Enzymology of the Mashing Step During Beer Production[1]

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ABSTRACT

α-Amylase, alkaline protease, neutral protease, and β-glucanase were extracted from a crude microbial enzyme preparation and partially purified. β-Amylase was extracted from soy flour. The effects of these enzymes on malt and barley were studied, using a modified infusion mashing schedule. The effects of α-amylase, β-amylase, and β-glucanase on corn and rice during mashing were also studied. Minimum effective levels of α-amylase, β-amylase, and β-glucanase on various substrates were determined. Neutral protease was significantly more effective than alkaline protease in hydrolyzing malt and barley protein. Data obtained from this study should allow calculation of the optimum mix of enzymes required for any specified mash bill.

Key words: *Alkaline protease, α-Amylase, β-Amylase, Cereal adjuncts, β-Glucanase, Neutral protease*

Use of adjuncts in brewing is not new. Corn and rice are used routinely and, with the increasing cost of malt, interest in barley as an adjunct has been renewed. Ehlers (5,6) has reviewed some of the technical and economic aspects of using unmalted cereals in brewing. At high adjunct levels, malt can no longer supply the enzyme concentrations necessary for satisfactory modification of the various substrates in the mash. Under these conditions, enzyme additions to the mash may be required.

The enzymes of most interest in brewing are α-amylases, β-amylases, proteases, and β-glucanases. A number of studies have been conducted documenting the effects of adding these enzymes to brew mashes (2,3,9,15–18). The advantages of this addition are the production of increased amounts of soluble protein and free amino nitrogen, increased fermentable carbohydrates, decreased wort viscosity, higher filtration rates, and increased wort yield, as well as the possibility of using larger amounts of unmalted adjuncts in the brewing process.

The purpose of this research was to clarify in a more quantitative manner the relationship between use level and effect on a specific substrate for various enzymes by 1) purifying the enzymes and adding them individually at progressively increasing concentrations to mashes containing only one raw material (malt, barley, corn, or rice), 2) constructing curves relating use level to effect, 3) predicting from the curves what would happen in mashes containing mixed mash bills, and 4) verifying the predictions through experimentation.

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EXPERIMENTAL

Assay Procedures

α-Amylase. α-Amylase was determined by a modified Wohlgemuth procedure (8). One unit of activity (MWU) was defined as the amount of enzyme that would dextrinize 100 mg of soluble starch in 30 min under the assay conditions.

β-Amylase. β-Amylase activity was determined by incubating 1 ml of enzyme solution with 4 ml of 1.25% soluble starch at 50° C and pH 5.8 for 6 min. Reducing sugars produced were determined by a modification of the Schoorl procedure (11). One unit of activity (DU) was defined as the amount of enzyme that would catalyze the production of reducing sugars equivalent to 100 mg of glucose in 6 min under the assay conditions.

β-Glucanase. Activity was measured in β-glucanase units (GU/g) detemined by the method of Denault et al (4).

Protease. Protease activity was determined by a modification of the Spectrophotometric Neutral Protease Assay described in the Food Chemicals Codex (8). The assays are identical except that in our procedure a temperature of 55° C was used and the assay was run at pH 5.8, 7.1, and 9.2. Also the unit of activity (MDU) was defined as the amount of enzyme that would solubilize 10 nmol of tyrosine per minute under the assay conditions.

Free Amino Nitrogen. Free amino nitrogen was determined with ninhydrin as described by Lie (12) and Wylie and Johnson (19).

Protein. Protein was determined on all samples by the Kjeldahl procedure. Liquid samples were also determined by the biuret method (10) and grain samples were checked by the modified biuret procedure of Noll et al (13).

Starch Determination. One gram of finely ground sample (~ 50 mesh) was added to 10 ml of ether and stirred for 10 min. The sample was then vacuum filtered through Whatman No. 1 paper and the cake washed slowly with 4 × 10-ml portions of ether. Next the cake was washed with 50 ml of 10% (v/v) ethanol, then with 10 ml of 95% ethanol.

The cake was transferred to a 125-ml Erlenmeyer flask. Aliquots of 0.5 ml of 0.17*M* $CaCl_2$, 0.04 ml of Kinase HT™ (Miles Laboratories, Elkhart, IN) (6,400 MWU), and 46 ml of distilled water were added. The flask was stoppered with a rubber stopper fitted with a thermometer and incubated at 60° C with stirring for 1 hr. The sample contents were then brought to a boil and held with stirring for 15 min. The temperature was lowered to 86–90° C, at which point 0.04 ml more Kinase HT was added and the sample held for 30 min with stirring. The sample was then cooled to 60° C and the pH adjusted to 4.2 with dilute HCl. Two units (0.02 ml) of glucoamylase (Diazyme L100™, Miles Laboratories, Elkhart, IN) were added and the sample incubated with shaking for 4 hr at 60° C. The sample was then vacuum filtered through Whatman No. 1 paper and the cake washed with 25–30 ml of distilled water. The filtrate was transferred to a 100-ml volumetric flask and diluted to volume with distilled water. Reducing sugars produced were determined on a 1-ml aliquot by a modification of the Schoorl procedure (11). Percent starch was calculated using the formula:

$$\% \text{ Starch} = 9.1 \times \text{reducing sugars, mg/ml}$$

Theoretical Wort Volume. Theoretical wort volume (TWV) was calculated according to the following formula:

$$\text{TWV} = \frac{\text{Total extract (g)} + \text{Added water (g)} + \text{Grain moisture (g)}}{\text{Theoretical specific gravity}}$$

in which total extract equals the percent starch times the weight of the grain.

Theoretical specific gravity is obtained from degrees Plato by referring to the tables published by the ASBC (1). Degrees Plato can be calculated from the weights in the numerator of the above equations.

Viscosity. Viscosity was measured at 20° C with a size 75 Cannon type of Viscometer.

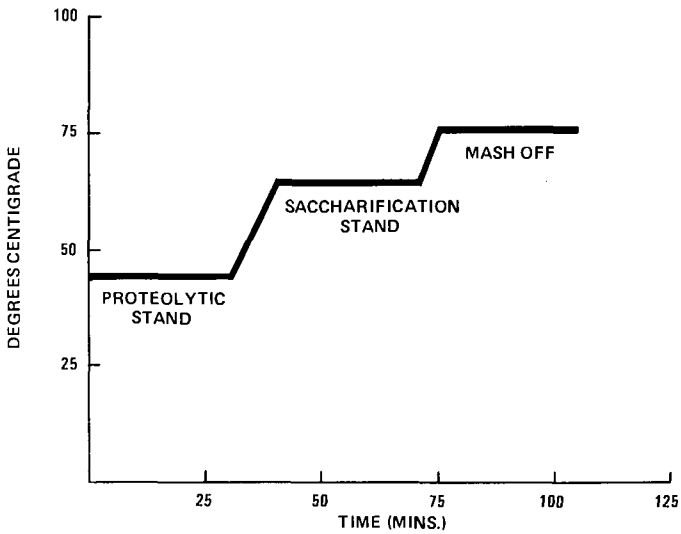

Fig. 1. Modified infusion mashing schedule. Proteolytic hold was 30 min at 45° C, saccharification stand 30 min at 65° C, mash off 30 min at 76° C. Temperature rise between stands was 2° C/min.

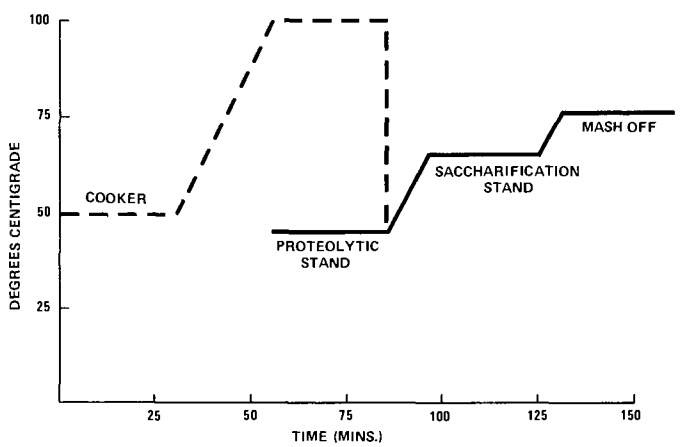

Fig. 2. Adjunct mashing schedule. Main mash was the same as the modified infusion schedule. The cooker was held for 30 min at 50° C in the presence of thermostable α-amylase (Kinase HT™), then brought to boiling for 30 min before being added to the main mash.

Enzyme Purification

α-Amylase. α-Amylase was purified from Brew-n-zyme™ (Miles

TABLE I
Effect of Alpha-Amylase Concentration of Barley during Mashing

α-Amylase Starch (units per gram of starch)	Iodine Reaction	Extract (° P)	Reducing Sugars (% as dextrose)	Filtration Rate Measured as Wort Volume (% of theory) After Filtration Time of		
				5 min	11 min	40 min
0	+	2.65	0.45	4	8.0	14
200	+	7.00	1.88	26	34	40
500	+	7.89	1.97	33	39	44
1,000	+	8.28	2.44	42	48	54
2,000	+	9.10	2.82	42	48	55
4,000	+	9.72	3.01	44	50	56
6,000	+	10.03	3.29	47	55	64
8,000	+	10.26	3.20	46	55	63
10,000	−	10.70	3.66	58	63	69
20,000	−	11.06	3.94	58	63	69

Laboratories, Elkhart, IN) by the method of Robyt and Ackerman (14). The purified enzyme assayed 20,000,000 MWU per gram.

Alkaline Protease. Crystalline alkaline protease was purchased from Sigma Chemical Company, St. Louis, MO. The enzyme (Sigma P5255) is produced by *Bacillus amyloliquefaciens.*

Neutral Protease. Neutral protease was purified from Brew-n-zyme. Brew-n-zyme (20 g) was cleaned by being mixed in 200 ml of pH 7.0, 0.1 *M* tris buffer with 400 g of diethylaminoethyl cellulose equilibrated at pH 7.0 with the same buffer. After being mixed in the cold for 2 hr, the slurry was filtered through Whatman No.1 paper on a 25-cm Buchner.

Celite 512 (20 g) was added to the filtrate and polish filtration carried out. The polished filtrate was freeze-dried, yielding 7.5 g of cleaned-up product. This process removed 86% of the color.

The cleaned-up Brew-n-zyme was then dissolved in 400 ml of 0.01 *M* tris buffer, pH 8.0, and 50-ml aliquots were placed on eight 2.3 × 27 cm diethylaminoethyl cellulose columns equilibrated at pH 8.0 with the same buffer. Elution was accomplished with the same buffer until a single beak at 275 nm of absorbing material had been washed from the column. The peaks from the eight columns were combined and freeze-dried, yielding 3.7 g of product. This product contained a mixture of neutral and alkaline proteases.

The freeze-dried product from the column runs was dissolved in 200 ml of 10% isopropanol containing 100 mg of phenylmethylsulfonylfluoride and the alkaline protease was inactivated (7). The final product (1.77 g) assayed 304,000 MDU per gram at pH 7.1.

β-Glucanase. β-Glucanase was separated from a fermentation product produced by a *Bacillus amyloliquefaciens* mutant. This mutant produced no α-amylase and only a small amount of

TABLE II
Comparison of Enzyme Preparations with Different Neutral Protease to Alkaline Protease (NP/AP) Ratios on Various Mash Bills

		Actual Percentage				
NP/AP	Predicted Percentage[a]	Malt/ Barley 50:50	Malt/ Barley/ Corn 30:15:55	Malt/ Corn 40:60	Malt/Rice 40:60	Average
AP	0.080	0.090	0.080	0.075	0.077	0.0805
1.8	0.028	0.030	0.032	0.027	0.029	0.0295
3.7	0.025	0.023	0.031	0.025	0.025	0.0260
4.2	0.022	0.022	0.025	0.022	0.023	0.0230
25.0	0.019	0.021	0.019	0.018	0.019	0.0193

[a]The predicted percentages represent the level of enzyme used as a percentage of the malt replaced by adjunct in the mash and were picked from Figs. 13 and 14 so as to give the same wort protein and free amino nitrogen for the same mash bill.

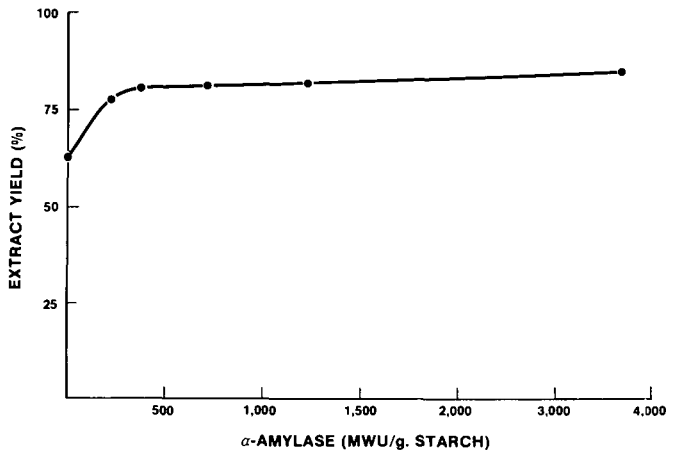

Fig. 3. Effect of α-amylase on corn during mashing. Corn slurry was carried through the adjunct mashing schedule. β-Amylase (20 DU/g of starch in the mash) and α-amylase were added at the beginning of the saccharification stand. MWU = α-amylase units measured by the modified Wohlgemuth procedure (7).

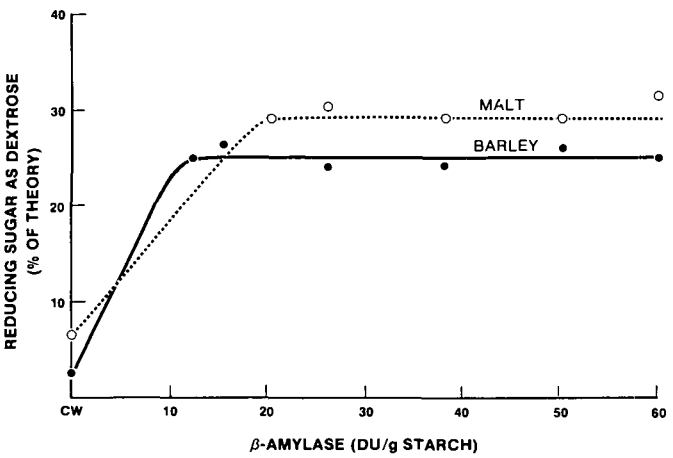

Fig. 4. Effect of β-amylase on malt and barley during mashing. The modified infusion mashing schedule was employed. α-Amylase was added to the barley mashes in an amount equal to that found in the malt. **CW** = cold water extract. β-Amylase concentration represents the total in the mash (barley or malt β-amylase + added β-amylase) and is expressed in β-amylase units (DU) per gram of starch in the mash.

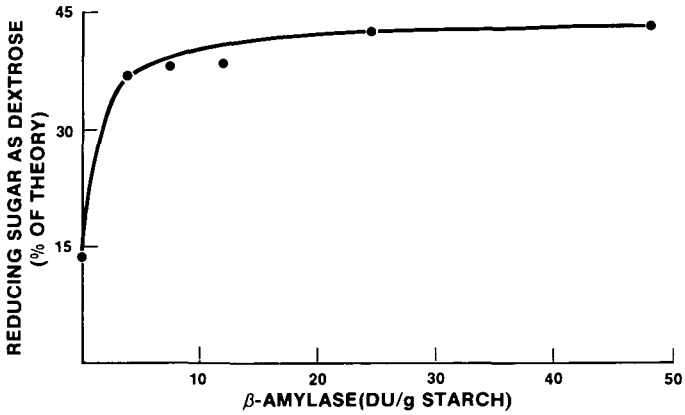

Fig. 5. Effect of β-amylase on corn during mashing. The adjunct mashing schedule was used. α-Amylase (368 MWU/g starch in the mash) and β-amylase were added at the beginning of the saccharification stand. MWU = α-amylase units measured by the modified Wohlgemuth procedure (7).

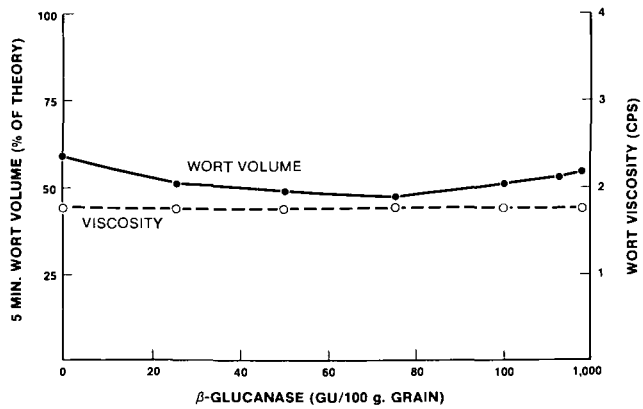

Fig. 6. Effect of β-glucanase on malt during mashing. The modified infusion mashing schedule was followed. β-Glucanase was added at the beginning of the proteolytic stand. β-Glucanase concentration (GU/100 g of grain) represents only β-glucanase added to the mash; β-glucanase activity of the malt is not considered. GU = β-glucanase units determined by the method of Denault et al (4).

protease but a considerable amount of β-glucanase. Purification consisted of inactivating neutral protease by treatment with ethylenediamine tetraacetic acid at pH 5.5 and 55° C for 1 hr and inactivating alkaline protease by treatment with diisopropylfluoro phosphoric acid (7).

This treatment yielded a product assaying 2,000 GU per gram.

β-Amylase. β-Amylase was purified from soy flour by the following procedure. Forty-five pounds of Nutrisoy flour (18–20 DU/g) was extracted with 55 gal of 40% ethanol for 60 min at room temperature. Sodium bisulfite (1 lb) was added to protect the enzyme against oxidation. After 60 min, the slurry was centrifuged on a basket centrifuge and the resultant cake washed with 15 gal of 40% ethanol.

The filtrate plus wash was cooled in a brine-cooled tank and 1.6 gal of cold 95% ethanol No. 35A added for each gallon of filtrate. After standing 2 hr, the settled precipitate was resuspended and separated from the mother liquor by centrifugation on a basket centrifuge. The cake was washed with 95% ethanol. The cake was then dissolved in water and the hazy solution polish filtered and freeze-dried. By assaying 376 DU/g, 243 g of product was obtained.

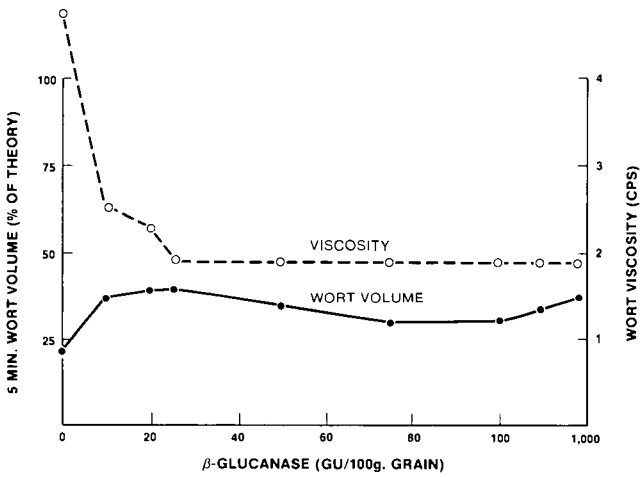

Fig. 7. Effect of β-glucanase on barley during mashing. The modified infusion mashing schedule was followed. β-Glucanase was added at the beginning of the proteolytic stand with α-amylase in an amount equal to that found in the malt. β-Glucanase (GU/100 g of grain) represents only β-glucanase added to the mash. GU = β-glucanase units determined by the method of Denault et al (4).

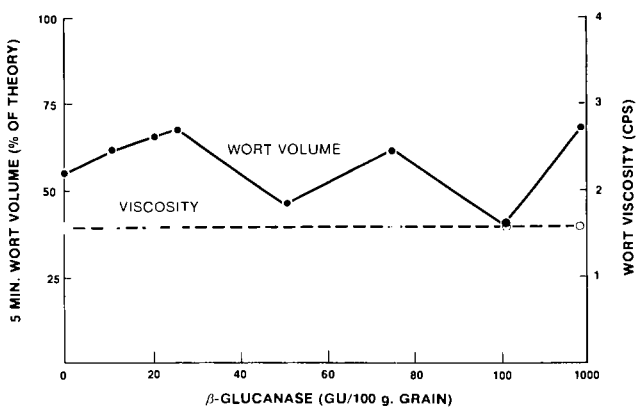

Fig. 8. Effect of β-glucanase on corn during mashing. The adjunct mashing schedule was followed. β-Glucanase (GU/100 g of grain), with α-amylase (368 units per gram of starch) and β-amylase (20 units per gram of starch), was added at the beginning of the saccharification stand. GU = β-glucanase units determined by the method of Denault et al (4).

Mashing Procedure

A modified infusion mashing schedule (Fig. 1) was used. This consisted of incubating the malt or barley mash at 45° C for 30 min with mixing. The temperature was then raised to 65° C at 2°/min and held for 30 min. Finally the temperature was raised to 76° C and held for 30 min. At the end of mashing, the mash was checked with I_2 to see if starch conversion was complete, then either vacuum filtered or gravity filtered through Reeve Angel No. 802 paper. When an adjunct such as corn or rice was employed, the cooker contents (Fig. 2) were held at 50° C for 30 min in the presence of Kinase HT; then the temperature was increased to boiling at 2° C/min where it was held for 30 min. While the cooker mash was being held at boiling, the main mash was undergoing the proteolytic stand at 45° C. Following the 30-min boil, the cooker contents were added to the main mash, helping to increase the temperature to 65° C. The remainder of the mashing schedule was the same as the modified infusion schedule. When the mash consisted of corn or rice alone, the cooker contents were cooled to 65° C, the appropriate enzymes (α-amylase, β-amylase, or β-glucanase) were added, and the 65 and 76° C holds conducted according to schedule.

Test of Thermostability

β-Glucanase samples extracted from malt and purified bacterial β-glucanase were dissolved in 12° P barley wort to give a

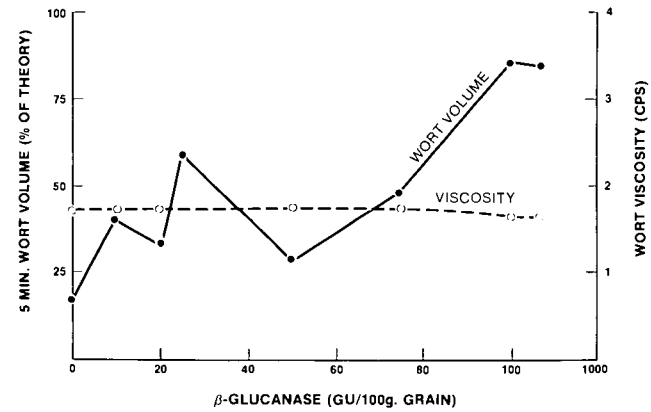

Fig. 9. Effect of β-glucanase on rice during mashing. The adjunct mashing schedule was used. β-Glucanase (GU/100 g of grain), with α-amylase (368 units per gram of starch) and β-amylase (20 units per gram of starch) was added at the beginning of the saccharification stand. GU = β-glucanase units determined by the method of Denault et al (4).

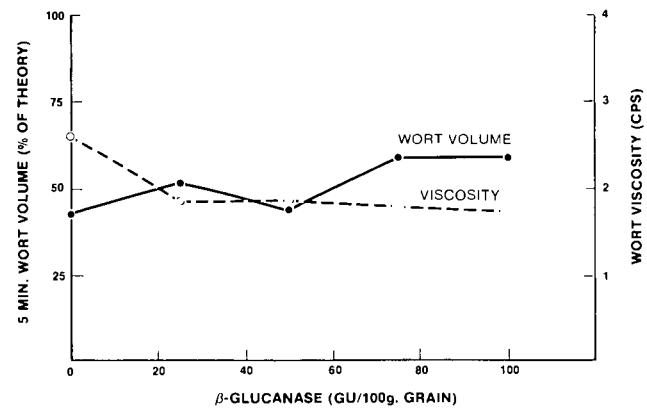

Fig. 10. Effect of β-glucanase on 50/50 malt/barley mash. The modified infusion mash schedule was used. β-Glucanase was added at the beginning of the proteolytic stand. β-Glucanase (GU/100 g of grain) represents only β-glucanase added to the mash. GU = β-glucanase units determined by the method of Denault et al (4).

concentration of 1 GU/ml. After 30 min at the test temperature, remaining activity was determined by adding 1 ml of the test solution to 9 ml of barley wort equilibrated at 40° C in a Cannon 100 viscometer and measuring the rate of change in the wort viscosity over a 20-min period. This rate of change was then compared with that of a control that had not undergone a 30-min hold before assay.

RESULTS AND DISCUSSION

Effect of α-Amylase on Malt and Barley during Mashing

To determine the minimum amount of α-amylase needed for maximum extract yield and fermentability, varying concentrations of α-amylase were added to malt and barley mashes. In the case of malt, which contained approximately 30,000 MWU/g of starch, addition of α-amylase did not increase extract yield, reducing sugars, or any of the other characteristics measured. Thus the malt appeared to contain sufficient α-amylase to obtain maximum extract yields.

In the case of barley (Table I), which contained no measurable α-amylase activity and about 60% as much β-amylase activity as the malt, addition of increasing levels of α-amylase produced a continuous increase in extract and reducing sugars over the entire range of α-amylase concentrations tested. At 10,000 MWU/g of starch, however, a leveling-off effect was observed. At this level of α-amylase, a negative iodine reaction was obtained on the mash, and extract yield, reducing sugars, and filtration rate were at or near maximum.

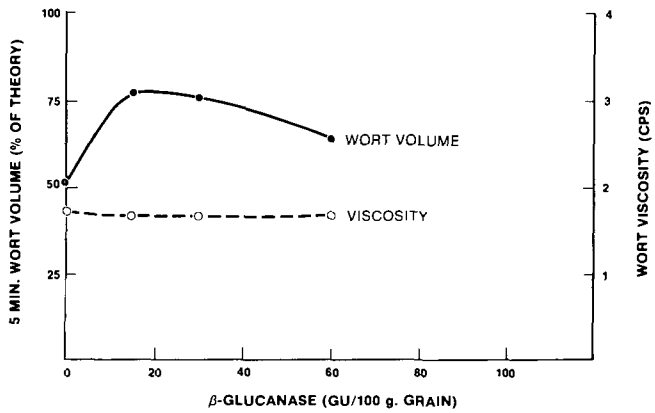

Fig. 11. Effect of β-glucanase on 40/60 malt/rice mash. The adjunct mashing schedule was used. β-Glucanase was added to the main mash at the beginning of the proteolytic stand. β-Glucanase (GU/100 g grain) represents only β-glucanase added to the mash. GU = β-glucanase units determined by the method of Denault et al (4).

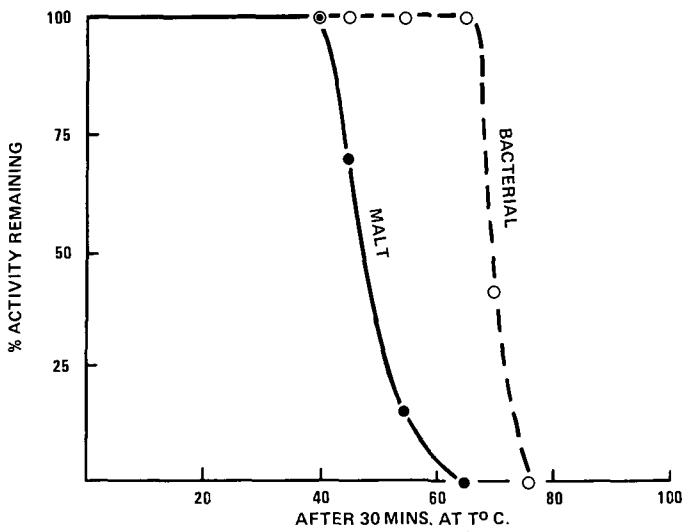

Fig. 12. Comparison of thermostability of malt and bacterial β-glucanases.

Effect of α-Amylase on Corn during Mashing

To determine the effect of α-amylase on corn during mashing, the corn was first run through the cooker as described earlier. Kinase HT, at a level of 450 MWU/g of starch, was used as the cooker enzyme. Following the cook, the temperature was lowered to 65° C and β-amylase was added at the same level found in malt (20 DU/g of starch). α-Amylase, at the appropriate levels under study, was then added and the modified infusion mash schedule conducted.

Figure 3 shows the results of these experiments. Maximum extract yield was obtained with approximately 368 MWU/g of starch. This concentration is less than 5% of the amount required for malt and barley.

Effect of β-Amylase on Malt, Barley, and Corn During Mashing

Figures 4 and 5 show the effects of varying the β-amylase concentration on malt, barley, and corn during mashing. In the case of barley, α-amaylase was added to the mash at the same level found in malt (30,000 MWU/g of starch). With corn the amount of α-amylase found to give maximum extract in the previous experiment (368 MWU/g of starch) was added to the mash. Malt contained 20 DU of β-amylase per gram of starch and Fig. 4 shows that this was sufficient to produce a maximum yield of reducing sugars. Further addition of β-amylase produced no increase in reducing sugars. As with α-amylase, malt apparently also contains an excess of β-amylase. Barley contained 10 DU of β-amylase per gram of starch, and this also appeared sufficient to produce maximum reducing sugar yields, although filtration rate and wort yield at this level were slightly lower than at higher levels of β-amylase.

Figure 5 shows that with corn about 20 DU of β-amylase per gram of starch are necessary for maximum reducing sugar yields. It appears from this study that a minimum of 10–15 DU/g of starch are necessary for maximum yield of fermentable sugars from malt, barley, or corn.

Effect of β-Glucanase on Malt, Barley, Corn, Rice, and Mixed Mash Bills During Mashing

Figures 6–11 show the results of adding various amounts of β-glucanase to mashes containing a single grain (malt, barley, corn, or rice) and to a mixed mash bill (malt/barley and malt/rice). In the barley mashes, α-amylase was added at the level found in malt (30,000 MWU/g of starch). In the corn and rice mashes, α-amylase was added at a level of 500 MWU/g of starch and β-amylase at a level of 20 DU/g of starch.

Figure 6 shows the effect of adding β-glucanase to malt mashes. In this study, the mashes were gravity filtered through Reeve Angel No. 802 paper and the rates of filtration as well as wort viscosities measured. Filtration was allowed to proceed for 40 min. Wort volumes were measured after 5, 11, and 40 min. This figure shows the wort volume as a percent of the theoretical volume after 5 min of filtration along with the final wort viscosity in centipoise. Addition of β-glucanase to a malt mash does not improve filtration or lower wort viscosity, probably because the β-glucans of malt are well modified during the malting process.

Figure 7 shows the effect of β-glucanase on barley during mashing. β-Glucanase has a striking effect on wort viscosity and filtration rate. Maximum filtration rate, along with a bottoming out of wort viscosity, is found at a β-glucanase level of 25 GU/100 g of barley. Filtration rate appears to fall off between 25 and 100 GU/100 g of barley, then begins to increase again.

Figure 8 shows the effect of β-glucanase on corn. Here, as with barley, we see a peak in filtration rate of about 25 GU/100 g of grain.

A second peak appears to occur at 75 GU/100 g of grain, and a third somewhere above 1,000 GU. β-Glucanase apparently has no effect on wort viscosity.

Figure 9 shows the action of β-glucanase on rice during mashing. Here three peaks were found—the first at 15 GU, the second at 25 GU, and the third at 100 GU/100 g of rice. A slight viscosity drop in the wort accompanied the 100-GU peak. At this level, over 90% of the TWV was obtained after only 5 min of filtration compared with 18% with the control. The peak at 25 GU/100 g of grain was common to all the substrates, so this appears to be a practical use level.

Figure 10 shows what happened when β-glucanase was added to a mash containing 50% malt and 50% barley. The pattern is very similar to that of barley, showing a rate peak and minimum viscosity at 25 GU/100 g of grain in the mash.

Figure 11 shows the effect of β-glucanase on a 40:60 malt/rice mash. Here we see a peak at about 18 GU/100 g of grain. A slight lowering of wort viscosity also occurred.

From the results of these studies, it is readily apparent that β-glucanase can be a very effective filtration aid in mashes containing barley, corn, or rice. Not only are the filtration rates significantly increased, and the wort viscosity usually lowered, but improved wort yields should also be expected.

One question that presented itself during the studies with β-glucanase was why the addition of such a small amount of bacterial β-glucanase (25 GU/100 g of grain) proved so effective when malt contained nearly 1,000 and barley nearly 500 GU per 100 g. Obviously the malt and barley enzymes differed from the bacterial enzyme. Study indicated two significant differences: 1) pH optima (the bacterial enzyme had an optimum pH at 6.5 and about 90% of its optimum activity at the mashing pH; malt had an optimum pH at 4.0–4.5 and only about 65% of its optimum activity at the mashing pH), and 2) heat stability (Fig. 12). In this study, the enzymes were held in wort at the temperature under study for 30 min, then assayed for remaining activity. The curves show that the bacterial β-glucanase is significantly more heat stable than the malt β-glucanase. The malt enzyme lost 30–35% of its activity after 30 min at 45° C and 100% of its activity after 30 min at 65° C, whereas the bacterial β-glucanase retained 100% of its activity after 30 min at 65° C and still had 40% of its activity after 30 min at 70° C.

Effect of Alkaline and Neutral Proteases on Malt and Barley during Mashing

Next the action of proteases during mashing was investigated. Figure 13 shows the action of alkaline and neutral proteases on malt and barley mashes. When mashing was done without addition of protease, we found that 17% of the protein in barley and 35% of the protein in malt was solubilized. Addition of increasing amounts of alkaline and neutral protease to malt and barley mashes produced the curves shown. The neutral protease was much more effective in solubilizing malt and barley protein than was alkaline protease. In fact, calculation of the slope over the linear portion of the curves shows that it took 695 MDU of alkaline protease to increase the amount of protein hydrolyzed by 1% (ie, from 35% of the available protein to 36%) but only 165 MDU of neutral protease to accomplish the same thing.

Figure 14 shows the relationship between free amino nitrogen and wort protein for these experiments. Free amino nitrogen is an important factor for proper yeast fermentation. Little difference appeared between neutral and alkaline protease on the individual substrates; however, a significant difference occurred between malt and barley. From the slopes of the lines, it can be determined that there are 37.5 mg/L of free amino nitrogen for each 0.1% wort protein when malt is used, and 21.7 mg/L of free amino nitrogen per 0.1% wort protein when barley is used.

The data in Figs. 13 and 14 allow calculation of the expected wort protein and free amino nitrogen for any mix of malt and barley and prediction of the increase in these levels upon addition to the mash of a protease of known neutral protease/alkaline protease ratio.

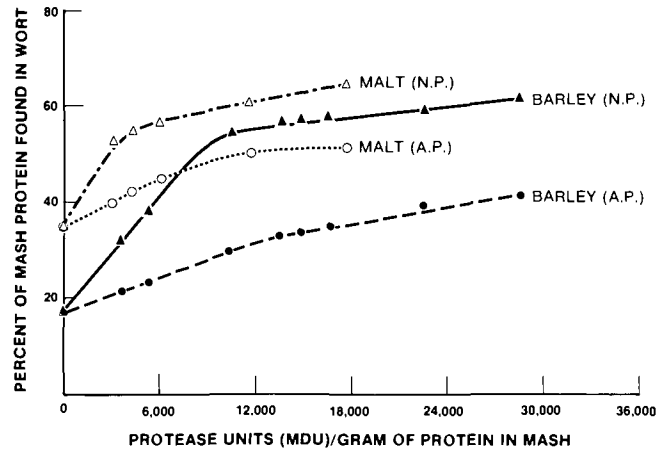

Fig. 13. Effect of adding alkaline and neutral protease to malt and barley mashes. The modified infusion mashing schedule was followed. α-Amylase was added to the barley mashes at the level found in malt. **A.P.** = alkaline protease, **N.P.** = neutral protease, MDU = amount of enzyme needed to solubilize 10 nmol of tyrosine per minute under assay conditions.

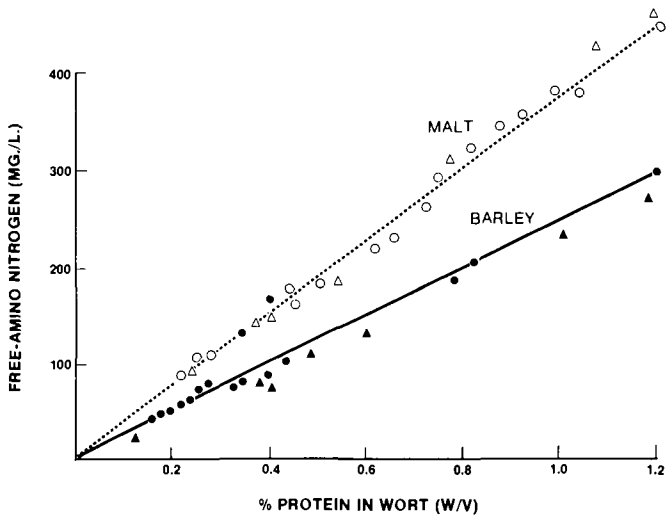

Fig. 14. Percent wort protein vs free amino nitrogen in malt and barley mashes treated with alkaline and neutral proteases. The modified infusion mashing schedule was followed. α-Amylase was added to the barley mashes at the level found in malt. o • = alkaline protease; Δ ▲ = neutral protease.

To test this hypothesis, enzyme preparations with varius neutral protease/alkaline protease ratios were prepared. Each preparation was adjusted to assay 500,000 MDU/g. The relative use levels of the enzyme preparations, calculated as a percent of the weight of malt replaced in the mash, were determined from the data in Figs. 13 and 14 so that all would give the same free amino nitrogen and wort protein values for any particular mash bill. The results obtained were then compared with those predicted and the required enzyme levels adjusted accordingly. The data in Table II show that the levels of the enzyme preparations actually required are in excellent agreement with those predicted.

Literature Cited

1. American Society of Brewing Chemists. Tables for Extract Determination in Malt and Cereals, 3rd ed. Am. Soc. Brew. Chem.: St. Paul, MN, 1940.
2. Bourne, D. T., Jones, M., and Pierce, J. S. *Tech. Q. Master Brew. Assoc. Am.* 13:3, 1976.
3. Bullon, A. H., and Palmer, J. R. *J. Inst. Brew.* 80:206, 1974.
4. Denault, L. J., Allen, W. G., Boyer, E. W., Collins, D., Kramme, D., and Spradlin, J. E. *J. Am. Soc. Brew. Chem.* 36:18, 1978.

5. Ehlers, G. M. *The Brewer, 1978* (Nov.), p. 423.
6. Ehlers, G. M. *The Brewer, 1978* (Dec.), p. 423.
7. Fahrney, D. E., and Gold, A. M. *J. Am. Chem. Soc.* 85:997, 1963.
8. Food Chemicals Codex (2nd ed., 1st suppl.). Nat. Acad. Sci.: Washington, DC, 1974.
9. Gorinstein, S., Kitor, S., Sorel, S., Berman, O., Berliner, M., Popovich, G., and Vermus, Ya. *J. Am. Soc. Brew. Chem.* 38:23, 1980.
10. Gornall, A. G., Bardawill, C. S., and David, M. M. *J. Biol. Chem.* 117:751, 1949.
11. Hodge, J. E., and Davis, H. A. U.S. Dept. Agric., Tech. Bull. ALC 333, 1952.
12. Lie, S. *J. Inst. Brew.* 79:37, 1973.
13. Noll, J. S., Simmonds, D. H., and Bushuk, W. *Cereal Chem.* 51:610, 1974.
14. Robyt, J. F., and Ackerman, R. J. *Arch. Biochem. Biophys.* 155:445, 1973.
15. Ke-Yin Shih, E., and Banasik, O. J. *Brew. Dig.* 35(12):48, 1960.
16. Slaughter, J. C. *Brew. Guardian, 1974* (April), p. 25.
17. Wieg, A. G. *Fermentatio* 71(4):175, 1975.
18. Witt, P. R., and Banasik, O. J. *J. Am. Soc. Brew. Chem., Proc. 1970,* p. 134.
19. Wylie, E. G., and Johnson, M. J., *Biochim. Biophys. Acta* 59:450, 1962.

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