ISSN 1330-7142
UDK = 639.112.2:637.5.07

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

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Original scientific paper

SUMMARY

The goal of this study was to develop calibration equations to predict the chemical composition of raw, homogenized rabbit meat by means of near infrared spectroscopy (NIRS). 44 Pannon White rabbits were housed in groups in three different pen types (16 anim./m^2), and were fed the same diet. Another 45 animals were housed in cages (12 anim./m^2) and 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 by NIRS using a NIRSystem 6500 equipment with small ring cup sample holder. The ether extract and protein content of all samples were determined chemically. Samples 44 of housing experiment were applied in producing LOCAL calibration equations tested on the 45 samples from the separate feeding experiment. Coefficients of determination (R^2) of the predictions were 0.89 and 0.99 for fat, 0.85 and 0.96 for protein in fresh and freeze-dried samples, respectively. Results are reassuring, because the equations were applicable, however the analyzed samples were from independent housing and feeding systems. Therefore the chemical compositions differed in the two datasets, i.e. 9.46%, and 11.79% for fat, 85.75% and 83.44% for protein content in calibration and prediction datasets, respectively. The average of NIRS predicted values for fat and protein was 11.36%, 83.88% or 11.54%, 83.45% when using fresh or freeze-dried samples, respectively.

Key-words: NIR, rabbit, meat, fat, protein

INTRODUCTION

Although many workgroups study several qualification methods of meat, only a few papers are available in the topic of analysis of raw rabbit meat by means of near-infrared spectroscopy (NIRS). Although NIR technique is a non destructive method that requires only little or no sample preparation, 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 meat analysis 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.

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The Local calibration system, as a good estimator, was first described by Shenk et al. (1997) and later it was tested for several matters (Berzaghi et al., 2000; Micklander et al., 2006).
The goal of our investigation was to set a NIRS calibration on fat and protein content of rabbit hind leg meat with a relatively low sample number, and to test it on an independent stock. Thus the testing of Local calibration system and the generating of a futurely applicable equation were appointed.

MATERIAL AND METHODS

Meat samples

The 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 (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 a*d libitum*. Other 45 rabbits were from a feeding experiment (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; restricted2: 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 used for chemical analyses. The 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

The fresh homogenized and freeze-dried meat samples were measured by a Foss NIRSystem 6500 monochromator (Foss NIRSystems INC., Silver Spring, MD, USA) equipped with a sample transport module and a small ring cup cuvette. Reflectance spectra were taken from 400 to 2500 nm region and recorded as log(1/R) at 2 nm intervals. The WinISI II version 1.5 spectral analytical sofware (InfraSoft International, Port Matilda, PS,USA) was utilized for the operation of the scanner and for the development of analytical 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 set Local equation 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 sample-to-sample baseline variation and to enhance the absorption peaks ("WinISI format": 2, 8, 6).
The samples 44 were applied to produce a calibration equation that was tested on 45 rabbit meat samples coming from the separate feeding experiment. The true chemical composition was known for the predicted dataset as well. Model performance during validation was reported as coefficient of determination (R^2), standard error of prediction (SEP), bias and SEP(C) corrected for bias when the program was terminated.

RESULTS AND DISCUSSION

Chemical analyses

The descriptive statistics for the two datasets – first from the housing experiment used for calibration and the other from the feeding experiment used for validation – are shown in Table 1.

Table 1. Descriptive statistics for the stocks of calibration and validation

	Calibration set (n=44)				Validation set (n=45)			
Variable	**Mean**	**SD**	**Min.**	**Max.**	**Mean**	**SD**	**Min.**	**Max.**
Fat (%)	9.46	2.04	5.53	14.06	11.79	2.15	7.70	16.67
Protein (%)	85.78	2.03	81.65	89.92	83.44	2.17	78.17	87.64

Significant differences ($p<0.001$) were found between the two groups concerning the dry matter based fat and protein content. Fat content was lower and protein content was higher in rabbits coming from the housing experiment. The differences in both traits were more than 2%.

NIRS prediction

Optimization was performed for finding the best wavelength interval for calibration and validation, but the best results were achieved when using the whole interval of 1100-2500 nm (Table 2).

Table 2. Prediction statistics for chemical composition

		Fresh (n=45)					Freeze-dried (n=45)				
Variable	**Lab**	**Pred**	**SEP**	**Bias**	**SEP(C)**	**R^2**	**Pred**	**SEP**	**Bias**	**SEP(C)**	**R^2**
Fat (%)	11.79	11.36	0.83	0.43	0.71	0.89	11.54	0.35	0.25	0.24	0.99
Protein (%)	83.44	83.88	0.95	-0.44	0.86	0.85	83.45	0.46	-0.01	0.47	0.96

Lab: average of laboratory values, Pred: average of predicted values, SEP: standard error of prediction, SEP(C): standard error of prediction corrected for bias, R^2: coefficient of determination

Coefficients of determinations (R^2) were 0.89 and 0.99 for fat, 0.85 and 0.96 for protein in fresh and freeze-dried samples, respectively (Figure 1).

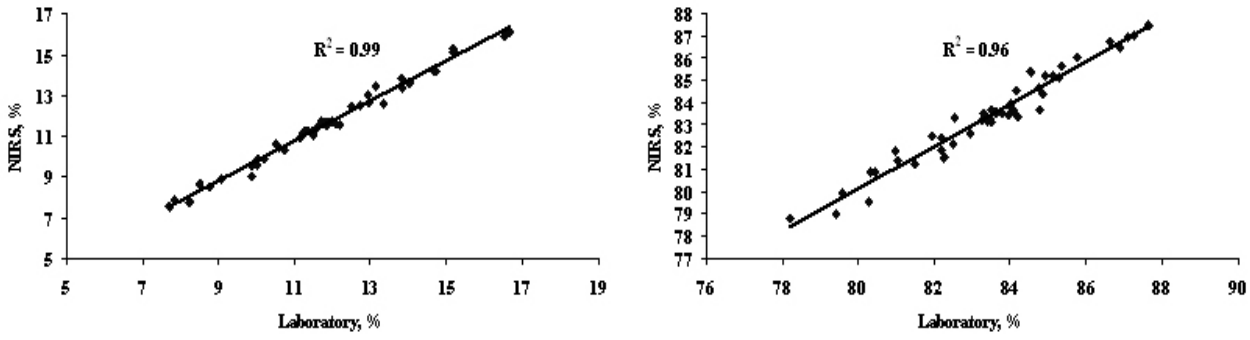

Figure 1. Validation line between NIRS-predicted and laboratory determined value of dry matter based fat and protein contents of freeze-dried meat samples

Results are reassuring, because the equations were applicable, however the validated samples were from an independent housing and feeding system so the average chemical compositions differed significantly from those of the calibration dataset. The difference between the average of observed and predicted values of both fat and protein were not higher than 0.5 % for both fresh and for freeze-dried samples.

Validation results for fat and protein content are highly similar to results reported for other species (Viljoen et al., 2005; Alomar et al., 2003).

CONCLUSION

NIR spectroscopy is an applicable technique for quick analysis of raw rabbit hind leg meat. It was established that after proper calibration, NIRS is highly sufficient in testing procedures for the estimation of fat and protein content of meat.
The dataset of 44 meat samples seems to be enough robust for generating Local calibration equation being highly useful for quick and massive prediction of the chemical composition of independent samples, either using fresh or freeze-dried samples. Accordingly, the dataset is convenient for husbandry experiments and meat investigations.

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(Received on 9 May 2007; accepted on 3 July 2007)