Application of near infrared reflectance spectroscopy against malnutrition and hidden hunger in developing countries

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Introduction

Vitamins and minerals are often seriously lacking in human diets with the highest incidence in developing countries where deficiencies of iron (Fe), zinc (Zn) and vitamin A are the most critical. Risk factors for micronutrient deficiencies are monotonous plant-based diets containing no vitamin A and only poor Fe and Zn bioavailability due to the presence of high concentrations of inhibitors such as phytates and polyphenols. Such diets represent a primary risk factor for micronutrient deficiency. Low intakes of animal foods, orange and yellow fruits and vegetables aggravate the deficiencies. Increased demands for micronutrients appear during growth, pregnancy, lactation, infection and disease. Micronutrient malnutrition, so called *hidden hunger*, diminishes the health and productivity of over half the world's population, impacting primarily on the well-being of women, infants and children.[1] About 37% of the world's population is deficient in Fe (Fig.1). In young children, Fe deficiency impairs physical growth, cognitive development and immunity that strongly affect school performance. Children are less strong due to decreased oxygen transport in blood, have decreased energy metabolism and increased risk of malarial morbidity. In pregnant women, Fe deficiency causes foetal growth retardation or low birth weight, and is responsible for a large proportion of maternal deaths. It is estimated that 100,000 maternal deaths/year during birth are due to Fe deficiency.[2]

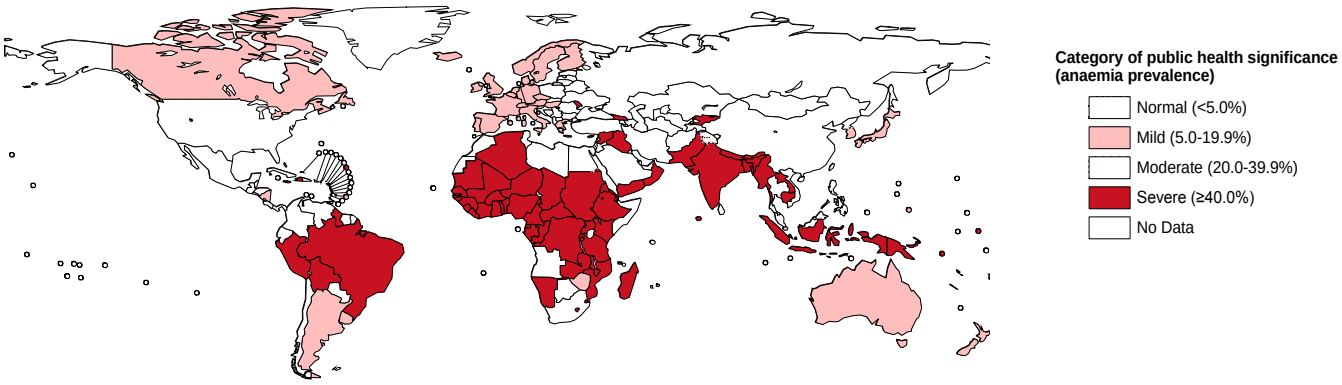

Figure 1. Fe deficiency as a public health problem in preschool children, 0-5 years.[1]

About 49% of the world's population is deficient in Zn (Fig.2). Health consequences of Zn deficiency in childhood are low weight gain and decreased immune defence resulting in increased digestion problems and respiratory, skin and neurological infections. Increased risk in pregnancy and increased prenatal mortality are reported.[2]

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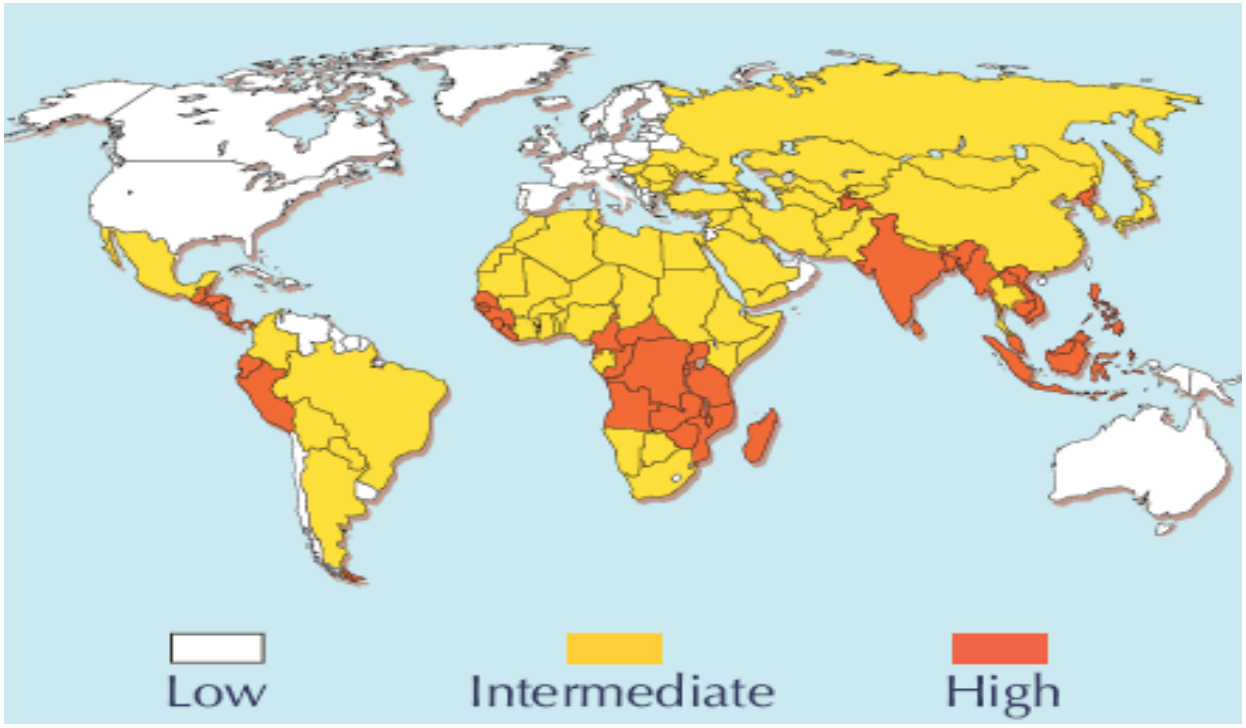

Figure 2. National risk of Zn deficiency based on combined information on childhood stunting by inadequate Zn intake.[3]

Vitamin A deficiency is the most important cause of total blindness in developing countries. It is estimated that 25% of preschool age children of the world's population have vitamin A deficiency. Worldwide, around 250 million children have subclinical vitamin A deficiency; 3 million have clinical vitamin A deficiency.[4] It is estimated that 20 million pregnant women have subclinical vitamin A deficiency; 7 million have clinical vitamin A deficiency and 6 million are night blind.[3] Other health consequences of vitamin A deficiency are reduced immune defence including increased risk of illness or death from childhood diseases.

Several initiatives (HarvestPlus, Agrosalud, SASHA) have been set up to increase the vitamin A, Fe and Zn concentration in staple food crops to help improve human nutrition status in developing countries. Plant breeding and genetic engineering techniques for development of new staple food crop varieties with increased mineral and vitamin content is known as biofortification. Biofortification seeks to improve human micronutrient status, an endeavour that entails merging plant breeding activities with nutrition and socioeconomic studies to enhance traits that have measurable value in health outcomes. Biofortification is complementary to other strategies for reducing malnutrition such as supplementation, fortification and diversification; nutritional benefits come directly from the biofortified crops with no additional costs.

To support biofortification programmes there is a need for high throughput techniques to screen macro- and micronutrient concentrations of germplasm and breeding populations in tens of thousands of genotypes in short time frames. High performance liquid chromatography (HPLC) is the common method used to determine vitamin A concentration while inductively coupled plasma spectrometry (ICP) is commonly deployed to determine mineral concentration in food crop samples. Although HPLC and ICP are very accurate, the high costs of these methods and the time required for the analysis limits their use to small numbers of samples relative to those required in extensive screening and biofortification programmes. Due to the requirement for simple sample preparation, NIR spectroscopy was selected to facilitate the analysis of several traits simultaneously. Within HarvestPlus Challenge Program (www.harvestplus.org), an evaluation of NIR for determining vitamin A in sweetpotato, cassava, maize and potato, Fe and Zn in rice, beans, wheat, pearl millet, potato and sweetpotato was performed . In this feasibility study, the development of calibrations to enable the application of NIR to biofortification of sweetpotato in Peru and in African countries is reported.

Materials and Methods

Samples

Freeze-dried and milled sweetpotato samples were used for calibration development (n=320) and external validation (n = 422) of models to predict vitamin A (ß-carotene) and minerals (Fe and Zn) respectively

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(Tables 1 and 2). The samples originated from white to deep orange fleshed sweetpotato cultivars grown in different Peruvian and African locations during the period 2006-2009.

Each sample was obtained from five unblemished medium sized roots that were collected at random from different plants from each accession. Roots of each sample were washed thoroughly with tap water, rinsed with deionised, distilled water, patted dry with paper towels and peeled with stainless knifes. Roots were quartered longitudinally and 2 opposite quarters were sliced to obtain a sample of approximately 50 g. The sample was immediately frozen at –20ºC, freeze-dried for 72h, milled with a 0.425-mm grid (40 mesh) and immediately scanned by NIR and analysed by reference methods to develop and extend the NIRS calibration models.

Reference analysis

Vitamin A

Vitamin A analysis in freeze-dried sweetpotato samples was carried out according to Kimura et al.[5] Briefly, 0.1 - 1g of the freeze-dried and milled sweetpotato sample was extracted with acetone by grinding in a mortar and pestle. Extraction was repeated until the residue was devoid of colour. The resulting extracts were brought to a volume of 25ml with petroleum ether. For HPLC analysis, 15 ml of the extract were dried with nitrogen gas , re-dissolved in 1 mL of HPLC grade acetone (Fisher) and filtered through a 0.22 µm PTFE syringe filter (Millipore). In total, 10 µL of the filtered extract were injected into a Waters HPLC machine, equipped with a separation system (model 2995), quaternary pump, autosampler, in-line degasser and photodiode array detector (model 2696) controlled by Empower software.

Fe and Zn

Analytical sub-samples of 0.6 g were taken from each of the three respective dried samples of each genotype from each site, and digested at 140ºC in 70% (w/w) $HNO_3/HClO_4$. Samples were analysed for Fe, Zn and aluminium (Al) by ICP–optical emission spectrophotometry using an ARL 3580B ICP (ARL, Switzerland). As Al is commonly found at higher levels in soil and very low levels in crops, it was measured to give an indication of contamination from soil or dust particles. Analyses were performed by Waite Analytical Services, Adelaide, Australia.

NIR analysis, calibration development and validation

Each freeze-dried and milled sample was scanned twice in reflectance mode (400 to 2500nm at 2 nm intervals) using a NIR monochromator instrument (model FOSS 6500 autochanger; NIRSystems Inc., Silver Spring, MD, USA) and small ring cups. Calibration equations for vitamin A were developed under WinISI II Project Manager 1.50, with spectral information from 400 to 2498 nm and using modified partial least squares (MPLS) regression and cross-validation techniques. Calibration equations for Fe and Zn were developed with spectral information between 1100 and 2500nm. The derivative treatments used were 2, 5, 5 ,1 (vitamin A) and 1, 4, 4, 1 (Fe and Zn). The first number is the derivative order, the second the gap, and the third and fourth numbers are the smooth. Results of the calibration calculations were checked observing the t-outliers with t > 2.0, GH- and X-outliers >8; the number of outlier elimination passes was two. Samples with t > 2.0 were deleted from the calibration file. A lower than usual t-outlier value of 2 was chosen because no extra care was taken during the reference analysis e.g. duplicate analysis of the same samples for Fe and Zn.

The 320 and 422 samples used for development and external validation of the calibration models for vitamin A, Fe and Zn, respectively were separated in different sample sets several times. Each time, the samples used for external validation were deleted from the calibration data file. Each new validation file consisted of ca. 20% samples of the complete dataset. New calibration equations models were developed, which showed only small deviations compared to the calibration equations developed for the complete sample set (results not shown). These calibration equations were used to predict the vitamin A, Fe and Zn concentrations of the samples of the accessions not represented in the calibration file.

Results and Discussion

NIR spectroscopy calibration development and validation

NIR spectroscopy calibration development

Mean values, standard deviations and ranges of the reference values and the statistics of NIR calibration and cross-validation are shown in Table 1. NIR calibration equations showed high coefficients of determination for the calibrations (R^2c) (0.81 to 0.98) with slightly lower coefficients of determination for cross-validations

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(R^2cv) (0.80 to 0.97). The highest R^2c and R^2cv were found for vitamin A (0.98 and 0.97 respectively). Standard errors of calibration (SEC) and in cross-validation (SECV) were low for all traits (Table 1).

The potential to estimate vitamin A concentrations by NIR spectroscopy has been demonstrated and applied for maize, potato and Chinese kale.[6-9] Because NIR measures light absorption by organic molecules, its successful application for inorganic minerals seems improbable. However, it is also reported that prediction of minerals in forages by NIR spectroscopy is possible through their association with the organic matrix of plant samples. [10, 11]

Table 1. Variation of concentrations as measured by reference methods, NIR calibration and cross-validation statistics for vitamin A, Fe and Zn in sweetpotato (complete calibration sets).

Analyte	Reference Values			Calibration		Cross-validation	
	Range [a]	Mean [a]	SD [a]	R^2_c	SEC [a]	R^2_{cv}	SECV [a]
Vitamin A (n=320)	0.0 – 157.2	33.7	37.9	0.98	4.25	0.97	5.69
Fe (n=422)	0.8 – 4.5	2.0	0.7	0.81	0.26	0.80	0.27
Zn (n=422)	0.5 – 3.1	1.3	0.5	0.91	0.14	0.89	0.15

SD = standard deviation, R^2_c = coefficient of determination in calibration, SEC = standard error of calibration, R^2_{cv} = coefficient of determination in cross-validation, SECV = standard error of cross-validation, [a] = mg 100 g^{-1} in dry weight, n = number of samples

Validation

Mean values, standard deviations, ranges of the reference values and the performance parameters of external validation are shown in Table 2. External validation of the NIR calibrations with 64-84 samples randomly selected and not represented in the calibration set confirmed the results obtained in cross-validation. Coefficients of determination in external validation (R2v) ranged from 0.77 to 0.92 and are comparable to those found for the calibration and cross-validation (Table 1).

Vitamin A and Zn showed high R2v (0.92 and 0.88, respectively) indicating that these analytes can be estimated by NIR spectroscopy with high accuracy. Fe showed a medium R2v value of 0.77, implying that NIR predictions for Fe are less precise than for vitamin A and Fe. Standard errors of prediction corrected for bias [SEP(C)] showed values (Table 2) only slightly higher than the corresponding SECVs (Table 1) for all analytes. These results demonstrate that NIR calibrations can estimate concentrations of all trait analytes investigated.

Table 2. Variation of concentrations as measured by reference methods for vitamin A, Fe and Zn in sweetpotato samples and NIR validation statistics for external validation samples.

Analyte	Reference Values			*External* Validation	
	Range [a]	Mean [a]	SD [a]	R^2_v	SEP(C) [a]
Vitamin A (n=64)	0 – 134.9	31.2	37.3	0.92	7.94
Fe (n=84)	0.8 – 3.8	1.7	0.7	0.77	0.35
Zn (n=84)	0.4 – 3.1	1.0	0.5	0.88	0.18

SD = Standard deviation, R^2_v = coefficient of determination in validation, SEP(C) = standard error of prediction corrected for bias, [a] = mg 100 g^{-1} in dry weight, n = number of samples

Application of the developed calibrations for estimating Vitamin A, Fe and Zn

The sweetpotato breeding program at the International Potato Center (CIP) is working to develop sweetpotato genotypes with good yield, resistance to biotic (virus and pest) and abiotic (drought, heat) stress under African conditions and high nutritional value. The NIR calibrations developed have been used to screen for high vitamin A, Fe and Zn sweetpotato clones in the breeding programme at CIP and a set of advanced clones with high vitamin A, Fe and Zn concentrations as well as good yield have been identified. To-date, more than 350,000 freeze-dried and milled sweetpotato samples have been analyzed by NIR spectroscopy at CIP and a NIR analytical network has been established to facilitate the analysis of sweetpotato samples in African target regions (Uganda, Mozambique and Ghana), based on NIR calibration models developed and maintained at CIP headquarters in Lima, Peru. The NIR models for vitamin A, Fe and

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Zn reported are applied together with other models for micro- and macronutrients such as total protein, total starch, starch quality, total and individual sugars (glucose, fructose, sucrose), calcium and magnesium (calibrations not shown).

NIR calibrations have also been used to estimate the vitamin A, Fe and Zn concentration of more than 1000 sweetpotato accessions from the germplasm held by CIP[12]and 90 sweetpotato farmers' varieties from East Africa grown in different East African environments.[13]. The use of NIR spectroscopy for screening a large germplasm collection has permitted the identification of accessions with high vitamin A, Fe and Zn concentrations at low cost and in reduced time compared to the chemical methods used for carotenoid and mineral analysis (HPLC and ICP).

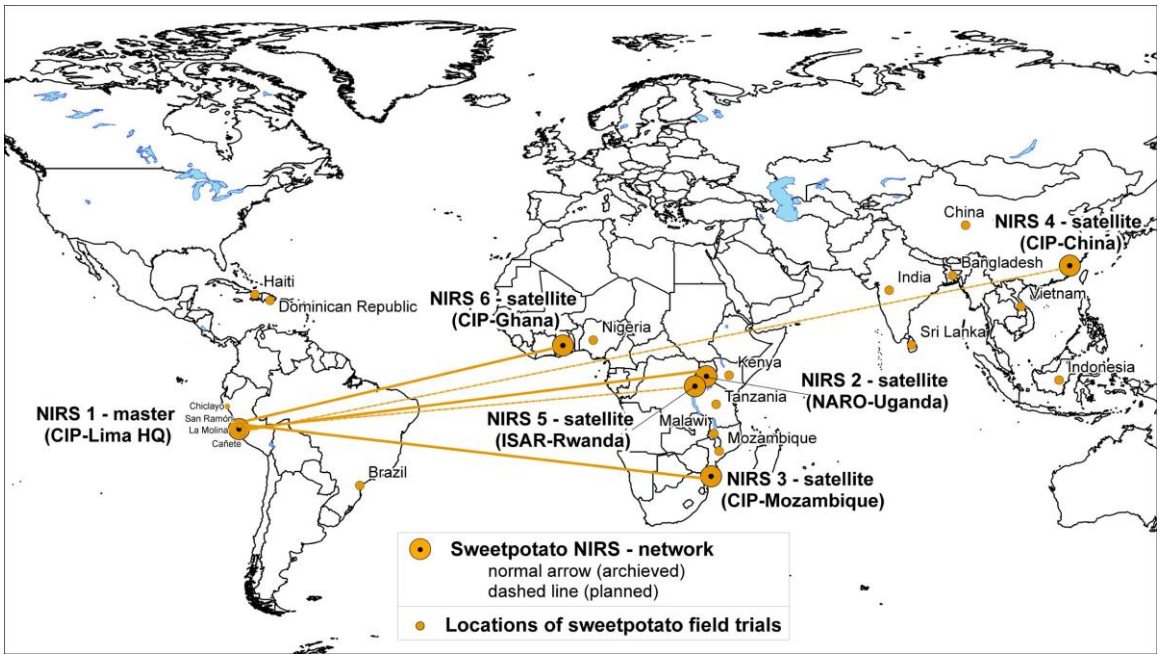

Figure 3. Locations of facilities in NIR spectroscopy analytical network established mainly for sweetpotato breeding in Africa.

Conclusion

Based on several hundred samples each, NIR calibrations to estimate vitamin A, Fe and Zn in freeze-dried and milled sweetpotato root samples were developed. These calibrations are extended on an on-going basis by including samples from different African and Peruvian environments. Fe and Zn calibrations for freeze-dried and milled sweetpotato material have good accuracy, close to that for vitamin A. Reported NIR models for vitamin A, Fe and Zn are applied together with other models for micro- and macronutrients in a NIR analytical network established mainly for sweetpotato biofortification in Africa.

Acknowledgements

This research was largely supported by the HarvestPlus Challenge Program.

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