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Comparison of MALDI-TOF Mass Spectrometric to Enzyme Colorimetric Quantification of Glucose from Enzyme-Hydrolyzed Starch

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Successful quantification of the glucose produced by enzyme hydrolysis of starch was achieved by a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) protocol, using sorbitol as an internal standard. The starch contents measured by MALDI-TOF MS of corn starch, fiber-enriched oat flour derivatives, oat and barley flours, and barley flour/corn starch composites were evaluated in comparison to a widely accepted and validated method of starch determination, which relies on enzyme colorimetry (EC). The average starch content measured in a series of corn starch samples of different masses was 93 and 101% for EC and MALDI-TOF MS, respectively, values that represent the estimated purity of the sample. There was an agreement of 99% between the starch contents determined by the two analytical methods for complex flour-derived samples. Starch values estimated by MALDI-TOF MS consistently showed a greater degree of variability than those determined by EC, but this limitation was readily compensated by rapid acquisition of multiple mass spectra. This study is the first to report the quantification of glucose by MALDI-TOF MS, and it offers new perspectives into the potential utility of MALDI-TOF MS as a definitive tool for monosaccharide analysis and rapid starch determination in complex samples.

KEYWORDS: Carbohydrates; monosaccharides; oligosaccharides; cereals; oat; barley; sugar alcohol; fiber residue; small molecules; sucrose; fructose; enzyme colorimetry

INTRODUCTION

Starch is the principal storage carbohydrate in cereals and many other foods. In plants, it is contained in starch granules and is composed of varying amounts of amylose and amylopectin, highly organized glucose polymers within the granules. The physicochemical properties of starch differ widely with plant origin and depend on the molecular structure. Amylose is a linear polymer of D-glucose linked by α-1→4 glycosidic bonds and is typically comprised of approximately 6000 glucose units (*1*). Amylopectin is a branched polymer of D-glucose, at least 10 times larger than amylose. It is not uncommon for amylopectin to contain up to three million glucose units, which are linked by α-1→4 glycosidic bonds with varying degrees of α-1→6 branching. Amylopectin is among the largest molecules found in nature. The size and complexity of these molecules offer many challenges for quantitative analysis. The definitive quantification and characterization of starch are very important to food and agricultural industries and to researchers in many other scientific disciplines. There are several methods for analyzing starch, including infrared techniques and numerous variations of enzyme colorimetry (EC) (*2*−*4*). Procedures for quantification affect the outcome of assay accuracy, reproducibility, susceptibility to interferences, sample size, complexity of analysis, cost, speed, and adaptability to other analytical procedures. Thus, researchers must be aware of and balance their priorities when selecting a quantitative method.

The availability of high-purity enzymes has enabled the widespread use of an EC approach in commercial total starch analysis kits. A very common EC procedure for starch analysis is AOAC Method No. 996.11, which is widely used. In this method, starch in cereal flours is solubilized and hydrolyzed by boiling in the presence of thermostable α-amylase to produce dextrins, followed by complete hydrolysis by amyloglucosidase to glucose. Glucose is measured colorimetrically by reaction with a glucose oxidase−peroxidase (GOPOD) reagent. This method is typically reported to have standard errors of ±2%. As with any method that relies on enzymatic processes, interference from sample matrix contaminants could confound the measurement. The assumption that starch is hydrolyzed completely cannot be easily verified for complex and unknown samples. Because colorimetric methods are indirect measurements of glucose, nonglucose chemical interferences may affect the absorption response and ultimately the accuracy of starch analysis.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has become a versatile and important tool for the determination of molecular masses of

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various fragile and nonvolatile samples such as peptides or proteins (*5*, *6*), oligosaccharides (*7*), oligonucleotides (*8*), and synthetic polymers (*9*). Cocrystallization of samples with a suitable matrix of low molecular weight UV-absorbing organic compounds has minimized the degree of fragmentation commonly associated with other mass spectrometry, allowing for simpler spectral interpretation. The ability to rapidly obtain accurate and highly resolved mass information with relative ease of sample preparation suggests the potential of MALDI-TOF MS to become a powerful routine tool in the analysis of all types of organic molecules. Coupling of MALDI with TOF instrumentation allows a virtually unlimited mass range to be monitored (*10*, *11*); thus, historically, MALDI-TOF MS applications have been focused toward large biomolecule analysis. Successes in the use of MALDI-TOF MS for the characterization of many low molecular weight compounds have been reported (*12*), but the field of small molecule analysis is still emerging. There is a valid concern that analysis of small molecules, especially those with molecular weights in the matrix region ($m/z < 500$), can be subject to interference from matrix peaks. However, if it can be demonstrated for a given methodology that no matrix peaks occur in the mass region of interest, then identification of certain small molecules is achievable.

Glucose is a biologically ubiquitous small molecule (molecular weight of 180), which forms the basic monomeric unit of a vast number of carbohydrates. Development of a MALDI-TOF MS methodology to identify glucose directly by molecular weight is an improvement over conventional approaches since molecular weight information is more definitive than indirect inference based on color development. Although MALDI-TOF MS has proven to be valuable for the analysis of many types of polymeric or oligomeric carbohydrates (*7*, *13*−*17*), we did not find any references reporting its successful use in direct determination of glucose or other monosaccharides in biological samples. Problems with sensitivity due to poor carbohydrate response are usually cited, which can be overcome with variable degrees of success by derivatization, often to include a more "ionizable" group. For example, Gouw et al. (*18*) were able to increase the sensitivity of responses of small oligosaccharides, including glucose, by a factor of 1000 by addition of a quaternary ammonium center. We suggest that glucose derivatization for the ultimate purpose of starch determination is unnecessary in that the high glucose concentrations resulting from enzyme hydrolysis of starch samples compensate for its inherently low response in MALDI-TOF MS.

Various strategies can be used in the development of MALDI-TOF MS quantification of carbohydrates. One could measure the intact carbohydrate, the oligomeric products of intermediate hydrolysis, or the monomeric products of complete hydrolysis. The insolubility of large native carbohydrate polymers such as starch makes them difficult to analyze by any analytical technique, including MALDI-TOF MS. Intermediate hydrolysis to characterize the structure of starch has been investigated (*15*, *16*). In a previous study from this laboratory, Wang and coworkers (*16*) analyzed enzymatically debranched maltooligosaccharides of corn and barley starch by MALDI-TOF MS, where chain length profiles of hydrolyzed amylopectin revealed structural information about the parent polysaccharides (**Figure 1**). This work highlighted the ability of MALDI-TOF MS to determine the degree of hydrolysis and potentially to monitor the completion of enzymatic digestion. However, one shortcoming of this study was that a quantitative relationship was not established between the masses of oligosaccharides determined by the MALDI-TOF MS methodology and the mass of sample analyzed. It is important to verify the relationship between the relative response observed in the mass spectrum and the sample composition because mass discrimination effects, where olymers of different masses ionize differentially, often confound attempts to accurately quantify oligosaccharides by MALDI-TOF MS (*19*).

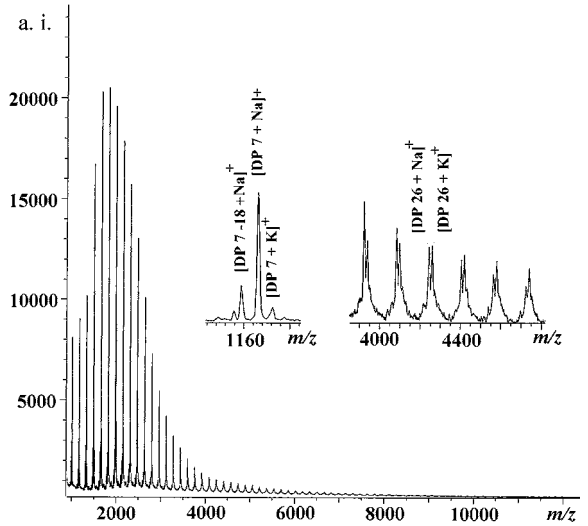

Figure 1. MALDI-TOF MS spectrum of a debranched waxy corn starch sample. Reprinted with permission from ref *16*. Copyright 1999 Wiley VCH.

Our main objective was to use MALDI-TOF MS for total starch determination in complex samples by quantifying glucose produced by complete starch hydrolysis. Because structural information was not a priority, quantification of the monomeric subunit produced by complete hydrolysis was deemed a better strategy than one based on partial hydrolysis. One commonly acknowledged limitation to quantification by MALDI-TOF MS is the high spot-to-spot and sample-to-sample variability, which can result from poor crystal homogeneity and variable incorporation of analytes into the crystal bed. However, use of an internal standard and the ability to rapidly acquire multiple spectra can compensate for this limitation and improve the accuracy of the method by reducing the error. In this paper, we evaluated MALDI-TOF MS as a tool for quantification of starch at various concentrations in corn starch, fiber-enriched oat flour derivatives, oat and barley flours, and barley flour/corn starch composites as it compares to a standard EC methodology. We chose AOAC Method No. 996.11 for comparison, because it is a widely accepted and validated assay used for total starch analysis in industry and research.

MATERIALS AND METHODS

Materials and Reagents. Samples were selected and prepared to represent a range of starch concentrations in a variety of matrixes. Corn starch (Staley 7350, waxy No.1) was obtained from A. E. Staley Manufacturing Co. (Decatur, IL). Flour derivatives enriched in soluble and insoluble fiber (fiber residues) were prepared by selectively removing varying amounts of starch according to a protocol described by Vasanthan and Temelli (*20*). The fiber residues used in this study were prepared from Marion oats (estimated 15 and 25% starch) and Common oats (estimated 35% starch).

Candle barley grains were obtained from Agricore United (Winnipeg, MB). The grains were pearled to remove 32% of the outer layers at the University of Saskatchewan (Saskatoon, SK) using a "Satake" testing mill (model-TM05, Satake, Tokyo, Japan) fitted with an abrasive roller and 1 mm screen. The pearled grains were pin-milled (Alpine Contraplex wide chamber mill Type A 250, Hosokawa Micron Systems, Summit, NJ) at the POS Pilot Plant (Saskatoon, SK) to obtain flour.

Phoenix barley grains were obtained from Dr. Brian Rossnagel from the Crop Development Center, University of Saskachewan. Antoine oat grains were obtained from Agri-Food Canada, Central Research Station, Ottawa. Both Phoenix barley and Antoine oat grains were pearled to remove 20.6 and 20.7% of the outer layer, respectively, using a pearler (Alberta Agriculture, Food and Rural Development) and milled into flour using a Retsch Ultra Centrifugal Mill (Type ZM 100, Retsch GmbH & Co. KG, Germany). The three flours were sieved through a $<250\ \mu$m mesh screen and were estimated to contain about 60% starch.

Two composite samples were prepared composed of Candle barley flour plus corn starch in a 7:3 or 3:7 wet weight ratio to provide samples with approximately 70 and 90% starch. All enzyme and buffer solutions were prepared as described by AOAC Method No. 996.11. Glucose and sorbitol standards and the matrix, 2,5-dihydroxybenzoic acid (DHB), were purchased from Sigma Chemical Co. (St. Louis, MO). The deionized water used in this study was from a Milli-Q water purification system (Millipore Corp., Bedford, MA).

Moisture Determination. Moisture contents of glucose, sorbitol, and corn starch were determined in duplicate by differential weighing before and after heating for 20 h at 80 °C in a forced air oven.

Enzymatic Starch Hydrolysis. Enzymatic conversion of starch into glucose was carried out according to a version of the standard assay procedure described by AOAC Method No. 996.11. Corn starch (20, 30, 40, 50, 60, 70, 80, and 100 mg) and 100 mg each of fiber residue, flour, and composite samples (subsequently referred to as flour derivatives) were weighed to ± 0.1 mg accuracy in duplicate. Each sample was wetted with 0.2 mL of 80% aqueous ethanol and incubated with thermostable α-amylase in a boiling water bath for 6 min. The samples were then incubated with amyloglucosidase for 30 min at 50 °C. Following this incubation, corn starch solutions were diluted to 10 mL, whereas flour solutions were not diluted. An aliquot of each solution was centrifuged for 2 min at 5000 rpm. From the supernatants, a 200 μL aliquot was taken for MALDI-TOF MS analysis and a 1.000 mL aliquot was taken for colorimetric starch determination.

Starch Quantification by MALDI-TOF MS. MALDI-TOF mass spectra were acquired on a linear Bruker Proflex III instrument equipped with a nitrogen laser (337 nm) (Bruker Analytical Systems Inc., Billerica, MA). Mass spectra were obtained in positive ion mode with an extraction potential of 20 kV and a delay of 6000 ns. All mass spectra were generated by collecting 100 laser shots. Laser strength and detector voltage were adjusted to obtain optimal signal-to-noise ratios and high resolution.

A 200 μL aliquot obtained from the enzymatic process was diluted 1:1 with water. To 200 μL of this solution, 40 μL of sorbitol solution (10.012 mg/mL in water) was added. A matrix solution of DHB was prepared (10 mg/mL) in 50% aqueous ethanol. The matrix solution was mixed 1:1 with sample solutions (100 μL each), the mixture was centrifuged for 2 min at 5000 rpm, and 1 μL was applied to a stainless steel target probe. The matrix and sample were cocrystallized on the probe by allowing the solvent to evaporate in ambient conditions, accelerated by a fan. Three spectra were generated for each sample duplicate, and peak heights of the sodium adducts (added to the peak heights of the respective potassium adducts when present) were measured. The glucose concentration was related to the starch content according to eq 1.

$$\text{starch (mg)} = [\text{sorbitol (mg/mL)}] \times (\text{pk ht glucose/pk ht sorbitol}) \times \text{response factor} \times \text{dilution factor (DF)} \times \text{conversion factor (CF)} \quad (1)$$

The concentration of sorbitol in the 240 μL solution made prior to dilution with matrix solution was corrected for moisture content ([sorbitol] = 1.66 mg/mL). The response factor was derived from the linear region of a standard curve of glucose and sorbitol, shown in **Figure 2**. The DF accounts for the proportion of starch in the original sample weighed (DF = (240 μL/200 μL) × (7.3 or 10.0 mL)). The CF adjusts for the conversion of glucose to anhydroglucose, as it occurs in starch (CF = 162/180).

Determination of MALDI-TOF MS Response Relationship between Glucose and Sorbitol. Standard solutions were prepared in aqueous NaCl (0.01 M) where the concentration of sorbitol remained constant at 0.5 mg/mL and the glucose concentration was either 0.1, 0.5, 1.0, 1.5, 2.0, 3.0, 5.0, or 10.0 mg/mL (glucose to sorbitol concentration ratios of 0.2, 1, 2, 3, 4, 6, 10, or 20). Each solution was mixed 1:1 with DHB matrix solution (10 mg/mL in 50% ethanol), and approximately 1 μL was applied to the probe. Six mass spectra per solution were obtained, and the results were averaged. Responses were based on the peak heights of sodium adduct ion signals associated with either glucose or sorbitol. The slope of the linear region of a graph showing the concentration ratio vs response ratio was taken to be the glucose:sorbitol response factor (**Figure 2**).

Starch Quantification by EC Method. A 1.000 mL aliquot prepared from the enzymatic starch hydrolysis was diluted with water to either 10.00 mL for corn starch samples or 15.00 mL for flour derivatives. From this solution, a 0.100 mL aliquot was mixed with 3.00 mL of GOPOD solution and incubated for 20 min at 50 °C, and the absorbance was read at 510 nm. The glucose concentration was related to the starch content according to eq 2.

$$\text{starch (mg)} = (A_{510}\ \text{sample}/A_{510}\ \text{glucose control (GC)}) \times \text{GC (mg)} \times \text{DF} \times \text{CF} \quad (2)$$

GC consists of 0.100 mL of glucose standard solution (1 mg/mL) and 3.00 mL of GOPOD reagent (GC = 0.100 mg of glucose). The DF accounts for the proportion of starch in the original sample weighed (starch DF = 100.0 mL/0.100 mL; sample DF = (15.00 mL/0.100 mL) × (7.3 mL/1.000 mL)). The CF adjusts for the conversion of glucose to anhydroglucose, as it occurs in starch (CF = 162/180).

RESULTS AND DISCUSSION

Two volumetric modifications of AOAC Method No. 996.11 for total starch analysis were necessary in the case of the flour derivative samples so that MALDI-TOF MS and EC methods could analyze sample replicates from a common source immediately following starch gelatinization and enzyme-catalyzed hydrolysis. A volumetric adjustment to 10.00 mL was readily performed for the corn starch samples, and this allowed for their valid and accurate quantitative assessment. However, the same volumetric adjustment was not possible for flour after hydrolysis, because they contained large amounts of suspended solids, which would necessitate a larger volume for quantitative transfer. Furthermore, a concentrated solution at this stage (≤10 mL) was required for optimal MALDI-TOF MS glucose analysis of the low starch samples. Calculations of starch content in the flour derivatives reflected both the volume modification at this stage (7.3 mL instead of 10.0 mL) and the volume modification in the final dilution (15.0 mL instead of 10.0 mL). We do not believe that these modifications would have affected the quantitative ability of the EC assay. Although changes in volume due to sample addition (i.e., corn starch, flour, etc.) were not factored in, these were assumed to be negligible and, in any case, consistent between both methodologies. The common initial steps to generate glucose from starch, followed by parallel analysis of the same sample by the two different techniques, guaranteed that sample preparation differences were not a source of discrepancy between the two methods. The internal standard sorbitol was added to the samples after the common initial stages to eliminate possible interference in the EC method. Ideally, sorbitol would be added at the beginning of the procedure, which would eliminate the need for time-consuming quantitative dilution steps.

The phenolic compound DHB was selected as the matrix for MALDI-TOF MS analysis of glucose because there were few matrix peaks evident in the *m*/*z* 180−250 range. More importantly, there were no interfering matrix peaks at *m*/*z* 203, 205, 219, or 221 (which would be identical to masses of glucose and sorbitol ions) even at high laser strength. Flour and corn

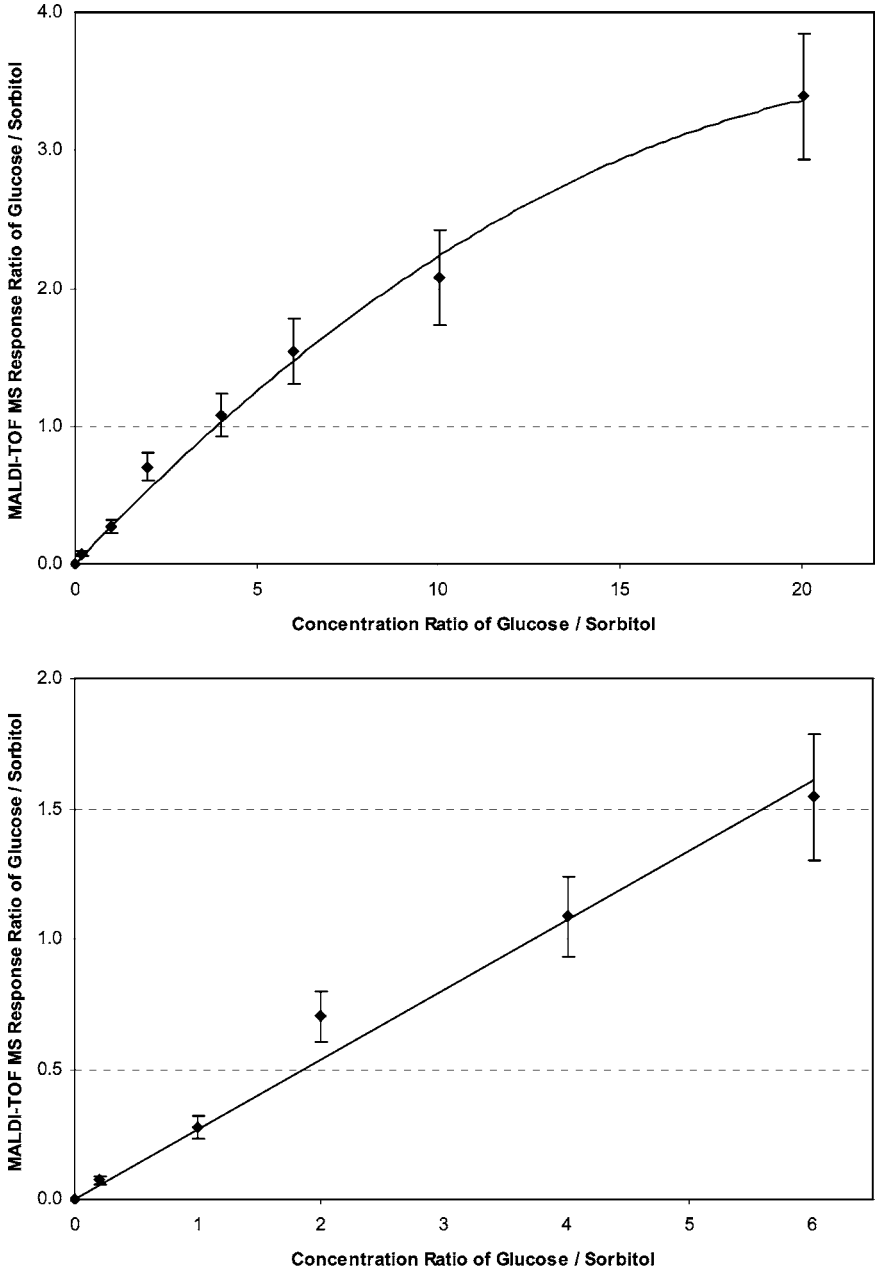

Figure 2. Standard curves generated from MALDI-TOF MS spectra of glucose and sorbitol where glucose concentration was varied from 0.1 to 10 mg/mL in 0.01 M NaCl and sorbitol concentration remained constant at 0.5 mg/mL in 0.01 M NaCl. The top graph shows the entire range of data as a polynomial function ($y = -0.0055x^2 + 0.2781x$; $R^2 = 0.9933$); the bottom graph shows an expansion of the region from concentration ratios of 0–6 as a linear function ($y = 0.2677x$; $R^2 = 0.9826$; relative average standard deviation = 16.0%). Each data point represents the average of six measurements.

starch samples, analyzed with DHB, with and without standard additions of glucose and sorbitol, produced good signal-to-noise ratios for both glucose and sorbitol and demonstrated a lack of interfering peaks when analytes were absent. Sorbitol was selected as an internal standard because it is structurally similar to glucose, is unlikely to occur naturally in cereals, has a different molecular weight than glucose, and responds consistently relative to glucose. The [M + 2] isotopic peak of glucose was negligible even at high glucose concentrations relative to sorbitol, so it did not interfere with the response of sorbitol, which also occurs at two mass units higher than glucose. Peaks corresponding to proton adducts for glucose or sorbitol were not observed, in agreement with observations by other authors (*21*), whereas sodium adducts were common and potassium adducts were rare and minor.

The average moisture contents for glucose and sorbitol standards were 1.22 ± 0.08 and 0.33 ± 0.05%. The MALDI-TOF MS response relationship, or response factor, between glucose and sorbitol was adjusted accordingly to reflect the dry weight ratios. The concentrations of glucose and sorbitol used to establish the response factor were selected to be in the same range as the concentrations derived from hydrolysis of the corn starch and flours. The absolute concentrations, as long as they remain within the analytical range of the instrument, are not as important as the ratio of the concentrations between the analyte and the internal standard. It is important to keep the concentration ratios in such a range that the response of analyte and internal standard is similar. In our case, if the concentration ratio of glucose/sorbitol exceeded 6, the response relationship became nonlinear, as shown in **Figure 2**. However, in a range where the concentration ratio was between 0 and 6, a linear relationship was established. All samples were targeted to this zone for analysis. From the slope of the linear regression, the response factor between glucose and sorbitol was determined

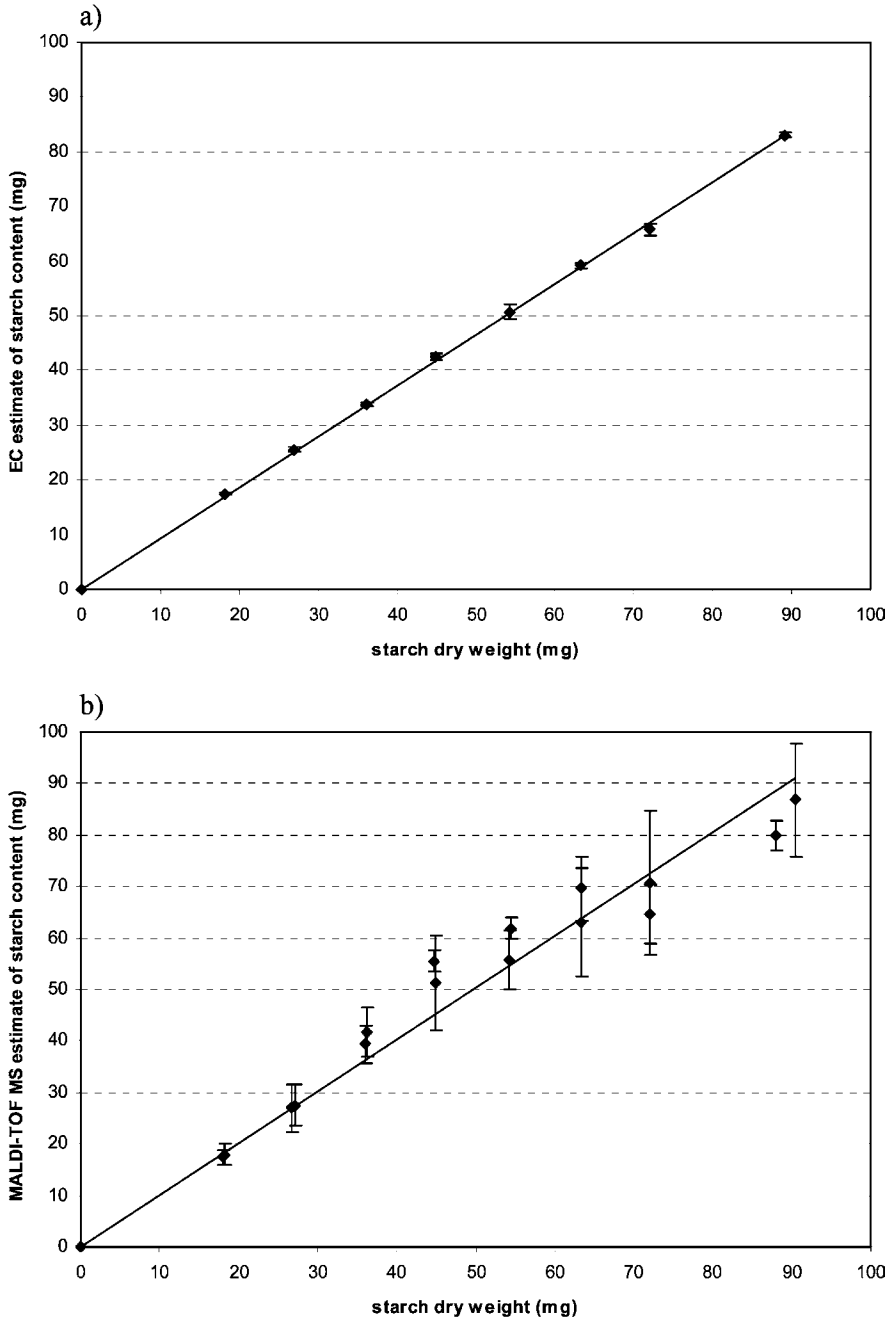

Figure 3. Relationship between dry weight of corn starch and measurement of starch content by (**a**) the EC method and (**b**) the MALDI-TOF MS method. Error bars in panel **a** represent the standard deviation of starch content in sample duplicates ($y = 0.9307x$; $R^2 = 0.9995$; relative average standard deviation = 1.3%). Error bars in panel **b** represent the standard deviation in starch content based on three mass spectra per sample. In panel **b**, the two data points per *x*-value are associated with duplicate samples ($y = 1.0061x$; $R^2 = 0.9551$; relative average standard deviation = 11.1%).

to be 0.2677 ($R^2 = 0.9826$). This means that the response of glucose is consistently about four times lower than sorbitol for a given concentration within the analytical range of the instrument. The considerable difference in response is surprising because these two compounds differ structurally by only two hydrogens. This finding further emphasizes the need for accurate determination of response factors when quantifying using MALDI-TOF MS.

On the basis of the linear regressions between starch weights and starch estimates by EC or MALDI-TOF MS shown in **Figure 3**, the MALDI-TOF MS starch estimates were 8.6% higher than the corresponding EC starch estimates at 100.6 and 93.1%, respectively. Although results of the EC starch measurement were not in agreement with a statement from the corn starch manufacturer, which lists the starch content on a dry weight basis to be greater than 99.9%, the validity and reliability of the EC method have been well-established (*22*). Thus, we assume that the EC measurement represents the true starch value in this sample (accurate to within a standard error of +2%, as the assay handbook describes). The presence of resistant starch, which is routinely measured in our laboratory to be between 1 and 1.5% in a starch sample, cannot explain the impurity of this commercial starch. It is possible that other contaminants (i.e., protein, lipid, ash, etc.) are also present. Variability in MALDI-TOF MS corn starch data was high as compared to the corresponding variability in EC data (relative average standard deviations = 1.3 and 11.1%, respectively), and MALDI-TOF MS accuracy on a sample-to-sample basis was only moderate as demonstrated by the lower correlation of the linear regression for the corn starch sample series ($R^2 = 0.9995$

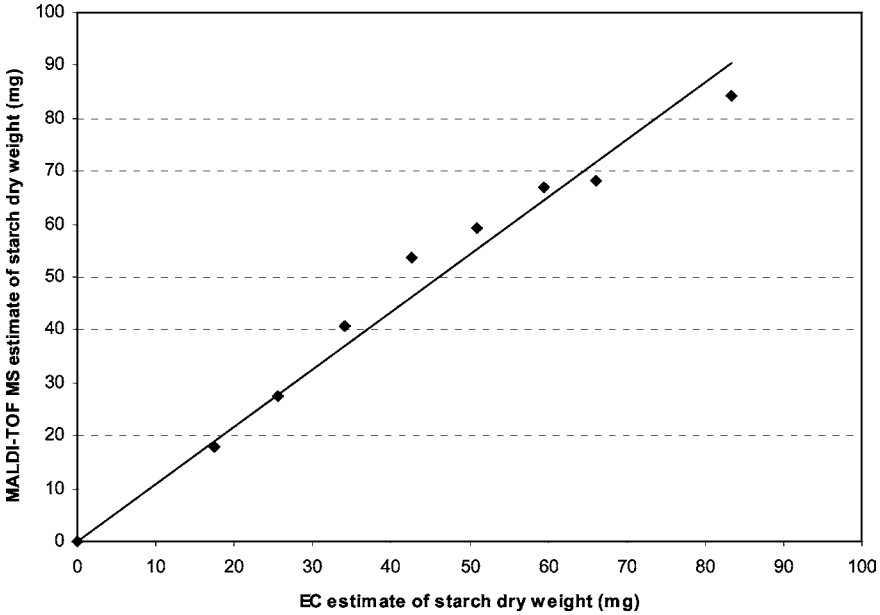

Figure 4. Correlation between MALDI-TOF MS and EC estimates of the starch content in corn starch samples of varying weights ($y = 1.0863x$; $R^2 = 0.9745$).

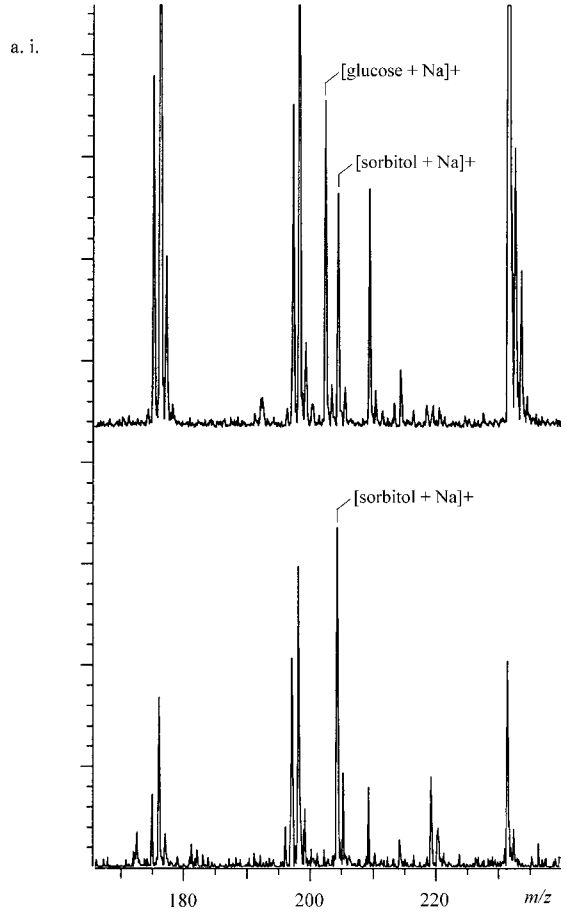

Figure 5. MALDI-TOF MS positive ion spectra of Antoine oat flour prior to enzyme hydrolysis (bottom) and after hydrolysis (top). Sorbitol was added to both samples at the same concentration. Sodium adduct peaks of glucose and sorbitol occur at *m/z* 203 and 205, respectively. These spectra are typical of all complex flour derivative samples. All unlabeled peaks are due to the DHB matrix.

and 0.9551 for EC and MALDI-TOF MS, respectively). However, within the degree of error, the MALDI-TOF MS starch measurements agree with the EC measurements.

Table 1. Comparison of EC and MALDI-TOF MS Measurements of Percent Starch in Fiber Residues, Flours, and Flour/Starch Composite Samples

sample	enzyme colorimetry	MALDI-TOF MS
fiber residue 1 (Marion oat)	18.9 ± 0.7	20.4 ± 3.4
fiber residue 2 (Marion oat)	28.5 ± 0.2	31.4 ± 8.0
fiber residue 3 (Common oat)	36.6 ± 0.8	37.7 ± 5.1
flour 1 (Antoine oat)	60.9 ± 0.8	55.5 ± 7.8
flour 2 (Phoenix barley)	64.6 ± 0.1	57.8 ± 6.9
flour 3 (Candle barley)	67.1 ± 0.3	58.4 ± 9.4
composite 1 (Candle flour/corn starch, 7:3)	73.6 ± 0.0	78.2 ± 14.2
composite 2 (Candle flour/corn starch, 3:7)	81.6 ± 0.1	89.3 ± 26.5

In comparing the two methods for a range of corn starch masses (20−100 mg), as shown in **Figure 4**, MALDI-TOF MS estimates of starch on a dry weight basis correlate well with the corresponding EC estimates ($R^2 = 0.9745$). The slope of the line ($y = 1.0863x$) showed that on average, MALDI-TOF MS slightly overestimated the starch content as compared to the EC measurement. The moisture content of corn starch was determined to be 10.02 ± 0.03%. Adjustments for free glucose were made in the EC starch estimates based on a finding of 0.3 mg glucose per 100 mg corn starch sample blank. Because no significant response corresponding to glucose or its isomers was observed in the MALDI-TOF MS spectra for the corn starch sample blank, no adjustment was made for free glucose in this case. The detection limit of MALDI-TOF MS for glucose was approximately 0.1 mg/mL, which corresponds in our methodology to 1 mg free glucose per 100 mg sample. Thus, free glucose at levels of 1% or less in the sample would not be measurable by our MALDI-TOF MS methodology.

A typical MALDI-TOF mass spectrum of the complex flour samples is shown in **Figure 5**. Flour derivative sample blanks did not show any response corresponding to glucose or its isomers in the MALDI-TOF MS spectra or in the EC analysis; therefore, no adjustment was made for free glucose in those samples. A comparison of EC and MALDI-TOF MS measurement of starch for the complex flour-derived samples is shown in **Table 1**. When these values were plotted against each other (not shown), on average, the two methods had a very close

Table 2. Comparative Evaluation of MALDI-TOF MS and EC Methods of Starch Quantification

concept	MALDI-TOF MS	EC
accuracy	moderate accuracy	good accuracy
reproducibility	low reproducibility; requires many replicate spectra to improve accuracy	high reproducibility among replicates and between assays
tolerance of complex sample impurities	tolerant	tolerant
sample preparation	minimal sample preparation; internal standard eliminates the need for quantitative volumetric dilutions	time-consuming volumetric dilutions necessary
directness of assay	direct measurement of glucose	indirect measurement of glucose
interferences	interference possible by other compounds of identical molecular weight to glucose	interference possible by enzymes or other compounds that interfere with production/measurement of color
simultaneous analysis of other compounds	many compounds can be simultaneously analyzed, including unhydrolyzed dextrins	only glucose is measured
speed and efficiency	fast and potentially automatable, both in sample preparation and in spectral data interpretation	automatable, but efficiency is ultimately limited by time-consuming sample preparation

agreement between their percentage starch estimates (slope of the linear regression = 0.9892). There was a good correlation between MALDI-TOF MS and EC estimates of starch in the flour derives ($R^2 = 0.9620$). However, as with the corn starch, starch values for flour derivatives determined by MALDI-TOF MS were much more variable than those determined by EC (relative standard deviations ranging from to 12 to 30% for MALDI-TOF MS as compared to a range of 0−4% for EC).

Table 2 summarizes the advantages and disadvantages of EC and MALDI-TOF MS methods of starch quantification. Good correlation between the two methods indicates that MALDI-TOF MS is able to reasonably predict the starch levels in both pure starch and complex flour derivative samples. Relatively higher variation in the MALDI-TOF MS system was partially reduced by averaging the results of triplicate spectra for each sample, whereas the EC method was so reliable that only a single colorimetric measurement was necessary for each sample. Together with the volumetric dilutions necessary in the EC procedure, the enzymatic color reaction and colorimeter measurement stages are inherently laborious and represent a "bottleneck" limitation of this procedure. Using the EC procedure and a manual spectrophotometer, an experienced technician can reasonably be expected to process 25 samples in duplicate (50 total) in an 8 h work day. In comparison, using MALDI-TOF MS with manual sample preparation, loading, and triplicate spectral acquisition, at least double this many samples could be processed in the same time. The lack of volumetric adjustments required for MALDI-TOF MS analysis would mean that the entire sequence of hydrolytic steps could be performed easily in one tube prior to analysis.

On average, determination of starch in simple and complex samples by MALDI-TOF MS agrees with the standard EC method, but the relatively large spectrum-to-spectrum variability between identical samples currently limits the practicality of adopting such a technique for routine analysis. Further work is necessary to reduce the high degree of variability, possibly by establishing more rigorous and dependable criteria for accepting or rejecting spectra. Also, improvements in sensitivity for free glucose detection in complex samples will be necessary for MALDI-TOF MS to compete with standard colorimetric methodology. Ongoing developments in MALDI-TOF MS technology and methodology will continue to improve the practicality and scope of this important analytical tool. Determination of glucose by MALDI-TOF MS has inherently more potential for efficiency than EC techniques and more easily lends itself to high-throughput analysis. Other important advantages of the MALDI-TOF MS method include its ability to directly identify glucose by molecular weight and its potential to monitor the completion of enzymatic digestion in starch determination at the level of oligosaccharides as well as monosaccharides.

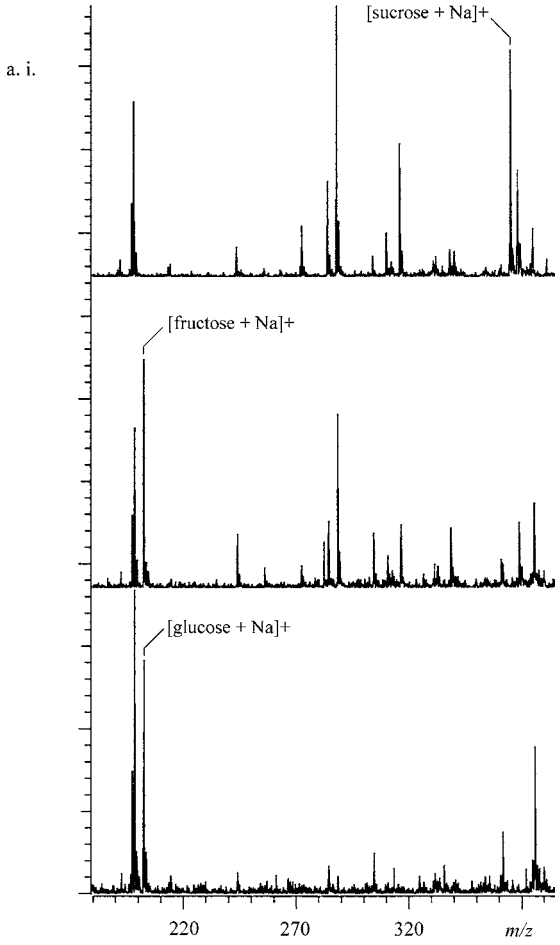

Figure 6. MALDI-TOF MS positive ion spectra of glucose (bottom), fructose (middle), and sucrose (top) dissolved at 1.0 mg/mL in 0.01 M NaCl. Sodium adduct peaks of glucose, fructose, and sucrose occur at *m/z* 203, 203, and 365, respectively. All unlabeled peaks are due to the DHB matrix.

There is potential to analyze other important small carbohydrates in addition to glucose, as we were readily able to detect fructose and sucrose by MALDI-TOF MS (**Figure 6**). Modified starches, β-glucan, cellulose, hemicellulose, other homogeneous or heterogeneous polysaccharides, and other carbohydrates could also potentially be characterized by MALDI-TOF MS determination of glucose or other constituent monomers using selective enzyme systems for hydrolysis.

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