Research Article

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Development of NIRS calibration curves for sugars in baked sweetpotato

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Abstract

Background: Variability in sugar content between raw and cooked sweetpotato storage roots impact nutritional and dietary importance with implications for consumer preference. High-throughput phenotyping is required to breed varieties that satisfy consumer preferences.

Results: Near-infrared reflectance spectroscopy (NIRS) calibration curves were developed for analysing sugars in baked storage roots using 147 genotypes from a population segregating for sugar content and other traits. The NIRS prediction curves had high coefficients of determination in calibration (R^2_c) of 0.96 (glucose), 0.93 (fructose), 0.96 (sucrose), and 0.96 (maltose). The corresponding coefficients of determination for cross-validation (R^2_{cv}) were 0.92 (glucose), 0.89 (fructose), 0.96 (sucrose) and 0.93 (maltose) and were similar to the R^2_c for all sugars measured. The ratios of the standard deviation of the reference set to the standard error of cross-validation were greater than three for all sugars. These results confirm the applicability of the NIRS curves in efficiently determining sugar content in baked sweetpotato storage roots. External validation was performed on an additional 70 genotypes. Coefficients of determination (r^2) were 0.88 (glucose), 0.88 (fructose), 0.86 (sucrose) and 0.49 (maltose). The results were comparable to those found for the calibration and cross-validation in fructose, glucose, and sucrose, but were moderate for maltose due to the low variability of maltose content in the population.

Conclusions: NIRS can be used for screening sugar content in baked sweetpotato storage roots in breeding programs and can be used to assist with the development of improved sweetpotato varieties that better meet consumer preferences.

Keywords: sweetpotato storage roots; NIRS; high-throughput phenotyping; consumer preferences; nutritional quality; storage root

INTRODUCTION

Sweetpotato is emerging from its traditional role of a classic food security crop to a more commercialized crop.[1] Studies on sweetpotato storage roots have revealed that over 80% of β-carotene, a vitamin A precursor, is retained when boiled or steamed and few food crops can match this level.[2] The storage roots of sweetpotato contain high levels of nutrients such as carotenoids, starch, dietary fiber, vitamins A and C, and minerals, and they can be boiled, steamed, baked, or fried. Sweetpotato storage roots can be used for diverse applications, such as table use, processed foods, starch and alcohol production, and animal feed. In developed countries, sweetpotato has recently been reevaluated as a health-promoting food because of its high and balanced content of nutrients and functional components such as anthocyanins, carotenoids, and phenolic compounds, which have anti-oxidant and other beneficial activities.[3,4]

Evaluation of sweetpotato germplasm for varietal release typically focuses on agronomic traits such as yield and pack-out

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performance. However, consumer preferences can determine the success (or failure) of a new variety. Subjective consumer assessments of quality often influence a consumer's initial decision to purchase sweetpotato in the marketplace. Willingness to purchase is often based on external attributes such as appearance, color, shape, and size.[5,6] However, the decision for subsequent purchases is dependent upon consumer satisfaction based on flavor and internal quality, and how these traits relate to the soluble solids content (mainly sugars), texture and appearance of the product in addition to other sensory related factors.[6–8]

The diversity of raw storage roots brings about high heterogeneity in cooked storage roots, which creates the need to evaluate nutritional quality of cooked products to facilitate breeding for consumer preferences. Sweetpotato nutritional assessments have generally been based on evaluations of the raw storage roots without paying much attention to assessment of the nutritional quality of cooked products. Cooking methods such as baking, change the sugar composition and starch morphology of sweetpotato, sometimes resulting in a sweet aroma and a soft texture.[9,10] Free sugars (namely fructose, glucose, and sucrose) are contained in both raw and baked storage roots.[11] However, during baking, amylases act upon gelatinized starch, resulting in formation of maltose.[12]

Relatively little breeding effort has gone into breeding staple crops as better sources of vitamins and minerals, and even less so when consumer preferences are considered. Generally, the sugar composition and starch morphology of sweetpotatoes change dramatically during baking.[9] Research has revealed that fresh sweetpotato storage roots contain sucrose, glucose, and fructose[10,13] but not maltose.[10] In a study of 11 released sweetpotato varieties in Ghana, large changes in sucrose and sweetness were observed based on the different cooking methods.[14] This study recommended that cooking treatment should be taken into consideration as an important criterion when evaluating quality attributes of sweetpotato storage roots for appropriate utilization. Several factors, including maturity period, storage length, amylase activity, curing, and baking significantly influence sugar content and sweetness of sweetpotato.[14,15]

Near-infrared reflectance spectroscopy (NIRS) has been widely used to predict the chemical and physical properties of a wide range of products rapidly and precisely with minimal to no sample preparation.[16] For measuring of nutritional quality without destroying or causing change to the shape or characteristics of macromolecules, non-destructive methods such as NIRS can be used. It is a method of choice among many spectroscopic methods for the assessment of agricultural produce due to its short measurement time, low cost, simple operation, high efficiency, and repeatability.[17–19] NIRS methods have been successfully developed for the measurement of the internal quality (moisture and starch content) of raw and dried sweetpotato.[18] It has also been successfully applied to simultaneously estimate starch, protein, sugars, carotenoids, and minerals such as iron and zinc content in raw storage roots of sweetpotato, but there are no published reports of its application for sugars in baked sweetpotato. The current research focused on development and validation of NIRS calibration equations for sugars in a reference set that was subjected to baking, which is a popular method of cooking sweetpotato globally.[10,20]

MATERIALS AND METHODS

Preparation of the calibration set

A set of 248 genotypes were planted in a single replication at a spacing of 0.6 within row × 1 m between rows in 2017 at the Horticultural Crops Research Station, Clinton, NC, USA as two plants per plot and harvested after approximately 4 months of growth. These genotypes were derived from a biparental genetic mapping population [Tanzania × Beauregard (TB)]. The storage roots harvested from each plot comprised of 233 of the 248 since some genotypes produced only pencil roots that could not be processed. Immediately after harvesting roots from each plot, the roots were split into three sub-samples, which were subjected to three pretreatments: (i) uncured; (ii) cured at ∼29°C and 85% relative humidity (RH) for 1 week from the time of harvest; (iii) and stored at ∼13°C and 85% RH for 11 weeks from the time of curing. A total set of 699 samples were collected (233 genotypes × three sub-sample pretreatments) out of which 147 genotypes were randomly selected for NIRS calibration curve development. Roots from one set of samples were baked at harvest, after curing and after postharvest storage for 11 weeks, whilst another set of selected roots from the same 699 genotypes were also dry matter processed at harvest, after curing and after postharvest storage for 11 weeks. The latter set of samples were not baked before freeze drying and labelled as raw samples. Storage roots used for this study were mainly US #1 size roots, ∼4.5–8.8 cm diameter and ∼ 7.6–22.8 cm long. Before baking, the storage roots were washed, pricked with a fork six times, and wrapped in aluminium foil. One to two roots of each genotype, depending on root availability, were placed on trays, and baked in batches in a conventional oven for 90 min at 204 °C.[21] After baking, the roots were cooled and stored at 5 °C overnight to become firm. The individual storage roots were cut into two longitudinal sections. The baked root was scooped into zip-lock bags using spoons and weighed. Approximately 100 g of the processed roots (fresh weight) were stored at −20°C until freeze drying. Samples were freeze dried to < 3–4% moisture content using a VirTis SP Scientific freeze drier (24DX48 GPFD 35 L EL-85; NY, USA) and milled on a Cyclotec 1093 sample mill (FOSS 123; Hillerød, Denmark) with a 0.1 mm screen.

Reference analysis, instrumentation, and conditions

Sugar extraction

Sugars were extracted by weighing 1 g each of duplicate freeze-dried sweetpotato flour samples (making a total of approximately 1398 samples) contained in 50 mL centrifuge tubes. Next, 25 mL of ethanol, heated until boiling point, was added to the sample and the mixture was vortexed, and then vortexed again at the end of a 15 min sugar extraction period and allowed to sit at room temperature after vortexing. The samples were then centrifuged at 6500 rpm for 10 min at 20 °C and the supernatant was poured through a plastic funnel with filtered glass wool into a 50 mL volumetric flask. Each funnel was rinsed with about 2 mL of hot ethanol using a transfer pipet. Another 20 mL of hot ethanol was added to the 50 mL centrifuge tube containing the pellet residue and extracted for 10 min. The samples were then centrifuged at 6500 rpm for 10 min at 20 °C and the supernatant was poured and combined into the corresponding labelled 50 mL volumetric flask with a plastic funnel filtered with glass wool. Glass wool was pressed with a transfer pipet, and hot ethanol was added until the glass wool was clear, to complete the extraction. Additional hot ethanol was then added to the 50 mL line on the volumetric flask, and the sugar extract was poured from the volumetric flask into a new 50 mL centrifuge tube for high-performance liquid chromatography (HPLC) analysis.

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Quantitation of sugars

Sugars were analysed using a Shimadzu HPLC system equipped with an SIL-20 AC HT autosampler, DGU-20a3 degasser, LC 20 AD pump, CTO-20A column oven, and CBM-20A controller connected to an Antec Leyden model Decade II electrochemical detector running in the pulsed mode and using a gold electrode equipped with LabSolutions/LC Solution Acquisition software (Shimadzu Corp., Kyoto, Japan). Sugar separation (glucose, fructose, sucrose, and maltose) was achieved using a 250 × 4 mm CarboPac-PA1 column attached to a 50 × 4 mm CarboPac guard column (Thermo Scientific, Waltham, MA, USA). The eluent was 0.2 N sodium hydroxide (NaOH) at a flow rate of 1 mL/min and temperature of 30 °C. External calibration curve method was used to quantify sugars, and all standard curves had a goodness-of-fit coefficient of at least 0.99 with the intercept being set to zero.

NIRS analysis of chemical constituents of raw storage roots

We measured glucose, fructose, and sucrose content in the raw (unbaked) storage roots. All traits were quantified based on NIRS scan measurements. Near-infrared reflectance (NIR) spectra of the milled samples were obtained using a FOSS XDS rapid content analyser (FOSS NIRSystems, Inc, Höganäs, Sweden) and WinISI software (FOSS NIRSystems, Inc., Laurel, MD, USA). Total sugar was determined as the total of the different sugars in raw and baked storage roots.

Determination of sweetness (sucrose equivalent)

Sweetness levels for all genotypes were calculated for uncured, cured and stored treatments in both raw and baked storage roots. For raw roots the equation was: Sweetness (= sucrose equivalent) = 1.2 fructose + 0.64 glucose + 1 sucrose (based on the recognition that maltose hardly exists in raw storage roots). For baked roots the equation was: Sweetness (sucrose equivalent) = 1.2 fructose + 0.64 glucose + 1 sucrose + 0.43 maltose.[18,22]

NIRS analyses, calibration development and validation

A total of 699 samples were NIRS scanned in this study. Each freeze dried and milled sweetpotato sample was scanned by NIRS within the range of 400–2498 nm, registering the absorbance values log(1/*R*) at 0.5 nm intervals for each sample using a NIRS monochromator (model FOSS XDS, solid module) and ring cup. Calibration equations were developed using WinISI 4 Project Manager software, with spectral information from 400 to 2498 nm and using modified partial least squares (MPLS) regression and cross-validation techniques. The derivative and mathematical treatments were 1, 4, 4 and 1, where the first number is the derivative, the second the gap, and the third and fourth numbers were smoothing factors. The results of the calibration calculation were checked observing the *t*-outliers with $t > 2.0$ and GH-outliers > 4.0. The number of outlier elimination passes was two. Samples with $t > 2.0$ were deleted from the calibration file.

One hundred and forty-seven samples randomly selected from 699 samples were used for the calibration. Each time, the samples used for validation were deleted from the calibration data file. Each new validation file consisted of about 20% of the samples of the complete dataset. To get the best calibration model out of the total 699 samples with variable sugar content, several calibration equations models were developed using 147 randomly selected samples. These models showed only small deviations compared to the overall calibration equations developed for the complete sample set.

To complete the validation process independent validation was done by selecting 70 other genotypes for regression analysis between measured reference (laboratory values) and NIRS predicted values for fructose, glucose, sucrose and maltose in baked sweetpotato storage roots using R version 3.4.1. We generated histograms, boxplots and bar graphs for comparison of variability of sugar components and sweetness in raw and baked sweetpotato in uncured, cured and stored samples also using R version 3.4.1.

RESULTS

Comparison of raw and baked sweetpotato sugars in uncured, cured, and stored roots

The comparisons of summary statistics of raw and baked storage root sugar components in uncured, cured, and stored roots are shown in Fig. 1. We observed changes in both reducing (glucose and fructose) and non-reducing (sucrose and maltose) sugars in both raw and baked storage roots for the three pretreatments. There was generally an increase in sugar content for stored roots compared to cured and uncured. Mean fructose content was 0.50% dry matter (DM) in raw storage roots and ranged from 0.60% DM (uncured roots) to 0.90% DM (stored roots) in baked roots. Mean glucose content in raw storage roots ranged from 0.20% DM (uncured roots) to 0.80% DM (stored roots) and 0.80% DM (uncured roots) to 1.00% DM (stored roots) in baked storage roots. Sucrose content in raw storage roots ranged from 4.00% DM (uncured roots) to 6.00% DM (stored roots) and 3.0% DM (uncured roots) to 4.00% (stored roots) in baked roots. We observed large differences in total sugar contents among the different genotypes present in the sample population. The mean of total sugars in raw storage roots ranged from 4.00% DM (uncured roots) to 8.00% DM (stored roots), those of the baked samples ranged from 14.00% DM (uncured roots) to 17.00% DM (stored roots) in the sample population.

In Fig. 2, we compare total sugar content and sweetness (sucrose equivalent) in raw and baked for uncured, cured, and stored storage roots. The range of mean total sugar content (4–8% DM) in raw storage roots was much lower than range of mean total sugar content in baked (14–17% DM) storage roots in uncured, cured and storage treatments as already reported previously in this section. However, sweetness slightly increased from 5% DM (raw) to 8% DM (baked) in uncured; 7% DM (raw) to 9% DM (baked) in cured; and 7% DM (raw) to 10% DM in storage treatments.

NIRS calibration development

Mean values, standard deviations (SDs) and ranges of the reference values obtained by HPLC and the statistics of the NIRS calibration and of the cross-validation are provided in Tables 1 and 2. For the whole population set, glucose, fructose and sucrose showed a large range of variation while maltose showed a relatively narrow range (Table 1). A subset of 147 sub-samples were selected to make a calibration curve for comparison purposes. NIRS calibration equations based on both the whole population and sub-population, resulted in high coefficients of determination for the calibration (R^2_c) for glucose, fructose, sucrose and maltose, with maltose having the lowest R^2_c value at 0.81 (Table 1). The NIRS calibration for maltose developed on the basis of the subpopulation resulted in higher R^2_c of 0.96 which was very close to those of glucose ($R^2_c = 0.96$), fructose ($R^2_c = 0.93$) and sucrose ($R^2_c = 0.96$), as shown in

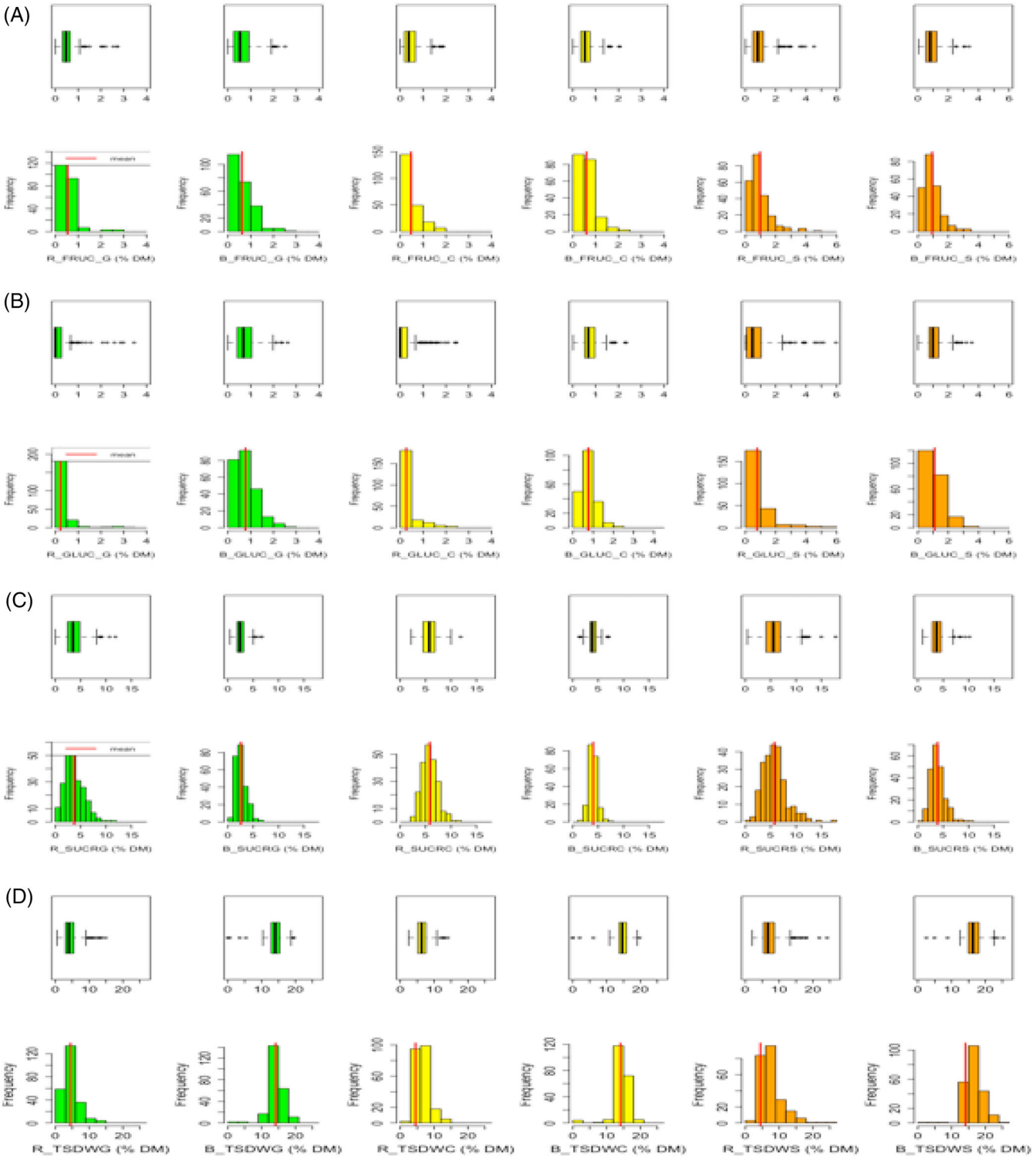

Figure 1. Boxplots and histograms comparing distribution of raw (R) and baked (B) storage root (A) fructose, (B) glucose, (C) sucrose, (D) and total sugar content in uncured (G), cured (C) and stored (S) samples of TB mapping population.

Table 2. The coefficients of determination for cross-validation (R^2_{cv}) for all traits were approximately equal to those of R^2_c as shown in Tables 1 and 2. The ratio of SDs to standard errors of cross-validation (SECVs) based on the sub-population were 3.41, 3.15, 4.76, and 3.97 for glucose, fructose, sucrose and maltose, respectively (Table 2).

External validation

When the validation of the NIRS calibrations using the equation of the 147 sub-samples to predict 70 samples outside of the calibration set was done, the coefficients of determination for glucose ($r^2 = 0.87$), fructose ($r^2 = 0.87$), sucrose ($r^2 = 0.88$) and maltose ($r^2 = 0.49$) in external validation (Fig. 3) were highly comparable

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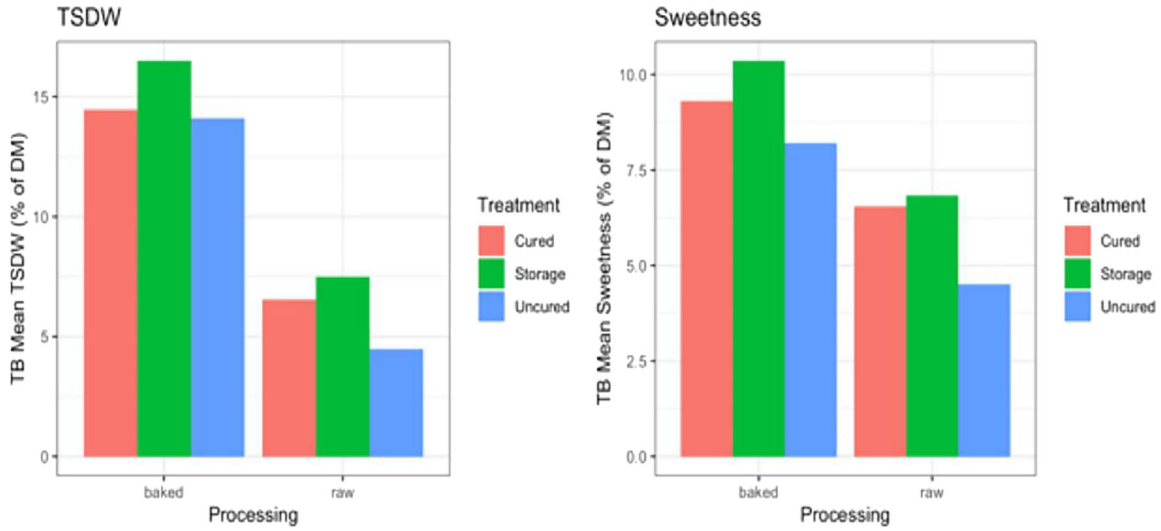

Figure 2. Bar graphs showing comparison of total sugars (TSDW) and sweetness (sucrose equivalent) in raw and baked (uncured, cured and stored) storage roots.

Table 1. Reference values, near-infrared reflectance spectroscopy (NIRS) calibration and cross-validation statistics for sugars in baked sweetpotato roots from the Tanzania × Beauregard (TB) mapping population

		Reference values			Calibration		Cross-validation		
Trait	*n*	Range (% DM)	Mean (% DM)	SD (% DM)	R^2_c	SEC (% DM)	R^2_{cv}	SECV (% DM)	SD/SECV
Glucose	561	0.00–2.14	0.80	0.45	0.97	0.08	0.96	0.09	5.00
Fructose	556	0.00–2.08	0.65	0.48	0.98	0.07	0.97	0.08	6.00
Sucrose	575	0.00–7.10	3.29	1.27	0.96	0.26	0.95	0.27	4.70
Maltose	569	6.57–13.86	10.22	1.22	0.84	0.48	0.81	0.53	2.30

Note: DM, dry matter; *n*, number of samples; R^2_c, coefficient of determination for calibration; R^2_{cv}, coefficient of determination for cross-validation; SEC, standard error of calibration; SD, standard deviation; SECV, standard error of cross-validation.

Table 2. Reference values, near-infrared reflectance spectroscopy (NIRS) calibration, and cross-validation statistics for sugars in baked sweetpotato roots from a subset of the Tanzania × Beauregard (TB) mapping population

		Reference values			Calibration		Cross-validation		
Trait	*n*	Range (% DM)	Mean (% DM)	SD (% DM)	R^2_c	SEC (% DM)	R^2_{cv}	SECV (% DM)	SD/SECV
Glucose	114	0.00–2.01	0.78	0.41	0.96	0.08	0.92	0.12	3.42
Fructose	125	0.00–1.86	0.62	0.41	0.93	0.11	0.89	0.13	3.15
Sucrose	122	0.00–7.44	3.31	1.38	0.96	0.26	0.96	0.29	4.76
Maltose	131	0.98–18.54	9.76	2.93	0.96	0.60	0.93	0.75	3.91

Note: DM, dry matter; *n*, number of samples; R^2_c, coefficient of determination for calibration; R^2_{cv}, coefficient of determination for cross-validation; SEC, standard error of calibration; SD, standard deviation; SECV, standard error of cross-validation.

to those found for the calibration and cross-validation except for maltose (Table 2).

DISCUSSION

There are great opportunities to expand consumer demand for sweetpotato both as a staple food and for value-added food product purposes. This requires the development of products from storage roots that meet consumer preferences. Screening for sugars in raw roots is useful, but chemical changes occur during and after cooking that influence consumer preference. To bridge this gap, breeders have been searching for ways to screen cooked sweetpotato to facilitate product development just as in the case of rice,[23,24] and potato.[25] NIRS is a rapid and non-destructive technology that does not require use of chemicals or reagents for estimation of the physical and chemical properties of samples.[16] The use of NIRS has been reported to be efficient in characterizing and estimating a large germplasm collection with relatively high

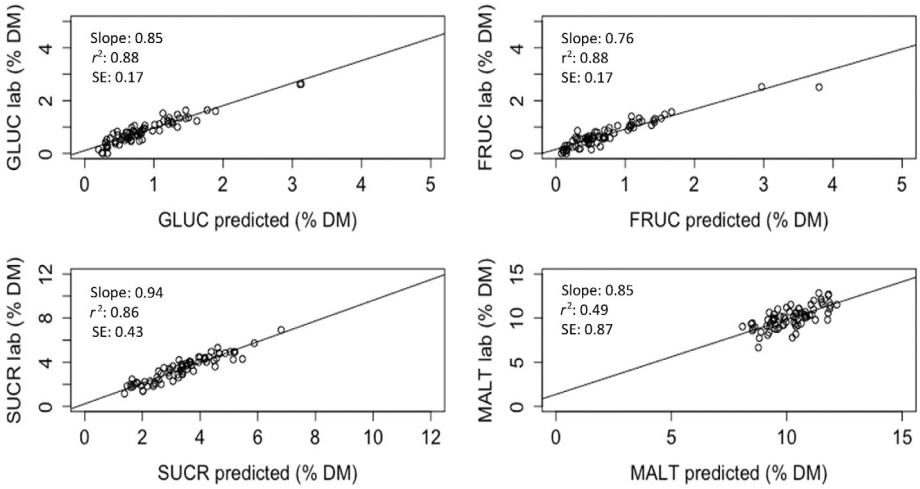

Figure 3. Relationship between reference (lab) and near-infrared predicted values of glucose (GLUC), fructose (FRUC), sucrose (SUCR) and maltose (MALT) for baked uncured, cured and stored storage roots of Tanzania × Beauregard (TB) genetic mapping population. Regressions are based on a set of 70 samples different from those used for the calibration equations.

beta-carotene concentrations and other traits (such as starch, protein, sugars, iron, and zinc) at low cost and in reduced time compared to HPLC and other chemical techniques.[26]

Major changes occurred in starch content composition in raw storage roots after baking, which demonstrates the fact that starch is hydrolysed to sugars in raw sweetpotatoes. The major product obtained after starch hydrolysis in cooked roots is maltose, which affects sweetness and hence flavour. Production of maltose caused a large difference in total sugars between raw and baked storage roots for all three treatments. Undetected levels of maltose have been reported in raw storage roots compared to high maltose content in baked roots.[9,10,27] Sucrose content was slightly lower in baked as compared to raw storage roots, which could be attributed to sucrose degradation because of thermal treatment.[9] It has been reported previously that caramelization of sucrose not only changed the colour of storage roots but also converted sucrose to form oligomers and polymers.[10] In our studies, sweetness in raw roots was largely dependent on sucrose content since it is the main sugar in raw storage roots. Maltose produced from baked storage roots because of starch hydrolysis increased sugar content, but only slightly increased the sweetness in baked roots based on our analyses (Fig. 2).

Using a segregating mapping population with genotypes of varying sugar, starch and α- and β-amylase levels, calibration curves with very high accuracy with reference to high coefficient of determinations in calibration of 0.96 for glucose, sucrose, and maltose and 0.93 for fructose were developed. The corresponding coefficients for cross and external validations were very high ranging from 0.89 to 0.96. Furthermore, the SD/SECV ratios obtained for the equations were all greater than three which establishes the usefulness and applicability of the developed calibration curves for the assessment of glucose, fructose, sucrose and maltose content in baked sweetpotato roots. Bonierbale *et al.*[26] developed NIRS calibration curves for total and individual carotenoid concentrations in *Solanum phureja* potatoes using 167 selected samples. They reported medium to high coefficients of determination for calibration set (R^2_c) (0.65–0.93) and approximate coefficients of determination for cross-validation (R^2_{cv}) (0.56–0.93) with higher values for total carotenoids and zeaxanthin, similar to the results obtained in this study. We anticipate that these methods will be very useful in sweetpotato breeding programmes focused on developing new cultivars with improved culinary and processing qualities. The SD/SECV ratios have been reported to show the usefulness of NIRS calibrations for sugars in baked storage roots in breeding programmes. If this ratio is greater than a value of three the calibration equation is considered very effective. However, if this is between two and three, the applicability is limited.[26,28,29] In the development of calibration curves for total and individual carotenoids, the values obtained for SD/SECV ratios were 3.3 and 4.2 for total carotenoids and zeaxanthin respectively[26] and they were similar to the SD/SECV ratio values (3.15–4.76) obtained for fructose, glucose, sucrose and maltose (Table 2). Generally, NIRS calibration model validation depends upon the modelling approach, the materials and the process used to develop the calibration set.[30] The research materials used in the development of our calibration models for sugars in baked samples were genotypes from the TB mapping population in which the parents significantly differed in sugars in both raw and baked sweetpotato, except in the case of maltose. This helped in the development of a robust calibration model and resulted in very strong coefficients of determination.

The development of NIRS calibration curves for glucose, fructose, sucrose, and maltose in baked sweetpotato is important for screening for genotypes with different levels of sugars in breeding and mapping populations. This is an important step forward in breeding for genotypes with end-user preference especially when working in large breeding populations. In varietal release pipelines, testing for sugars in both raw and baked samples is a pre-requisite for the release of varieties tailored toward the needs of the end-user. The NIRS calibration curves developed from this study will facilitate the screening of genotypes with varying levels of fructose, glucose, sucrose, and maltose that affect sweetness in baked sweetpotatoes. These curves should enable breeders to evaluate the differences between sugar levels of both raw and baked roots of sweetpotato to determine their variability in sugar content without resorting to wet chemistry procedures such as HPLC which is very expensive and time-consuming especially when dealing with very large sample numbers. However, there is the need to improve the calibration curves

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developed from time to time by testing on different breeding populations and inclusion of new genotypes and environments. More emphasis on improvement of the developed NIRS calibration curve for maltose is needed due to the moderate coefficient of determination values obtained for external validation as a result of the relatively low variability of maltose content of genotypes in the TB mapping population.

In conclusion, this study represents a major step forward for rapid evaluation of sugar content in baked sweetpotato storage roots using the NIRS calibration curves developed. However, the calibration curves developed are not applicable for the determination of sugar content in other baked root and tuber crops.

AUTHOR CONTRIBUTIONS

Field establishment, baking, dry matter processing and NIRS analysis were conducted by Victor A. Amankwaah, Sharon Williamson, Rong Reynolds, Xiaofei Zhang, Ragy Ibrahem, and Kenneth V. Pecota. Victor A. Amankwaah, Ragy Ibrahem, and Thomas Zum Felde worked on the NIRS calibration and data analyses. Victor A. Amankwaah wrote the manuscript as part of his doctoral dissertation. Reuben Ssali, George Craig Yencho, Bode A. Olukolu, Van-Den Truong, and Edward Carey reviewed and gave guidance on manuscript preparation, George Craig Yencho conceived the study, supervised, coordinated, managed and guided it.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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