# Ethanol Determination via Immobilized Enzyme

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## CONCLUSION

Ethanol was determined in three sample pairs via an immobilized enzyme method (4) and via distillation (1). Results from and precision of the enzyme method were comparable to the distillation method. However, some difficulty was encountered with the durability of the immobilized enzyme membrane.

## RECOMMENDATIONS

Because of the rapid analysis time and the good agreement with the reference procedure, the immobilized enzyme method should be considered for adoption as an alternate method for measurement of ethanol in beer. However, the method should be studied for one more year, with minor modifications, in hopes of improving accuracy and precision still further.



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This subcommittee was charged with evaluating an immobilized enzyme method (1E) and comparing it with the reference method (RM) for the determination of ethanol in beer. In accordance with the Youden Unit Block design, three sample pairs of bottled beer were sent to the collaborators. These contained approximately 3.5, 4.5, and 6.5% ethanol (v/v), the range of values generally encountered in most breweries. Collaborators were provided with a YSI model 27 analyzer, immobilized enzyme membranes, buffer, and ethanol standards.

## PROCEDURE

Ethanol determination via distillation was performed as described in the ASBC Methods of Analysis (1). In the immobilized

**TABLE I**
**Ethanol in Beer (%, v/v): Distillation Reference Method (RM) vs Immobilized Enzyme Procedure (IE)**

| | Pair 1 | | | | Pair 2 | | | | Pair 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sample A | | Sample C | | Sample B | | Sample E | | Sample D | | Sample F | |
| Lab No. | RM | IE | RM | IE | RM | IE | RM | IE | RM | IE | RM | IE |
| 1 | 6.60 | 6.50 | 6.56 | 6.58 | 4.60 | 4.70 | 4.62 | 4.72 | 3.56 | 3.67 | 3.55 | 3.65 |
| 2 | ...[a] | 6.55 | ...[a] | 6.49 | ...[a] | 4.66 | ...[a] | 4.66 | ...[a] | 3.72 | ...[a] | 3.72 |
| 3 | ...[a] | 6.45 | ...[a] | 6.29 | ...[a] | 4.59 | ...[a] | 4.59 | ...[a] | 3.63 | ...[a] | 3.57 |
| 4 | 6.45 | 6.46 | 6.45 | 6.46 | 4.55 | 4.59 | 4.54 | 4.59 | 3.52 | 3.59 | 3.50 | 3.59 |
| 5 | 6.86 | 6.72 | 6.69 | 6.66 | 4.62 | 4.60[b] | 4.50 | 4.86[b] | 3.55 | 3.57 | 3.57 | 3.65 |
| 6 | 6.60 | 6.62 | 6.60 | 6.58 | 4.63 | 4.76 | 4.65 | 4.73 | 3.61 | 3.75 | 3.61 | 3.75 |
| 7 | 6.60 | 6.52 | 6.54 | 6.52 | 4.58 | 4.60 | 4.60 | 4.47 | 3.60 | 3.50[b] | 3.58 | 2.99[b] |
| 8 | 6.44 | 6.86[b] | 6.44 | 6.49[b] | 4.42 | 4.63 | 4.42 | 4.60 | 3.50 | 3.53 | 3.36 | 3.53 |
| 9 | 6.81 | 5.87[b] | 6.92 | 6.63[b] | 4.68 | 4.43 | 4.67 | 4.47 | 3.57 | 3.71 | 3.58 | 3.53 |
| Mean | 6.62 | 6.55 | 6.60 | 6.51 | 4.58 | 4.62 | 4.58 | 4.60 | 3.56 | 3.65 | 3.54 | 3.63 |
| Grand mean | | | | | | | | | | | | |
| RM | 6.61 | | | | 4.58 | | | | 3.55 | | | |
| IE | 6.53 | | | | 4.61 | | | | 3.64 | | | |

[a]These collaborators did not use distillation as a reference method.
[b]Outlier by Dixon's ratio test. Values excluded from calculation of mean.

**TABLE II**
**Ethanol in Beer: Statistical Summary[a]**

| Method[b] | No. of Labs. | Sample Pair | Grand Mean (%) | Within-Lab. Error, $S_r$ | Between-Lab. Error, $S_b$ | Combine Error, $S_c$ | Coefficient of Variation, c.v. | Calculated F-Ratio[c] |
|---|---|---|---|---|---|---|---|---|
| RM | 7 | 1 | 6.61 | 0.061 | 0.153 | 0.165 | 2.49 | 13.4 ** |
| RM | 7 | 2 | 4.58 | 0.035 | 0.079 | 0.086 | 1.88 | 10.96** |
| RM | 7 | 3 | 3.55 | 0.038 | 0.054 | 0.066 | 1.86 | 5.05* |
| IE | 9 | 1 | 6.52 | 0.216 | 0.00 | 0.216 | 3.32 | 0.87 |
| IE (two outliers removed) | 7 | 1 | 6.53 | 0.052 | 0.094 | 0.108 | 1.65 | 7.53* |
| IE | 9 | 2 | 4.62 | 0.073 | 0.082 | 0.109 | 2.38 | 3.47* |
| IE (one outlier removed) | 8 | 2 | 4.61 | 0.036 | 0.091 | 0.098 | 2.12 | 13.5 ** |
| IE | 9 | 3 | 3.59 | 0.125 | 0.117 | 0.171 | 4.76 | 2.74 |
| IE (one outlier removed) | 8 | 3 | 3.63 | 0.053 | 0.062 | 0.081 | 2.23 | 3.74 |

[a]Calculations made according to the report of the ASBC Subcommittee on Statistical Methodology (3).
[b]RM = reference method. IE = immobilized enzyme method.
[c]* = Significant at 95% confidence level. ** = Significant at 99% confidence level.

enzyme method, samples were degassed and then diluted 25:1 with volumetric glassware. The model 27 was calibrated using a 200 mg/100 ml ethanol standard. A diluted sample was injected into the instrument and the result recorded. For a more detailed description of the method, see Ref. (4).

## RESULTS AND DISCUSSION

The raw data are given in Table I. Table II is a statistical summary.

The average c.v. for the distillation method was 2.08%, while the c.v. for the enzyme method (with four outliers removed) was 2.00%. The statistics for RM are somewhat similar to those reported in another collaborative study on ethanol determination, comparing RM with gas chromatography (2). The short time required for one analysis and the accompanying statistics make the enzymatic method promising.

As mentioned above, some collaborators encountered problems with the stability of the immobilized enzyme membrane. In the middle of the study, the manufacturer, having encountered similar difficulties with some of its customers, changed the membrane and buffer formulation. No problems were encountered after this change.

Collaborator 9 had an outlier value for sample pair 1. From the collaborator's report on the performance of the model 27, it appears that the instrument was not functioning properly. There was no obvious reason for the other outliers.

Greater precision and accuracy might be achieved for the IE method with a minor modification of the procedure. Diluting beers such that their concentration will be as close to the calibration value (200 mg/100 ml) as possible, rather than diluting all beers 25:1, has increased accuracy and precision in the chairman's laboratory. Alternatively, three calibrator solutions could be used. The beer in question could be diluted 25:1 and assayed against a 200 mg/100 ml calibrator. If the value fell below 150 mg/100 ml the beer would be assayed again against a 100 mg/100 ml calibrator; if it were above 250 mg/100 ml it would be assayed against a 300 mg/100 ml calibrator. These modifications of the procedure will be investigated by the subcommittee.

Since this instrumentation is new to most brewery laboratories, a field representative from Yellow Springs Instrument Co. spent at least one day at laboratories of most collaborators, demonstrating the operation of the instrument. Time restrictions made it impossible for all collaborators to receive individual instruction. The statistics at the six facilities where individual instruction was given were significantly improved. The overall c.v. and the between-laboratory error improved considerably.

## LITERATURE CITED

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