Fast and accurate prediction of the amino acids contents in maize seeds by near-infrared reflectance spectroscopy

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Introduction

The evaluation of the nutritional value of a feedstuff is traditionally performed by analysing the feedstuff for moisture, crude protein (nitrogen), fat, fibre and possible energy. Particularly with respect to protein, it has become clear that simply evaluating the nitrogen content in a feedstuff is inadequate. Animals are in need of amino acids for the synthesis of body protein and as intermediates in metabolic pathway.

Accurate knowledge of the amino acid contents of feedstuffs is very important for a successful feed compounding because a lack of methionine, lysine, threonine, and other essential amino acids can limit the nutritional efficiency of the feed.[1]

Chromatographic amino acid analysis requires oxidation and hydrolysis of the protein followed by ion exchange chromatography. This wet chemical procedure is quite complicated and needs a minimum of 3 days of processing time.

Near-infrared reflectance spectroscopy (NIRS) calibrations were developed to enable the accurate and fast prediction of the total contents of 17 amino acids (methionine, cystine, lysine, threonine, arginine and other essential amino acids), protein and moisture in the samples of commercial maize hybrids, belonging to different FAO maturity classes.[2]

The purpose of this study is to evaluate whether NIRS calibrations of good accuracy can be obtained for maize samples.

Materials and methods

A set of 97 maize samples was ground with a laboratory centrifugal Retsch model ZM-1 mill (Retsch, Haan, Germany) using 0.5 mm sieve, analyzed by chemical and chromatographic methods. The nitrogen content of the samples was determined by Kjeldahl method using Kjeltec Auto 1030 (Tecator, Höganäs, Sweden) – protein was obtained using the conversion factor of 6.25; water was determined by drying in a ventilated oven for 4 h at 103˚C. Maize samples for amino acids were analyzed using a HPLC Beckman System Gold amino acid analyzer (Beckman Coulter, Inc., Fullerton, California, USA) after hydrolysis for 24 h with 6 M hydrochloric acid. For methionine and cystine analysis samples were subjected to performic acid oxidation before hydrolysis.[3] All measurements are the average of two replicates.

The calibration samples were scanned in a closed sample cup taking reflectance readings every 2 nm between 1100 and 2500 nm from an InfraAlyzer 500 spectrophotometer (Bran+Luebbe GmbH, Norderstedt, Germany). The reflectance at each wavelength was expressed as log (1/R) using a ceramic plate as reference. Different calibrations algorithms on spectra or derivatives such as multiple linear regression (MLR), full spectra principal component regression (PCR) and partial least squares regression (PLS) were tried with the aid of a Sesame software ver.2.1 (Bran+Luebbe GmbH, Norderstedt, Germany).

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The following procedure gave the best results: the spectra were smoothed over four data points (8 nm), and the second derivatives of the calibrations spectra were calculated using a gap of four data points. These procedures allow to obtain the optimal information coming from the spectrum and to reduce the particle size effect. The multiple linear regression (MLR) technique was used for calculating the calibration equations. These calibrations were then applied to a separate set of 30 samples, which for validation purposes, were also analyzed by wet chemistry.

Results and discussion

Table 1 summarizes the performance parameters obtained for the calibration equations. Based on Table 2, it can be concluded that – with the exception of the sulfur-containing amino acids – methionine and cystine, validation showed that 88 – 98% of the amino acid variance in the maize samples could be explained using NIRS.

Table 1. NIRS calibration statistics of maize seeds.

Variable	Content (g/kg) of variables in the sample population				NIRS calibration performance data	
	Mean	SD	Min	Max	SEC	RSQ
Lysine	2.42	0.19	2.07	2.74	0.11	0.924
Methionine	1.69	0.26	1.09	2.25	0.14	0.895
Cystine	1.81	0.24	1.25	2.48	0.16	0.811
Arginine	3.41	0.32	2.89	4.02	0.16	0.906
Glutamic acid	17.37	2.14	14.72	22.34	0.89	0.931
Aspargic acid	6.52	0.61	5.68	7.68	0.31	0.893
Glycine	3.19	0.23	2.85	3.66	0.11	0.923
Histidine	2.52	0.21	2.23	2.94	0.09	0.922
Isoleucine	3.24	0.34	2.79	4.01	0.12	0.944
Leucine	12.11	1.62	10.10	15.91	0.64	0.989
Phenyloalanine	4.71	0.61	3.97	6.16	0.28	0.913
Proline	8.46	0.91	7.07	10.27	0.37	0.934
Serine	4.15	0.45	3.54	5.39	0.16	0.951
Threonine	2.85	0.27	2.49	3.44	0.12	0.906
Thyrosine	1.77	0.36	1.22	2.53	0.15	0.896
Valine	4.31	0.39	3.73	5.16	0.16	0.931
Alanine	7.21	0.82	6.11	9.06	0.24	0.955
Water (%)	8.68	1.02	6.37	10.47	0.21	0.963
Protein (%)	9.52	1.01	8.06	11.73	0.31	0.936

The higher error of the reference values for methionine and cystine affected the accuracy of NIRS calibrations (Figure 1). Reasons for this are as follows: (1) the prior oxidation of the sulfur amino acids enlarges the sample preparation error; (2) due to their low contents and to baseline interferences at the peak position of cysteic acid in the HPLC chromatogram, the peak integration is more difficult than for other amino acids.

The best results were obtained for alanine and leucine, the amino acids with the best reproducibility in the chromatographic assay (Figure 2). NIRS predictions compared to reference result agree excellently, with relative mean deviation below 5%.

Table 2. NIRS validation statistics for independent samples of maize seeds

Variable	Content (g/kg) of variables analysed with the reference method			NIRS performance data of independent validation	
	Mean	Min	Max	SEP	RSQ
Lysine	2.49	2.06	2.65	0.14	0.911
Methionine	1.64	1.05	2.27	0.18	0.846
Cystine	1.92	1.29	2.54	0.19	0.808
Arginine	3.29	2.84	4.07	0.18	0.896
Glutamic acid	17.01	14.62	22.95	0.92	0.921
Aspargic acid	6.21	5.61	7.55	0.37	0.879
Glycine	3.08	2.82	3.75	0.16	0.914
Histidine	2.29	2.26	2.99	0.12	0.908
Isoleucine	3.11	2.68	4.07	0.15	0.936
Leucine	12.64	10.22	16.02	0.68	0.940
Phenyloalanine	4.56	3.85	6.19	0.31	0.903
Proline	8.25	7.05	10.63	0.41	0.917
Serine	4.33	3.59	5.84	0.19	0.932
Threonine	2.59	2.41	3.21	0.14	0.899
Thyrosine	1.82	1.32	2.58	0.18	0.891
Valine	4.52	3.78	5.27	0.19	0.915
Alanine	7.56	6.25	9.42	0.27	0.939
Water (%)	8.73	6.34	10.43	0.23	0.959
Protein (%)	9.46	8.11	11.94	0.35	0.929

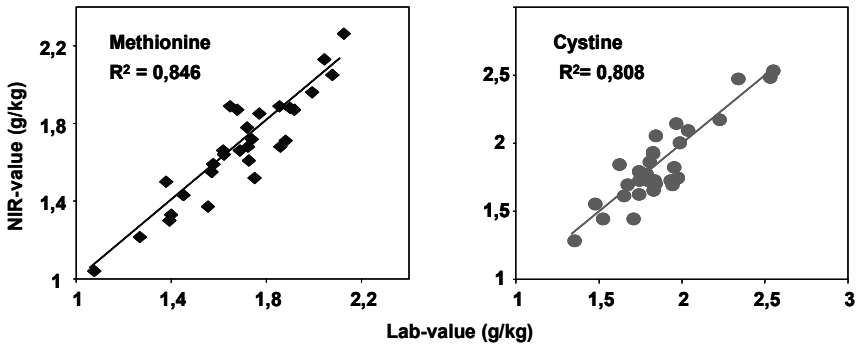

Figure 1. Validation of the NIRS amino acids prediction for maize seeds: methionine and cystine compared to reference analysis (30 samples).

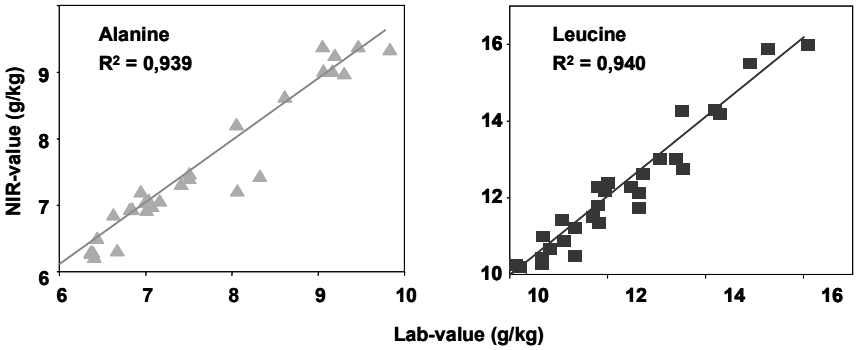

Figure 2. Validation of the NIRS amino acids prediction for maize seeds: alanine and leucine compared to reference analysis (30 samples).

The results show that NIRS calibration equations are mostly able to give very meaningful predictions of the amino acids contents in maize samples. By enabling the amino acid analysis of many samples to be completed in a short time, NIRS can improve the accuracy of feed formulation and thus the quality and production costs mixed feeds.

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