Determining the amino acid content of dry beans in ground form using NIR reflection spectroscopy

Sándor Turza,[a] Mária Váradi[a] and György D. Bisztray[b]

[a]*Analytical Department, Central Food Research Institute, H-1022, Herman O út 15 Budapest, Hungary*

[b]*Department of Plant Genetics and Plant Breeding, Szent Isván University, H-1118, Ménesi út 44/A Budapest, Hungary*

Introduction

The quality and nutritional value of bean proteins are greatly determined by their amino acid composition and their relative amount to each other. A number of methods has been elaborated and used for amino acid analysis in the agriculture,[1–3] food and feed[4,5] industry. The wet chemical analysis is quite complicated, labour intensive and requires at least two days of processing. NIR has been applied to amino acids analysis successfully since the late seventies, early eighties by a number of authors.[6–8] The fast and accurate determination of protein and amino acid composition could help breeders in selection programs.

Therefore our aim was to determine the amino acid composition of dry beans commercialised as sowing seeds in Hungary.

Materials and methods

The 53 dry bean varieties were provided by the gene bank of the Szent István University. Samples were ground on a Lab-Mill-I QC-114 grinder using a 1 mm sieve, then were stored in screw cap containers in a dark place until measurement.

Chemical analysis

Twenty mg of the samples were measured into a 10 mL injection vial and 4 mL of 3 mol/l p-toluene sulfonic acid containing 0.2% triptamine was added, which was then bubbled with N_2 for 30 s and the vial was then sealed. Samples were then hydrolysed at 110°C for 24 h in a drying cupboard. After cooling to room temperature, they were neutralised with 4 mL of 2 mol L^{-1} NaOH, and then were made up to 10 mL with distilled water. Samples were then centrifuged (10000 rpm) for 15 min and were filtered through a 0.2 µm pore size membrane filter (Millipore). They were stored frozen until measurement.

Amino acids were analysed by a Biotronik LC 3000 (Biotronik, Frankfurt, Germany) analyser equipped with a cation-exchange column. Samples were eluted with Na-acetate and detected by ninhydrin post column derivatisation at 570 and 440 nm.

Spectral measurement

Spectra were recorded on an NIRSystems 6250 instrument in the 1100–2500 nm range with 2 nm steps using standard powder cuvette.

Near Infrared Spectroscopy: Proceedings of the 11th International Conference.

Calibration and validation

After spectral transformation multiple linear regression (MLR) and partial least squares (PLS) analysis were used to set up calibration equations that were tested with cross-validation.

Results and discussion

The chemistry results of the bean varieties are presented in Tables 1 and 2 separately for non-essential and essential amino acids.

Table 1. Descriptive statistics for the non-essential amino acids determined.

	N	Max.	Min.	Av.	*SD*	*Var.*	*CV*%
ALA (mg g^{-1})	53	12.16	6.19	9.38	1.22	1.49	13.01
ASP (mg g^{-1})	53	35.67	16.59	26.90	3.93	15.44	14.61
CYS (mg g^{-1})	**53**	**3.25**	**0.67**	**1.70**	**0.59**	**0.35**	**34.71**
GLU (mg g^{-1})	53	48.53	20.13	25.23	5.50	30.25	21.80
GLY (mg g^{-1})	53	10.32	5.53	8.31	1.05	1.10	12.64
PRO (mg g^{-1})	53	26.99	7.79	14.39	4.15	17.22	28.84
SER (mg g^{-1})	53	16.32	8.30	12.83	1.82	3.31	14.19
TYR (mg g^{-1})	53	12.16	4.37	8.29	1.39	1.93	16.77

Table 2. Descriptive statistics for the essential amino acids determined

	N	Max.	Min.	Av.	*SD*	*Var.*	*CV*%
ARG (mg g^{-1})	53	24.94	7.08	14.54	3.44	11.83	23.66
HIS (mg g^{-1})	53	11.69	5.04	8.88	1.25	1.56	14.08
ILE (mg g^{-1})	53	13.78	6.79	9.99	1.51	2.28	15.12
LEU (mg g^{-1})	53	25.33	11.20	17.56	2.74	7.51	15.60
LYS (mg g^{-1})	53	20.18	9.30	14.85	2.13	4.54	14.34
MET (mg g^{-1})	**53**	**5.74**	**1.86**	**2.73**	**0.87**	**0.76**	**31.87**
THR (mg g^{-1})	53	12.21	5.88	9.67	1.43	2.04	14.79
TRP (mg g^{-1})	**53**	**2.96**	**0.74**	**1.95**	**0.57**	**0.32**	**29.23**
VAL	53	15.08	7.41	11.53	1.62	2.62	14.05

var = variance, *CV* = coefficient of variability

Amino acids with bold case letters are the limiting amino acids in beans. The tables also show that Aspartic and Glutamic acids are present in the highest amounts, which conforms well to literature data. The *CV* values for cysteine, proline, methionine and tryptophan are the biggest, meaning that their determinations are less precise with the method described in the previous section. For spectral

treatment a second segment-gap (1,6 point) derivative proved to be the best. These spectra can be seen in Figure 1.

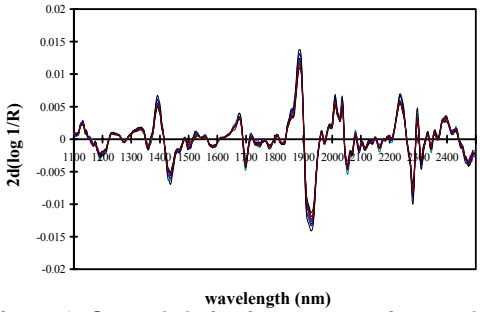

Figure 1. Second derivative spectra of ground beans measured on the NIRSystems 6250.

Table 3 shows the strength of the relation between protein and amino acids and their linear association. Amino acids with bold case letters vary the most with protein content and have the highest linear association, indicating strong, linear relation to the amount of protein. If both values in Table 3 for certain amino acids are high and they are present in relatively high amounts (Asp, Glu, etc.) in beans, then in this case we assumed that selected wavelengths would perhaps correspond to protein absorption bands, or fall in the vicinity of them. At first calibrations were done with MLR and PLS, but only the MLR results are reported (Tables 4 and 5) since they are far better then those of PLS.

Table 3. Covariance and correlation of amino acids to protein.

	Cov-matrix	Corr-matrix
ALA	0.27	0.76
ARG	**0.85**	**0.85**
ASP	**0.96**	**0.84**
CYS	–0.005	–0.02
GLU	**1.36**	0.84
GLY	0.25	0.81
HIS	0.20	0.56
ILE	0.31	0.75
LEU	**0.64**	**0.80**
LYS	**0.51**	**0.82**
MET	0.13	0.51
PHE	**0.52**	**0.79**
PRO	0.21	0.17
SER	**0.43**	**0.80**
THR	0.28	0.68
TRP	0.03	0.19
TYR	0.24	0.60
VAL	0.36	0.77

Table 4. Calibration and validation results for the essential amino acids..

	N	R_{cal}	R_{val}	*RMSEC*	*RMSEP*	Bias	Term	Wavelength
ARG (mg g^{-1})	49	0.92	0.91	1.50	1.60	0.00	2	2084, 2410
HIS (mg g^{-1})	49	0.94	0.92	0.54	0.61	0.00	4	1770, 2178, 2418, 2426
ILE (mg g^{-1})	52	0.71	0.62	0.39	0.44	0.00	4	1150, 2174, 2266, 2410
LEU (mg g^{-1})	49	0.93	0.91	0.44	0.50	0.00	4	1252, 1770, 1870, 2488
LYS (mg g^{-1})	52	0.91	0.90	0.42	0.46	0.00	3	1770, 2320, 2402
MET (mg g^{-1})	**48**	**0.84**	**0.79**	**0.70**	**0.78**	**0.00**	**4**	**1508, 2354, 2418, 2452**
PHE (mg g^{-1})	50	0.93	0.91	0.73	0.91	0.00	3	1568, 1770, 2172
THR (mg g^{-1})	49	0.95	0.94	0.51	0.55	0.00	3	1144, 2296, 2428
TRP (mg g^{-1})	**51**	**0.92**	**0.90**	**0.53**	**0.59**	**0.00**	**4**	**1220, 1326, 1914, 2418**
VAL (mg g^{-1})	50	0.88	0.85	0.61	0.68	0.00	4	1344, 1622, 1770, 2426

RMSEC, *RMSEP* = root mean square error of calibration and validation

F-value = the result of the Fisher-test

Table 5. Calibration and validation results for the non-essential amino acids

	N	R_{cal}	R_{val}	*RMSEC*	*RMSEP*	Bias	Term	Wavelength
ALA (mg g^{-1})	50	0.95	0.94	0.96	1.07	–0.01	4	1284, 1510, 2160, 2396
ASP (mg g^{-1})	50	0.96	0.95	0.54	0.72	0.02	4	1980, 2320, 2404, 2436
CYS (mg g^{-1})	**48**	**0.85**	**0.83**	**0.52**	**0.55**	**0.00**	**2**	**2156, 2178**
GLU (mg g^{-1})	48	0.95	0.94	0.72	0.79	0.00	4	1256, 1770, 2328, 2404
GLY (mg g^{-1})	51	0.94	0.93	0.59	0.65	0.00	4	1284, 1414, 1770, 2034
PRO (mg g^{-1})	49	0.77	0.70	0.39	0.44	0.00	4	1720, 2120, 2366, 2460
SER (mg g^{-1})	47	0.77	0.73	1.85	2.00	0.01	3	2152, 2452, 2456
TYR (mg g^{-1})	49	0.82	0.78	0.29	0.32	0.00	4	1376, 2178, 2256, 2444

From Tables 4 and 5 it is apparent that some samples were removed from the calibration set. The best results were achieved for Ala, Asp, Glu, Arg and Thr. For these five amino acids, the last two being essential, the R-values were over 0.9, the errors are small compared to the calibration range and the F-values are over a 100. The worst figures are shown for Cys, Met, Pro, Ser and Ile. It is not the error, but the linearity and the robustness (*F*-value) that have to be improved. The terms were selected by the NSAS MLR algorithm and their significance was tested by the regression analysis in Unscrambler, and thus some terms were eliminated to make calibrations more robust. It is strange to observe that there is so much difference in accuracy between Leu and Ile, which is hard to explain. An other interesting feature is that there are no repeating wavelengths found by program; there is no single wavelength which, in general, helps explain the relation between spectra and amino acid concentrations. The PLS (NIPALS) algorithm didn't perform as well as MLR, so they are not reported. In Figures 2–5 we try to elucidate the relationship between pure protein and amino acids.

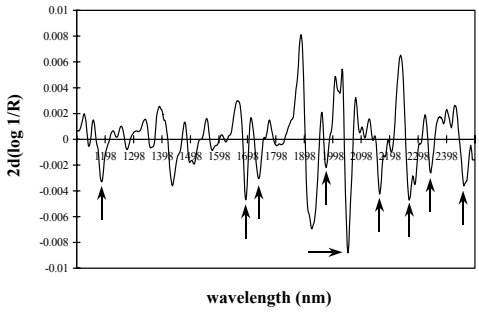

Figure 2. Second derivative spectrum of the water-soluble fraction of bean protein with characteristic absorption bands.

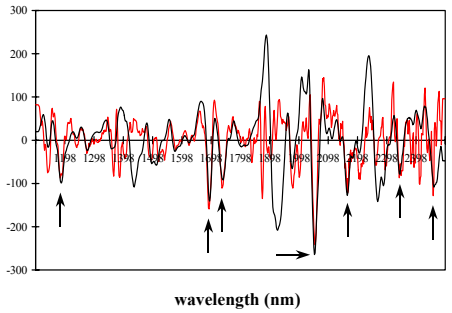

Figure 1. The spectrum of water soluble protein fraction (black line multiplied by a factor of 30.000) and the regression coefficient vector for Asp with PLS.

In Figure 2 the spectrum of water-soluble part of bean protein is seen with characteristic absorption bands. The 2nd figure shows the similarity between 2nd derivative spectrum and regression coefficient vector for ASP PLS regression. Though there are wavelengths that correspond very well and ASP is in high amount, MLR results don't support the assumption that amino acid determination is related to or can be unambiguously associated to protein absorption bands. The same phenomenon is visible in Figure 4 for His, which has smaller covariance and correlation coefficient value to protein. Figure 4 indicates that the shape of both regression coefficient vectors

are essentially the same, which suggests why there is no big difference between the shapes of curves in Figure 3 and Figure 4.

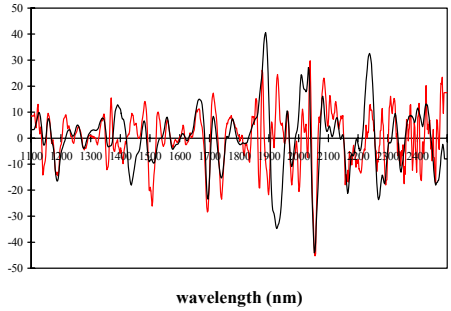

Figure 4. The spectrum of water soluble protein fraction (black line multiplied by a factor of 5.000) and the regression coefficient vector for His with PLS).

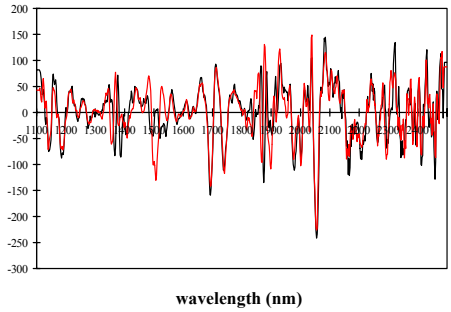

Figure 5. The regression coefficient vector for Asp PLS regression (black line) and that of His with PLS (multiplied by a factor of 5).

Around half the amino acids, some of them essential, can be determined with acceptable accuracy using NIR. MLR performed better than PLS (NIPALS). There is no apparent relationship between protein absorption bands and those found for amino acids by MLR.

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