

## DETERMINATION OF REPEATABILITY AND REPRODUCIBILITY OF A NEW RAPID ENZYME METHOD FOR THE DETERMINATION OF GLYCOSIDE NITRILE IN MALTED BARLEY

SUBMITTED ON BEHALF OF THE MALTSTERS ASSOCIATION OF GREAT BRITAIN BY A. T. BROWN AND P. MORRALL

**A new rapid method for the determination of malt glycosidic nitrile, using an enzymatic incubation with beta-glucosidase, was tested in an inter-laboratory collaborative trial. Repeatability ( $r_{95}$ ) and reproducibility ( $R_{95}$ ) values are reported. A limited comparison is made with the existing determination of malt combined nitrile using methods involving laboratory fermentations.**

**Key Words:** Collaborative test, malt analysis, malt, glycosidic nitrile, combined nitrile, ethyl carbamate, urethane precursor

### INTRODUCTION

Ethyl carbamate, which has been identified as a potential carcinogen, occurs in foods and fermented beverages in varying amounts. Whisky contains low concentrations of ethyl carbamate and certain countries have set legal limits, e.g. Canada, 150 ppb in distilled spirits. The formation of ethyl carbamate in a malt whisky distillery is by complex mechanisms<sup>5,7</sup>. Malting conditions and more importantly the variety of barley used, greatly influence the level of ethyl carbamate precursors in malt and thus the potential level in the final whisky<sup>4</sup>. Cyanogenic glycosides in malt have been identified as precursors of ethyl carbamate. Routine methods for the determination of nitriles in malt have been developed but have not been evaluated by the Institute of Brewing.

Methods for combined nitrile based on laboratory mashes followed by fermentation and distillation are currently in use. Unfortunately these methods take at least 48 hours to complete. An alternative technique based on rapid enzyme conversion has been developed and recently used by distillers. This method does not require lengthy fermentations but uses a mash in which cyanogenic glycosides are hydrolysed with beta-glucosidase<sup>2,3</sup>.

This paper reports the results for a collaborative trial to determine the repeatability ( $r_{95}$ ) and reproducibility ( $R_{95}$ ) values for the determination of glycosidic nitrile using the rapid enzyme method.

### METHODS

#### Rapid Enzyme Method<sup>2</sup>

Malt (51 g) was ground at setting 2 in a Buhler-Miag laboratory mill. Grist (50.0 g) was weighed into a plastic beaker.  $\beta$ -Glucosidase solution (200 ml of an aqueous solution containing 0.5 g/litre  $\beta$ -glucosidase (EC 3.2.1.21, Sigma Chemical Co. Product No. G-0395) in 0.1 M acetate buffer, pH 5.0) was added and the contents transferred to a 500 ml round-bottomed, long-neck flask. The flask was incubated at 60°C for 1 hour with occasional swirling. The sample was then distilled using an electric heating mantle and approximately 100 ml of distillate were collected in a volumetric flask (200 ml) wrapped in aluminium foil. During distillation charring must be avoided. The distillate was made up to volume (200 ml) with distilled water. An aliquot (20.0 ml) of the distillate (in order to keep the final absorbance below 1.0, this may be diluted by factor A if necessary) was placed in a Quickfit tube and 1.0 ml of freshly prepared Chloramine T reagent added (N-chloro-p-toluenesulphonamide 0.5 g/100 ml) followed by 1.0 ml of a barbituric acid-pyridine solution (1,3-dimethyl barbituric acid, 6 g plus pyridine, 30 ml per 100 ml

aqueous solution). The barbituric acid-pyridine solution must be stored in the dark. The tube was sealed with a ground glass stopper, vortex mixed and incubated in a water bath at 25°C for 5 minutes. The absorbance of the solution was read at 590 nm against a reagent blank. A calibration curve was prepared using aqueous potassium cyanide solutions (20 ml, 0–200 ppb cyanide) in place of the distillates. The result was calculated as follows:

$$\begin{aligned} \text{Let sample reading be } X \text{ ppb CN}^- \\ \text{Result (Y) as g CN}^-/\text{tonne malt (as is)} \\ = X \times 4 \times 10^{-3} \times \text{dilution factor A} \end{aligned}$$

#### Existing Fermentation Method

Variants of a basic method proposed by Cook *et al.*<sup>4</sup> were used by the respondents.

#### Organisation of the Collaborative Trial

This was carried out according to ISO 5725<sup>6</sup>. A uniform experiment with two replicates per sample was employed. Each laboratory received three homogeneous test malt samples covering the normal range of glycosidic nitrile encountered commercially and full, detailed, written instructions for the new rapid enzyme method. Each collaborating laboratory purchased its own reagents. Twelve laboratories participated.

Laboratories were also requested to analyse the malts for glycosidic nitrile if they were routinely using an existing “in-house” fermentation method. Seven of the twelve laboratories participated.

### RESULTS AND DISCUSSION

#### Rapid Enzyme Method

The primary results are given in Table I. In accordance with ISO 5725, Cochran's maximum variance test was applied to detect poor repeatability. One pair of results, from laboratory 1, for the high glycosidic nitrile malt sample, was identified as an outlier (at the 99% confidence level). This pair of results was not used in the calculation of  $r_{95}$  and  $R_{95}$ . The Dixon's test for all means showed no outliers or stragglers.

#### Fermentation Methods

The results are listed in Table I. No stragglers or outliers were identified using Cochran's test for the repeatability of duplicates. Dixon's test showed outliers from laboratory 5 for the means at all three levels of glycosidic nitrile content. These results were not used in the calculation of  $r_{95}$  and  $R_{95}$ .

#### Repeatability ( $r_{95}$ ) and Reproducibility ( $R_{95}$ ) Values

The repeatability and reproducibility values are given in Table II. At all three levels of glycosidic nitrile examined and



