observed on the Coleman Junior spectrophotometer. These wave lengths were chosen because nitric oxide hemoglobin is reported (*3*) to have absorption peaks at 545 and 575 mμ. Hence, an increase in absorbance at 545 and 575 mμ and a decrease at 555 mμ would be indicative of the formation of nitric oxide myoglobin and the disappearance of myoglobin. The absorbance at 545 and 575 mμ increased as expected; however, instead of a decrease at 555 mμ, there was an increase. The maximum absorbance change was obtained within 5 minutes after addition of nitrite.

When the reaction was apparently complete, the spectral curve was determined using the Beckman DU spectrophotometer (Figure 2). The spectral curves for the isolated metmyoglobin and myoglobin (formed after treatment of metmyoglobin with ascorbic acid) are also presented. These data show that nitric oxide myoglobin has absorption peaks at 544 and 575 mμ and the increase in absorption at 555 mμ observed with the Coleman Junior spectrophotometer was due to the inability of this instrument to resolve the peaks of maximum absorption. While the absorption peak at 544 mμ agrees with that presented by Kiese and Kaeske (*7*) for nitric oxide myoglobin from horse heart, the peak at 575 mμ is at variance with the position they present for it at 584 mμ. Further studies on these and other chemical reactions of myoglobin and related derivatives are being carried out.

**Summary** A procedure slightly modified from that described by Theorell, Morgan, and Drabkin has been used for the preparation of purified metmyoglobin. The conversion of metmyoglobin to myoglobin was investigated by following spectral changes after the addition of ascorbic acid. No difference in the rate of conversion was observed when the reaction was carried out at pH 5.8 as compared to pH 7.0. Myoglobin was converted to nitric oxide myoglobin in less than 5 minutes at pH 5.8 when nitrite was added. Absorption curves for metmyoglobin, myoglobin, and nitric oxide myoglobin are presented.

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## EVALUATION OF MALT

# Relation of Alpha-Amylase and Limit Dextrinase of Barley Malt to Production of Ethyl Alcohol from Grains

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THE DISTILLING AND MALTING INDUSTRIES have long recognized the desirability of predetermining the performance of barley malts used in the production of ethyl alcohol from grain. Barley malt potentialities have usually been measured in terms of the activity of one of the enzymes involved in the hydrolytic degradation of starch. There is little or no agreement at present as to the total number of enzymes involved in this degradation, but two principal types have been accepted generally—β-amylase, a saccharifying enzyme, and α-amylase, a dextrinizing enzyme. Recent evidence has revealed a third enzyme with an action that appears to be additive to those of α- and β-amylase. Kneen (*3*) in 1945 termed this enzyme "limit dextrinase" and hypothesized that it hydrolyzes the complex dextrins which remain after α- and β-amylase have acted on grain starch.

The diastatic power test, which is principally a measurement of β-amylase, was at one time the most commonly used criterion for measuring the potential performance of barley malts used in the distilling industry. Four research laboratories (*2*) collaborated in a program to study the relationship between alcohol yield and such factors as diastatic power, α-amylase, wort nitrogen, Kjeldahl nitrogen, and proteolytic activity. The data showed that the diastatic power test is not a reliable index and that, of the factors investigated, α-amylase is the most significant criterion for predicting alcohol yield. Limit dextrinase activity was not measured in those studies, be-

**Table I. Distribution of Malt Samples According to Enzyme Activities**

| α-Amylase (Sandstedt Method) | | α-Amylase (ASBC Method) | | Limit Dextrinase | |
|---|---|---|---|---|---|
| Units | No. of samples | Units | No. of samples | Units | No. of samples |
| 75–80 | 3 | 50 and above | 2 | 300 and above | 4 |
| 70–74 | 2 | 45–49 | 16 | 280–299 | 6 |
| 65–69 | 8 | 40–44 | 16 | 260–279 | 9 |
| 60–64 | 9 | 35–39 | 28 | 240–259 | 10 |
| 55–59 | 12 | 30–34 | 18 | 220–239 | 14 |
| 50–54 | 20 | 25–29 | 8 | 200–219 | 19 |
| 45–49 | 20 | 20–24 | 6 | 180–199 | 12 |
| 40–44 | 10 | 15–19 | 4 | 160–179 | 9 |
| 35–39 | 6 | 10–14 | 1 | 140–159 | 4 |
| 30–34 | 4 | | | 120–139 | 6 |
| 25–29 | 4 | | | 100–119 | 7 |
| 20–24 | 3 | | | Below 100 | 1 |

**Distillers need a rapid reliable test for evaluating the performance of barley malt. Analyses of 101 experimental and commercial barley malts for α-amylase, limit dextrinase, and alcohol production from grain starch show that the measurement of α-amylase or limit dextrinase provides a reliable index of performance. The correlation coefficients of the reciprocals of α-amylase units (SKB) and limit dextrinase units *vs.* alcohol production are —0.847 and —0.800, respectively. Either test, therefore, may be used to ascertain in advance, without the usual laborious fermentation tests, the suitability of a specific malt for grain saccharification.**

cause a suitable method of analysis was not available. Later (1948) Kneen and Spoerl (*4*) published a method for determining limit dextrinase activity in barley malts. These investigators analyzed samples of barley malt for α-amylase, diastatic power, and limit dextrinase; alcohol production was checked on grain starch. They pointed out that their data were limited, but concluded that "it is significant that for the malts used, limit dextrinase is the only one of the three carbohydrase activities determined that would be of value in predicting potential efficiency in alcohol production."

The purpose of the present investigation was to evaluate a sufficient number of barley malts to permit calculation of the relationship of limit dextrinase to alcohol yield and a comparison of α-amylase and limit dextrinase as indexes of potential malt performance. β-Amylase was not included in these studies because of the negative results demonstrated in the earlier collaborative project.

### Materials and Methods

Eighty-two samples of experimental barley malts were supplied by six malt manufacturers. The experimental malts were used to obtain a wider variation between α-amylase and limit dextrinase than is normally found in commercial malts, so that the effect of each enzyme could be separated statistically from the effect of the other. In addition to the experimental malts, 19 typical commercial distiller's malts from 16 maltsters were also included. Each sample was analyzed for moisture, α-amylase, and limit dextrinase activity; production of alcohol was checked on grain starch.

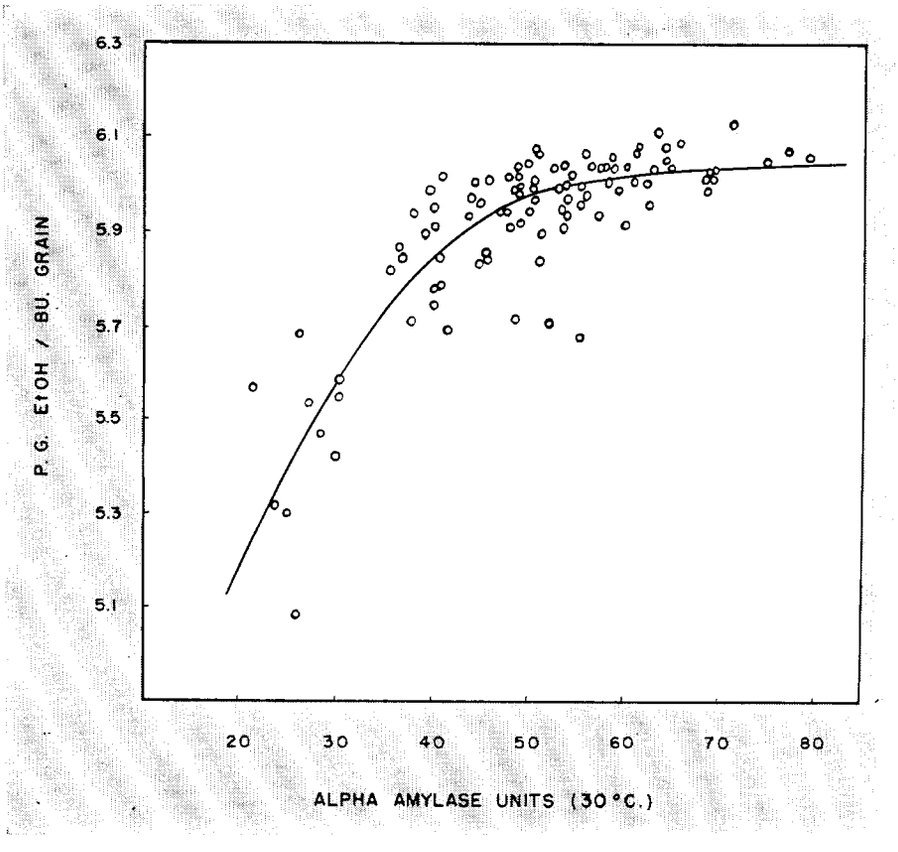
α-Amylase activity was determined by two methods: the method of Sandstedt and associates (*7*) as modified by Redfern (*6*) for use with the Hellige comparator, and the official method of the American Society of Brewing Chemists (*1*). The principal differences in the procedures are in the methods of extraction and in the reaction and extraction temperatures (30° C. for the Sandstedt method; 20° C. for the ASBC method). The α-amylase activity of each barley malt sample was determined twice by each method. The malt-substrate reaction was performed in duplicate, yielding a total of four such reactions for each sample.

Limit dextrinase activity was determined by the Lowry and associates (*5*) modification of the method of Kneen and Spoerl (*4*). The modified procedure specifies the use of a commercial limit dextrin prepared by the Wahl-Henius Co.; this avoids the errors inherent in individual preparation of the limit dextrin.

The samples of barley malt were tested in laboratory grain fermentations according to the method of Stark and associates (*8*). A suboptimal level of conversion malt (5%) was used in these studies in order to accentuate differences in starch-converting power. The grain bill for these mashes consisted of 92% corn, 1% premalt, 5% conversion malt, and 2% malt added at temperatures sufficiently high to destroy enzyme activity. This inactivated malt was included in order to prevent a deficiency in yeast nutrients.

The samples of barley malt were mashed in duplicate and three fermentations were prepared from each mash, giving a total of six fermentations for every malt. A portion of the uninoculated mash was filtered and analyzed for balling (approximate per cent sugar), total acid, and pH. After inoculation with yeast, the fermenters were incubated for 68 hours in a water bath controlled at 30° C. At the end of the incubation the beer (fermented mash) from each fermenter was divided into two portions. One portion was used to determine the

Figure 1. α-Amylase units (30° C.) *vs.* ethyl alcohol production



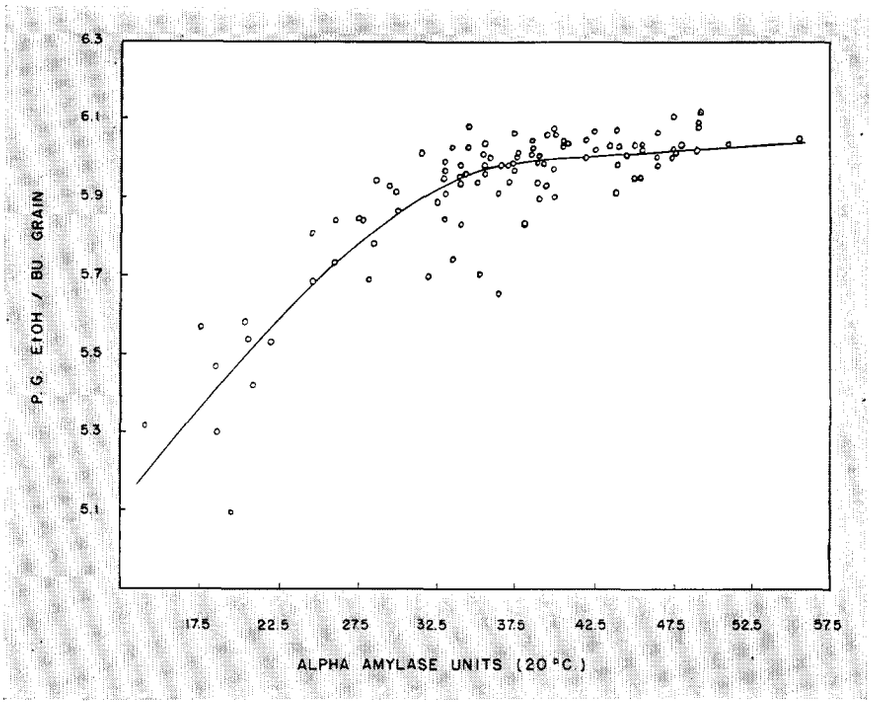



Figure 2. α-Amylase units (20° C.) *vs.* ethyl alcohol production

yield of alcohol, expressed in terms of proof gallons per 56 pound bushel of grain. (A proof gallon of ethyl alcohol is a standard U.S. gallon measured at 60° F. and containing 50% alcohol by volume.) The other portion of the beer was filtered and analyzed for balling, total acid, pH, and reducing substances (calculated as glucose equivalents). These analyses were made to determine whether the fermentations were normal, but the data are not presented here.

The results were evaluated graphically and statistically. The simple correlation coefficients were determined according to Pearson's product moment formula. This formula is valid only if the relationship between the two measured values is linear. Because the relationships, α-amylase *vs.* alcohol yield and limit dextrinase *vs.* alcohol yield, are curvilinear, the enzyme values were transformed and expressed as 1000/enzyme units.

### Results

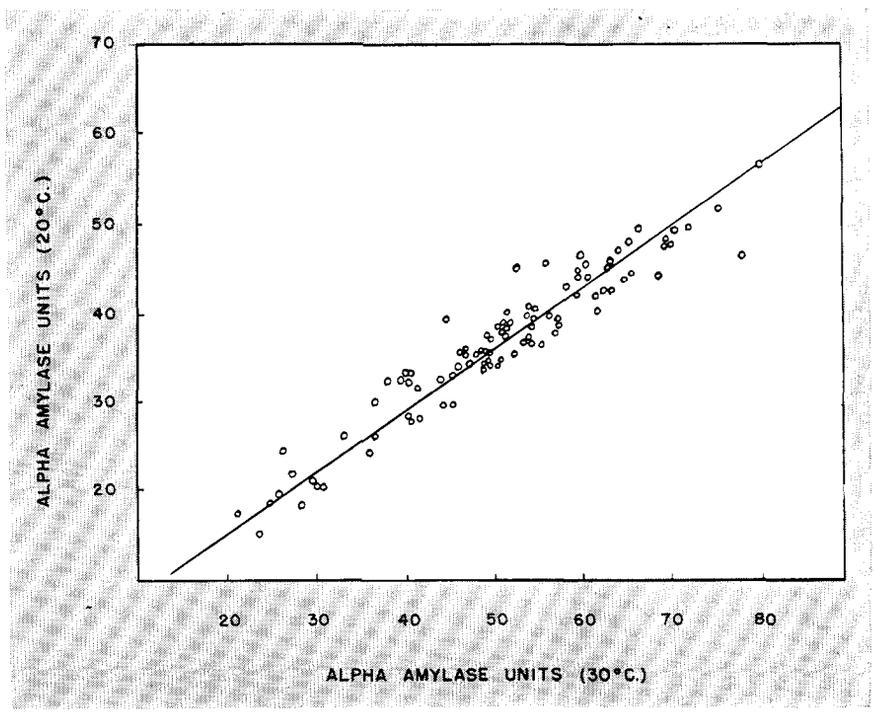
The α-amylase activities of the various malts as determined by the Sandstedt method ranged from 21 to 80 dextrinizing units and were distributed into arbitrary groups according to α-amylase activity as shown in Table I. The correlation coefficient of α-amylase activity determined at 30° C. *vs.* alcohol yield is −0.847. This value is significant at the 1% confidence level. This relationship is illustrated graphically in Figure 1. From this curve it can be concluded that a minimum of 53 to 55 α-amylase units is necessary to assure a maximum alcohol yield at a 5% level of conversion malt.

α-Amylase values as determined by the ASBC method ranged from 14.1 to 56.7 units and were distributed into arbitrary groups according to α-amylase activity as shown in Table I. The relationship between the α-amylase activity of barley malts and alcohol yield, as analyzed by this method, is presented in Figure 2. The correlation coefficient of α-amylase activity measured at 20° C. and alcohol production is −0.880, which is significant at the 1% confidence level. This value is not significantly different from that of the correlation of α-amylase determined at 30° C. and alcohol yield.

The correlation coefficient between α-amylase values determined at 30° and 20° C. is 0.953. This value is remarkably high and strongly suggests that a conversion factor may exist for the two methods. A linear relationship between the enzyme values determined by the two methods is indicated by Figure 3, in which the α-amylase units at 30° C. have been plotted against the corresponding values determined at 20° C. Calculation of the ratio $\frac{30^\circ \text{ units}}{20^\circ \text{ units}}$, however, gave a wide range of values extending from 1.10 to 1.69. Approximately 73% of these values fall between 1.30 and 1.49, with an over-all average of 1.39. Therefore, the factor 1.39 may be used to calculate approximate α-amylase values at 30° C. from analyses made at 20° C., and conversely the reciprocal of the factor may be used to calculate 30° C. values from 20° C. analyses.

The values for limit dextrinase ranged from 39 to 384 units. The samples were distributed into arbitrary groups according to limit dextrinase activity, as shown in Table I. The relationship between limit dextrinase activity of barley malt and alcohol yield is presented graphically in Figure 4. There is a rapid rise in alcohol yield from approximately 40 to 180 limit dextrinase units and at 220 units the yield becomes independent of enzyme value. It can be concluded from this evidence that under the conditions of these experiments a minimum of approximately 220 limit dextrinase units

Figure 3. α-Amylase units (30° C.) *vs.* α-amylase units (20° C.)



in malt is necessary to assure maximum alcohol yield. The correlation coefficient for limit dextrinase *vs.* alcohol yield is −0.800; this is significant at 1% confidence level (Table II).

**Table II. Statistical Analyses of Data**

| | |
|---|---|
| A. Simple Correlation Coefficients | |
| α-Amylase units (20° C.) *vs.* alcohol yield | −0.880 |
| α-Amylase units (30° C.) *vs.* alcohol yield | −0.847 |
| Limit dextrinase units *vs.* alcohol yield | −0.800 |
| Limit dextrinase units *vs.* α-amylase units (30° C.) | 0.891 |
| α-Amylase units (30° C.) *vs.* α-amylase units (20° C.) | 0.953 |
| B. Multiple Correlation Coefficient | |
| Limit dextrinase units and α-amylase units (30° C.) *vs.* alcohol yield | 0.850 |
| C. Partial Correlation Coefficients | |
| α-Amylase units (30° C.) *vs.* alcohol yield, limit dextrinase units constant | 0.492 |
| Limit dextrinase units *vs.* alcohol yield, α-amylase units (30° C.) constant | 0.187 |

The multiple correlation coefficient was calculated for α-amylase (30° C) and limit dextrinase *vs.* alcohol yield in order to determine whether measurement of the two enzyme values would provide a more accurate criterion of malt potential than either enzyme alone. The multiple correlation coefficient is 0.850. This value is high, but does not vary significantly from the correlation coefficients of the individual enzymes and alcohol yield.

Partial correlation coefficients were calculated to determine the effect of each enzyme upon alcohol yield while the other enzyme value is held mathematically constant. The partial correlation coefficients are 0.492 for α-amylase *vs.* alcohol yield with the limit dextrinase held constant and 0.187 for limit dextrinase *vs.* alcohol yield with α-amylase held constant. α-Amylase has a significant relationship to alcohol yield at the 3% confidence level, whereas the relationship of limit dextrinase to alcohol yield is not significant. The high simple correlation between limit dextrinase and alcohol yield is probably due to the close association between limit dextrinase and α-amylase activities. This close association is substantiated by their linear relationship when plotted against each other and the highly significant correlation coefficient of 0.891. Therefore, it can be assumed that a malt having a high α-amylase activity will also have high limit dextrinase activity and, conversely, a high limit dextrinase value indicates a high α-amylase activity. A measurement of either enzyme activity is a reliable criterion for selecting barley malts for the production of alcohol from grain.

## Summary and Conclusions

Eighty-two samples of experimental barley malt and 19 of typical commercial distiller's malt were analyzed for α-amylase and limit dextrinase activity. In addition, each sample of malt was tested in laboratory fermentations to evaluate the action of malt enzymes on grain starch in terms of alcohol yield. These data were analyzed statistically. The correlation coefficients were significantly high at the 1% confidence level for alcohol yield *vs.* α-amylase or *vs.* limit dextrinase and for α-amylase *vs.* limit dextrinase activity. The multiple correlation coefficient for limit dextrinase and α-amylase with alcohol yield was no more significant than the correlation coefficients for the individual enzymes and alcohol yield.

The partial correlation coefficient was significant at the 3% confidence level for α-amylase *vs.* alcohol yield with limit dextrinase held constant but was not significant for limit dextrinase *vs.* alcohol yield with α-amylase held constant.

This statistical analysis shows that the measurement of α-amylase activity provides a reliable index of malt performance, and that limit dextrinase, because of its direct proportionality to α-amylase, may also be used as a reliable index of performance. As alcohol yield is independent of limit dextrinase activity, the utility of the limit dextrinase determination is apparently dependent upon its relationship to α-amylase activity.


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Figure 4. Limit dextrinase units *vs.* ethyl alcohol production