KLOTZ, KAREN L.[1*], and DANIEL N. MARTINS[2], [1]USDA-ARS, Northern Crop Science Laboratory, 1307 18th St. N., Fargo, ND 58105, and [2]Universidade Federal de Viçosa, 36571-000, Viçosa, MG, Brazil. **Microplate assay for rapid determination of sucrose, glucose, fructose and raffinose.**

ABSTRACT

Current methods for the quantification of carbohydrates in sugarbeet roots have limitations. Polarimetry and refractometry measure only sucrose content and are inaccurate with deteriorated roots. High performance liquid chromatography (HPLC) and gas chromatography (GC) quantify all simple carbohydrates regardless of root quality, but are time-consuming, costly (HPLC) or require the use of noxious reagents (GC). Research was conducted to develop a rapid, high-throughput microtiter plate assay for accurate determination of sucrose, glucose, fructose and raffinose in sugarbeet roots. The assay modifies a previously described enzyme-based microtiter plate assay for quantification of sucrose, glucose, and fructose (J. Sci. Food Agric. 82:80, 2001) to decrease sample preparation and analysis time, and increase the range of sugar concentrations that can be accurately quantified. In addition, the assay was expanded to quantify raffinose in addition to sucrose, glucose and fructose. Using 10 to 15 minute, enzyme-coupled reactions and commercially available reagents, sucrose, glucose, fructose and raffinose were quantified with high reproducibility. The assay was linear for samples containing 4 to 200 mM sucrose, 0.4 to 20 mM glucose, 0.4 to 20 mM fructose, and 0.03 to 3 mM raffinose. Aluminum sulfate concentrations typically used to clarify beet extracts did not affect the assay indicating that sample preparation methods currently used for polarimetry measurements can be used with this assay. The assay was used to quantify sucrose, glucose, fructose and raffinose concentrations in clarified extracts from healthy, stored, rotted and frost-damaged roots. Comparison of these results with those obtained by HPLC and a spectrophotometric assay was used to validate the assay.