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Near infrared spectroscopy determination of sucrose, glucose and fructose in sweet sorghum juice☆

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ABSTRACT

Sweet sorghum is a very robust crop which has the potential to be used in ethanol production due to its high fermentable sugar content present in its stem juice, very similar to sugarcane. Therefore, for breeding purposes it is relevant to analyze sugar composition in the juice to characterize sweet sorghum genotypes and their period of industrial utilization within different environments for maximum ethanol yield. In this work we developed a rapid, low cost and efficient method to determine the profile of sugars (sucrose, glucose and fructose) in sorghum juice by near infrared spectroscopy and partial least square regression, and validation of the method was performed according to the high-performance liquid chromatography method. Developed models provided root mean square error of prediction of 4, 1 and 0.6 $mg \cdot mL^{-1}$ and ratio performance deviations of 8, 5 and 5 for sucrose, glucose and fructose, respectively. Relative standard deviations of three sweet sorghum juice samples were reported with content variation (low, medium and high) 0.2, 0.3, 0.8% for sucrose; 1, 2, 2% for glucose; 1, 2, 3% for fructose. Sugar profile is an asset for crop breeders to take decisions for the development of more productive cultivars and higher sugar content.

1. Introduction

Sweet sorghum is one of the most promising alternative crops to sugarcane for ethanol production due to the presence of sweet juice in its stem [1].

Sugar content in sweet sorghum juice varies between 14 and 23% Brix and may be extracted by protocols similar to those used for sugarcane [2]. The juice from the fresh stem contains sucrose, glucose and fructose, with sucrose being the main sugar [3,4].

One of the measures undertaken by the sugar industry to assess sweet sorghum quality is the determination of the contents of soluble solids (Brix) of the juice extracted. However, the Brix is an indirect measure that relates the soluble solids dissolved in water based on refractive index changes. It is a measure widely used in the technological qualification of sugarcane juice [5], fruit juice [6] without specifying the sugar present. Brix in sweet sorghum samples has been strongly correlated with sucrose content, albeit not correlated with glucose and fructose [7].

Since the sugar extracted from sweet sorghum is a function of biomass yield, fiber content and juice quality, it is important to know the composition of the sugars in sorghum juice to better qualify the sweet sorghum genotypes and their period of industrial utilization (PIU) in different environments to provide maximum yield of ethanol during the fermentation process [2]. PIU should be the longest possible, with a minimum threshold of 30 days. In fact, PIU comprises the period in which the cultivar may remain in the field maintaining productivity and quality at optimal levels, according to the minimum standards established to ensure the viability of the crop until it is harvested and processed by the ethanol industry.

Chromatographic techniques, such as high performance liquid chromatography (HPLC) [8], ion chromatography (IC) [9], gas chromatography (GC) [10] or enzymatic methods [11], are commonly used to determine the chemical composition of sugars in sorghum juice.

However, all these techniques, coupled to several chemicals and inputs needed for sample preparation allow only a few analyses per day.

The Embrapa Sorghum Breeding Program requires a great number of sugar content analyses of sweet sorghum juice during the harvest period. The method we stablished in this work allowed a faster and low-cost alternative to the HPLC method to detect hybrids with high sugar yield potential during their PIU. The method employs near infrared spectroscopy (NIR) associated to the development of multivariate chemometric regression models. PLS regression is a multivariate method and uses information of the NIR spectrum to establish the calibration equation. NIR

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Table 1
Sucrose, glucose and fructose contents as determined by HPLC from 160 samples of sweet sorghum juice.

Component	Sucrose	Glucose	Fructose
Minimum	26.50	6.60	4.21
Maximum	169.52	36.16	17.5
Mean	89.40	17.58	9.97
Standard deviation	3	5	2

Units: mg mL^{-1}.

region contains information on the relative proportions of C—H, N—H and O—H bands which are the primary structural components or organic molecules [12].

This approach has been widely used in numerous agricultural and food products [13] and offers decisive advantages over traditional methods, such as little sample handling, no chemicals, high precision and accuracy, inexpensiveness and faster results [12].

The evaluation of sugar quality by near infrared spectroscopy has been reported in the literature for fruit juice [14], sugar beet [15], sugarcane [16] and sweet sorghum in dry samples [17,18]. Chen et al. [17] extracted sucrose and glucose from dry sorghum stalks using distilled water and autoclave at 121 °C for 15 min. Mid-infrared spectroscopy was used to predict sucrose, glucose and fructose contents in juice samples of sweet sorghum [4].

This work aimed at developing a multivariate calibration-based method using near infrared transflectance spectroscopy as a source of analytical information to determine sucrose, glucose and fructose contents in sweet sorghum juice with the minimal pretreatment of samples for high-throughput screening phenotyping.

2. Materials and methods

2.1. Preparation of samples

The experiment was conducted in the field experimental area of Embrapa Maize and Sorghum, in Sete Lagoas (19°28′S, 44°15′08″W), MG, Brazil, using cultivars of Embrapa's sweet sorghum breeding program.

One hundred sixty juice samples, from eight genotypes of sweet sorghum (BRS 508, BRS 509, BRS 511, CMSXS643, CMSXS646, CMSXS647, CV 198, CV 568 with similar flowering patterns were harvested, at different stages of maturation, 72 days after sowing with an interval of seven days approximately. The samples were collected during 2015 and 2016.

Normal cultural practices were maintained to conduct the experiment, following May et al. [19].

2.2. Sugar analysis

Stalk panicles were removed and eight stalks were crushed in a forage chopper machine (Irbi, Araçatuba SP Brazil). Further, 500 g of the material were taken to the hydraulic press (Hidraseme, Ribeirão Preto SP Brazil) for 1 min with minimal constant pressure of 250 $kgf \cdot cm^{-2}$. An 80 mL aliquot of juice extracted from each sample was stored in a polyethylene vial and frozen at −4 °C for later analysis, totaling 160 samples. Sucrose, glucose and fructose contents were analyzed by HPLC as follows: sorghum juice samples were thawed at room temperature and 3 mL of each sample were diluted 15 times with deionized water. The samples were then shaken at 45 rpm for 15 min and centrifuged at 3000 rpm for 15 min. Samples were filtered through a C18 cartridge, previously conditioned with 2 mL acetonitrile and 2 mL deionized water. After this process, 2 mL of the solution were filtered with 0.45 μm membrane filters (PTFE) and analyzed by HPLC (2695 Alliance Waters, Milford, MA, USA) using a Phenomenex column (RCM-Ca). The mobile phase used was ultrapure water flux 0.6 mL min^{-1}, column temperature 65 °C. The detector was the Refractive Index (Milford MA, USA) working at 40 °C. Analytical curves were produced by using sucrose, D-glucose and D-fructose as standards (Sigma-Aldrich) with 99.5% purity, respectively. Sucrose, glucose and fructose in the samples were detected by comparison to standard retention time. Three calibration curves ($R^2 \geq 0.999$) were established for sucrose, glucose,

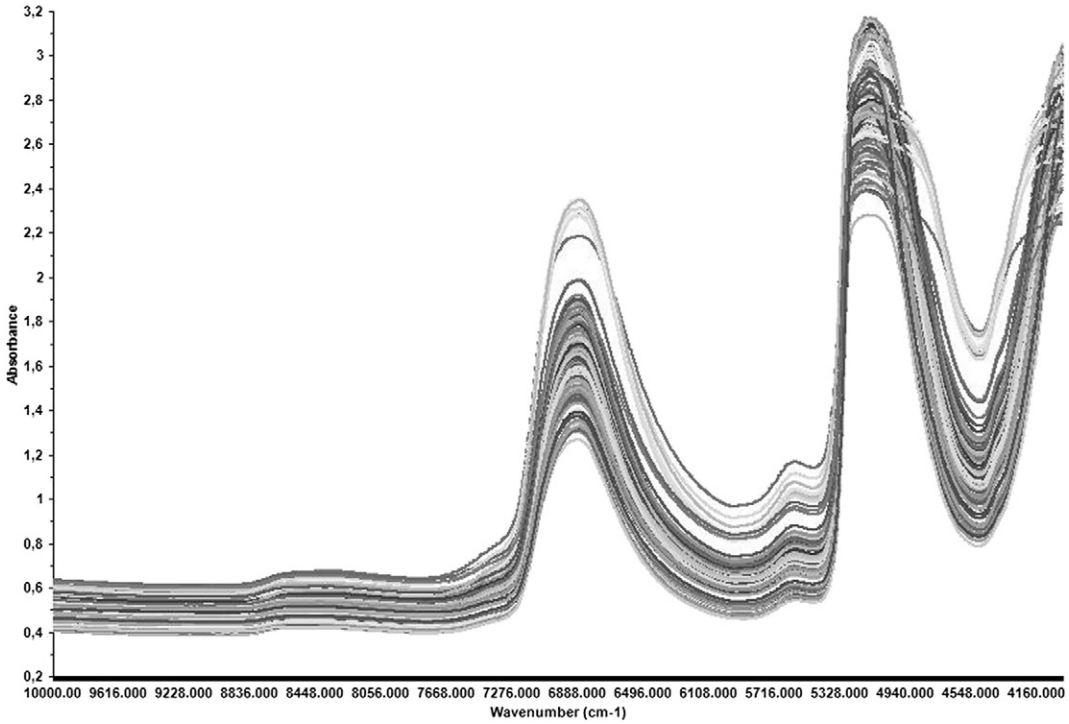

Fig. 1. Set of raw NIR spectra of 160 sorghum juice samples.