Optimizing the Use of Near Infrared Reflectance Spectroscopy (NIRS) to Predict Nutritional Quality in Cowpea (*Vigna unguiculata*) Leaves for Human Consumption

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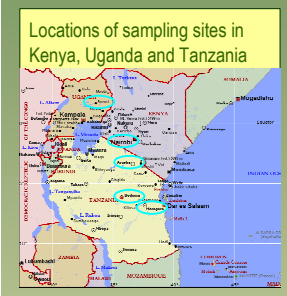
Introduction

- Reliable laboratory services are scarce and relatively expensive in eastern Africa.
- Near infrared reflectance spectroscopy (NIRS), as a non-destructive, rapid mass-screening technique, has shown an impressive throughput of analyses, once robust calibration equations are developed.
- Tefera (2006) developed a calibration equation (**Equation 1**) for crude protein (CP) in young cowpea (*Vigna unguiculata*) leaves based on 107 samples selected from a broad spectrum of accessions (n=939) from Tanzanian environments during 2003-2005 within the **ProNIVA** project.
- When **Equation 1** was applied to cowpea and lablab (*Lablab purpureus*) samples resulting from previous studies of the ProNIVA I project grown under field conditions in Malawi (Malidadi, 2006), or greenhouse and outdoor conditions at the Institute of Agronomy in the Tropics, University of Göttingen, Germany (Magesa, 2006), CP predictions were not satisfactory.



Results

- Principal Components were plotted in 3-D to give an overview of the spectral population used to develop the calibration equations (**Fig. 3**).
- Large spectral variability has been added to that available when **Equation 1** was developed due to different growing seasons, further genetic materials, environmental conditions and sample processing.

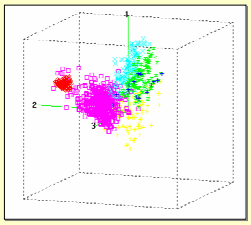


Fig.3: Comparison of different sample sets by their first three principal components based on spectral variability of ground cowpea & lablab leaves. Yellow (+) = Tefera (n=107; used to develop equation 1); magenta (□) = Towett (n=561; main population); red (◊) = Malidadi (n=126); light blue (x) = Magesa freeze-dried (n=145); green (z) = Magesa oven-dried (n=117); dark blue (+) = Magesa sun-dried (n=14).

- When adding new samples to develop Equations 2, 3A or 3B, the precision of prediction remained (R^2 >0.97, SEC <0.8); and the predictive capacities increased (RPD >6.8) (**Tab. 1**).

Tab. 1: Modified partial least-squares (MPLS) statistics of calibration and cross-validation for different calibration equations developed using NIRS for crude protein (%) based on different sample batches and combinations.

Eqa No.	N	Mean	SD	SEC	R^2_{cal}	SECV	R^2_{cval}	RPD
1	103	30.99	5.45	0.486	0.992	0.596	0.988	11.21
2	261	32.11	4.52	0.661	0.979	0.739	0.973	6.84
3 A	283	32.65	4.76	0.688	0.979	0.779	0.973	6.91
3 B	280	32.19	4.91	0.647	0.983	0.725	0.978	7.59

Key: Eqa No. = equation number; N = number of spectra in the calibration set; Mean = estimated by NIRS (expressed as %); SD = standard deviation; SEC = standard error of calibration; R^2cal = determination coefficient of calibration; SECV = standard error of cross-validation; R^2cval = determination coefficient of cross-validation; RPD = ratio performance deviation.

Methodology

- Sample processing involved sun-drying and freeze-drying; for milling, a standard lab grinder and a coffee grinder were used.
- All samples collected from experimental fields, farms and markets representing a wide range of environments in Tanzania and Uganda as well as genotypic variation were scanned using a FOSS 6500 spectrophotometer.
  - The first-derivative plot of log 1/R is useful to resolve overlapping bands, to minimize the effect of particle size, and to show only component absorption (**Fig. 1**).

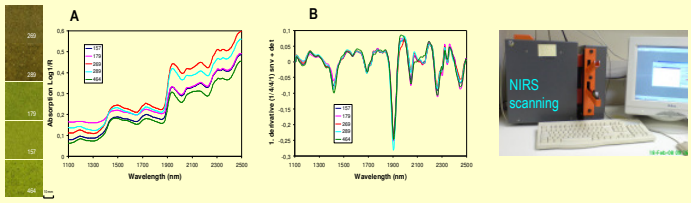


Fig. 1: **A**. Visual and spectral variation in five extreme cowpea leaf samples; and **B**. First derivative (D1) for five extreme cowpea leaf samples calculated for NIRS-work using mathematical treatment (1.4.4.1) (first derivative, gap over which derivative was calculated, number of data points used in first smoothing and in second smoothing). Sample ID: 157 = UG-CP-1; 179 = UG-CP-4; 269 = UG-CP-9 (KOL 42); 289 = Dakawa; 464 = Ex-Iseke.

- Samples were selected for reference analysis based on their spectral characteristics, with a 26 PCA-Factor-model using WinISI II software V.1.50
- A modified partial least-squares (MPLS) regression with cross-validation was used
- Successive calibration equations were developed using different batches of leaf samples (**Fig. 2**).

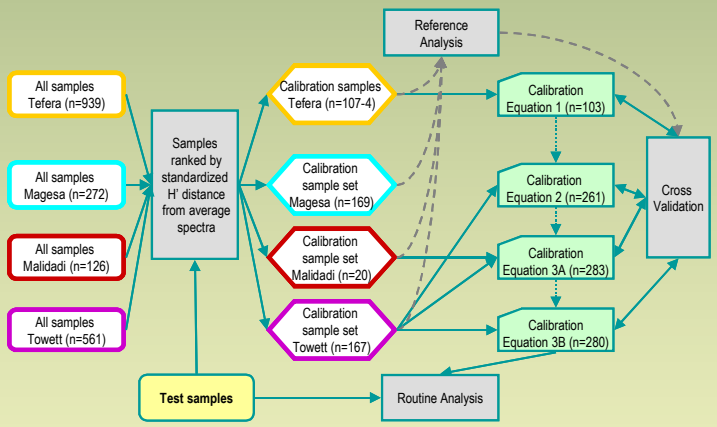


Fig. 2: Scheme of developing a robust crude protein calibration for cowpea leaves and applications on various sample sets.

Discussion & Conclusions

- NIRS predicted crude protein in a wide range of cowpea leaves from different agro-ecological zones of East Africa, including different genetic materials, processing, seasons & growth stages of the plants with high accuracy (broad-based calibration) (**Tab. 1**).
- NIRS is applicable under African conditions as long as a spectrophotometer is available and samples can receive adequate processing (drying & milling); this will save resources for laboratory analysis while obtaining reliable values for nutritional quality of this leafy vegetable.
- Nevertheless, it seems challenging to cover the broad variability existing in an apparently simple plant product, i.e. cowpea leaves, and the developed calibration equation(s) can be further improved upon with additional spectral data.

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