Gillian Eggleston[1*] and Jean-Marc Huet[2], [1]USDA-ARS-SRRC, 1100 Robert E. Lee Boulevard, New Orleans, LA 70124 and [2]Groupement D'Echanges Techniques Sucrerie de Sainte-Emilie, Ste. Vermandoise Industries, 80240 Villers-Facuon, France. T**he measurement of Mannitol in a sugar beet factory to monitor deterioration and processing problems.**

ABSTRACT

Sugar beet deterioration can still be a major technological constraint in processing. The major (but not sole) contributor to deterioration in the U.S. and many other countries, particularly when warm and humid conditions prevail, is infection by hetero-fermentative *Leuconostoc mesenteroides* lactic acid bacteria. In recent years it has emerged that mannitol is a major product of *L. mesenteroides* deterioration of sugar beet and a sensitive marker that can predict processing problems. An enzymatic factory method that is rapid, simple, accurate, and inexpensive is now available to measure mannitol in juices and is also applicable to downstream products. The method recently became an Official ICUMSA (International Commission for Uniform Methods in Sugar Analysis) method GS8-12 "The Determination of Mannitol in Beet Juices, Thin Juices and Syrups by an Enzymatic Method." Numerous factories in Europe, particularly France, Germany, and Belgium are now using the method to monitor for *Leuconostoc* activity in beets, press water, raw and thin juice. In two Belgian factories steam disinfections of juice/cossettes heat exchangers are applied when critical levels (>160 ppm) of mannitol are detected. At a German factory, heaters are treated regularly with sodium hydroxide when mannitol content becomes greater than 50-60 ppm. In numerous French factories, mannitol levels are helping to control filtration difficulties. Mannitol balances undertaken by Groupement D'Exchanges Techniques during the 2009 beet campaign are discussed.

OBJECTIVES

The overall objectives of this study was to test an Official ICUMSA enzymatic method GS8-12 for determining mannitol in beet sugar processing products, particularly cossette, diffuser, and thin juices in sugar beet factories, and determine how they can help prevent and/or alleviate processing problems.

IMPORTANT RESULTS

The major contributor to sugar beet and sugarcane deterioration is *Leuconostoc mesenteroides* infections, particularly when humid and warm environmental conditions prevail. L. mesenteroides deterioration in moderate and severe cases can disrupt normal processing operations, particularly first and second carbonation filtration. Previously, the sugar industry has considered dextran polysaccharide, as the major deterioration product of a *Leuconostoc* infection, but it is now known that mannitol, a sugar alcohol, is also a major degradation product of *Leuconostoc* sugar beet and sugarcane deterioration. Unfortunately, current factory methods to determine dextran are either too time consuming and complicated (ASI enzymatic method) not specific enough (haze method), too expensive (antibody method), too imprecise (antibody method), or too difficult in the interpretation of results (haze method). As chromatography techniques are too sophisticated for use at the factory, very expensive, and a high level of expertise is required by the operator, an enzymatic method (Eggleston, 2009) to measure mannitol in sugarcane juices at the factory was developed. The method utilizes mannitol

dehydrogenase (MDH) to convert mannitol to fructose in the presence of co-enzyme NAD^+. The NADH formed can be easily measured spectrophotometrically at 340 nm:

$$\textbf{Mannitol} + \textbf{NAD}^+ \xrightarrow[\textit{Dehydrogenase}]{\textit{Mannitol}} \textbf{Fructose} + \textbf{NADH} + \textbf{H}^+$$

The current cost per analysis of mannitol in a sugarcane load at the factory is only ~60 US cents (US$0.60), with the largest cost being NAD at 45 cents per analysis (Eggleston, 2009). Kits, for example, by Biosentec™ are now also available to measure mannitol in juices, but they cost over US$4 per analysis.

Huet (2009, 2011) tested, adapted, and improved the Eggleston enzymatic method for determining mannitol in beet sugar processing products, particularly cossette, diffuser, and thin juices. Sensitivity, precision, accuracy were acceptable to the sugar beet industry. Recovery tests were also undertaken at three laboratories after adding 10, 20, 50 and 100 ppm of mannitol. There was an excellent linear correlation between the amounts of mannitol added and recovered for both raw and thin juices. Recovery and precision (coefficient of variation; CV) were acceptable for concentrations of mannitol higher or equal to 20 ppm. At 10 ppm the recovery and CV were unacceptable (Huet, 2009). Thus, method sensitivity for raw juices was between 10-15 ppm. Results were even better for thin juices. Although CVs were still high at 10 ppm recovery was 94.3%. Thus, for thin juices the method sensitivity is lower than 10 ppm (Huet, 2009). In 2010, the reproducibility and repeatability limits of the enzymatic method were accepted by ICUMSA and it became an Official ICUMSA method.

At the Euskirchen factory of Pfeifer and Langen in Germany, mannitol determinations were used to monitor for dextran formation and *Leuconsotoc* deterioration within the raw juice heaters where they often had problems in former sugar beet campaigns. Mannitol concentrations in the inlet and outlet juices of the heaters were determined. When mannitol was >50-60 ppm the heaters were successfully treated with sodium hydroxide and the factory reported they had no more problems with dextran formation within the heaters. In sugar beet factory extraction plants, the greatest amounts of mannitol are always found in diffuser and juice/cossette heat exchanger areas (Huet, 2009). At the Raffinerie Tirlemointoise Company in Belgium, mannitol determinations were undertaken to follow *Leuconostoc* activity within juice stations at Hollogne and Longchamps factories, which send raw juice to the central factory of Wanze. Steam disinfections of juice/cossette heat exchangers were applied when critical levels (≥ 160 ppm) of mannitol were detected. Mannitol determinations are now being routinely measured (at least once a week) in all factories in France. Greater than 60 ppm mannitol predicted filtration problems at first carbonation filtration plants. At one French factory, mannitol is determined ever day. Preliminary investigations of mannitol have also occurred at Amalgamated Twin Falls factory in Idaho during diffuser processing. Mannnitol concentrations were low in most products but sometimes high mannitol concentrations (> 100 ppm) were detected in raw juice and mid tower diffuser juice.

MAJOR CONCLUSIONS

- Mannitol is a relevant tracer of *Leuconostoc* bacteria
- An enzymatic method is now an Official ICUMSA GS8-12 (2009) method: "The Determination of Mannitol in Beet Juices, Thin Juices, and Syrups by an Enzymatic Method"
- Mannitol measurements at a beet factory allow
- ✓ Predicting and controlling processing problems (infections, carbonation filterability, cleaning)
- ✓ Adapting anti-bacterial treatments during processing
- ✓ Undertaking process improvements (dead end pipes elimination etc.)
- Routine mannitol determinations are necessary to define critical thresholds at each factory
- All French factories are now measuring mannitol, and one factory is measuring mannitol daily
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Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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