ABSTRACT

The infection of sugarcane and cane milling streams with dextran-forming bacteria is of concern to the sugar industry. The microbial biosynthesis of dextrans occurs via the action of extracellular dextranucrase enzymes on sucrose. Consequently, infections of sugarcane and cane milling processing streams with these bacteria cause loss of sucrose to dextran. Furthermore, high dextran concentrations in process streams effect an increase in viscosity that reduces sucrose recovery and factory efficiency. Finally, dextrans and α-glucan oligosaccharides (the products of dextran hydrolysis) are dextrorotatory, and their presence in raw sugar at the point of sale to refiners increases the polarimetric measurement of sucrose purity.

Currently, two methods for analyzing dextran in raw sugar hold some favor in the sugar industry:

- **Haze method.** Involves enzymic removal of high molecular weight starch, ion-exchange removal of inorganic salts (in some versions), precipitation of proteins with trichloroacetic acid, and measurement of turbidity of a 50% aqueous ethanol solution. The haze method is not sensitive at low dextran concentrations and is specific for high molecular weight, relatively linear dextran (i.e., dextran that precipitates in 50% aqueous ethanol). A version of the haze method was recently accepted by the International Commission for Uniform Methods of Sugar Analysis (ICUMSA) for measuring dextran in raw sugars.

- **Roberts’s copper method.** The official AOAC method involves quantitative precipitation of total polysaccharides in 80% aqueous ethanol; the precipitate is redissolved and selective precipitation of dextrans in alkaline copper solution is followed by colorimetric determination of sugars with the phenol-sulfuric acid reagent. While the haze method is specific for high molecular weight dextran, this method does not appear to be specific to a molecular weight range. Hence the results are usually significantly higher than those of the haze test. In addition to the total dextran, the copper precipitate of the AOAC method may contain 1-4% nondextran polysaccharides, as does the alcohol precipitate in the haze test.

Recently, Galea & Inkerman (1993) reported the development of an enzyme (dextranase)-HPLC analysis for dextran in raw sugars. Although these methods have existed since 1974, they have not been used routinely as they are technically difficult and time consuming. The current enzyme-HPLC method requires a minimum of two days per batch of samples. However, the primary intent of Galea & Inkerman was to establish a reference method (rather than a routine analysis) by which currently favored methods (viz., AOAC and haze methods) could be compared.

The enzyme-HPLC method involves quantitative precipitation of total polysaccharides in 80% aqueous ethanol, digestion of the precipitate with dextranase from *Chaetomium gracile* and HPLC analysis of the isomaltose product of dextranase hydrolysis. There is no doubt that the *C. gracile* enzyme is specific for dextran; however, the calculation of dextran concentration in the raw sugar is based on a dextran-to-isomaltose conversion factor, which is determined by the action of the dextranase on Pharmacia T-series dextrans (Pharmacia Fine Chemicals) and on cane dextran standards that have been purified by precipitation in 50% aqueous ethanol. These dextran standards are therefore comparatively linear with a narrow molecular weight range; the method fails to take into account the polydispersivity and heterogeneity of naturally occurring dextrans (i.e., the method underestimates dextrans with higher branching frequencies). Consequently, it is not valid to refer to (Galea & Inkerman) enzyme-HPLC analysis as a reference method.

Spectroscopic methods of dextran analysis offer a new, more rigorous approach to an absolute determination of dextran. This poster presentation reports the development of a reference method for analyzing dextran in raw sugars that is based on a physical measurement of dextran (using 1H NMR) with little wet chemical preparation of the raw sugar sample (Edye et al 1995). The poster also explores differences in the conventional analytical methods (viz, haze and AOAC) by the analysis of alcoholic precipitates of raw sugar solutions using gel permeation chromatography and 1H NMR spectroscopy.
REFERENCES
