Determining the total protein and trypsin inhibitor content of dry beans in ground and whole kernels with NIR

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Introduction

Dry beans are important sources of food proteins, because on average they contain 18–32 % protein,[1] and are very popular in Hungary. One of the major roles of breeding is to produce varieties that have the highest possible protein content. However, the digestibility of proteins is inhibited by antinutritive factors[2] such as proteinase inhibitors, which are widely distributed in legume seeds. Among legumes, mostly soybean and pea composition was investigated with NIR and NIT technique in ground, bulk kernel[3] and single kernel form; a considerably smaller scientific effort has been made to characterise dry beans.

Therefore, our aim was to determine the total protein and trypsin inhibitor content of dry beans commercialised as sowing seeds in Hungary.

Materials and methods

Fifty nine dry bean varieties were provided by the gene bank of Szent István University. The intact beans were stored in paper bags. Fifty grams of each variety were ground on a Lab-Mill-I QC-114 grinder using a 1 mm sieve, and then was put in glass containers with a screw cap until spectral measurement and chemical analysis.

Chemical analysis

Total protein content was determined by the Kjeldahl method as Nx6.25 in three replicates. Trypsin inhibition was determined according to the method of Kakade *et al*.[4] using BAPA as substrate, also in three replicates. Prior to this, samples were dissolved in 0.1 M, pH 9.5 tris-hydrochloride buffer spiked with $CaCl_2$ and a 2% suspension was made, which was followed by 1 h extraction, filtration and dilution based on presumed activity.

Spectral measurement

Spectra were recorded on an NIRSystems 6250 instrument in the 1100–2500 nm range using standard powder cuvette and on the Tecator FFA in the 850–1050 nm range with 2 nm increments both as powder and whole kernels with 4 mm and 30 mm rings, respectively.

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Calibration and validation

After spectral transformation MLR and PLS algorithms were used to set up calibration equations that were tested with cross-validation as there were not enough representative samples for a separate test set.

Results and discussion

Table 1 presents the statistics for protein and trypsin inhibitor.

Table 1. Descriptive statistics for protein and trypsin inhibitor.

	N	Max.	Min.	Av.	*SD*	Var.	*CV*%
Protein	59	29.56	14.81	22.91	2.81	7.89	12.2
Trypsin (TUI mg^{-1}	59	36.86	3.39	19.29	6.47	41.86	33.5

Var = variance, CV = coefficient of variability

It is obvious from the table that the *CV* for trypsin is almost three times that of protein, which indicates that the spread of data is larger compared to the average value. After spectral measurement the raw spectra for the NIRSystems 6250 are shown in Figure 1 and the second derivative spectra, with a segment of one and a gap of six points in Figure 2. In this case derivative treatment was enough, no scatter correction was required.

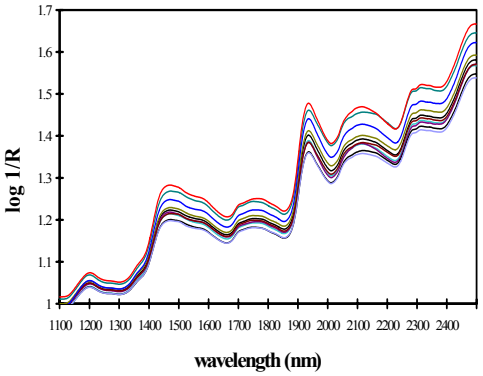

Figure 1. Raw spectra of beans measured on the NIRSystems 6250 in ground form

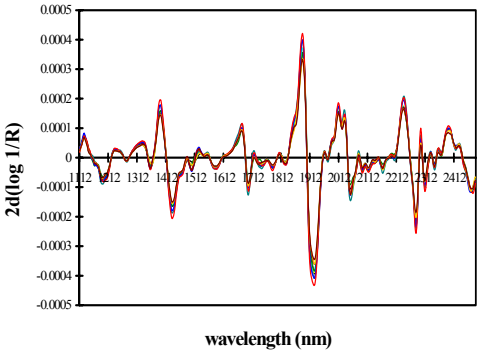

Figure 2. Second derivative spectra of beans measured on the NIRSystems 6250.

There are no unusual features in the reflection spectra as the sample presentation was quite uniform. The second derivative treatment nicely enhanced peaks of interest. However, we can see a much larger variability in the transmission spectra, even for ground samples. The spectra lying apart from the rest belong to very dark samples (Figure 3); the hull particles are very much visible even in the powder. This spectral phenomenon is even more pronounced in bulk form (Figure 4). The "top-to-bottom running" spectra are samples of very dark coloured varieties (*Vigna mungo*). The white coloured varieties have smaller absorbance by 1 to 1.5 OD in the ground case and 1.5 to 2 OD in the bulk case. In general, the Tecator spectra have very high OD values, especially in the intact kernel case, reaching almost six, therefore non-linearity becomes an issue, making analysis more difficult. It also has to be said that the FFA 1255 was not built to measure bulk samples of that large

a variability in size, shape and colour. The large spectral difference prompted us to separate the white and colour varieties and run separate calibrations with them. This feature was also explored by PCA, which supported the statement of visual inspection.

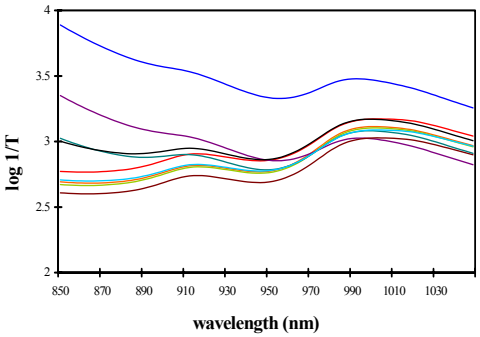

Figure 3. Raw spectra of ground samples on the FFA 1255.

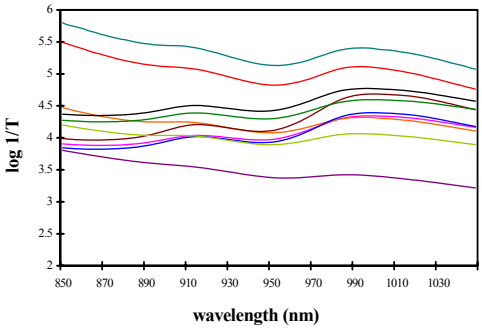

Figure 4. Raw spectra of bulk samples on the FFA 1255.

Figures 5 and 6 show the second derivative (segment = 1, gap = 10 points) of the corresponding raw Tecator spectra. The two sets of spectra differ much due to particle size differences, shape and colour. This region is more sensitive to colour information reaching over from the visible region, so these spectra were split into a white and a colour group, based on visual spectral inspection and PCA analysis. The Tecator intact kernel spectra were MSC treated for the entire sample set as well as for the two separate groups. With these treatments calibrations were performed for total protein and trypsin inhibitor content, the results of which are seen in Table 2.

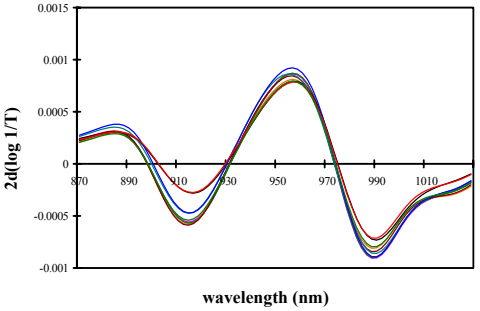

Figure 5. Second derivative spectra of ground samples on the FFA 1255.

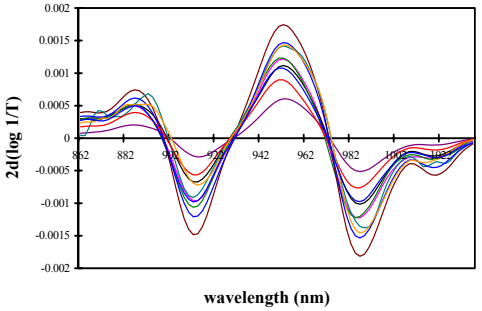

Figure 6. Second derivative spectra of bulk samples on the FFA 1255. This set of spectra is not MSC-treated to show the difference to Figure 5.

Table 2 PLS regression results for protein and trypsin inhibitor

	N	R_{cal}	R_{val}	*RMSEC*	*RMSEP*	Bias	F
NIRSystems	Protein, powder						
1100–2500 nm	59	0.98	0.98	0.45	0.54	0.00	3
sel. range	59	0.99	0.99	0.35	0.44	0.01	3
Tecator							
850–1050 nm	58	0.98	0.98	0.49	0.55	0.00	3
sel. range	58	0.98	0.98	0.46	0.51	0.00	3
white	34	0.99	0.98	0.34	0.39	0.00	4
sel. range	34	0.99	0.98	0.31	0.38	0.00	4
coloured	24	0.99	0.99	0.34	0.49	0.03	4
Tecator	Protein, bulk						
850–1050	52	0.94	0.88	0.78	1.12	0.09	6
white	33	0.93	0.89	0.75	0.95	0.02	4
sel. range	32	0.94	0.92	0.70	0.84	0.01	3
coloured	23	0.93	0.87	1.21	1.61	0.00	4
Tecator	Trypsin, bulk						
white	31	0.90	0.76	2.33	3.47	0.09	6

sel. = selected range, F = Number of factors

Table 2 shows the PLS regression results of protein and trypsin inhibitor. There is only a little difference in accuracy for protein between Tecator and NIRSystems results in case of ground samples. Wavelength range selection, based on the regression coefficient vector improved results, but not by much. For the separate groups the errors are smaller, however the calibrations are not so robust, due to the smaller number of samples. For bulk samples errors are much higher and more factors were necessary, moreover some samples were removed from the calibration set. In case of trypsin we only had an acceptable result for the white samples, which is still not as good as found by others for soybean,[5] however, the validation linearity is much worse than that of the corresponding calibration. Table 3 illustrates MLR results for protein and trypsin inhibitor.

Table 3. MLR regression results for protein and trypsin inhibitor.

	N	R_{cal}	R_{val}	*RMSEC*	*RMSEP*	Bias	Term
Protein, powder							
NIRSystems	59	0.99	0.99	0.29	0.32	0	4
Tecator	58	0.699	0.99	0.41	0.44	0	2
Tecator	Protein, bulk						
white	32	0.94	0.92	0.74	0.85	0	3
Trypsin, powder							
NIRSystems	53	0.83	0.80	3.10	3.30	0.03	3

The NIRsystems results are better, but need more terms for protein than the Tecator instrument. Bulk samples have considerably larger errors and we only had acceptable results in case of white samples for protein. The trypsin determinations need to be improved.

Conclusions

Protein content can be determined with high accuracy for powdered and acceptable for bulk samples, respectively, both with PLS and MLR and with both instruments. Determination of trypsin inhibitor content needs to be improved to have practical use.

References

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