylose content. The highest being two Compana Freak varieties, which contained 25.6% and 24.9% amylose. The next highest variety found was Dekap, from Turkey,

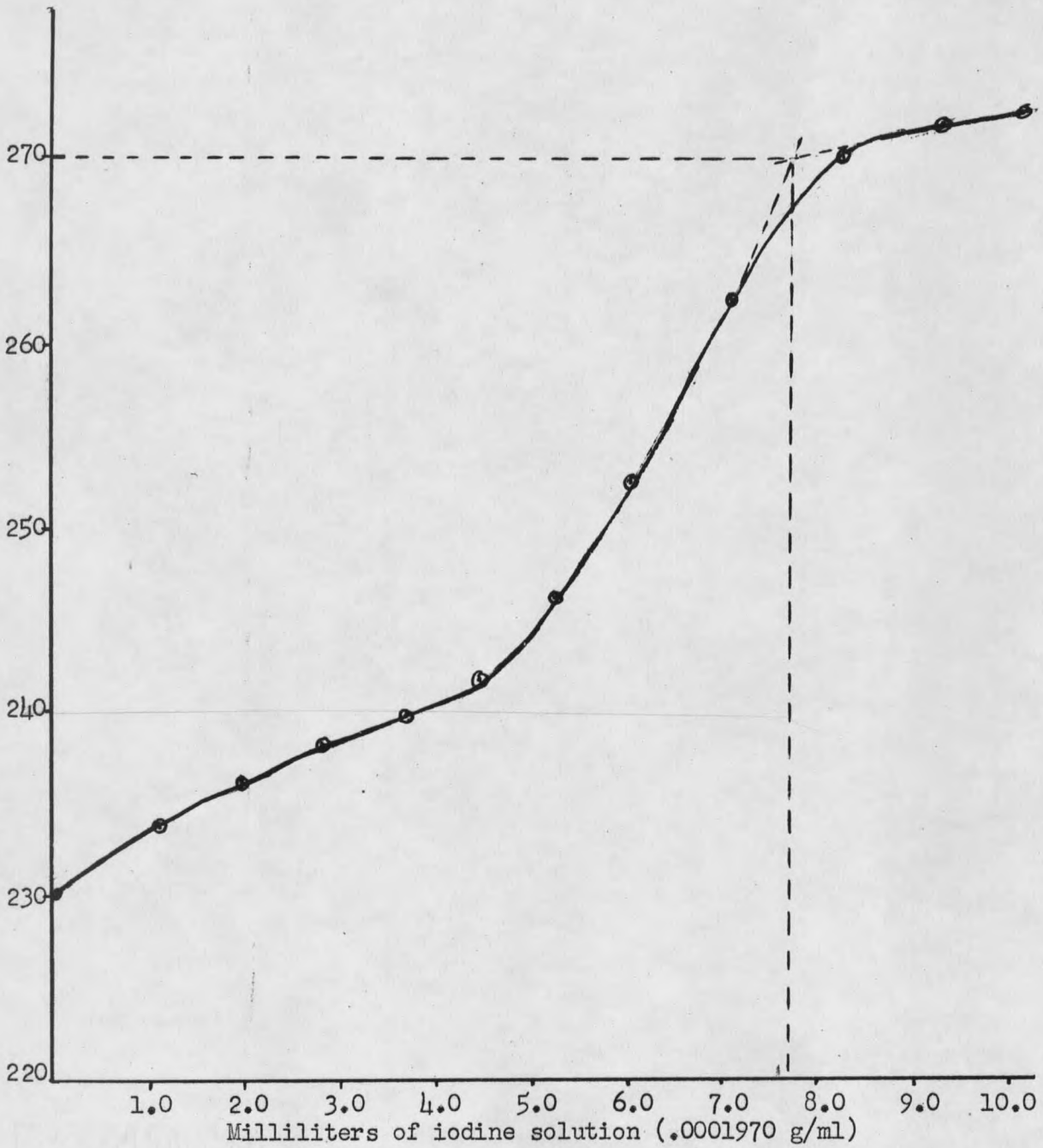

Figure 1. A typical potentiometric titration curve of Compana amylose using iodine in a potassium iodide solution.

containing 24.2% amylose. The low was PI 220069 from Pakistan with 12.4%. The average was 18.0% amylose (Tables II, III and IV).

If cross-breeding is to be attempted on the basis of this survey, it is suggested that Dekap and the two Compana Freaks be used, and possibly Bulk (23.4%), Otis (23.0%) and Barbless (23.0%) varieties.

TABLE I

ENVIRONMENTAL INFLUENCE ON PERCENT AMYLOSE IN COMPANA BARLEY STARCH

	% Protein	% Starch	% Amylose in the starch fraction
1. Years			
1950	12.8%	75.1%	21.2%
1951	14.7%	75.3%	20.4%
1952	14.6%	75.4%	21.1%
1953	16.9%	73.1%	21.0%
1954	9.2%	80.8%	21.0%
1955	9.7%	80.3%	20.6%
2. Fertilizers			
NH_4NO_3	12.6%	77.4%	20.8%
$(NH_4)_2SO_4$	12.4%	77.5%	19.5%
16-20-0	9.3%	80.7%	19.1%
0-43-0	8.1%	81.9%	21.8%
11-48-0	6.7%	83.3%	22.2%
11-48-0	6.7%	83.3%	22.4%
3. Planting Dates			
May 4, 1954	6.6%	83.4%	20.2%
May 18, 1954	6.6%	83.4%	21.1%
June 3, 1954	7.2%	82.8%	20.4%
June 17, 1954	7.2%	82.8%	22.0%
4. Locations			
Creston			
Dryland	7.2%	82.8%	21.1%
Irrigated	7.2%	82.8%	21.1%
Sidney			
Dryland	10.1%	79.9%	21.4%
Irrigated	8.8%	71.2%	22.6%
Huntley	12.1%	77.8%	21.6%
Havre	9.4%	80.6%	20.1%
Stevensville	7.6%	82.4%	21.8%
Springhill	11.1%	78.9%	20.1%
Missoula	10.1%	79.8%	20.2%
Ronan	11.7%	78.3%	21.5%
5. Growth Stage When Harvested			
Late Milk	12.1%	77.8%	20.7%
Soft Dough	9.4%	80.6%	21.6%
Hard Dough	9.4%	80.6%	22.0%
When Ripe	8.5%	81.5%	21.5%
Average			20.6%

TABLE II

PERCENT AMYLOSE IN BARLEY STARCHES OF U.S. ORIGIN

Variety	State	% Protein	% Starch	% Amylose in the starch fraction
Lico	Colorado	9.6%	80.4%	23.0%
Otis	Colorado	10.7%	81.3%	14.3%
Bulk	California	8.7%	81.3%	23.4%
Atlas 46	California	8.1%	81.9%	22.9%
Hooded Atlas	California	9.6%	80.4%	16.2%
Velvon II	Utah	8.6%	81.4%	21.4%
Bonneville	Utah	8.0%	82.0%	18.0%
Prosser #6	Washington	8.6%	81.4%	18.9%
Kindred Titan	N. Dakota	8.6%	81.4%	19.5%
Custer	Nebraska	13.0%	77.0%	14.6%
Hiland	Wyoming	9.2%	80.8%	16.5%
Spartan	Montana	13.3%	76.7%	20.4%
Compana Glossy Mutant	Montana	13.2%	76.8%	19.5%
Compana Glossy Seedling Striped Mutant	Montana	8.6%	81.4%	19.5%
Compana Margenot	Montana	10.7%	79.3%	19.1%
Barbless	Montana	16.8%	73.2%	23.0%
Barbless Glossy Mutant	Montana	9.2%	80.8%	22.3%
Mars	Montana	10.7%	79.3%	14.3%
Mars Glossy Mutant	Montana	8.1%	81.9%	16.7%
Glacier Titan (47-7405-V)	Montana	8.6%	81.4%	15.5%
Glacier Titan (50-5639-12)	Montana	10.7%	79.3%	21.0%

TABLE III

PERCENT AMYLOSE IN BARLEY STARCHES OF FOREIGN ORIGIN

Variety	Country	% Protein	% Starch	% Amylose in the starch fraction
Harlan	Canada	8.8%	81.2%	19.9%
Titan	Canada	9.5%	80.5%	22.3%
Husky	Canada	8.7%	81.3%	22.8%
Sanatta	Canada	9.6%	80.4%	17.6%
Morovian	Morovia	9.5%	81.5%	18.9%
P.I. 221377	Yugoslavia	12.2%	77.8%	19.1%
P.I. 221304	Yugoslavia	13.7%	76.3%	15.7%
P.I. 221309	Yugoslavia	12.5%	77.5%	15.9%
P.I. 221322	Yugoslavia	12.1%	77.9%	16.9%
P.I. 221327	Yugoslavia	13.3%	76.7%	14.5%
P.I. 211598	Afghanistan	11.9%	78.1%	13.9%
P.I. 212845	Afghanistan	15.3%	74.7%	12.6%
P.I. 212847	Afghanistan	15.0%	75.0%	15.1%
P.I. 212850	Afghanistan	11.3%	79.7%	13.9%
P.I. 219860	Afghanistan	14.8%	75.2%	14.7%
P.I. 221422	Afghanistan	11.1%	79.9%	14.4%
P.I. 220069	Pakistan	13.7%	86.3%	12.4%
P.I. 717532	Pakistan	13.8%	76.2%	17.5%
P.I. 220853	Sweden	17.3%	72.7%	16.6%
P.I. 219757	So. Africa	12.4%	77.6%	17.6%
P.I. 215708	Peru	15.5%	74.5%	15.9%
P.I. 221072	Germany	15.2%	78.4%	15.0%
Tammi	Finland	8.5%	81.5%	16.7%
P.I. 214326	India	13.7%	76.3%	14.8%
P.I. 216035	India	14.9%	75.1%	19.7%
Dekap	Turkey	8.2%	81.8%	24.2%
Trebi	Turkey	8.2%	81.8%	20.2%
Int. C.I. 7837	Turkey	8.4%	81.6%	21.8%

TABLE IV

PERCENT AMYLOSE IN STARCHES OF COMPANA SHRUNKEN ENDOSPERMS AND COMPANA FREAKS

Shrunken Endosperm	% Protein	% Starch	% Amylose in the starch fraction
Ag. Number			
7152	16.6%	73.4%	12.6%
7154	16.2%	73.8%	17.0%
7155	16.0%	74.0%	14.6%
7157	11.6%	78.4%	19.3%
7158	10.4%	79.6%	20.1%
7159	11.2%	78.3%	17.4%
7160	12.1%	77.9%	15.2%
7162	11.0%	79.0%	18.1%
7163	10.2%	79.8%	18.0%
7164	11.2%	79.8%	16.4%
7165	12.2%	77.9%	16.7%
7166	10.6%	79.4%	17.6%
7167	10.7%	79.3%	15.6%
7168	9.2%	80.8%	15.9%
7169	11.5%	78.5%	16.4%
Freaks			
Ag. Number			
7217	14.6%	75.4%	18.3%
7218	13.8%	76.2%	10.7%
7219	10.5%	79.5%	25.6%
7220	7.0%	83.0%	18.4%
7223	10.7%	79.3%	24.9%

IV. EXPERIMENTAL PART II

METHODS

The process developed by Dimler (6) essentially involves the dissolving of the flour protein with dilute alkali and centrifuging, thus leaving the starch fraction as the residue. The alkaline protein solution is then acidified to precipitate the alkali soluble protein. The starch and protein can then be processed as desired.

Dimler's process has been developed for the production of wheat starch, using sodium hydroxide as the solvent, and sulfuric acid for the acidification and precipitation of the protein.

In order for this process to work efficiently on barley flour the maximum solubility and the pH for the maximum precipitation of the barley protein must be determined.

Compana barley flour, prepared in a Buhler mill was used for the testing. The protein in the flour was found to be 9.12% by the Kjeldahl method. The apparent protein solubility was based on this figure.

Applying Dimler's work on wheat to barley, various normalities of sodium hydroxide were used to find the apparent maximum solubility. Twenty gram samples of barley flour were mixed with two hundred ml portions of sodium hydroxide varying from 0.015 N to 0.035 N until a homogeneous mixture resulted. The samples were left to stand with occasional stirring for thirty minutes to insure solubility. They were then centrifuged for five minutes and decanted. The pH of the solutions were determined and ten ml aliquots taken for Kjeldahl analysis. The milligrams of nitrogen were divided by twenty to find milligrams of nitrogen dissolved per gram

of flour. The procedure was carried out between 20°C and 30°C to prevent gelatinization of the starch.

After the pH for maximum solubility was found, the pH for maximum precipitation of the protein was sought.

Protein recovery was carried out on six alkaline solutions which were acidified with 0.30 N sulfuric acid. The solutions were then centrifuged for five minutes and a protein analysis was run on the filtrate. The difference between the percent protein per ml in the filtrate, and the percent protein per ml in the original alkaline solutions was taken as percent protein precipitated. This assumption is not strictly valid as some protein may adhere to the starch when the pH is lowered. However total protein recoveries on this material by a co-worker using the same method were in good agreement with these values.

RESULTS

In applying Dimler's process to barley flour it was found that barley protein has an apparent solubility of 100% at a pH of 11.4 and above (Table V). This indicates that barley starch of high purity could be produced with this process depending upon the purity of the flour used. Wheat protein has an apparent solubility of 100% at a pH of 11.7.

It was noted during centrifugation that the starch was quite heavy and appeared to settle more rapidly than either corn or wheat starch. It was readily obtained in 70% yield but due to lack of time this fraction was not investigated.

The protein was 91% precipitated at a pH of 6.0 (Table VI). This was quite high as compared to Dimler's results of 77% precipitation of wheat

protein at a pH of 5.5. This would mean that over 90% of the original protein in barley flour could be recovered by this process.

Although much work must yet be done to further explore the possibilities of this process with barley flour, the basic solubility pattern of barley protein gives favorable indications for its use.

TABLE V

THE APPARENT SOLUBILITY OF BARLEY PROTEIN

Normality	pH	Apparent Solubility
0.015N	9.8	35.0%
0.020N	10.3	73.0%
0.025N	11.1	80.0%
0.030N	11.4	100.0%
0.035N	11.7	100.1%

TABLE VI

PRECIPITATION OF BARLEY PROTEIN FROM ALKALINE EXTRACT

pH	% Protein Precipitated
4.0	72.8%
4.5	77.7%
5.0	85.3%
5.5	90.2%
6.0	91.0%
6.5	74.0%
7.0	52.6%

V. EXPERIMENTAL PART III

METHODS

The amino acid analyses on barley protein samples were carried out chromatographically according to the method of Moore and Stein (13). Using this method most of the common amino acids and several of the infrequently occurring amino acids in protein material can be separated. Tryptophan, cysteine and cystine are notable exceptions since they are destroyed by acid hydrolysis in the presence of carbohydrate material.

The samples of barley protein used were prepared from the flour of Compana and Vantage varieties with a modification of Dimler's alkali process. The protein of the two was not purified and contained 73.0% and 69.3% protein respectively by Kjeldahl analysis. The remainder of the sample was carbohydrate material.

Each protein sample was dried for twenty-four hours in a vacuum oven at 75°C and approximately 50 mg was hydrolyzed by refluxing with 200 ml of 6 N hydrochloric acid for 24 hours. This method (14) for hydrolysis permits the presence of large amounts of carbohydrate material in the hydrolysate without altering the values of the amino acids on analysis.

The hydrolysate was then filtered through a sintered glass filter and evaporated to dryness in a vacuum dessicator. The residue was taken up with 10 ml of distilled water and a one ml aliquot was used for each analysis. For the hydrolysis of Compana, 50 mg of crude protein was used, or 36.5 mg of pure protein. For the Vantage analysis, 52.3 mg of crude protein were hydrolyzed, or 36.2 mg of pure protein.

The chromatographic separations were made with equipment already in

use at the Experiment Station of Montana State College. The equipment consisted of two chromatographic columns packed with Dowex 50, a sulfonated polystyrene resin. One column was 0.9 cm by 100 cm, while the other was 0.9 cm by 25 cm. The former column was jacketed to permit temperature control with a regulated water bath. The water was circulated through the jacket from the bath with a small pump. The short column was not jacketed, as all determinations with this column were carried out at 25°C.

A rotating wheel which held eighty-eight collecting tubes around it's perimeter was used to collect one ml samples as they were eluted from the column. The wheel rotated the distance between the centers of adjacent collecting tubes every fifteen minutes. The long column was adjusted above the tubes and by means of a tank of nitrogen exerting pressure on the buffer in the column, a constant flow of one ml per fifteen minutes was achieved. The flow of the short column was adjusted simply by raising or lowering the buffer reservoir which is held near the level of the top of the column on an iron ring.

The long column was used for the determination of all but the basic amino acids in the hydrolysates. The short column was used to determine the basic amino acids, arginine, lysine, and histidine.

The buffer sequence for the long column was as follows (13); the column was begun with a pH 3.42 citrate buffer at 37°C until valine was eluted, then a pH 4.25 citrate buffer at 50°C was used, being raised to 75°C before tyrosine and phenylalanine came out to insure good separation of the two. This sequence worked very well for the acidic amino acids of both barley protein determinations.

The short column buffer sequence began with a citrate buffer of pH 5.0 and after 25 ml of this buffer had been passed through the column, it was followed by a pH 6.5 citrate buffer to finish the separation.

One ml per 100 ml of buffer of a detergent, BRIJ-35, was used to give better separation of the amino acids (13).

From the collecting tubes, 0.7 ml of solution was transferred to test tubes for determination of the amino acids. This solution was brought to pH 5.0 by addition of sodium hydroxide or hydrochloric acid, and one and one-half ml of ninhydrin solution was added. The test tubes were placed into a boiling water bath for thirty minutes to develop color (15). Two moles of ninhydrin combine per mole of amino acid when held at the boiling temperature of water for thirty minutes to produce a color which is a quantitative measure of the amino acid. The solution was diluted to 10 ml with 50% ethyl alcohol, transferred to a colorimeter tube, and the optical density was read at 570 mu except for proline which was read at 440 mu on a Beckman Model B Spectrophotometer.

The transferring was accomplished with a tuberculin syringe. A standard curve was made using solutions of leucine between 0.05 mMolar and 0.3 mMolar concentrations and developing color with ninhydrin as above. The standard was read on the colorimeter and the optical density was plotted against concentration. From this curve the concentrations of amino acids from the columns were found using their optical densities. This concentration was divided by a color yield for each amino acid which corrected for the difference in optical density reading of different amino acids of the same concentrations.

From the total concentrations of all of the readings of a particular amino acid and the volume in which it was contained, the percent in the hydrolysate was found using the following formulas (15):

$$(1) \quad \% = \frac{\text{conc. of amino acid x volume x molecular weight}}{\text{color yield x wt. of protein used for analysis in mg}}$$

Some amino acids were eluted overlapping one another. The following formulas were used in these cases (15):

$$(2) \quad A_1 = \frac{P_1 T}{F_1(P_1(V_2/V_1)P_2)}$$

$$(3) \quad A_2 = \frac{(V_2/V_1)\, P_2 T}{F_2(P_1(V_2/V_1)P_2)}$$

Where:

A_1, A_2 quantities in u moles of amino acids in 1st and 2nd peaks when optical density is plotted vs. conc.
P_1, P_2 height of each peak in u moles
V_1, V_2 effluent volumes at each peak
F_1, F_2 color yields
T leucine equivalents of combined peaks

When A_1 and A_2 are found the percentages can be calculated using formula (1).

It was noted when analyzing the basic amino acids that an amino acid appeared that is not commonly found in protein analyses. This particular amino acid was overlapping the tyrosine, phenylalanine combination which appears in the short column. In order to separate the amino acid, it was necessary to run the long column past the pH 4.25 buffer at 75°C by cooling it to 25°C and passing a pH 6.8 citrate buffer to elute it (16). Its presence was verified in both protein determinations. The presence of this amino acid is discussed in the Experimental Part III Results.

The percentages for all the amino acids found are reported in Table

VII. The total percentage is almost 100% even though tryptophan, cysteine, cystine and ammonia were not present in the calculations. This is due to the addition of water to the amino acids as they are broken from the protein on hydrolysis, thus increasing the total weight of hydrolyzed material in the hydrolysate.

The experimental error due to the technique is about 10%. This is increased somewhat in using the factor 5.7 convert mg of nitrogen to percent protein during the Kjeldahl analysis. This figure is not an absolute value, but has been accepted as the conversion factor by cereal chemists.

RESULTS

Of the amino acids analyzed only one, proline, was found to be of a noticeably lower percentage in Vantage than in Compana. The percentage of proline was 9.5% and 18.9% respectively. This difference of 9.4% is well above the experimental error involved. Although no conclusions can be drawn from this one comparison, it will be of interest when analyzing other malting barley varieties. This large difference indicates that amino acids in different barley proteins can be quite varied. Other amino acids were of differing percentages between the two proteins, but none so outstanding as proline. The addition of amino acids to malt, as mentioned in the Introduction, did not include proline.

Several of the amino acid percentages did not correlate with the analyses available in the literature (17,18) which were microbiologically performed on unnamed barley varieties. The impression given from these articles was that barley protein does not vary with variety. From the present analysis this does not appear to be the case (Table VII).

During the analysis two amino acids appeared that were not positively identified. Glutamine (19) and gamma aminobutyric acid (2) have lately been identified in barley protein and it is probably these acids that are present. Further work is planned to positively identify these acids.

The high percentage of glutamic acid, 36.5% in Compana and 37.3% in Vantage, suggests the possible use of barley protein for the commercial production of sodium glutamate. The chief source of sodium glutamate at this time is wheat protein, which contains 31.8% glutamic acid (21).

The amino acid percentages are presented in Table VII. These values are compared to available percentages of amino acids in wheat (22,23). No complete amino acid analysis was found for wheat protein although the percentages were available for the essential amino acids and glutamic acid. These analyses were made microbiologically.

From the standpoint of the basic amino acids and glutamic acid, the proteins appear somewhat alike from the overall ranges of amino acids reported, although the two barley proteins reported here are higher in glutamic acid and valine, while being somewhat lower in methionine. It is possible when developing new varieties for feed purposes that more attention should be given to the amino acid content of the protein than to yield per acre.

TABLE VII

COMPOSITION OF THE PROTEIN IN COMPANA AND VANTAGE BARLEY

Amino Acid	Compana	Vantage	Others (17,18)	Wheat (21,22,23)
Aspartic	1.7%	2.9%	0.8- 1.2%	----
Serine	2.0%	3.6%	0.1- 3.2%	----
Threonine	1.6%	2.5%	1.9%	2.8- 3.3%
Glutamic	36.5%	37.3%	22.8-23.9%	31.8%
Proline	18.9%	9.5%	4.2-15.3%	----
Glycine	1.5%	2.4%	0.0- 1.7%	----
Alanine	2.1%	4.0%	0.4- 1.2%	----
Valine	6.3%	6.3%	0.1- 3.5%	3.6- 4.5%
Tyrosine	3.2%	2.9%	0.8- 1.8%	----
Phenylalanine	5.1%	5.3%	2.5- 3.6%	3.7- 5.7%
Histidine	2.1%	1.4%	0.8- 3.2%	1.0- 2.5%
Lysine	3.5%	2.6%	0.0- 0.8%	2.7- 2.9%
Arginine	3.6%	3.0%	4.0- 6.0%	0.5- 4.4%
Methionine	1.1%	0.5%	0.8%	1.3%
Leucine	9.1%	6.8%	3.5- 4.6%	5.8- 8.3%
Isoleucine	3.4%	3.4%	3.5- 4.3%	3.3- 6.9%
Gamma aminobutyric*	.24%	.33%	----	----
Glutamine*	.25%	.30%	----	----
Totals	99.19%	95.03%		

*not positively identified

VI. SUMMARY

1. Compana Freaks, numbers 7219 and 7223, Dekap, Bulk, Otis, and Barbless varieties of barley are suggested for use in cross-breeding to attempt to raise the percent amylose in their starches.

2. Preliminary results indicate that barley may be used as an economically favorable substrate for the production of starch and glucose syrup in the Intermountain area.

3. Two barley protein samples from Compana and Vantage varieties were analyzed chromatographically and the percentages of amino acids in the two proteins were found to differ.

4. The outstanding variation in the amino acids of the two barley varieties, one of which is a good malting barley, the other poor, was in the 9.4% difference in proline.

5. Both varieties were high in glutamic acid which might make barley protein commercially feasible for the production of sodium glutamate.

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