Enzymatic conversion of barley carbohydrate into syrup
by Martin J Houle

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

Abstract:
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By using a portion of the beta amylase naturally present in barley, good syrup yields can be obtained with as little as 0.5% of a wheat bran culture of A. oryzae or by the use of 0.1% commercial enzyme. Preliminary results indicate enzymatically converted barley syrup should compete favorably with corn syrup in Montana.

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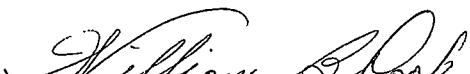

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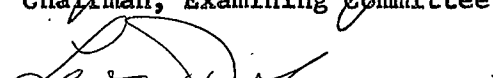

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I ABSTRACT

Various combinations of amylolytic enzymes have been used as a means for the conversion of whole barley into syrup. The hydrolysis products have been determined quantitatively and indicate that the sugar composition of the syrup can be varied over wide limits by the proper selection of enzymes and variation of conversion time.

By using a portion of the beta amylase naturally present in barley, good syrup yields can be obtained with as little as 0.5% of a wheat bran culture of A. oryzae or by the use of 0.1% commercial enzyme. Preliminary results indicate enzymatically converted barley syrup should compete favorably with corn syrup in Montana.

II INTRODUCTION

The production of syrup from the carbohydrate fractions of various grains, tubers and fruits, has been periodically investigated for the last 150 years. In 1811, the Russian chemist, Kirchoff, reported the basis for a commercial method of production of starch syrup and crude starch sugars (1). His discovery was accidental for he was not after sugar or syrup but gum arabic for use in porcelain manufacturing. When he subjected his mixture to the action of sulfuric acid for too long a period of time, he obtained a syrup. A short time later, Proust, found a way to produce a sugary substance from grapes (2). The French chemist, de Saussure, determined that both were identical hydrolysis reactions (3). The sugar obtained from these two methods was glucose. A considerable amount of chemical research was concentrated in this field at this time because of a 100,000 franc reward offered by Napoleon I to the first individual to produce sugar in quantity from native plants (4). France was at war with England and her sugar imports had been cut off. However, this young industry collapsed in 1814 when the war was concluded.

The use of starch for syrup and sugar production was introduced into the United States in 1842 but it was not an important industry until 1857. The acid hydrolysis procedure was used as described by earlier investigators. In 1939, Langlois found that the use of molydenum as a catalyst increased the rate of acid hydrolysis (5). The following year Dale and Langlois introduced the use of enzymes with neutralized acid hydrolyzate. This combination further improved the yield of syrup and reduced the time necessary to complete the reaction (6). This process is being used today

for the production of certain kinds of corn syrup. This was not the first time that enzymes had been used in the sugar industry. In 1928, Weichherz used malt to make a high maltose syrup. In order to achieve good conversions, it was found necessary to use high concentration of malt which made the method impractical. In 1944, Baker was able to produce crystalline maltose by using malt with partially acid hydrolyzed starch (7).

The restriction on wheat acreage in the last decade has resulted in increased barley production in Montana because this crop was best suited for these diverted acres. In 1958, Montana produced 48,000,000 bushels of barley on 1,618,000 acres, but was only able to market a fraction of this through the normal grain markets. Montana ranked third in the nation in barley production but last in marketing (8). Due to this limited outlet, a large surplus has accumulated in this state. Nationally, a large amount of barley is used in the malting industry. However, since the major part of the malting industry is located in Minnesota and Wisconsin, its barley is obtained primarily from North Dakota and South Dakota. These two states enjoy a definite freight rate advantage over Montana. The limited usage of Montana barley for malt production has forced most of the barley grown here to be fed directly to livestock, stored as government surplus, or shipped out for feed.

Due to the fact that Montana grain prices are determined by the price at Minneapolis, less freight, Montana barley offers one of the least expensive sources of carbohydrate in the United States.

In searching for outlets to utilize this surplus grain, it appeared

that the conversion into syrup offered the greatest potential since a vast market for this material is available in the Pacific Coast States. It is used for the production of glazed fruit, candy and ice cream as well as for the packing of berries, fruits and for the fortification of wine. In order to compete with corn which is now supplying the syrup demand it would be necessary to have either a less expensive material or one which offered some advantage over corn syrup.

Since the general procedure for making corn syrup involves the acid hydrolysis of corn starch, Goering and Imsande investigated the possibility of producing barley starch for this purpose (9). Although high quality starch was obtained, the yield and processing difficulties did not make this process look too attractive. Therefore the acid hydrolysis of barley starch did not appear practical.

Work by Goering indicated that acid hydrolysis of whole grain gave unsatisfactory yields of sugars and off flavors. Even if flavor difficulties were not encountered, the low sugar yields would make this method impractical (10).

Since barley contains substantial amounts of beta-amylase, it was considered possible to produce a syrup competitive with corn syrup by utilizing this natural enzyme source in combination with other commercial enzymes for the conversion of whole grain. Furthermore, it should be possible to obtain a wide variation in sugar composition by proper selection and variation of the enzyme sources. Recently there has been an increased interest in high maltose syrup because it is less hygroscopic than is glucose and thus better suited for producing hard candy (11).

Since maltose sugar can not be made by acid hydrolysis which yields primarily glucose, the possibility of making such a syrup by direct enzyme conversion seemed worthy of investigation.

If either an economical method of enzyme conversion is found which will allow for direct competition with acid hydrolyzed corn starch, or if an effective procedure is developed for production of maltose syrup, large quantities of Montana barley possibly could be used for this purpose.

III EXPERIMENTAL

The barley varieties used throughout this investigation were of known history and produced by the Agronomy Department at Montana State College. Betzes and Compana varieties were used because of their availability in Montana.

The barley was ground in a laboratory Wiley mill and thoroughly mixed. Whole grain was used instead of flour for reasons of economy.

Both barley varieties were investigated for their proximate feed value and carbohydrate content. The moisture, crude fiber, protein and fat were determined by the official A.O.A.C. methods (12, 13, 14, 15). Total carbohydrate was determined by refluxing with HCl as outlined in Cereal Laboratory Methods (16). The sugar content of the acid hydrolyzate was determined by the colorimetric method of Dubois et.al. and read at 490 mμ in a Beckman Model B Spectrophotometer (17). A standard glucose curve was made by making appropriate dilutions from a glucose solution containing 1000 μg./ml. of the sugar. The readings obtained from the acid hydrolyzate were compared to this curve. The percent of glucose was calculated using the following formula;

$$\frac{A \times \text{dilution factor} \times B \times 100}{\text{Sample weight}} = \% \text{ carbohydrate}$$

Where A = ug. of glucose obtained from the standard curve
B = Total volume of the acid hydrolyzate.

Both strains of barley were checked for beta-amylase contents in the following manner. Exactly 1 gram of barley was added to 100 ml. of distilled water containing 1 gram of papain and the mixture allowed to stand for 3 hours at 20° C. with occasional mixing. It was then filtered, the

filtrate being referred to as a barley infusion. The papain releases bound beta-amylase from the protein moiety and in this way total beta-amylase can be determined. A standard soluble starch solution at a pH of 4.5 was made up by dissolving 20 grams of starch in 100 ml. of boiling water, boiling for 2 minutes, adding 20 ml. of standard acetate buffer and making up to 1 liter. One ml. of barley infusion was thoroughly mixed with 200 ml. of starch solution in a 250 ml. volumetric flask and incubated for exactly 30 minutes at 20° C. At the end of this time 20 ml. of 0.5 N NaOH was rapidly added with thorough mixing and the total volume made up to 250 ml. A blank was made up in the same manner except 0.5 N NaOH was added before the addition of barley infusion. The reducing sugar released in this reaction was determined by the colorimetric method of Bernfeld and read on a Beckman Model B Spectrophotometer at 540 mμ (18). Because the addition of papain would be impractical in the commercial preparation of syrup, a water infusion was made in a similar manner except papain was not added. The activity of the beta-amylase was expressed as degree of Linter (°L) which is an expression of maltose equivalent. By definition, a maltose equivalent is the number of grams of reducing sugar, calculated as maltose, that is produced by 100 grams of malt under certain standard conditions (19). The following formula was used for its calculation;

$$\frac{\mu g \text{ reducing sugar} \times 25{,}000 \text{ ml.}}{\text{Sample weight}} = \mu g \text{ maltose/g barley}$$

$$\frac{\mu g \text{ maltose} \times 100 \text{ g malt}}{1{,}000{,}000} = \text{grams maltose}$$

$$\text{grams maltose}/4 = L$$

In addition to water infusion, the other beta-amylase sources used

were malt and Wiszyme, both produced by the Wisconsin Malting Company. The activity of these two enzymes were determined as described above. All enzyme preparations except the water infusions were checked for alpha-amylase activity by a modified Wohlgemuth method (20). A bacterial alpha-amylase source called HT-44, which has considerable resistance to heat, was used as a starch liquifier. This enzyme is produced by the Takamine Division of Miles Chemical Company.

Two laboratory cultures, Aspergillus oryzae and Aspergillus niger were grown on wheat bran using the method devised by Hao et al (21). In addition to the above analysis these enzymes were analyzed for maltase activity using the procedure of Sumner (19).

Three other commercial enzymes prepared by Rohm and Haas Company were also used and compared to the previously mentioned commercial enzymes.

Barley was "mashed" as follows; 25 g. finely ground whole barley was mixed with 225 ml. water in a 1000 ml. graduated Erlenmeyer flask. Varying amounts of the liquifying enzyme, HT-44, were added to this mixture and heated to 75-80° C., held at this temperature for one or two hours and then autoclaved for 30 minutes at 15 p.s.i. The mash was cooled to 55° C. and varying amounts of the enzyme were added. The mixture was placed in a constant temperature bath and held at 55° C. $\pm$ 1° C. for 16 to 18 hours. After conversion the mash was autoclaved for 30 minutes at 15 p.s.i., centrifuged, suspended in an equal amount of water and centrifuged a second time. The washings were added to the original filtrate and reducing sugars determined on the filtrate using the 3,5, dinitrosalicylic acid reagent (22).

When it was found that pressure cooking did not improve sugar yields, this step was eliminated. The effects of various combinations of commercial and laboratory enzymes and/or water infusion on sugar yields were investigated. In addition, the effects of mash strength, conversion time and certain selected enzyme combinations were investigated.

The enzyme additions were expressed as percent of the total dry barley used in each run. Conversion efficiencies were determined on each and calculated as follows:

$$\frac{A \times \text{dilution factor} \times B \ 100}{\text{Sample weight} \times \% \text{ carbohydrate}} = \% \text{ of total carbohydrate converted}$$

Where A = µg maltose in sample taken as compared to a standard maltose curve

B = Total liquid volume of filtrate from the enzyme extract.

All results were calculated as maltose.

The water infusion was extracted from the barley by mixing the ground barley with cold water and allowing it to stand for 12 hours with occasional stirring. Then various percentages were removed by decantation and used in combinations with other saccharifying enzymes. These solutions were added after the liquifaction step had been completed and the mash cooled to 55° C. since the temperature at which the liquifier operates would denature this crude beta-amylase. Later studies indicated 12 hour extractions were unnecessary and essentially the same enzyme concentration could be obtained in 2 - 4 hours time.

The liquifying enzymes, HT-44 and Rohzyme H-39 were studied to determine the concentrations necessary to give maximum liquifaction. The barley was mixed with different amounts of the two enzymes and held at 75 - 80° C. for two hours. At the end of this time an aliquot was taken

out in a special pipette and flow rate was determined. The viscosity reduction was used as the criteria of maximum liquifaction.

The effect of mash strength was studied by varying the concentration of grain in 250 g. of mash using different enzyme concentrations.

The effect of time of conversion was studied by making up the mash in the normal way and liquifying for the prescribed two hours. The saccharifying enzyme was allowed to act on the carbohydrate for varying lengths of time. The optimum practical time was based on total reducing sugar activity.

The enzymatic hydrolysis of barley produces quite an array of sugars. Some of the more promising enzyme combinations were chromatographed on Whatman No. 1 chromatography paper. Each spot of sugar was spotted on the paper in approximately 4 μmole quantities based on reducing power in the original filtrate. Two spots were made on each strip, one for color development and used for location of a sugar in a undeveloped strip, the other for the elution of the sugar to determine its concentration. The chromatograms were developed in a butanol, pyridine and water system. (6:4:3 v/v). The spots on the chromatogram were produced by the use of the CD-1 spray of Gordan et al (23). From this strip the various sugars could be identified either by a characteristic color or by the Rf values determined by Gordan and his group. If no Rf values were available or if the colors of two spots were the same or if both of these difficulties were encountered, the sugars were subsequently eluted, concentrated, subjected to the action of alpha-amylase and acid hydrolysis and the products rechromatographed. This procedure was used in determination of structure of some of the

disaccharides and trisaccharides. From this data sugar size could be determined and in most cases the linkage could be established. Isomaltose was determined using the starch phosphorylase method devised by French et al (24). The starch phosphorylase procedure is used to determine the number of glucose units in the molecule. The Rf value of isomaltose was also used along with its characteristic CD-1 color.

The quantity of sugar incorporated in each spot was measured by eluting with water and using the method of Dubois et al (17).

The proximate feed values and the total carbohydrate was determined in the grain residue remaining after the enzymatic hydrolysis (13, 14, 15, 16, 17).

IV RESULTS AND DISCUSSION

In order to obtain a semi-complete picture of the Betzes and Compana barley samples used in these analyses, the proximate feed values were determined as well as the total carbohydrate and natural diastatic power. These results are shown in Table I. Compana barley is not considered a good malting variety, while Betzes has met with favorable acceptance in the malting industry. To fulfill malt requirements, barley grain must have certain inherent physical and chemical characteristics which are not required in feeding varieties. The samples selected to represent these two varieties were purposely chosen for low protein, high carbohydrate content. It was felt that a malting barley may produce syrup either in somewhat larger quantities or with a reduced amount of added commercial enzyme than would be the case from the non-malting variety. Although a good malting barley must contain high amounts of natural beta-amylase activity, the Compana variety, which is a non-malting variety, contained slightly more beta-amylase than the Betzes which is the malting barley. Because of the very similar chemical, physical and agronomic characteristic of these two barleys, the results obtained did not show significant differences between malting and non-malting barley. Possibly if greater differences had been shown in the two strains, some basis for selecting barleys would have been established. This factor must be kept in mind in future studies or if this procedure is ever used commercially.

Potential diastatic power is determined by using a proteolytic enzyme, papain, to release more protein than is normally available by

Table I

Preliminary Analysis of the Two Barley Samples Used

Assay	Betzes	Compana
Protein	11.04%	11.57%
Crude fiber	3.70%	5.72%
Moisture	6.30%	7.40%
Extractable fat	1.66%	1.60%
Ash	2.55%	2.60%
Total carbohydrate	73.75%	68.10%
Diastatic activity (Papain)	130.00°L	140.00°L
Diastatic activity (Water)	87.70°L	93.75°L

The proximate feed values and carbohydrate accounts for 99% of the total Betzes and 97% of the Compana.

simple water extraction. In view of the cost of this enzyme it was considered desirable to measure the availability of beta-amylase by a simple water extraction. From the results indicated in Table I it was apparent that a sizable portion of the natural enzyme could be extracted in this manner.

In order to determine the maximum amount of beta-amylase available, a time study was undertaken. Water and sufficient barley were mixed and stirred for various periods of time after which the beta-amylase activity was determined. The results are shown in figure 1. Although the 12 hour extraction appeared to be necessary to obtain the maximum amount of enzyme, spontaneous fermentation was encountered under these conditions. The data presented in figure 1 indicated that 50% of the total beta-amylase was extracted in 2 to 4 hours time.

The enzymatic activities of the laboratory and commercial enzymes used were determined and this information is provided in Table II. The commercial additives were HT-44, malt, Wiszyme, Rhozyme H-39, Rhozyme K-2 and Rhozyme H-33. The two laboratory enzyme sources were Aspergillus oryzae and Aspergillus niger. Malt, Wiszyme, Rhozyme K-2 and Rhozyme H-33 were checked for both alpha- and beta-amylase activity. Malt is a good source of both alpha- and beta-amylase. It is obtained from barley germinated under carefully controlled conditions. Its alpha-amylase operates effectively at 55 - 63° C. Wiszyme is a mixture of beta- and alpha-amylase with a temperature optimum near 55° C. It was found to be far more effective than malt and was used in much lower concentrations. Rhozyme H-33 is also a good combination enzyme. The difference between

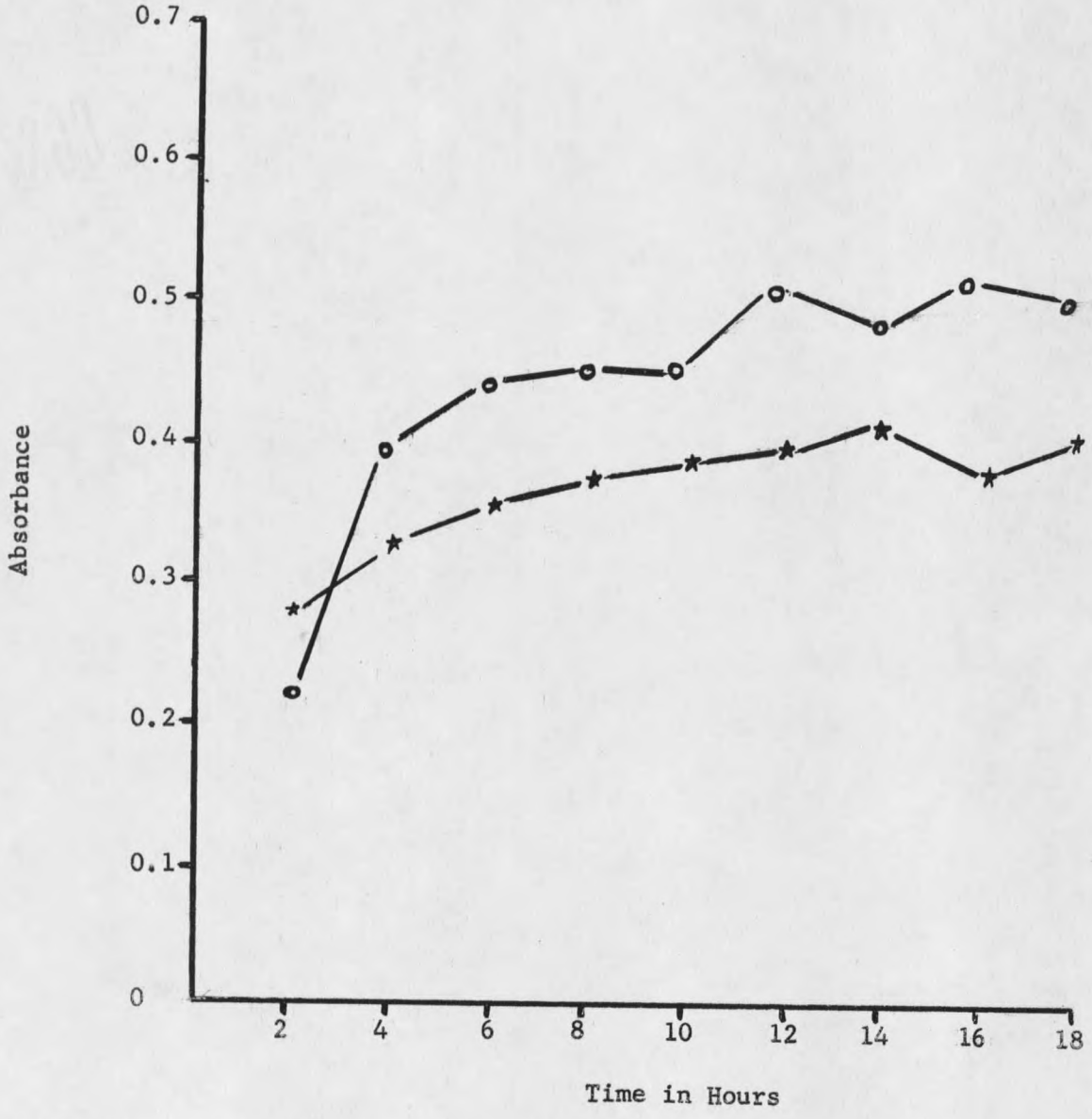

Figure 1. Effect of time on extraction of beta-amylase from barley

Legend: ★ Betzes
o Compana

Table II

Enzyme Activity of Preparations Used

Enzyme source	Alpha units	Beta units[1]	Maltase[2]
Malt	33	75	--
HT-44	105	--	--
Wiszyme	150	208	--
A. oryzae	100	--	28
A. niger	25	--	17
Rhozyme H-39	1920	--	--
Rhozyme K-2	203	400	--
Rhozyme H-33	105	183	--

1 Expressed as mMoles of maltose produced/g. of enzyme.
2 Expressed as time in minutes required for 1 g. or enzyme to hydrolyze 50% of the maltose.

this enzyme and Wiszyme is insignificant. Rhozyme K-2 is a concentrated enzyme source with a very high saccharifying power. Only the Aspergillus oryzae and Aspergillus niger were checked for maltase content because these were the only preparations used in this research that were known to contain substantial amounts of maltase. Although HT-44 had a lower alpha-amylase activity than did Wiszyme, Rhozyme K-2 or Rhozyme H-33 it was essential in this study because it is a high temperature stable alpha-amylase source. This is necessary to reduce the viscosity of the barley starch during its gelatinization. Rhozyme H-39 is extremely high in alpha-amylase activity and is stable at temperatures of 75 - 80° C. The Rhozyme enzymes were not studied as extensively as the other enzymes because they were received when this study was nearly completed. Aspergillus oryzae was used where a good saccharifying alpha-amylase was needed along with some maltase activity. Aspergillus niger was found to be the best source of maltase used in this research and when high glucose syrup was desired, this was used effectively in varying amounts.

The necessary weight of liquifying enzyme needed to produce maximum thinning was determined and these results are expressed in Table III. Rhozyme H-39 can be used in concentrations approximately 1/25 that of HT-44. This advantage is offset however by the cost of this concentrate. For this reason HT-44 was used as the standard liquifier.

Preliminary studies indicated that barley starch was readily converted into sugars. This suggested that pressure cooking, which is routinely used in enzymatic conversions of corn starch, might not be necessary. The validity of this assumption was established by the data presented in

Table III

Effect of Thinning Agent on the Mash Viscosity

Enzyme	% by weight	Flow time[1]
H-39	0.03	7.5
H-39	0.04	7.0
H-39	0.05	7.0
HT-44	0.25	10.8
HT-44	0.50	9.5
HT-44	0.75	9.5
HT-44	1.00	9.0

Mash strength 10%
1 Flow time is expressed in seconds.

Table IV. This was probably due to the continued action of the liquifying enzyme carried over from the liquification step.

The effect of mashing strength and liquifying enzymes were studied together and these results tabulated in Table V. Although the results shown were somewhat lower than normally produced, it would seem that 15% mash strength yields maximum results. Again it was apparent that 0.5% HT-44 was sufficient to produce satisfactory results. It was obvious that mash strengths greater than 15% were undesirable. However, when the HT-44 level was increased to 1%, the maltose equivalents dropped below that obtained with 0.5% HT-44 at the highest mash strength. The cause of this apparent inhibition is unknown but possibly it may be due to an initial rapid production of maltose in the presence of high substrate concentration. It has been reported that maltose in high concentration will inhibit beta-amylase (25). This could account for the observed results. The liquifier will produce more dextrins as its concentration is increased which would allow more non-reducing ends to appear. This in turn will present more sites of hydrolysis for the beta-amylase action causing a more rapid production of maltose. A similar effect was encountered with infusion and reduced amounts of malt and Wiszyme.

After determining the optimum concentration of the liquifying agent and maximum mash strength, the investigation was directed towards the best possible combination of saccharifying enzymes. The first studies did not include water infusion and are reported in Table VI. The use of 10% malt which was used as a control gave slightly better results than 4% Wiszyme. This was expected from the analytical data reported in Table II.

Table IV

Effect of Pressure Cooking Procedure on Sugar Production

Enzyme combination % of barley used					Pressure M.E.[2]	No Pressure M.E.[2]
A. oryzae	A. niger	Malt	Wiszyme	Infusion[1]		
--	--	--	4	--	61	74
0.5	0.5	--	3	--	66	73.1
1.5	0.5	2	--	--	77.5	80.6
0.5	0.5	--	2	30%	75	85
0.5	0.5	2	--	30%	70	82.5

Mash strength was 10% and 0.5% HT-44 was used in all cases as the liquifier enzyme. Barley was Compana.

1. Water extract of ground barley.
2. M.E. is maltose equivalent.

Table V

The Effect of Mash Strength and Liquifying Agent of Syrup Yield

Mash strength %	Syrup yields in M.E. using indicated amounts of HT-44		
	0%	0.5%	1.0%
10	22.7	57.0	61.2
15	26.2	60.6	61.5
20	20.0	55.7	55.7
25	-*	49.0	47.0

Conversion agent 0.5% A. oryzae, 0.5% A. niger and 20% infusion.
Barley was Compana variety.
* Too thick with starch.
M.E. equals maltose equilalent.

Table VI

Effect of Enzyme Combination on Yield of Syrup

% of enzyme used[1]				M.E. of Syrup[2]	
Malt	Wiszyme	A. oryzae	A. niger	Betzes	Compana
10	--	--	--	67.0	65.5
--	4	--	--	61.0	61.5
--	3.5	0.5	--	67.5	67.0
--	3.5	--	0.5	69.0	70.0
--	3.0	0.5	0.5	68.5	69.0
2	--	2.0	--	68.0	72.0
2	--	--	2.0	69.0	74.5
2	--	1.0	1.0	75.0	77.5
2	--	0.5	1.5	78.0	82.0
2	--	1.5	0.5	80.0	78.5

Mash strength 10% and 0.5% HT-44 as liquifying agent.
1 Percent of enzyme used equals percent by weight of 25 g. whole barley.
2 Maltose equivalent.

When malt was combined with Aspergillus oryzae and Aspergillus niger, very satisfactory results were obtained. The high maltose equivalent obtained was probably the result of the maltase action of Aspergillus oryzae and Aspergillus niger.

Utilization of naturally occurring beta-amylase extracted with water from barley and combined with other enzymes, was next studied. These results are reported in Table VII.

An attempt was made to determine the minimum concentration of malt and Wiszyme required to obtain good results. Some surprising results were observed. An inhibitory effect similar to that demonstrated in Table V was observed. These are illustrated in figure 2. Because of this inhibition, it appeared desirable to make a more thorough study of the infusion percentage required to produce syrup efficiently. In the following experiments, a bare minimum of other saccharifying enzymes were added with the infusion. This was done to accentuate the role natural beta-amylase plays in starch hydrolysis. It was concluded from the data in Table VIII that 20 - 30% infusion gave maximum conversion. Again the inhibition effect was observed.

From the data obtained it was obvious that certain combinations were necessary to get good syrup production.

Throughout this research, maltose equivalent was used as the guide post for conversion. When acid is used for conversion the term dextrose equivalent is used. Because in most cases it was found that maltose was produced in much higher concentrations than glucose this expression was used as a standard. In practice, any values which depend upon reducing

Effect of Enzyme Combinations and Infusion on Yield

% of enzyme used					M.E. of Syrup	
Malt	Wiszyme	A. oryzae	A. niger	Infusion	Betzes	Compana
--	2	--	--	--	69.0	69.0
--	2	--	--	30	71.0	72.5
--	2	--	--	60	60.0	74.0
--	2	--	--	75	74.0	74.5
--	1.5	--	--	0	54.0	62.5
--	1.5	--	--	30	58.0	64.5
--	1.5	--	--	60	59.0	66.0
--	1.5	--	--	75	60.5	67.5
--	1.0	--	--	0	59.0	55.0
--	1.0	--	--	30	61.0	65.0
--	1.0	--	--	60	69.5	63.0
--	1.0	--	--	75	67.0	65.0
2	--	--	--	0	60.0	64.5
2	--	--	--	30	68.0	65.0
2	--	--	--	60	52.0	64.0
2	--	--	--	75	51.5	65.0
2	--	0.5	--	30	66.5	67.0
2	--	--	0.5	30	65.0	64.5
--	2	0.5	--	30	72.0	75.0
--	2	--	0.5	30	75.5	78.0

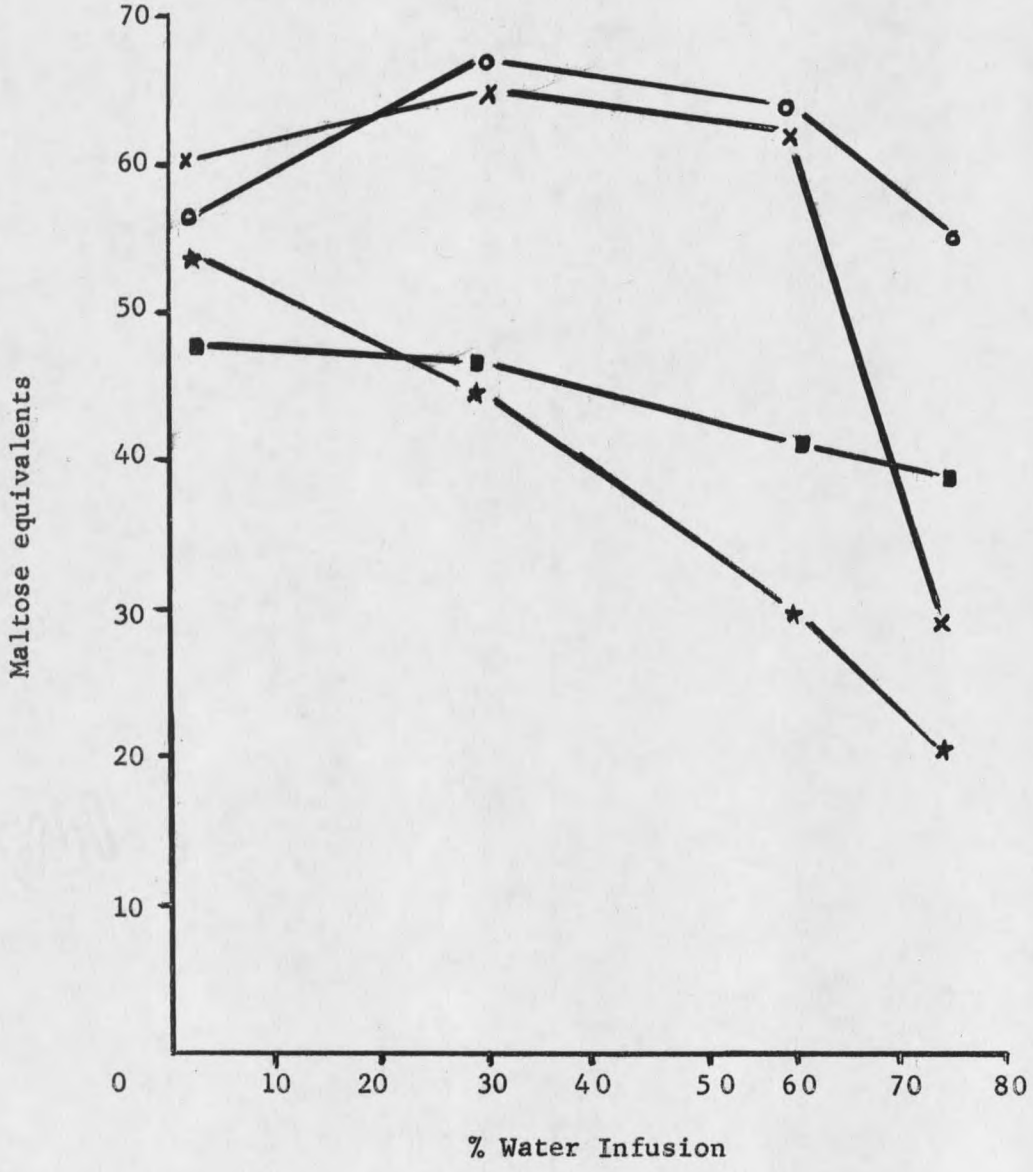

Figure 2. Inhibitory Effect of Water Infusion

Legend:
- ——×—— Betzes, 0.5% Wiszyme + infusion
- ——■—— Betzes, 1.5% malt + infusion
- ——★—— Compana, 1.5% malt + infusion
- ——o—— Compana, 0.5% Wiszyme + infusion

Table VIII

Effect of Barley Infusion on Syrup Yield

% of infusion added	M.E.
0	41
10	52
20	53
30	55
40	50
50	49

Mash concentration 10% with 0.5% HT-44 as liquifier, and saccharified with 0.5% Wiszyme.

sugar analysis do not completely show the effectiveness of the hydrolysis. To really observe the extent of a hydrolysis, the quantity of the various sugars produced must be measured. Some of the more promising combinations were selected and investigated with this in mind. It was determined that slight variations in enzyme concentrations will produce most any type of product desired as indicated in Table IX. A typical chromatogram of the hydrolysis products is shown in figure 3. It must be stated at this time that although the 20% infusion used as the only saccharifying source produces a high maltose syrup, the yields are very poor due to the high concentration of dextrins left in the residue.

The effect of conversion time upon sugar distribution was studied and is reported in Table X. Preliminary work had indicated that 16 hours was satisfactory and results obtained here confirm this assumption.

From the data obtained in Table IX, three enzyme combinations were selected for closer appraisal. These were 0.5% HT-44, 0.5% Aspergillus oryzae, 0.5% Aspergillus niger plus 20% infusion; 0.5% HT-44, 0.5% Aspergillus oryzae plus 20% infusion; 0.5% HT-44, 0.5% Aspergillus niger plus 20% infusion. The results of this study are shown in Table XI. The length of time of development of the chromatograms subsequently made on the hydrolysis products, was increased to 36 hours. This gave a much better separation than the normal 20 hour development. In this case the disaccharide nigerose appeared. This was in part due to the lengthened development time of the chromatogram. Another interesting point was the increase in concentration of nigerose when infusion and Aspergillus niger were used as the conversion agents. When Aspergillus oryzae and

Table IX

Sugars Produced in Wort by Various Enzyme Combinations

Enzyme combination in %					% of total sugar in wort					
A.O.	A.N.	I.	M.	W.	G_1	G_2	i-G_2	G_3	i-G_3	G_n
0.5	0.5	30	--	2	19.3	29.7	11.5	18.7	9.9	10.9
0.5	0.5	30	2	--	20.3	35.3	6.4	10.7	9.0	14.0
0.5	0.5	--	2	--	17.0	32.4	11.8	12.5	7.7	14.5
1.5	1.5	20	--	--	40.5	19.6	10.0	8.3	11.2	6.9
0.5	0.5	--	--	3	18.7	35.0	13.2	7.5	8.1	12.5
0.5	0.5	20	--	--	19.5	30.0	6.6	12.3	6.6	19.0
1.0	1.0	20	--	--	19.2	21.5	12.0	11.0	10.0	19.2
--	--	--	10	--	5.0	50.8	8.0	5.9	7.0	17.7
--	--	20	--	--	3.5	63.0	3.0	T[1]	T[1]	30.0[2]

Mash strength 10% liquified with 0.5% HT-44.

1 T = trace present.

2 A considerable amount of high molecular weight dextrins were found in this fraction.

G_1 = glucose, G_2 = maltose, i-G_2 = isomaltose, G_3 = maltotrios, i-G_3 = panose, G_n = higher oligosaccharides, A.O. = A. oryzae, A.N. = A. niger, I = infusion, M = malt, W = Wizyme.

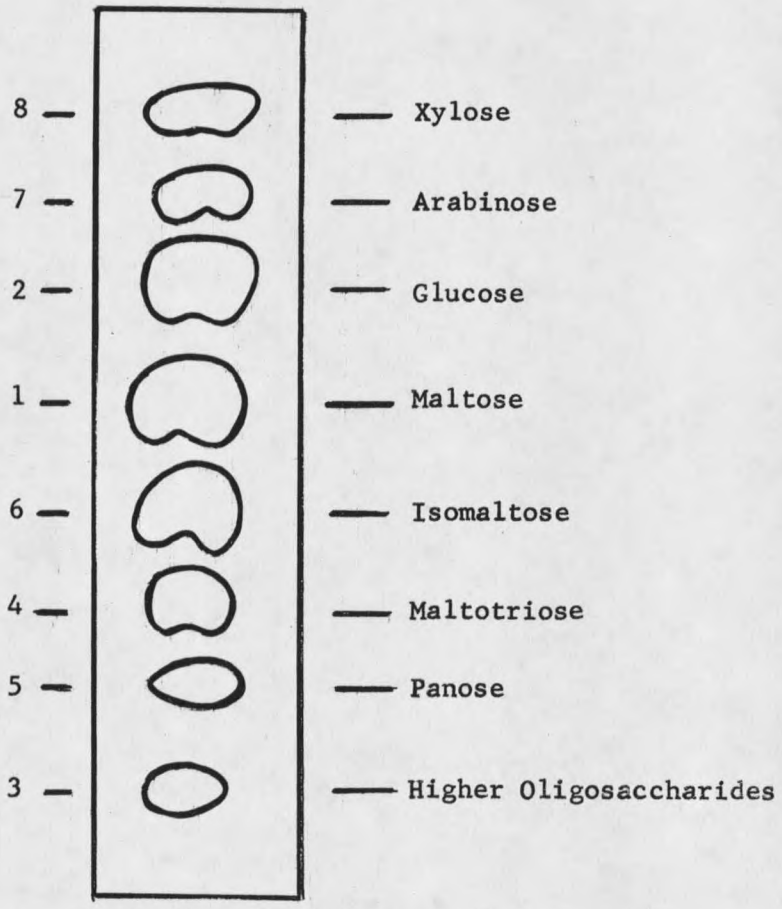

Figure 3. A Typical Chromatogram From Starch Hydrolysis
Numbers indicate relative concentration of sugars.

Table X

Effect of Conversion Time on Sugar Distribution

Conversion time in hours	% of total sugar in wort[1]					
	G^1	G^2	i-G^2	G^3	i-G^3	G^n
4	1.1	40.3	3.3	7.7	1.1	44.0
8	14.5	30.0	7.1	10.2	3.9	31.0
12	15.7	28.5	5.3	11.8	9.1	28.0
16	19.5	30.0	6.6	12.3	6.6	19.0

Mash strength 10%, 0.5% HT-44, 0.5% A. oryzae, 0.5% A. niger and 20% infusion.

1 See Table IX for nomenclature.

Table XI

Sugars Produced by Selected Enzyme Combinations

Sugars	% yield with indicated supplementary enzymes		
	0.5% A.O. 0.5% A.N.	0.5% A.N.	0.5% A.O.
Arabinose	1.5	4.3	2.0
Xylose	1.2	1.6	2.3
Glucose	18.6	14.7	7.5
Nigerose	1.2	8.0	2.0
Maltose	33.5	31.0	51.3
Isomaltose	7.0	9.8	6.0
Maltotriose	10.5	9.8	11.2
Panose[1]	8.0	4.9	5.7
Higher Oligosaccharides	18.5	14.1	15.7

Mash strength 10%, 0.5% HT-44 plus 20% infusions.

1 This compound gave three molecules on hydrolysis and was not acted on by alpha-amylase but not identified in any other manner.

A.O. equals A. oryzae.

A.N. equals A. niger.

Aspergillus niger were used together, the concentration decreased. It was also found that when malt and Wiszyme were used no appreciable concentration of nigerose could be detected. A similar effect was found by Peat et al (26) and Pazur et al (27). They found evidence of a 1→3 transglucosidase enzyme in extracts made from Aspergillus oryzae and Aspergillus niger that is capable of synthesizing nigerose from maltose and glucose.

The disappearance of nigerose in the presence of Aspergillus oryzae was surprising. Aspergillus oryzae is a high alpha-amylase source and is also fairly high in maltase activity. This would mean that although the transglucosidase was active in Aspergillus niger, the increased activity of alpha-amylase from Aspergillus oryzae was hydrolyzing the 1→3 glucosidic linkage. This is very contrary to that reported in the literature about the action of alpha-amylase. This oddity was also reported by Curran during his study of barley pectin (28). Recent work has indicated an impurity in alpha-amylase that may be a 1→3 glucosidase (29).

The sugar tentatively identified as maltotriose, was eluted from the chromatogram, concentrated and subjected to acid hydrolysis. Care was taken in determining the amount of reducing power in the original eluate. After the acid hydrolysate had been neutralized, the reducing sugar activity was again measured and the increase in reducing power gave approximately 3 glucose units. The original sugar was collected from various chromatograms, concentrated and placed with alpha-amylase. The hydrolysis products were rechromatographed along with some of the original sugar. These results are shown in figure 4. It was observed that

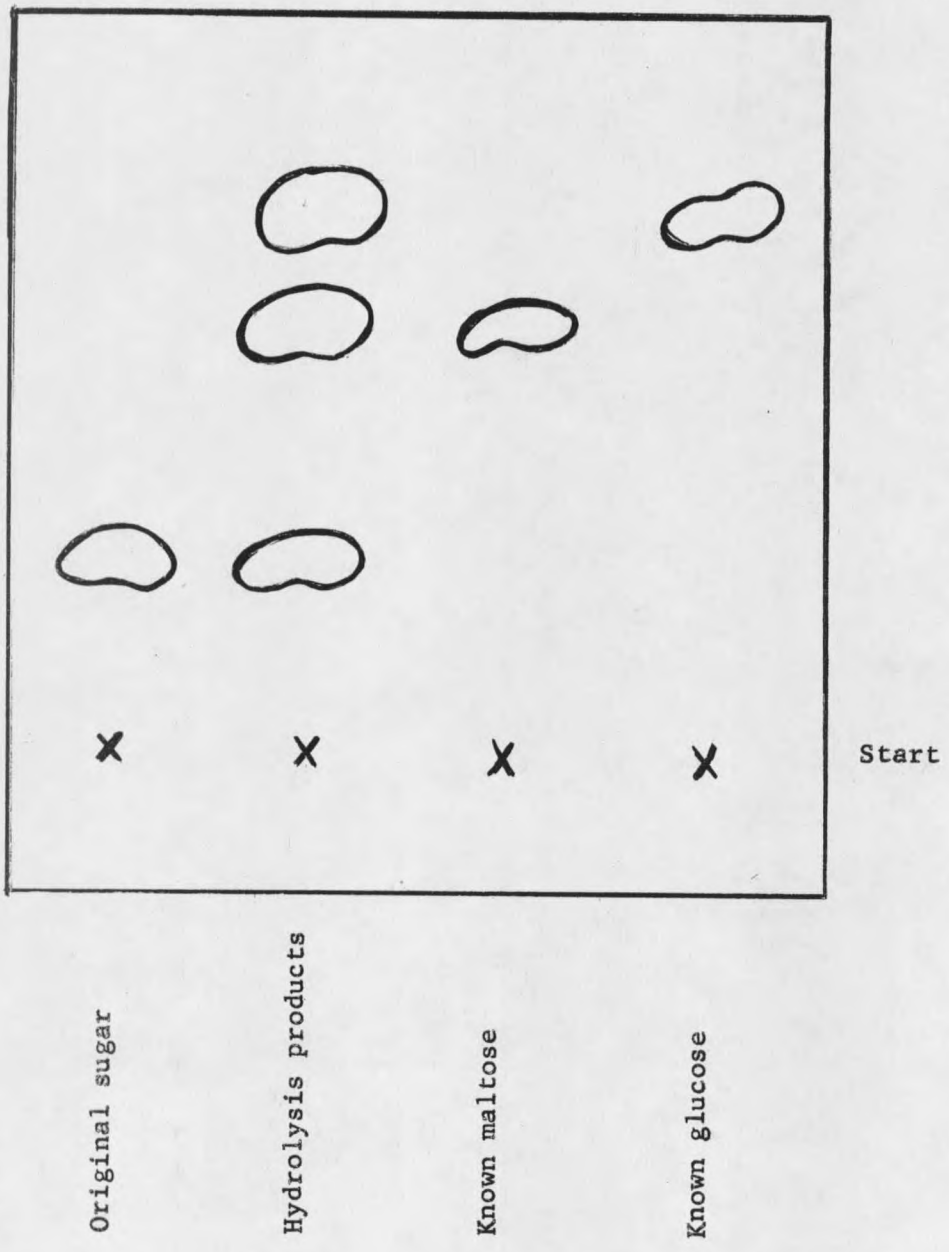

Figure 4. Chromatogram from Maltotiose Identification Work

only glucose, maltose and original sugar were obtained. From these experiments it was concluded that the sugar was maltotriose.

Panose was tentatively identified from limited identification work. When the sugar was acid hydrolyzed, 3 molecules of glucose were obtained. Alpha-amylase seemed to have no effect. This sugar could possibly be isomaltotriose. It was concluded from the known structural linkages of starch from other sources, that the 1→6 and 1→4 linkage combination of panose was more probable than the 1→6, 1→6 combination of isomaltotriose. For this reason the sugar was identified as panose.

The higher oligosaccharide fraction was checked for color with iodine solution and very little could be detected. This indicated that most of this fraction was 7 glucose units or less in length.

The enzymes furnished by the Rohm and Haas Company were used in comparable concentrations found optimum for HT-44, Wiszyme, and malt to investigate the possibility of other commercial enzyme sources. These results are shown in Table XII. It was obvious that both Rhozyme H-33 and K-2 will give very good results when a high maltose syrup is desired.

The residue obtained from the enzymatic treatment of whole barley was analyzed for the proximate feed values and for total carbohydrate content and these figures can be seen in Table XIII. The remaining carbohydrate plus the loss in weight from the original barley can be used as a method for determining efficiency of hydrolysis. From one sample calculated in this way it was determined that the hydrolysis was approximately 90% complete. If care is taken to wash out residual sugars, this percentage could be improved.

Table XII

Sugars Produced by Commercial Enzymes of Rohm and Haas

Sugar	% yield with indicated supplementary enzymes	
	0.3% H-33	0.1% K-2
Arabinose	4.0	2.4
Xylose	1.6	T[1]
Glucose	9.0	9.5
Maltose	56.0	50.0
Isomaltose	7.3	9.5
Maltotriose	4.9	4.0
Panose	T[1]	3.0
Higher Oligosaccharides	16.3	21.5

10% mashing strength, 0.5% HT-44 and 20% infusion.
1 Trace

Table XIII

Proximate Feed Value Analysis on Enzyme Treated Barley Residue

Assay	Results
Moisture	13.5 %
Protein	24.3 %
Fat	2.26%
Ash	3.01%
Fiber	17.3 %
Total Carbohydrate	39.6 %

All results are calculated "as is" basis.

V CONCLUSION

From results presented here, it appears that the enzymatic conversion of barley carbohydrate into syrup is economically sound, especially when the natural beta-amylase is utilized. The crude syrup obtained in this way has to be further treated to remove color and some taste factors. This possibly can be accomplished by de-hulling the barley or by milling it to flour before treatment. The crude extract obtained here has been successfully used in a pilot plant study for yeast production (30). The wort was used as the carbon source and in conjunction with other added nutrients has produced high protein yeast. The residue from the hydrolysis furnishes a high protein feed in itself. It also furnishes a potential raw material for the production of glutamic acid. This has been studied and shown to have real commercial possibility (31). If all of the above processes could be used in conjunction with one another it would insure the success of an industry in this field.

Several interesting problems were seen in the course of the investigation. The demonstrated inhibitory effect of malt and Wiszyme should be studied as well as the appearance and disappearance of nigerose when Aspergillus niger and Aspergillus oryzae are used. The starch itself has shown some differences from corn starch in its behavior towards syrup production in that the barley starch gives higher conversion efficiency. It is hoped that this work, investigations that preceeded it and those to follow will ultimately bring a new industry to Montana.

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