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NIRS Predictions, Phenotypic Variability and Optimization of Cooking Time for Evaluation of the Root Softness of Boiled Cassava

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Abstract. This study aimed at quantifying the extent of genetic variability of softness in cassava germplasm across varied cooking times and root sections. It also examined the possibility of using Near Infrared Spectroscopy (NIRS) for measurement of cassava root softness. Softness was evaluated using a penetrometer. This was done at 15, 30 and 45minutes cooking time, all across proximal, middle and distal root sections. These measurements were done on 57 accessions. For each sample, spectra were acquired using NIRS Benchtop (FOSS DS2500) on a composite of each root section of mashed fresh cassava sample. Modified Partial Least Squares regression (MPLS) was used for NIRS calibration development using WINISI software. Significant ($P < 0.001$) variability in softness was established. Cooking time significantly influenced softness and there were significant accession and root part interaction ($P < 0.001$). Wide variability and high heritability (H = 0.8) were found for softness at 30 minutes cooking time. Highest association was found with 30- and 45-minutes cooking time (r = 0.58). Strong association was observed between middle root section with distal (r = 0.74) and proximal (r = 0.73). NIRS softness calibration (R^2c) were 0.445, 0.413 and 0.521 for 15-, 30-, and 45-minutes cooking time respectively. NIRS prediction (R^2p) were 0.322, 0.192, and 0.390 for 15-, 30-, and 45-minutes cooking time respectively. These results suggest that 30 minutes cooking time and middle root section are optimum for softness phenotyping.

Keywords: Cassava, Cooking Time, Root Sections, Penetrometer, Softness.

Introduction

Cassava is cultivated as a major crop in tropical areas around the world as primary a source of carbohydrate feeding millions of people (Adjei, 2021). Cassava has the ability to grow with minimum inputs, adapt to drought-prone and low fertility regions, and can be easily propagated using stem-cuttings. Furthermore, the possibility of piece-meal harvesting makes it popular among many small-scale farmers (Waisundara, 2018), as these provides a way to store food *in-situ*. Cassava roots, the most economic part of the plant are processed into a variety products that are hinged on the processing method such as drying, milling, roasting, frying, steaming and boiling (Balagopalan, 2002).

In Uganda it is boiled cassava roots that are widely consumed. Accordingly, the main concern for consumers of boiled cassava roots is the final texture of boiled roots. In certain cases, boiled roots fail to soften or soften poorly even after prolonged cooking (Ngeve, 2003; Padonou & Mestres, 2005). Consistently, cassava consumers have desired for cultivars whose boiled roots are characterized by softness, mealiness (i.e., ability to disintegrate between fingers and in the mouth) and short boiling times (Lorenzi, 1994; Iragaba *et al.,* 2021). It is for these reasons that breeding for consumers and/or end-user quality traits has recently gained great **importance.**

Breeding varieties mindful of end-user trait preferences enhances variety approval in society and thus adoption (Chiwona-karltun *et al*., 2015; Bechoff *et al*., 2018). It's for these reasons that investments and studies have been undertaken to get more insights into cassava root quality traits and consequently the development of methods for undertaking routine quantification of texture and softness of boiled cassava roots (Iragaba *et al*., 2019; Tran et al. 2021)

The study by Iragaba *et al*., 2019, examined the possibility of using penetrometer for routine use. While that study observed moderate heritability ($H^2 = 0.37$), it couldn't provide adequate insights into the extent of genetic variability for softness, the best cooking time and which root part is most appropriate for softness and/or texture assessment. It also suffices to note that currently available softness assessment methods are laborious and time consuming (Iragaba *et al*., 2019). For example, the sensory-based consumer acceptability method involves assessing consumer's perception of boiled cassava products **(Iragaba et al., 2019)**. This method does not lend itself to evaluations that involve many **accessions** i.e., during early breeding stages **(Ceballos et al., 2004)**. Furthermore, it requires significant amounts of resources in terms of time, funds and personnel, further complicating the selection process.

On the other hand, the penetrometer assessment involves boiling roots at specific cooking time, followed by undertaking penetrometer reading to determine the pressure required to penetrate the boiled cassava root (Iragaba *et al*., 2019). While this method is quantitative, it **is** costly and tedious and thus of low throughput (Iragaba *et al*., 2019). These short comings justify the urgent need to develop alternative phenotyping methods that combine high throughput and precision. Such applications include Near-infrared spectroscopy (Agelet & Hurburgh, 2010 **;** Qiao et al, 2012).

Near-infrared spectroscopy (**NIRS**) has become a tool of choice in the food processing industry (Huang et al., 2008). It is a rapid, cost-effective and nondestructive technique, and thus allowing the simultaneous determination of many samples within a short time. **NIRS** has already been used to measure some cassava root quality traits e.g. dry matter content (DMC), total carotenoid content (TCC) and hydrogen cyanide content (Davrieux *et al*., 2016; Ikeogu *et al*., 2017). **NIRS** accurately predicts trait measurements with almost 90% prediction accuracy. Whether or not **NIRS** can accurately predict softness of boiled roots is unknown.

Thus, the aims of this study were to assess extent of genetic variation of boiled root softness in a diverse set of cassava germplasm that comprised of local **cultivars and** elite white-fleshed **accessions and** to **access** the potential of NIRS for high throughput measurement of softness of boiled cassava root.

Materials and Methods

Genetic materials and experimental set up

Genetic materials used in the study comprised of landraces, and elite white-fleshed accessions, all sourced from Uganda's cassava breeding population. In 2018, a total of 79 cassava accessions comprising, 21 landraces and 51 elite accessions were established in randomized complete block design field experiment at Namulonge (0.5333° N, 32.6167° E), central region. The site is characterized by moderate temperature and rainfall with an elevation of 1160m. Each plot comprised three rows of five plants/row. A plant spacing of 1m x 1m was adopted. The trial was replicated twice and kept weed-free throughout the growth period.

At 12 months after planting (MAP), all plants were uprooted and their roots bulked. Subsequently, three to four intact uniformly sized roots (i.e., 25cm in length and 15 cm in diameter) were randomly sampled from each plot, and appropriately labeled. The sampled roots were immediately washed with tap water to remove soil and debris. In the end, sampling requirement was only possible for 57 accessions (20 landrace accessions and 37 elite accessions).

Spectral data acquisition

Spectra were collected on mashed root sample using the NIRS Benchtop (FOSS DS2500). Accordingly, three roots per accession were peeled and then sectioned using a knife and ruler into three root portions of relatively equal size (i.e., proximal, middle and distal portions measuring ~5 cm). The three root portions were mashed to make one composite sample per root. About 20g of mashed sample in quartz sampling cups was placed in NIRS Benchtop (FOSS DS2500) and scanned using IS Scan Nova. Three scans per accession were collected. A total of 171 spectra were generated.

Softness evaluation

Samples used for spectra acquisition were the same samples used for softness evaluation. Three roots per plot (accession) were peeled and then sectioned into three root portions of relatively equal size: proximal, middle and distal disks about 5 cm length each were made using a knife and ruler. For each accession, the remaining portions; proximal, middle and distal per each root that remained after taking off mashed sample for NIRS scanning, were then loosely wrapped in aluminum foil and appropriately labelled. These were then boiled in laboratory water bath set at a constant near-boiling temperature of 96°C. Representative samples per accession (each root section) were boiled at three time points; 15-min, 30-min and 45 minutes. After cooking, at each respective boiling time point, each root section per accession, was removed and softness measured using a penetrometer (Model number: FHT-1122, Vetus Industrial Company Limited, Hefei, China). Thus, the 7.9 mm diameter tip of a digital penetrometer was pushed to a depth of 1 cm into each cooked root section. This was done at four different positions i.e., four measurements taken per root section. Hence, a total of 36 observations were obtained per accession.

Statistical Analyses

Generated data was subjected to statistical analyses using the R software suite (R Core Team, 2021). Analysis of variance (ANOVA) was done to determine mean differences of softness among accessions. A linear model was fitted where accession, cooking time, root part and technical replicates and their interaction were fixed effects. ANOVA was generated using the *anova* and *lm* function available in *Agricole* package (Mendiburu, 2020). The Tukey-Kramer honest significance (HSD) test (P-value < 0.05) was used to determine if accessions were significantly different from each other using the *hsd.test* function (Mendiburu, 2020).

Repeatability/Broad sense heritability of softness (H^2) was obtained by fitting mixed linear models that considered accession, cooking time and root part as random effects, while technical replicate considered as a fixed effect using *lmer* function in lme4 package in R (Kuznetsova et al., 2017). Accordingly, variance components obtained were used to estimate broad sense heritability (H^2) on an entry-mean basis (Holland et al., 2010). Best linear unbiased predictors (BLUPs) for all accessions were generated using the *ranef* function lme4 package in R.

Relationships among cooking time points as well as root sections were analyzed using the *cor* function of the *stats* package in R (R Core Team, 2021; Harris, 2018). The significance of the Pearson's correlation coefficient was declared at $\alpha = 0.05$ with the *cor.test* function.

Model development

NIRS models for softness were developed using Win-ISI 4.6 software (Infrasoft International and FOSS, Hillerod, Denmark). Modified-Partial Least Squares (MPLS) algorithm was used to develop prediction models (Agelet & Hurburgh, 2010). Effect of two light-scatter correction methods, Standard Normal Variate and De-trending (SNVD) (Barnes et al., 1989) and Multiplicative Scatter Correction (MSC) (Roggo et al., 2007) were tested on four derivative and smoothing options.

The options are given by four digits (D, G, S1, S2): where D indicates the derivative order number (0 indicates no derivation, 1 means the first derivative, and so on), G indicates the gap (the number of data points over which derivation is computed), S1 indicates the number of data points in the first smoothing (1 means no smoothing) and S2 indicates the number of data points in the second smoothing, where 1 means no smoothing. Ten pre-treatment methods (SNVD+1,2,2,1, SNVD+1,4,4,1, SNVD+2,2,2,2, SNVD+2,4,4,1, SNVD+2,5,5,1, MSC+1,2,2,1, MSC +1,4,4,1, MSC+2,2,2,2, MSC+2,4,4,1 and MSC+2,5,5,1) were compared to no treatment (none+0,1,1,1 and none+1,2,2,2).

The best model was selected based on: a) coefficient of determination of calibration (R^2c) i.e. high value desired; b) internal cross-validation (one minus the variance ratio, 1-VR) i.e. higher value desired; and c) the difference between standard error of calibration (SEC) and internal cross-validation (SECV) i.e. lower value desired (Yi et al., 2017).

The specific factors for each MPLS model were optimized according to WinISI 4.6 software, a maximum of six terms were used. The passes used were dependent on observation for passes that provided the highest R^2c and lowest difference between SEC and SECV. Scatter plots were generated to show relationships between predicted and reference data. In addition, highest coefficient of determination of prediction (R^2p), the ratio of performance to deviation (RPD = SD/SECV) as well as standard error of prediction (SEP) and bias were used to evaluate quality of the prediction models (Sánchez et al., 2014).

Results and Discussion

Softness diversity

There were significant differences ($P < 0.001$) in softness of boiled roots among cassava **accessions** at all cooking time points (Table 1). At 15 min cooking time softness varied from 0.87N to 10.91N, whereas at 30 minutes cooking time softness varied from 0.544N to 5.88N. On the other hand, at 45 min cooking time softness varied from 0.45N to 4.22N, (Figure 1).

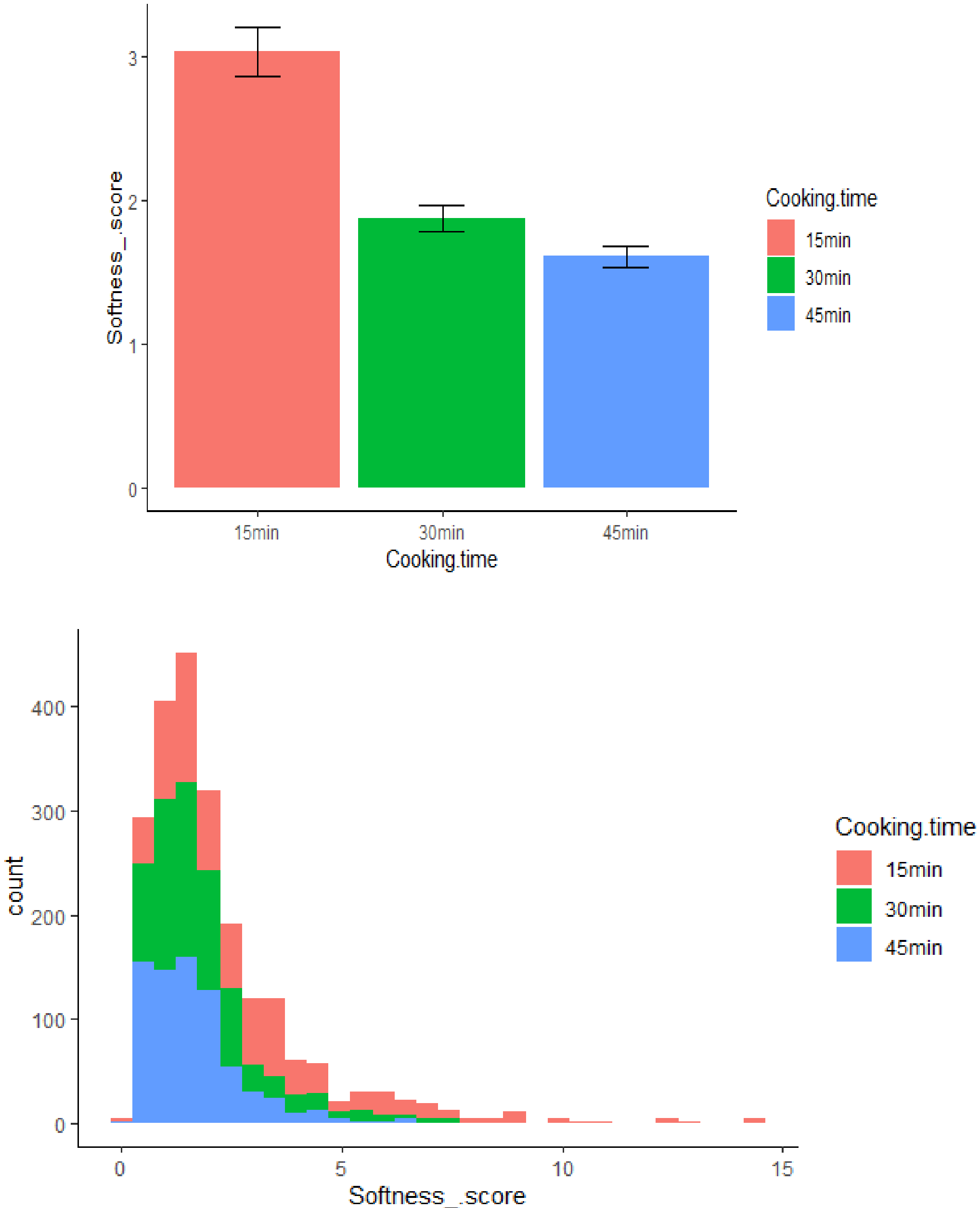

Figure 1. Variability in softness of boiled roots with mean error per cooking time

Furthermore, there was a highly significant accession by cooking time interaction ($P < 0.001$), and thus indicating that softness of boiled roots was dependent on cooking time (Table 1).

Table 1. Mean squares associated with boiled root softness

SOV	Df	Mean Square
Accession	56	63.38***
Cooking time	2	421.02***
Root part	2	12.93***
Tech_rep	3	0.11^{NS}
Accession*cooking time	112	11.10***
Accession*Root part	112	2.38***
Cooking time*Root part	4	8.32***
Accession*cooking time* Root part	224	3.61***
Error	1680	0.09
Mean		2.17
CV		13.63
H^2		0.44

SOV = source of variation; Df = degrees of freedom; *** represents significance at P = 0.001; CV = coefficient of variation. H^2 = Broad sense heritability. Roots were evaluated at 15-, 30-, and 45-minutes cooking time

Penetration force reduced with increased cooking time (Figure 1). The lowest mean force (1.57N) was observed at 45 minutes, whereas highest was observed at 15 minutes (3.42N). This is consistent with findings by Iragaba *et al.,* (2019), where highest average amount of force (3.33 N) required to penetrate roots was recorded after cooking for 15 minutes and the lowest average force (2.20 N) recorded after roots cooked for 60 minutes.

Related studies have suggested that amount of pectin and/or intercellular cell-wall adhesions play a critical role in softness of boiled roots (Favaro *et al.*, 2008). Softness variability could also be due to chemical composition i.e. physico-chemical properties, morphology and molecular structure of starch, quality and quantity of other root components (Sajeev et al., 2010). Correlations for all cooking time though significant and positive were moderate (Figure 2).

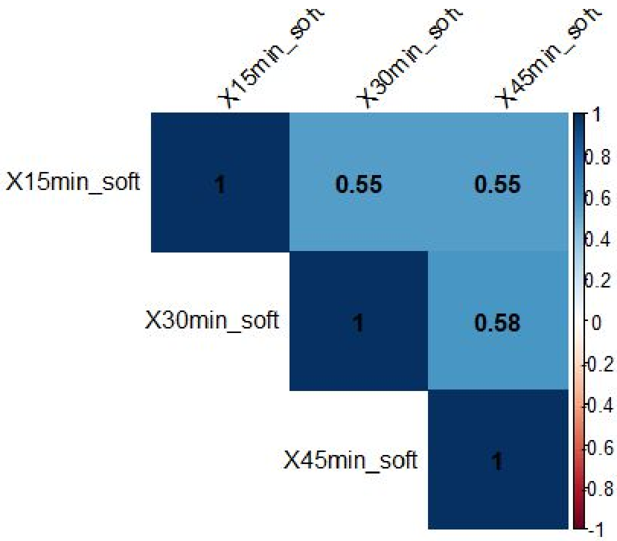

Figure 2. Pearson correlation coefficients (r) for cassava root softness measured by a penetrometer at 15-, 30- and 45-minute cooking time

These findings suggest moderate similarity between softness datasets generated at 30- and 45-minutes time points. Repeatability (H^2) for softness across the three cooking times was generally high (0.66 to 0.80). Overall broad sense heritability for softness at all the three cooking times was high (H^2 = 0.44), (Table 1). These results are consistent with findings by Iragaba *et al.*, (2019) where broad sense heritability of 0.37 was recorded at 45 minutes cooking time.

High heritability for softness 15 min cooking time, and at 30 minutes cooking time was registered (same, H^2 = 0.80), whereas at 45 minutes cooking time, it was moderate heritability (H^2 = 0.66). Considering that beyond 15 minutes cooking time most **accessions** are cooked, it suffices to note that widest variability in softness was obtained at 30 minutes cooking time. Thus, it is at this time point, that highest softness diversity can be achieved. Hence, we recommend 30 minutes cooking time for softness phenotyping.

According to Franck *et al.*, (2011), cassava genotypes were grouped for firmness or softness; the groupings were as follows; group 1; very cohesive (hard) softness/firmness value > 3N; group 2; cohesive (a bit hard), softness/firmness value 2.5N- 3N; group 3; friable (soft), softness/firmness value 1.5N-2.4N; group 4; very friable (very soft), softness/firmness value < 1.5N. Presented in Table 2 are the soft and very soft **accessions**, which are most suitable for end-users (Table 2). Generally wider variability in softness was found in land race **accessions** than elite **accessions.** However elite **accessions** were softer than land races (Table 2).

Table 2. Best Linear Unbiased Predictors for clones ranked the best performing for softness at all cooking times

	Very soft (< 1.5N)		Soft (1.5N -2.5N)	
	Accession name	*Softness(N)*	*Accession name*	*Softness(N)*
Land races	RUGOGOMA	0.81	KAKWALE	1.73
	HOIMA-1	0.90	GALWANDA	1.79
	EGABU	1.07	MACUNDE	1.82
	NYARABOKE	1.15	MAGANA	2.03
	KANYALI	1.15		
	BAO	1.26		
	KWATAMUMPALE	1.45		
	ALADO	1.49		
Elite clones	UG15F118P023	0.80	UG15F276P015	1.52
	UG15F117P001	0.86	UG15F364P001	1.54
	UG15F278P001	0.91	UG15F155P005	1.75
	UG15F306P006	1.06	UG15F117P009	1.81
	UG15F047P004	1.08	UG15F188P004	1.92
	UG15F109P503	1.09	UG15F172P004	1.93
	UG15F233P048	1.17	UG15F271P008	1.96
	UG15F233P052	1.27	UG15F276P014	2.11
	UG15F254P502	1.28	UG15F176P002	2.20
	UG15F258P004	1.29	UG15F251P006	2.30
	UG15F096P002	1.29	UG15F119P006	2.33
	UGC1710896	1.38	UG15F226P071	2.33
	UG15F168P002	1.41		
	UG15F117P014	1.45		
	UG15F154P005	1.49		

Middle root section most suitable for softness phenotyping

At all cooking times, there were significant variation ($P < 0.001$) in softness among root parts and accession-by-root part-interaction (Table 1). Highest variability was observed with the proximal section (0.47N - 6.76N) and least variability with distal section i.e., softness ranging from 0.61N to 5.83N (Figure 3). On average, the middle section was the softest i.e., 2.08N, and the proximal the hardest i.e., 2.32N (Figure 3).

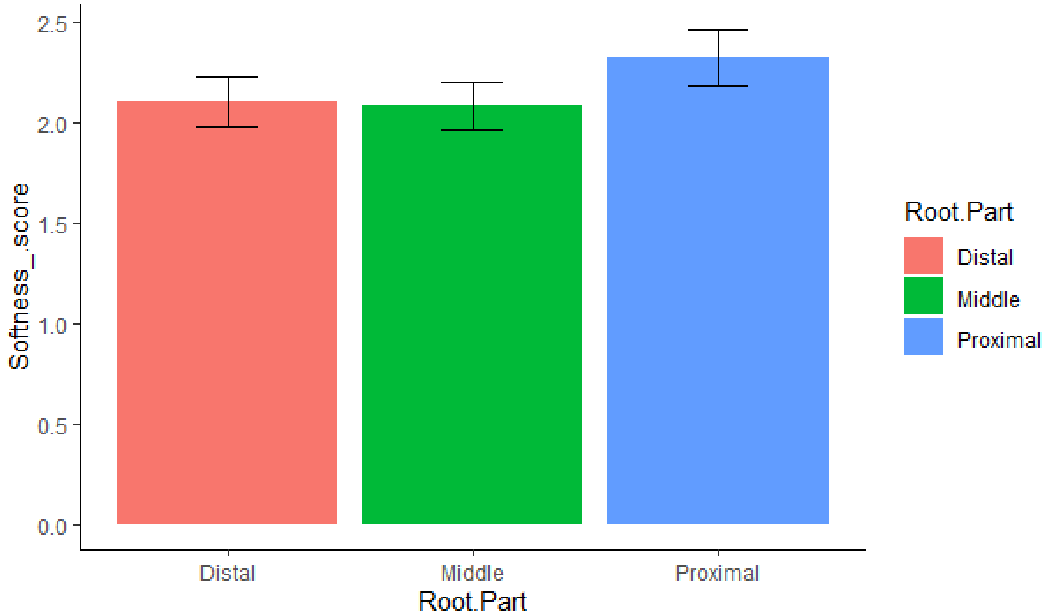

Figure 3. Softness of boiled roots across distal, middle and proximal root section with mean errors associated with the root sections across 15, 30, and 45 minutes cooking times

This patterns is likely due the fact that proximal section tends to be more fibrous than other root parts (Chisenga *et al*, 2019). Related studies by Safo-kantanka and Owusu-nipah, (1992), reported that softer (mealy) varieties were associated with higher dry matter. Furthermore, studies by (Chávez et al. (2008), reported that dry matter content was higher in the proximal section and much lower in the central or the distal sections of the root. Consequently, these differences may be associated with softness variability among root sections

Relatively higher heritability was observed in the middle ($H^2 = 0.56$) than proximal ($H^2 = 0.50$) and/or distal ($H^2 = 0.47$) root sections. Furthermore, higher correlations were observed between softness of middle root sections with other root parts i.e., $r = 0.73$ and $r = 0.74$, respectively for proximal and distal sections (Figure 4). These findings further justify the middle root sections as the most optimal sampling unit for softness assessment.

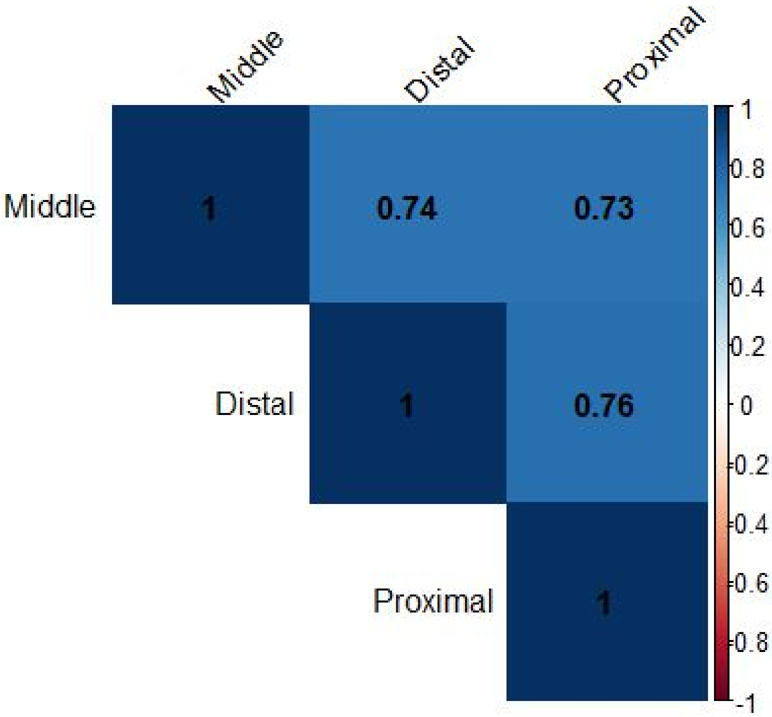

Figure 4. Pearson correlation coefficients for softness of boiled roots across the proximal, middle, and distal root parts at 45 minutes cooking time

NIRS has potential for screening softness in cassava roots

NIRS calibrations for root softness were done for root softness assessed at 15-, 30- and 45-minutes cooking time (i.e., each sample subjected to boiling treatment had an associated spectra acquired). Generally, the coefficient of determination of calibration (R^2c) for softness ranged from 0.413 to 0.521 (Table 3). For softness at 15 minutes cooking time, the pretreatment none +1,2,2,1 was the best, while at 30 minutes cooking time, the pretreatment standard MSC + 2,2,2,2, was the best. However, at 45 minutes cooking time, the pretreatment SNVD + 2,5,5,1 was the best (Table 3). Overall, the standard MSC and SNVD, were good mathematical treatments softness calibrations (Table 3).

Table 3. NIRs calibration assessment of root softness at 15- , 30- and 45 cooking times

Softness	Math trt	Scatter	SEC	R^2C	SECV	1-VR
15 minutes	None	1,2,2,1	1.263	0.445	2.416	0.202
30 minutes	MSC	2,2,2,2	0.526	0.413	1.215	0.158
45 minutes	SNVD	2,5,5,1	0.514	0.521	0.931	0.205

Math trt = math treatment; SNVD = Standard Normal Variate and Detrend; MSC = Multiple Scatter Correction; R^2C= coefficient of determination; SEC = Standard error of calibration; SECV = standard error of cross validation; 1-VR = one minus variance ratio

Elsewhere, it has been reported that multiplicative scatter correction (MSC) and standard normal variation (SNV) are highly effective pretreatments when using **NIRS** for routine measurements(Santos Panero *et al.* 2013; Zhao *et al.*, 2015). Furthermore, SNVD has also been successfully utilized when undertaking measurements of starch content in cassava (Sánchez *et al.*, 2014b), starch in corn (Qiao *et al.*, 2012) and shea nut profiles (Davrieux *et al.*, 2010) . Similarly, studies by Ikeogu *et al.*, (2017) reported good calibration for total carotenoid content in cassava using SNVD pretreatment with $R^2c = 0.90$. Thus, SNVD pretreatment is considered as optimal in developing softness calibrations.

Results of the model equations selected as applied to external independent set of samples are presented in Table 4. At 15 minutes cooking time, the coefficient of determination for calibration (R^2c) was 0.445, the coefficient of determination for prediction (R^2p) was 0.322, SEP was 2.556 while RPD was 1.21. In addition, the predicted average softness value was 3.06N similar to the actual average of 3.58N, (Table 4). At 30 minutes cooking time, the R^2c was 0.413, while R^2p was 0.169; SEP and RPD were respectively 1.576 and 1.11. The predicted average softness value was 2.02N, an estimate that was comparable to the actual average of 2.17N (Table 4). Finally, at 45 minutes cooking time, R^2c was 0.521, while R^2p was 0.390; SEP and RPD were respectively 0.602 and 1.22 (Table 4). Again, the predicted average softness value was (1.67N) was comparable to the actual average of 1.45N (Table 4). Thus, best calibrations were obtained at 45 minutes cooking time i.e., $R^2c = 0.521$ and $R^2p = 0.390$.

Table 4. Predictability of developed NIRs models

Softness	SEP	SEP (C)	R2C	R2p	Bias	Slope	RPD	Actual Average	Pred Average
15minutes	2.566	2.556	0.445	0.322	-0.519	0.983	1.21	3.06	3.58
30minutes	1.576	1.596	0.413	0.169	0.151	1.243	1.11	2.17	2.02
45 minutes	0.602	0.572	0.521	0.390	-0.217	0.957	1.22	1.45	1.67

SEP = standard error of prediction: SEP(C) =standard error of prediction corrected for bias; R2c =coefficient of determination of calibration; R2P =coefficient of determination of prediction, RPD: Ratio performance of deviation

Overall, the low to moderate **NIRS** prediction estimates for softness indicate that softness measured as a physical parameter by force, did not relate well with NIR spectra, which relates more with biochemical constituents (Yi *et al.*,(2016); Marten *et al.,* 1989). Nonetheless, these findings offer great promise and thus justify further improvements in softness phenotyping methods.

Conclusion

From the study three important conclusions are apparent. Firstly, that 30 minutes cooking time point is optimal for softness assessment in boiled cassava. Secondly, that cassava root **parts or sections across the root length** exhibit varying softness, with highest variability present in proximal and least with the middle root part. Thus, the middle section is most optimal for routine softness assessments. Thirdly, that **NIRS** has great potential to be used for high throughput assessment of cassava root softness, as witnessed with $R^2c = 0.521$ and $R^2p = 0.390$. Overall, these findings form the basis of development of protocols to be used for routine softness assessment in breeding programs and/or model improvements to increase **NIRS** precision.

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