NIR PREDICTION FOR PROTEIN AND INTRAMUSCULAR FAT CONTENT OF RABBIT HIND LEG MEAT

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ABSTRACT

Global calibration equations were developed to predict the chemical composition of raw, homogenized rabbit meat by means of near infrared reflectance spectroscopy (NIRS). Forty-four Pannon White rabbits were housed in groups in three different pen types (16 animals/m^2) and fed the same diet. Forty five animals were housed in cages (12 animals/m^2) and divided by groups fed by different feeding regimes. Rabbits were slaughtered at the bodyweight of 2.4-2.5 kg. Homogenized fresh and freeze-dried left total hind leg muscles were investigated using Foss NIRSystem 6500 spectrometer with small ring cup sample holder. The ether extract and protein concentrations of all samples were determined by wet chemistry (dry matter based fat and protein concentrations were 10.7±2.39% and 84.6±2.38%, respectively). Calibration equations were developed for the two separate series of samples (n=44 or 45) and for the entire dataset (n=89). Calibration was performed for 1100-2500 nm wavelength interval, and for its two half intervals (1100-1900 nm and 1800-2500 nm). Leave-one-out cross validation was applied to test the calibrations. Best results were gained by using 89 freeze-dried samples and analysing the 1100-2500 nm wavelength range (R^2=0.990 and 0.973, 1-VR=0.984 and 0.966 for fat and protein content, respectively).

Key-words: NIR, Rabbit, Meat, Fat, Protein.

INTRODUCTION

While meat qualification is an expanded science, only a few papers are available in the topic of analysis of raw rabbit meat by means of near-infrared spectroscopy (NIRS). NIR technique is a non destructive method that requires only little or no sample preparation, but its precision can be very high (Pla *et al.*, 2007). As opposed to conventional chemical analysis, NIRS requires no reagent, thus no waste is produced. The method has been developed as a rapid and accurate technical tool for quantitative analysis such as estimating chemical composition of different foods and feeds (Kaffka *et al.*, 1982; Xiccato *et al.*, 2003). The ability of NIR spectroscopy in analysis of meat was reviewed by Prevolnik *et al.* (2004). From qualitative aspect, discriminant analysis of samples, by their NIR spectra, makes it possible to control quality (Murray *et al.*, 2001), identify different meats by species (Mc Elhinney *et al.*, 1999) or by feeding sources (Berzaghi *et al.*, 2005). Pla *et al.* (2007) investigated the use and feasibility of NIRS to discriminate between rabbit meats, produced in conventional or organic systems. They successfully calibrated the technique for the fatty acid composition of rabbit meat, and their discriminant model classified correctly (98%) between rearing systems.

The goal of our investigation was to develop NIRS calibrations on fat and protein content of rabbit hind leg meat. Testing of the effect of sample number and finding the most appropriate wavelength interval was also appointed. Thus, generating of futurely applicable equations was aimed.

MATERIALS AND METHODS

Meat samples

Investigation was carried out on 89 Pannon White rabbits that were reared at the University of Kaposvár. The rabbits 44 were from a housing experiment (Experiment 1) (Princz *et al.*, 2006) in which five-week-old weaned rabbits were housed in three different systems (small cage: 0.12 m^2; large cage: 0.5 m^2; large pen: 1.72 m^2; 16 animals/m^2 for each). A commercial diet was fed *ad libitum*. Other 45 rabbits were from a feeding experiment (Experiment 2) (Radnai *et al.*, 2005), where weaned animals were housed in cages (0.17 m^2, 12 animals/m^2). All rabbits were fed commercial diet, but three different feeding regimes were applied (i.e. control: *ad libitum* feeding during the whole fattening period; restricted1: 60% of the feed consumption of the control group during the first week after weaning, 75% in the second week, 90% in the third, 100% in the forth week and *ad libitum* afterwards; restricted: 70% in the first, 80% in the second, 90% in the third, 100% in the forth week and *ad libitum* till slaughtering). Water was offered *ad libitum* from nipple drinkers. Rabbits were slaughtered at 11 weeks of age at the bodyweight of 2.4–2.5 kg. Total deboned left hind leg muscles were homogenized (Retsch Grindomix 200) and freeze-dried (Christ Alpha 1-4) after scanning. Freeze-dried samples were homogenized (IKA A11 basic) before repeated scanning.

Chemical analyses

All of the freeze-dried samples were analysed by wet chemistry. Fat content of samples was determined according to Folch *et al.* (1957). Hydrochloride acid digestion and a Kjel-Foss Fast Nitrogen Analyzer was used for the determination of the nitrogen content; protein content was obtained by multiplying these data with 6.25. Chemical data were used and are given on a dry matter basis.

NIRS analyses

Homogenized fresh and freeze-dried meat samples were measured by a Foss NIRSystem 6500 spectrometer (Foss NIRSystems INC., Silver Spring, MD, USA) equipped with a sample transport module and a small ring cup cuvette. Reflectance spectra were taken from 1100 to 2500 nm region and recorded as log(1/R) at 2 nm intervals. WinISI II version 1.5 spectral analytical software (InfraSoft International, Port Matilda, PS, USA) was utilized for the operation of the scanner and for data handling and evaluation procedures. Samples were scanned twice – fresh homogenized and freeze-dried homogenized. Data analyses were suited on both fresh and freeze-dried spectra. By knowing both spectral and chemical data, partial least squares (PLS) regression was used in order to develop Global equations (Sinnaeve *et al.*, 1994) for quantitative analysis. Different wavelength intervals were used for generating the calibration equations for the chemical components. When using NIR spectroscopy, excessive background often exists within the NIR spectra. Standard normal variance (SNV) and Detrend were applied for correction of the scattering effect. The sloping background was removed by the second derivative of the spectra (Tahboub and Pardue, 1985). A gap (8 nm) and a smoothing interval (6 nm) was used to reduce noise, sample-to-sample baseline variation and to enhance the absorption peaks ("WinISI format": 2, 8, 6). Model performance was reported as standard error of calibration (SEC), coefficient of determination (R^2), standard error of cross validation (SECV) and the estimate of the fraction of explained variance during cross validation (1-VR). SEC and SECV represent the uncertainty of the measurement, thus indicate the accuracy, while R^2 and 1-VR give the extent of precision.

RESULTS AND DISCUSSION

Chemical analyses

Descriptive statistics for the entire dataset and the two sample groups (Experiment 1 and 2) are shown in Table 1.

Table 1: Descriptive statistics of chemical data of sample sets

	Experiment 1 (n=44)				Experiment 2 (n=45)				Entire dataset (n=89)			
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
Fat (%)	9.49	2.06	5.53	14.1	11.8	2.15	7.70	16.7	10.6	2.39	5.53	16.7
Protein (%)	85.7	2.05	81.7	89.9	83.4	2.17	78.2	87.6	84.6	2.38	78.2	89.9

Significant differences ($P<0.001$) were found between the two groups concerning the dry matter based fat and protein content. Intramuscular fat content was lower and protein content was higher in rabbits coming from Experiment 1.

NIRS calibration

Optimization was performed for finding the best wavelength interval for calibration and validation. Results for three intervals – i.e. the whole scanned range (1100-2500 nm) and two spectral interval of it (1100-1900 nm and 1800-2500 nm) – are presented in Table 2 and Table 3.

Table 2: Calibration and cross validation statistics by using Experimental dataset separately

			Experiment 1 (n=44)					Experiment 2 (n=45)				
Type	Interval	Constituent	Factors[1]	SEC[2]	RSQ[3]	SECV[4]	1-VR[5]	Factors[1]	SEC[2]	RSQ[3]	SECV[4]	1-VR[5]
Fresh	1100 - 2500 nm	Fat	4	0.33	0.975	0.52	0.938	2	0.68	0.901	0.83	0.853
		Protein	4	0.34	0.973	0.53	0.934	2	0.82	0.858	1.00	0.793
	1100 - 1900 nm	Fat	3	0.44	0.955	0.49	0.945	2	0.65	0.907	0.80	0.863
		Protein	3	0.48	0.944	0.53	0.933	2	0.80	0.863	0.96	0.808
	1800 - 2500 nm	Fat	4	0.31	0.977	0.57	0.925	5	0.36	0.973	0.84	0.851
		Protein	6	0.21	0.990	0.53	0.934	2	0.81	0.861	1.05	0.772
Freeze-dried	1100 - 2500 nm	Fat	3	0.32	0.976	0.38	0.967	6	0.14	0.996	0.21	0.990
		Protein	4	0.33	0.974	0.43	0.957	3	0.41	0.964	0.48	0.951
	1100 - 1900 nm	Fat	3	0.32	0.976	0.37	0.968	4	0.18	0.993	0.21	0.991
		Protein	4	0.34	0.973	0.43	0.957	3	0.42	0.963	0.48	0.952
	1800 - 2500 nm	Fat	5	0.26	0.984	0.40	0.963	4	0.19	0.992	0.22	0.990
		Protein	4	0.34	0.972	0.43	0.957	4	0.41	0.964	0.49	0.949

[1]Factors: number of PLS factors used, [2]SEC: standard error of calibration, [3]RSQ: coefficient of determination, [4]SECV: standard error of cross validation, [5]1-VR: fraction of explained variance during cross validation

Table 3: Calibration and cross validation statistics by using the entire dataset

			Entire dataset (n=89)				
Type	Interval	Constituent	Factors[1]	SEC[2]	RSQ[3]	SECV[4]	1-VR[5]
Fresh	1100 - 2500 nm	Fat	5	0.38	0.975	0.58	0.941
		Protein	4	0.53	0.950	0.71	0.913
	1100 - 1900 nm	Fat	4	0.50	0.956	0.61	0.935
		Protein	3	0.67	0.921	0.73	0.909
	1800 - 2500 nm	Fat	2	1.01	0.820	1.17	0.763
		Protein	2	1.09	0.793	1.26	0.725
Freeze-dried	1100 - 2500 nm	Fat	5	0.24	0.990	0.30	0.984
		Protein	4	0.40	0.973	0.44	0.966
	1100 - 1900 nm	Fat	3	0.29	0.985	0.31	0.983
		Protein	8	0.28	0.986	0.42	0.968
	1800 - 2500 nm	Fat	4	0.31	0.983	0.36	0.978
		Protein	4	0.43	0.968	0.47	0.961

[1]Factors: number of PLS factors used, [2]SEC: standard error of calibration, [3]RSQ: coefficient of determination, [4]SECV: standard error of cross validation, [5]1-VR: fraction of explained variance during cross validation

It is a common problem that sample number is limited during calibration. As above results show, 44 samples are enough for generating appropriate calibration equations for the investigated constituents, however results are better when larger database is used (Figure 1).

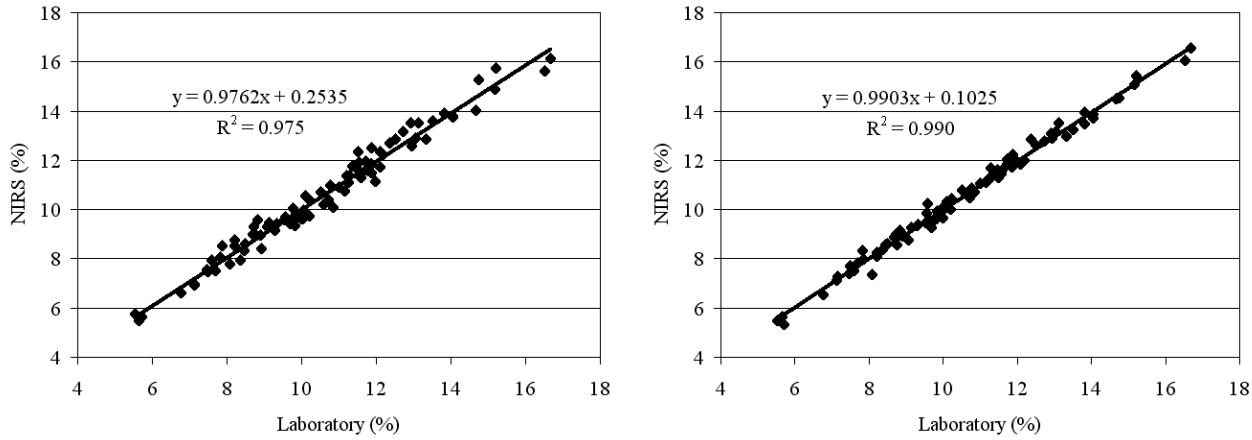

Figure 1: Calibration line between NIRS-predicted and laboratory determined value of dry matter based fat content of freeze-dried meat samples (1100-2500nm, n=89)

It is highly important in practical application of NIRS to find feasible intervals which are short but are characteristic for certain properties of samples investigated. Shorter interval means quick scanning and fast calculation. By this point of view it is shown that using only the half of the recorded wavelength interval is satisfactory for correct calibration, especially by using freeze-dried samples, where peaks of water do not affect, thus important peaks of fat and protein get subservient.

Calibration and cross validation results for fat and protein content are highly similar to results reported for other species (Viljoen *et al.*, 2005; Alomar *et al.*, 2003). Results gained with Global equation are more accurate then results achieved when applying Local method for calibrating on fat and protein content of rabbit meat (Bázár *et al.*, 2007). By using Global method in analysis of fresh meat, SEC for fat content is only 62% of SEC reached with Local method (n=89).

CONCLUSIONS

NIR spectroscopy is applicable for quick analysis of raw rabbit hind leg meat. The technique is sufficient in testing procedures for the estimation of fat and protein content of meat. The dataset of 44 meat samples seems to be enough for generating robust Global calibration equations. Best results were achieved by using 1100-2500 nm wavelength interval but shorter ranges gave also accurate and precise results. The expensive and time consuming procedure of freeze-drying is not necessary for proper output.

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