

A high throughput phenotyping technique for banana cultivar Sukali Ndizi based on internal fruit quality attributes

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Abstract

Background: *Sukali Ndizi* quality traits such as Total soluble solid (TSS) content, pulp texture and sugar/acid (S/A) ratio are critical in quality assessment. Screening very large numbers of fruit genotypes has prompted the development of a high throughput method using Near Infrared spectrometry (NIRS).

Results: The calibration procedure for the attributes of TSS, pulp texture and S/A ratio was optimized with respect to a reference sampling technique, scan averaging, spectral window, data pre-treatment and regression procedure. Calibration equations for all analytical characteristics were computed by NIR Software ISI Present WINISI using Modified Partial Least Squares (MPLS) and Partial Least Squares. The quality of calibration models were evaluated by Standard Error of Calibration and coefficient of determination parameters between the measured and the predicted values. The results obtained with FOSS NIR systems 2500 spectrometer (model DS 2500) using the 350-2500 nm range, showed good prediction of the quality traits TSS content, pulp texture and S/A ratio. The MPLS method produced satisfactory Calibration model performance for TSS, texture and S/A ratio, with typical R^2 of 0.73%Brix, 0.69kgf and 0.7; and root mean squared standard error of calibration of 0.73%Brix, 0.25kgf and 5.36 respectively. This is a good set of quality traits predicting *Sukali Ndizi* quality with NIRS with robustness, as it was obtained by using diverse *Ndizi* populations.

Conclusions: This can be a useful tool to phenotype large numbers of *Ndizi* hybrids per day, making it possible to reduce on the resources spent when utilizing organoleptic evaluation selection technique.

Keywords: *Sukali Ndizi*; Phenotyping; Platform; Quality traits

1 Introduction

Banana (*Musa spp*) is one of the world's most important fruits. In 2011, 145 million metric tons, worth an estimated \$44 billion were produced in over 130 countries [1]. It is widely consumed, with about ninety percent of production consumed in or around the production areas in Asia, Latin America and Caribbean (LAC) and Africa [2], and LAC representing 80% of global exports [3]. Dessert Banana has been used in other forms such as the production of purees, jams, wines, pastries, desserts, sorbet ice-creams, dried slices, and cream products [4, 5, 6].

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Apple banana (*Musa spp.*, AAB genome group) is the most widely distributed dessert banana cultivar in Uganda [7]. It is locally known as *Sukali Ndizi* and Kabaragara in the central and Western regions of Uganda respectively [8]. Apple banana is known for its small fruit, a thin peel, a slightly acidic apple-like taste of the pulp, an orange appearance and a firm pulp texture which are the unique characteristics [5, 9]. The dessert banana *Sukali Ndizi*, has been commonly sold and consumed fresh, but of late, it is being processed by a number of private sector and other development partners to improve shelf life and value addition for export [5].

The emergence of new pests and diseases (like fusarium wilt), need of high yielding genotypes to feed the ever increasing population as well as changes in consumption habits from rural to more urban lifestyles, compel breeders of bananas (*Sukali Ndizi*) to develop new varieties better adapted to biotic and abiotic stresses at farm and at post-harvest levels, consumers, exporters and agro-industrial value chains [10]. For the last two decades, many new *Sukali Ndizi* substitute varieties FHIA-01, FHIA-17, FHIA-23 and FHIA-25, have been developed and deployed [11]. Significant and relevant progress has been made in improving yields and the stability of performance through resistance to biotic and abiotic stresses; these include FHIA-01, FHIA-17, FHIA-23, and FHIA-25, SH-3640/10, Yangambi km5 and kikundi [5]. The deployments of new varieties have met with strong resistance both on farm and market levels due to the weaknesses in terms of end-use quality. Due to allelic segregation and independent assortment, this process requires making many crosses to be able to come up with a single plant that could have all the combination of the required fruit quality as demanded by the consumers, thus need for effective selection from a wide segregating population. Although assembling the necessary genetic resources is a challenge, conventional phenotyping the population is widely recognized as the most laborious and technically challenging part of this process [12]. Because sorting large numbers of fruit in a minimum of time and efforts is a concern for breeders and wholesalers, thus non-destructive techniques such as near infrared spectrometry (NIRS) have received lots of attention in the two past decades [13]. A strong interest for spectrometry, as a non-destructive technique, is that information can be collected on the same fruit, at different times. This means that it gives access, not only to punctual information, but also to the dynamics of the measured characteristics [14]. Regarding bananas, Vis/NIRS has been used to measure carotenoid contents in fruit from 28 *Musa* (banana and plantain) varieties [15]. These outcomes have obvious selection applications as correlating a spectral signature to *Sukali Ndizi* quality has a great importance for breeders. However, [16] reported on banana fruit quality and maturity stages using hyperspectral imaging, the coefficient of determination was found to be 0.85, 0.87, and 0.91 for total soluble solids, moisture and firmness of the banana fruits, respectively. Wahyuni et al [17] worked on Bananas moisture content prediction system using the Visual-NIR imaging technique and the prediction error between predicted and measured data with PCR was 0.58 % and produce correlation coefficient R^2 of 0.79. The PLSR model of banana content prediction system had RMSE 0.25% and R^2 0.96.

Germplasm screening relies on the accurate phenotyping of the trait in question. The available phenotyping procedures such as Organoleptic/Sensory evaluation are inefficient in terms of costs, time consuming, labor intensive and considerable subjective faults [12]. Breeding programs need to screen large numbers of genotypes for agronomic, nutritional quality and end-product quality traits to select the best ones for the next breeding and selection cycles. To consistently assess end-product quality, it is important to apply high-through put, indirect phenotyping methods that efficiently predict end-product quality traits economically and timely, thus successful inclusion of end user traits in the selection process.

A strong interest for spectrometry (as a non-destructive phenotyping technique), is that information can be collected on the same fruit, for all the quality traits at the same times, which means that it gives access, to punctual information and wholesomeness. The banana breeders can therefore benefit from non-destructive technology that rapidly and precisely predicts the quality parameters of *Sukali Ndizi* fruits. Current technologies that applied non-destructive quality measurement in bananas include Magnetic Resonance Imaging (MRI) [18], Fourier Transform Infrared (FTIR) [19], Laser-Induced Fluorescence spectroscopy (LIFS) [20], Time-Resolved Reflectance Spectroscopy [21], Proton Transfer Reaction Mass Spectrometry (PTR-MS) [22] and by Capacitance technique [23]. However, these are only on proof of concept and have not reached practical applications. Measurement of quality parameters based on NIRs can be promising with a wide range of applications.

NIR spectroscopy is uniquely qualified to provide analysis capacities to the fruit and related industries, through its interaction with the organic molecular material of fruits. Fruit is organic, containing C-H, C-OH and C-N-H and others physical features like firmness. These bonds and physical features interact in a measurable way with the NIRS portion of the spectrum. NIRS absorption bands are produced when NIR radiation at specific frequencies resonates at the same frequency as the molecular bond in the test sample. This allows association of a specific wavelength with a specific chemical bond /physical matrix vibration generating a specific spectrum that in turn is related to concentration of a specific component. NIRs creates a faster, safer work environment and do not require chemicals [24, 25].

In the current work, the interest was in developing methodologies for the high-throughput analysis of fruit Total soluble solutes (TSS) contents, Fruit pulp texture and sugar/Acid ratio as encountered in breeding and germplasm-screening programs. For this, a study was carried out to evaluate the potential of near-infrared spectroscopy (NIRS) to screen for TSS, pulp texture and sugar/acid ratio contents in fruit from a wide variety of *Sukali Ndizi* ecotypes. The research provides a useful reference and suggests a variety of uses for NIRs on agricultural products. The NIR spectroscopy has been observed as a useful new technique for internal quality evaluation and assessment of *Sukali Ndizi* fruit. This operation is of interest to breeders when selecting *Sukali Ndizi* highbred with consumer acceptable qualities (traits) at early evaluation trail and Preliminary yield trail level of selection process. Therefore, the objective of the present work was to develop NIRs calibration models for prediction of *Sukali Ndizi* quality parameters which are Pulp texture, Total soluble solutes, and Sugar acid ratio.

2 Material and methods

2.1 Fruit sample sets

Four sample sets of *Sukali Ndizi* (*Musa* genome AAB) fruits were organized for this study, each having a different region of origin (Table 1). The *Sukali Ndizi* used in the reported work were selected from the four major markets selling *Sukali Ndizi* from the major producing regions of Uganda, based on the need to cover a range of *Sukali Ndizi* composition content as wide as possible.

Table 1 Overview of the different *Sukali Ndizi* ecotypes analyzed and general descriptors

Source No.	Source (Ecotype)	Variety	Age of bunch at harvest	Period when harvested	Size band (grams)	No. of fruits analysed per bunch	Ripening stage	Scans per sample	Number of bunches
1	NARL Kawanda NBRP fields	Ndizi hybrids (NAMU1, NAMU2, NAMU3, NAMU4)	Two/three finger ripen of the first hand	From November-August 2019 from different fields	78g-98g	4 fruits randomly selected	7	6	9
2	Western Uganda (Mbarara, Ntungamo, Nyihanga and Buyanja)	Landrace Sukali Ndizi	18 weeks (physiological maturity with rounded edges)	Purchased in the retail market period of November-August 2019 probably from different farms	68g-85g	4 fruits randomly selected	6 & 7	6	10
3	Central Uganda (Maska, Kiboga, and Luwero)	Landrace Sukali Ndizi	17-18 weeks (physiological maturity with rounded & sharp edges)	Purchased in the market period of November-August 2019 probably from a different plantation	55g-77g	4 fruits randomly selected	7	6	19
4	NBRP fields at NARL Kawanda	Landrace Sukali Ndizi	Two/three finger ripen of the first hand	From November-August 2019, all from the same field	69g-80g	4 fruits randomly selected	7	6	13

2.2 Spectral acquisition

One thousand two hundred twenty four (1,224) scans were taken using spectrum FOSS NIR systems 2500 spectrometer (Model DS2500) to acquire absorbance spectra in the range of wavelength 350-2500 nm. Each peeled banana was cut into three sections, the proximal, middle and distal. Each of the three parts were sliced into discs of unequal sizes because of the shape of the fruit and placed into the small ring cup for better packaging and interception of all the light from the source. For every single sample, 6 sub spectra were collected.

2.3 *Sukali Ndizi* Physio-Chemical analysis

2.3.1 *Fruit pulp texture*

Ten (10) disease free ripe bananas were randomly selected from each bunch and peeled. The peeled pulp firmness was measured using a Texture analyzer (TMS-Pilot Food Technology Corporation) using a kraft knife probe. Firmness, defined as maximum force (kgf) required until tissue failure [23], measurements were taken at the middle part between proximal and distal end of the banana.

2.3.2 *Total Soluble Solutes assessment*

To determine the TSS of the scanned banana, pilled fruits were juiced using a commercial fruit blender (8011E Model 38BL41 Made USA). Fresh banana sample fifty (50g) grams were diluted in 50 mls of distilled water and blended for 1min until homogeneous juicy pulp was obtained. The juice pulp was centrifuged at 6000 rpm for 6 min using a Hitachi centrifuge (Hitachi Germany). A drop of supernatant from each sample was placed onto a refractometer to measure the soluble solids levels in %Brix. The value was corrected for sample dilution to give the final TSS measurement, done by multiplying the reading by a factor of 2 [26].

2.3.3 *Titrateable Acidity*

Titrateable acidity (TA) was determined from the above juice, using 10mls mixed with 50mls of deionized water [27]. Three drops of phenolphthalein was added to the juice/water solution in a beaker from a dropping pipette. The solution thereof was titrated with 0.1M NaOH until the solution turns pick, which marked the end point and the amount of NaOH used (titre) was recorded. The TA was expressed as malic acid equivalent (g malic per 100g fresh weight) (AOAC. 1990). The sugar/acid was calculated using equations 1 & 2.

$$\text{Acid (\%)} = \frac{\text{Titre X Acid Factor X 100}}{10 \text{ (ml Juice)}} \dots\dots\dots 1$$

$$\text{Sugar acid ratio} = \frac{\text{°Brix Value}}{\text{Percentage Acid}} \dots\dots\dots 2$$

2.3.4 *NIRS Calibration and Statistical Analysis*

Data sets of NIRS and constituents data were created for combined four sample sets with 6 subsamples (different banana parts, the proximal, middle and distal) pooled or averaged, and this dataset was used for calibration models. NIRS calibration models were established by using WinISI software version— (Infrasoft International, Port Matilda, PA, USA). To improve the accuracy of the calibration, before the analysis of the samples by removing the outliers from the dataset, the whole dataset was taken through detection of anomalous spectra by using the Mahalanobis distance (Global H statistics, GH). To optimize the calibration equations, various scattering pre-treatments, which include Standard normal variant + detrending, (SNV + DT), SNV only and DT only, were carried out to eliminate the influence of scatter phenomena. The mathematical pre-treatment used to fit the better calibration were “1, 2, 2, 1” & “2, 4, 4, 2”, where the first digit is the number of the derivative, the second one is referred to the gap over which the derivative is calculated, the third one is the smoothing segment, and the last one is the secondary smoothing segment [28]. Modified partial least square loadings (MPLS), partial least square loadings (PLS), and principal component analysis (PCA) were evaluated for the three *Sukali Ndizi* quality parameters. The best models were selected on the basis of the highest coefficient of determination (R²), and the lowest standard error of calibration (SEC) [29].

3 Results

3.1 *Sukali Ndizi* Physio-chemical parameters composition

A statistical summary of the range, average (mean), SD, and coefficient of variation (CV) for TSS, pulp texture and sugar/acid ratio contents determined by conventional methods are presented in Table 2.

The measured values varied considerably in the three parameters for all examined samples as shown by the range and CV given in Table 2. It was observed that variation in TSS was smaller than those observed in sugar/acid ratio and pulp texture parameters. The total measured TSS content varied from 22.8%Brix to 29.2%Brix, sugar/acid ratio 74.32 to 123.02 and pulp texture from 1.64kgf to 3.88kgf.

Table 2 Physio-chemical parameters of analyzed Sukali Ndizi fruit samples

Parameters	Range	Mean	SD	CV	Samples analyzed
TSS (%Brix)	6.4	26.212	1.398	5.333	51
Texture (kgf)	2.866	2.24	0.442	19.732	510
Sugar/Acid ratio	48.7	95.96	9.761	10.172	51

SD: Standard deviation of data; CV: Coefficient of variation.

3.2 NIRS Calibration Models

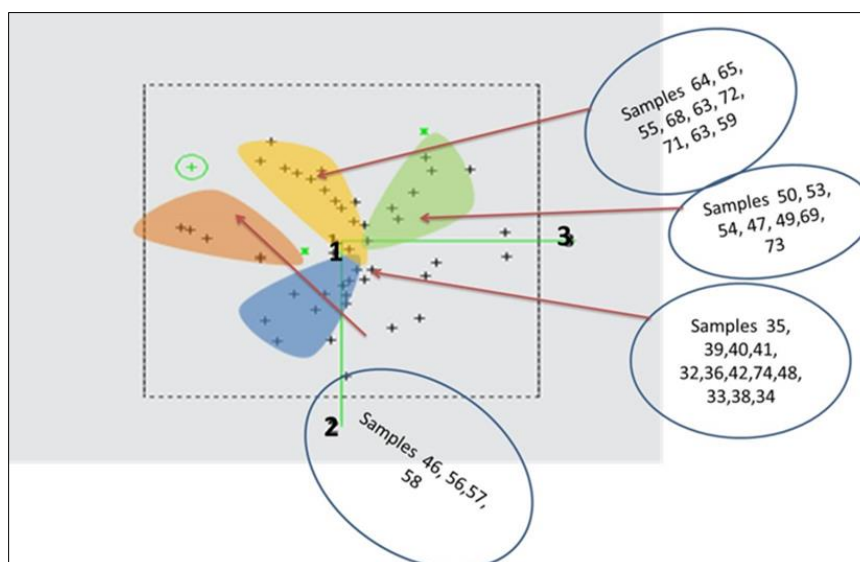
3.2.1 Spectra Analysis

The typical NIR spectra for all the 51 *Sukali Ndizi* was generated (Fig 3s). When the raw spectra was subjected to 1st derivative pretreatment with accompanying mathematics, and also 2nd derivative pretreatment with the accompanying mathematics the clarity of the peaks differed (Table 3 & Fig 3s). When first derivative was applied, the intensity of the absorption peaks was not coming out clearly and models were not good. When second derivative was applied, the intensity of the absorption peaks was very clearly standing out and the models improved (Table 3 & Fig 3s). The spectrum for the respective ecotypes were similar and all show 6 broad absorption peaks around the 438, 963, 1188, 1450, 1788 and 1915 nm regions. The band at 1188 nm is related to the C-H second overtone from carbohydrates [31] an absorption band at 1450 nm relating to the second overtone of O-H of water [32], absorption bands at 1450 and 787 nm are related to glucose, fructose and sucrose [33]. Lastly, the 1915 nm band represents the O-H stretch and O-H deformation combination and O-H bend second overtone related to moisture [34].

3.3 Spectra pretreatment methods

3.3.1 Results Based on Full Wavelength

PCA analysis was conducted for fruit pulp spectra to explore and visualize their trends based on full wavelength spectra. The principal component analysis was performed on the raw spectra; the score plot of the three principal components coming from the analyses of the 51 banana fruits raw spectra is presented in Figure 1.

**Figure 1** Scattering data of PCA Scores of 51 averaged samples of *Sukali Ndizi* Bananas showing four distinct clusters

PCA scores plot shows the position of each *Sukali Ndizi* fruit in the determined PC1–PC2 space. Spectra that had similar spectral characteristics were close to each other (Fig. 1). For all the constituents namely TSS, S/A ratio and texture, and the 2,4,4,2 was found to give good results and the best model was achieved with MPLS (Table 3) which can be applied in field selection. The quality of the calibration processes was assessed by looking at two statistical parameters (SEC and r) and used to determine the calibration equation (Table 3).

Table 3 Data pretreatments and calibration model for quality parameters

Parameters	N	mathematics	Scattering pretreatment	Regression method	SEC	Rc ²	N Cal	PLS terms retained	SE Laboratory
TSS	278	2,4,4,2	SNV + DT	MPLS	0.73	0.73	278	10	0.196
TSS	278	2,4,4,2	SNV+DT	PLS	0.94	0.54	265	10	0.196
Texture	278	2,4,4,2	SNV + DT	MPLS	0.25	0.69	278	10	0.020
Texture	278	2,4,4,2	SNV+DT	PLS	0.32	0.32	278	10	0.020
Sugar/acid ratio	278	2,4,4,2	SNV + DT	MPLS	5.36	0.70	275	10	1.367
Sugar/Acid ratio	278	2,4,4,2	SNV+DT	PLS	7.13	0.44	264	10	1.367

SEC: standard error of calibration; Rc²: coefficient of determination of calibration; SNV+DT: standard normal variate + detrending; MPLS: modified partial least square loadings, NCal; Calibration sample after PCA algorithm

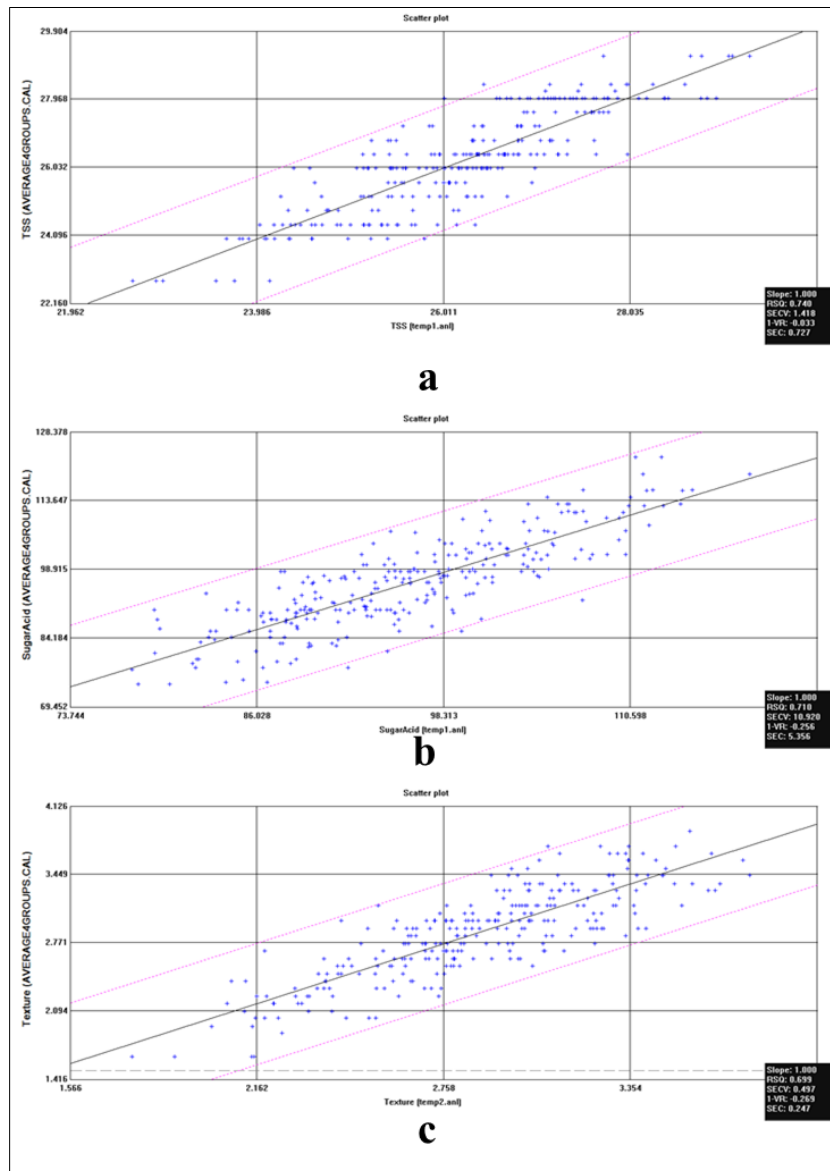


Figure 2 Calibration plots for: TSS content of the juice (^oBrix) (a); S/A ratio (b); and texture of whole fruit in Kgf (Nmm⁻¹) (c)

The calibration models for TSS, S/A ratio, and texture based on the optimal mathematical pretreatments, scattering processing, and regression method are summarized in Table 3, showing R_c^2 values corresponding to the predictions for the TSS, Pulp texture, and sugar/acid ratio were ≤ 0.70 , were higher, and their SEC equivalents were lower when MPLS technique was applied.

For all three quality parameters (reference data), the R^2 values were higher when using MPLS for calibration model development (Fig 2a, Fig 2b and Fig 2c) than when using PLS. Similarly, the SEC values were lower when MPLS was applied compared to PLS where the same values were relatively high, showing that MPLS gave better results than PLS for calibration model development.

4 Discussion

The distributions of the obtained physio-chemical values for each component measured by conventional methods shows that the distributions of the three parameters were a normal distribution and covered the full range of the constituents investigated (data not shown), meaning that the overall collected samples was suitable for constructing NIRS calibration equation (Table 2). In general, the TSS, pulp texture, and sugar/acid ratio contents exhibited by the samples in this study are consistent with those reported in [30].

As far as sample preparation was concerned, the non-uniformity of the discs could have made packing the sample in an uneven manner thus creating some air spaces and varying tissue sizes of the samples. Small sample-to-sample differences of a sample series can cause some spectral differences and these differences may have negative effects on the calibration models

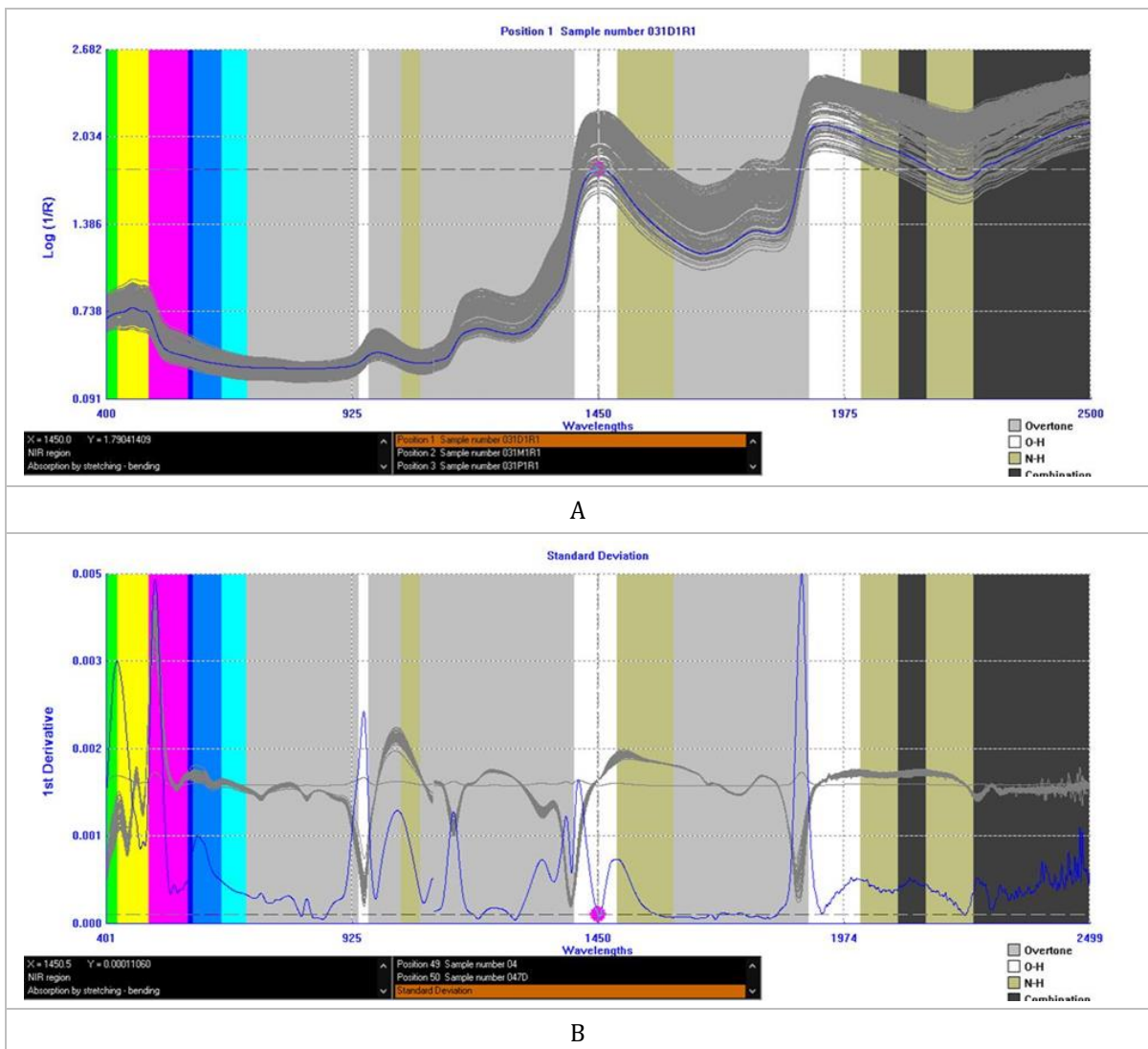
For PCA, only the first PC accounted for majority of the total variance, with second one and third PCs explaining a small portion. It was clearly shown in the scores plot that application of PCA yielded a distinct separation with obvious clustering of four well defined groups of fruits according to the general descriptors mentioned in Table 1. As expected, a part of some samples overlapped in all the clusters, and some of the samples could not be clustered together. This illustrated that the PCA was not sufficient for diversity (corresponding to size, harvest time, geographical region and cultivar/ or ecotype) classification and more complex supervised classification methods should be applied to improve classification accuracy. But it can be observed that this population used to derive the calibration equation had sufficient variability within the population in respect to the parameters of interest (Table 1).

For all quality parameters assessed (Table 3), the MPLS lower SEC and high R^2 values than for PLS signifying the efficiency of MPLS technique for model building. It is widely accepted that to have a good model, the SEC value should be kept low and R_c^2 which is correlation between predicted and measured should be the highest [35].

In this study, lower results for the SSC and pulp texture predictions were obtained, probably due to the pretreatments applied to the data 2,4,4,2, SNV+DT and MPLS regression method. The calibration processes were getting closer to the best series published so far on Cavendish bananas by [26] except for sugar/acid ratio prediction which was not tested before in their work. Improvement on the results can be achieved by including other pretreatment methods, mathematical treatment with derivative and smooth and the combination of scatter correction and mathematical treatment. For example [36] while working on rice cooking characteristics amylose content (AC), gel consistency (GC), alkali spread value (ASV), the NIRS models developed using the first derivative were generally superior to those developed using the second derivative for \log_1/R of rice flour spectra. For AC, the effect of the '1, 4, 4, 1' was better than the control and the '2, 4, 4, 1', which was similar to SNV+D. The combination method, with SNV+D/'2, 4, 4, 1', showed better effects than their two individuals. Furthermore, the methods of '1, 4, 4, 1' and SNV+D/'1, 4, 4, 1' showed the best effects on the AC models. MLR, partial least square (PLS) or other multivariate techniques are typically employed in the non-destructive assessment of fruit quality through correlation with the NIR spectra [26]. Many variables like temperature, geographic region, harvesting time, cultivar, data pre-treatment and model algorithm could affect the performance of a predictive model [37, 26]. A calibration model is deemed useful when the predictions within the population which it was developed can be transferred to new populations i.e., the calibration is robust [38]. In this study, the combined sample set contains a large variation in the constituent being modeled as well as other fruit properties, other than the constituents measured. For example, the fruits in the combined set varied in the fruit size, maturity age at harvest, originated from different agro-ecological zones within different plantations, cultivar used were *Sukali Ndizi* (landrace) and *Sukali Ndizi* hybrids harvested over a period of four months (August-November) same year. Here, the study presented calibration data set that exhibit large variations in fruit origin, harvest age, cultivar and size, as well as large variation in the constituent being modeled. The robust model for prediction of the attributes of intact fruit requires the use of calibration populations that exhibit large variation in fruit origin, storage age and size, as well as large variation in the constituent being modeled [39]. This implies that the calibration models developed with this kind of

population characteristics, which was because of combining the four distinct populations described earlier are robust in respect to time/season, across cultivar, ecotypes/growing districts in general the calibration models are perfectly robust in the prediction of the attributes and breeders can comfortably employ this technique in phenotyping. Basing on the above augments, the models may be robust enough to accurately predict the quality attributes in samples of *Sukali Ndizi* hybrids. In this study, the performance of the calibration model for texture was not as good as TSS and S/A (Fig 2a, Fig 2b and Fig 2c). [40] Stated that the accuracy of calibration models for firmness is usually worse than for SSC. This is because the firmness is a physical parameter that has limitation due to the changes in pectin and water absorbance bands compared to chemical parameter of SSC [26]. Nicolai et al. [41] suggested that better prediction for firmness can be obtained by separating the contributions of scattering and absorption based on time or spatially resolved techniques. [42] Developed calibrations to predict SS using 4 cultivars as combined calibration populations for 3 seasons and concluded that the cultivar or season or season calibration can be successfully employed to predict the SS content of fruits from different cultivars in different seasons.

Supplementary material



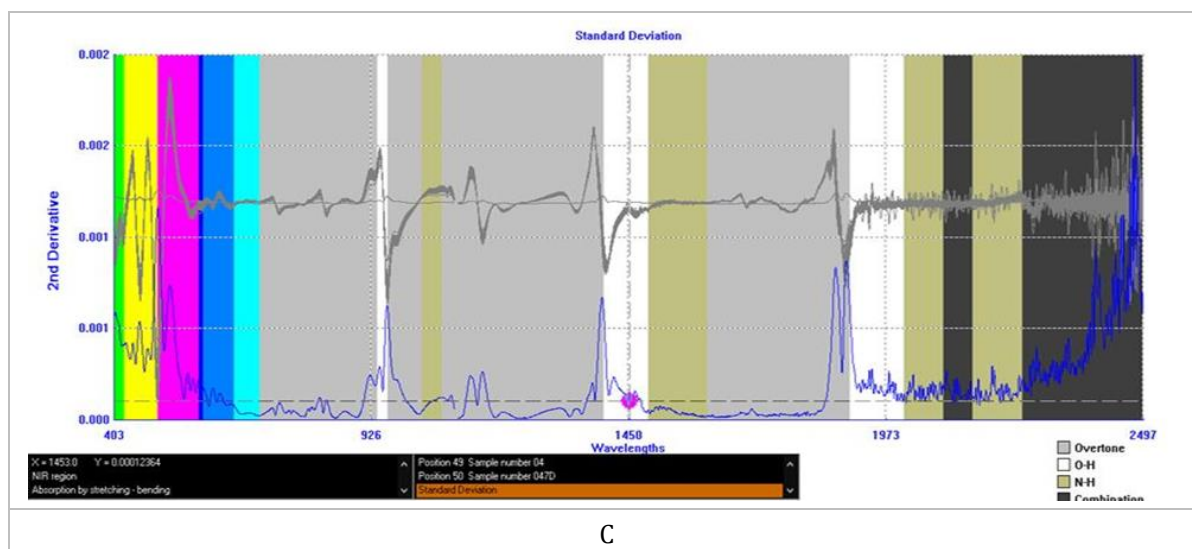


Figure S3 Typical *Sukali Ndizi* absorbance ($\log (1/R)$) spectra between 350-2500nm (a) on Raw spectra (b) Spectra after treatment with first derivative at 1,2,2,1 (c) Spectra for second derivative at 2,4,4,2

5 Conclusion

In the current study, the feasibility of NIRS to predict the quality traits determining parameters in *Sukali Ndizi* has been evaluated. Results have demonstrated that NIRS technology has good predictive performance for some quality traits determining parameters TSS, pulp texture and sugar/acid ratio, and can be employed in breeding selection (phenotyping) of *Sukali Ndizi* hybrids based on consumer preferred quality traits as an alternative to traditionally organoleptic methods. Considering the spectra information that it provides, NIRS technology could be very useful for the classification of *Sukali Ndizi* based on same qualitative aspects, such as TSS, pulp texture and sugar/acid ratio, which is crucial to *Sukali Ndizi* consumers. The present findings also indicate that the calibration is satisfactory for screening the *Sukali Ndizi* quality traits and to a lesser extent for S/A ratio prediction. From the results obtained during calibration development it can be observed that the parameters are to some extent adequately predicted ($r > 0.7$) when using NIR technique. It must be mentioned, however, that the obtained correlative results can never be taken with 100% reliability, thus, the method can never completely substitute sensory evaluations but complement it. However, it is absolutely applicable for screening processes at early evaluation trail level when each plant is genotype thus large population and the sensory measurement would take a lot of time and personnel. It can be applied successfully in fields, and promotes quick decisions making thus speed up the banana breeding process by shortening the selection process. This technique can be effectively deployed in a breeding program to facilitate high throughput phenotyping to mainstream quality trait in the selection process.

There is a need to perform further studies to confirm the results achieved for the estimation of the parameters of the *Sukali Ndizi*, since the low calibration results which are approximately ($R_c^2 = 0.7$) for all the parameters are not the best. From the results obtained in this study it can be stated that the accuracy of the calibration models pertaining the parameters can further be improved by working on the uniform physical presentation of the samples and including in other data pre-treatments.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors have no conflicts/competing of interest to declare that are relevant to the content of this article. All the authors have consented for the publication.

All authors contributed significantly to this research. The first and corresponding author contributed in all aspects of study design, data collection, data analysis and manuscript writing. Ephraim Nuwamanya conducted spectra data analysis; Steven Kashub Tumwesigye participated in analysis, manuscript writing and preliminary review; Moses Matovu and Priver Namanya did wet chemistry; Kephas Nowankunda, and Patrick Rubaihayo are the university supervisors; and Wilberforce K. Tushemereirwe participated in designing the experiment.

Declarations

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