INVESTIGATION OF PECTORALIS MUSCLE BY MEANS OF NEAR INFRARED SPECTROSCOPY IN BROILER AND MEAT TYPE TURKEY

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Summary

Pectoralis muscle of Ross308 broilers (n=34) and BUT8 turkeys (n=40) were investigated by means of near infrared spectroscopy, in both sexes. Pectoralis muscles were taken apart for identical scanning of superficialis and profundus parts, thus altogether 148 samples were applied. In broilers, classification of muscle types by NIR spectra was successful, while identification of sexes failed, both in fresh and freeze-dried samples. Identification of turkey muscle types was less proper but improved when freeze-dried samples were used, while discrimination by sexes of turkey meats was successful in both muscle types, showing that sex has greater impact on meat quality properties in turkey than in chicken. As a pilot study, calbiration equations were developed to predict the intramuscular fat content of chicken meat samples. Coefficient of determiantion was 0.91 or 0.88 when using fresh or freeze-dried samples, respectively.

I. INTRODUCTION

Determination of the origin of food raw materials and the detection of adulteration are major issues in food industry, and are attracting research topics (Al-Jowre *et al*., 1997). Species identification is generally not problematic when the meat is seen as whole cuts, but it does become impossible once meat has been minced or even chopped. Thus it seems to be a challange to determine whether a comminuted meat sample is of the species declared (McElhinney *et al*., 1999).

While many of the available techniques for detection of meat adulteration have been successful, near infrared (NIR) spectroscopy seems to be one of the most progressive methods which is used frequently for discriminating between different kinds of meats (Barai *et al*., 1992). NIR technique is a rapid and non destructive method requiring little or no sample preparation and its precision can be high. Contrary to wet chemistry, no reagents are required and no waste is produced (Pla *et al*., 2007). The ability of NIR spectroscopy in analysis of meat was reviewed by Prevolnik *et al.* (2004). According to fraud of meat and qualitative aspect, Thyholt *et al*. (1998) used dry extracts of homogenised meats for discriminating between beef, pork, mutton and poultry. McElhinney *et al*. (1999) reported species identification in raw homogenised meats originated form chicken, turkey, pork, beef and lamb.

The method has been developed also as an accurate technical tool for quantitative analysis such as estimating chemical composition of chicken meat (Cozzolino *et al*., 1996). Berzaghi *et al*. (2005) predicted successfully the chemical composition of chicken breast meat and discriminated between groups fed different n-3 feeding sources.

The goal of our study was to establish a method to discriminate meat samples concerning species of chiken and turkey. In order to test the sensitivity of the system, the possibility of dicrimination of different muscle types of each species was also investigated. Furthermore, NIR calibration for fat content of chicken pectoralis muscle was aimed.

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II. METHODS

1. Meat samples

Pectoralis muscle of 34 Ross308 broilers (6 weeks of age, both sexes) and 40 BUT8 turkeys (16 and 20 weeks of age, female and male, resp.) were investigated. Birds were kept on deep litter under closed, controlled conditions at the Experimental Farm of the University of Kaposvár, water and feed was offered *ad libitum*. Detailed compositions of the feeds for broilers and turkeys are shown in Table 1 and Table 2, respectively. Left breast meats were dissected in order to investigate superficialis and profundus pectoralis muscles respectively, thus, altogether 148 meat samples were used. Total amount of muscles originated from broilers and 200 g of turkey muscles were homogenized (IKA Basic A11) and freeze-dried (Christ Alpha 1-4).

Table 1. Diet composition fed by Ross308 broiler strain

	Starter	**Rearing**
weeks of feeding	0-3	4-6
chemical composition		
Dry matter [%]	90.3	88.4
Crude protein [%]	21.5	18.8
Ether extract [%]	7.4	8.2
Crude fiber [%]	3.2	3.3
Crude ash [%]	5.6	5.0
ME [MJ/kg DM]	13.2	13.4
NaCl [%]	0.46	0.34
Ca [%]	0.79	0.70
Phosphorus [%]	0.64	0.58

Table 2. Diet composition fed by BUT8 turkey strain

	Starter	**Rearing1**	**Rearing2**	**Finishing**
weeks of feeding (female)	0-4	5-8	9-14	15-16
weeks of feeding (male)	0-4	5-8	9-14	15-20
chemical composition				
Dry matter [%]	89.1	88.7	88.9	86.0
Crude protein [%]	29.2	26.2	21.9	16.7
Ether extract [%]	3.3	3.6	3.2	3.4
Crude fiber [%]	1.6	1.8	2.6	2.4
Crude ash [%]	7.2	7.1	6.1	5.7
ME [MJ/kg DM]	13.5	13.6	13.5	12.8
NaCl [%]	0.47	0.35	0.47	0.47
Ca [%]	1.44	1.36	1.18	0.98
Phosphorus [%]	0.99	0.87	0.77	0.61

2. Chemical analyses

All freeze-dried broiler meat samples (n=68) were analysed by wet chemistry. Fat content of samples was determined according to Folch *et al.* (1957). Chemical data were used and are

given on a dry matter basis, thus obtained values can be applied correctly both for fresh and freeze-dried samples.

3. NIRS analyses

Homogenized fresh (approx. 7 g) and freeze-dried (approx. 2 g) 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. Cuvette was washed and wiped dry after each sample.

Data analyses were suited on both fresh and freeze-dried spectra. In broilers, 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. 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 full 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.

As for qualitative aspect, PLS based discriminant analysis (PLS-DA) was obtained for classification of different muscle types and species (Berzaghi *et al.*, 2005). First derivatives of spectra were used for discriminantion and no scatter correction was applied ("WinISI format": 1, 4, 4). Accuracies of discriminant equations are presented as the percent of the successfully classified samples during the cross-validation.

III. RESULTS

Figure 1 and Figure 2 show the raw and second derivative NIR spectra of all investigated meat samples regarding broilers (n=68), in fresh and freeze dried conditions.

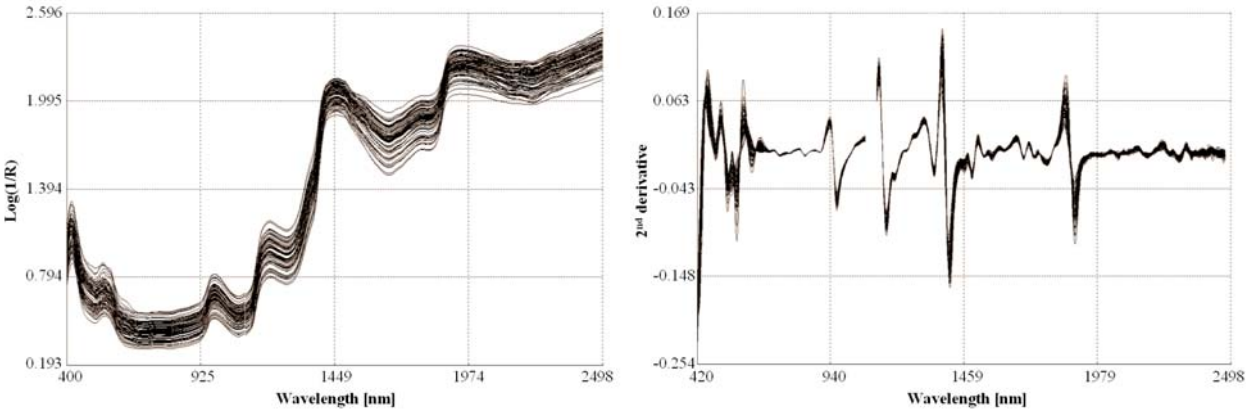

Figure 1. Log(1/R) and 2nd derivative spectra of fresh chicken meat samples (n=68)

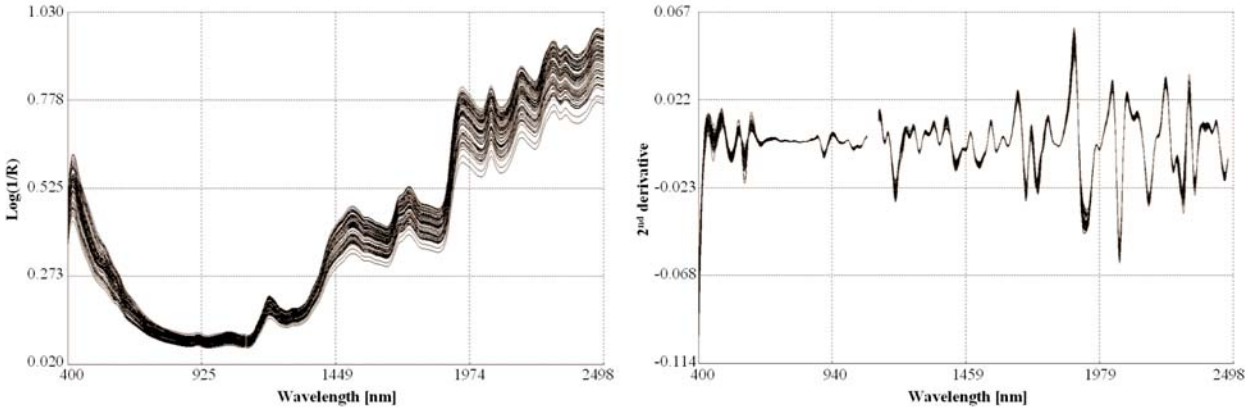

Figure 2. Log(1/R) and 2nd derivative spectra of freeze-dried chicken meat samples (n=68)

PLS-DA was applied for classifying the breast meat samples of the two species. During cross validation, 97.3% of the samples was identified correctly by genotype, when NIR spectra of both pectoralis superficialis and profundus muscles were used together for classification (n=148). When using freeze-dried samples, classification was faultless (100%), just like when involving only one type of muscle for identication of genotpyes. In case of identifing not only genotype, but also muscle type, 83.8% of fresh, and 95.3% of freeze-dried samples were placed into the correct class during the cross-validation (Table 3, Figure 3).

Table 3. Results of discriminant analysis for identifying genotype and muscle type of chickens or turkeys (n=148)

		Prediction							
		Fresh				Freeze-dried			
Genotype		Ross308		BUT8		Ross308		BUT8	
	Muscle type	Superficialis	Profundus	Superficialis	Profundus	Superficialis	Profundus	Superficialis	Profundus
Ross308	Superficialis	33	1	3	0	30	0	0	0
	Profundus	0	33	0	2	4	34	0	1
BUT8	Superficialis	0	0	29	9	0	0	38	0
	Profundus	1	0	8	29	0	0	2	39
	Total	34	34	40	40	34	34	40	40
	Missclassified	1	1	11	11	4	0	2	1

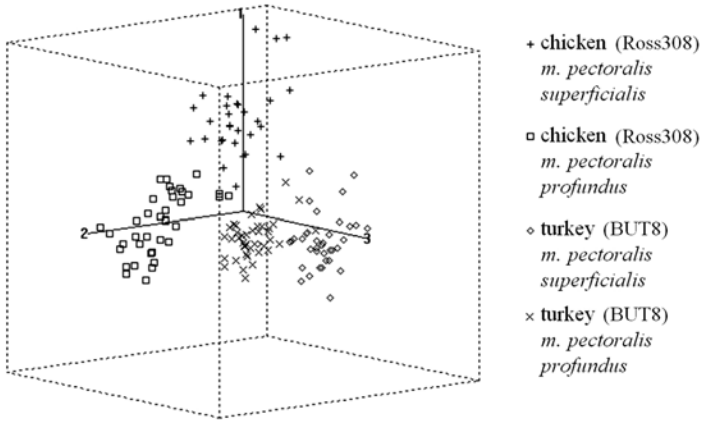

Figure 3. Discrimination of muscles by genotype and muscle type

Further data analyses were carried out in order to investigate the possibility of discrimination of muscle types of the breast meat in both genotypes, then to identify the sexes. Identification of muscle type in broilers was successful (100%) in both fresh and freeze-dried forms. In turkey, 77.5 and 98.8% of samples was classified correctly in fresh and freeze-dried conditions, respectively. When identifying sex of broilers, using pectoralis superficialis or profundus muscles, 67.6 or 75.5% of fresh, and 88.2 or 76.5% of freeze-dried samples were classified correctly. In turkeys, involvement of pectoralis superficialis muscle resulted faultless discrimination by sex, both in fresh and freeze-dried form, while there was one missclassified sample if using profundus muscle, resulting that 97.5% of samples were classified to correct sex category both in fresh and freeze-dried forms.

Results of chemical analyses are reported in Table 4, for the total broiler sample set (n=68) and for muscle types and sexes, respectively.

Table 4. Chemical data of the chicken meat samples (n=68)

	Total (n=68)			
	Mean	SD	Minimum	Maximum
Ether extract [%]	3.18	2.28	0.75	9.10

	m. pectoralis superficialis (n=34)				*m. pectoralis profundus* (n=34)			
	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
Ether extract [%]	5.01*	1.88	1.30	9.10	1.35**	0.28	0.75	1.80

	m. pectoralis superficialis								*m. pectoralis profundus*							
	Male (n=18)				Female (n=16)				Male (n=18)				Felmale (n=16)			
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
Ether extract [%]	5.77*	1.59	3.41	9.10	4.16**	1.85	1.30	6.79	1.33	0.30	0.75	1.80	1.36	0.26	0.79	1.78

*, **: different superscript in identical rows indicate significant difference ($P < 0.05$)

PLS regression based calibration equations were generated to investigate the relationship of spectral and chemical dataset. Estimation was run also using muscle types together or separately, for fresh and freeze-dried form. Results of calibrations and full cross-validations are shown in Table 5.

Table 5. Results of calibration and cross-validation for ether extract content of chicken meat samples

		SEC	RSQ	SECV	1-VR
Fresh	Total (n=68)	0.70	0.91	0.92	0.84
	Superficialis (n=34)	0.82	0.81	1.04	0.70
	Profundus (n=34)	0.24	0.23	0.26	0.17
Freeze-dried	Total (n=68)	0.78	0.88	0.82	0.87
	Superficialis (n=34)	0.99	0.73	1.10	0.67
	Profundus (n=34)	0.20	0.51	0.23	0.36

SEC: standard error of calibration

RSQ: coefficient of determination in calibration

SECV: standard error of full cross-validation

1-VR: estimate of the fraction of explained variance during cross-validation

IV. DISCUSSION

Results obtained during species identification show, that rapid, non destructive NIR technology gives an appropriate method to investigate the origin of a meat (consonant with McElhinney *et al*., 1999). Identification not only by species but also by muscle type (pectoralis superficialis or profundus muscle) was successful, showing the sensitivity of the system applied. Freeze-dried samples always gave better results for discriminant analyses, but the thechnique is also applicable using fresh meat samples, especially by using it for species identification during quick quality control. Freeze drying is still useful before re-checking misscleassified samples.

According to sex identification of broiler chickens, the percent of correctly classified meat samples was relatively low, but in turkey strain it was very successful. The reason of this may be the considerable gender associated difference between male and female turkeys (Sütő, 1997), additionally there was a one month difference in rearing period.

Calibration for ether extract of fresh homogenized or freeze-dried meat samples gave weak results, as compared to work of Cozzolino *et al.* (1996) or Berzaghi *et al.* (2005). The reason can be the very low fat content of the total breast meat, but especially the extreme low intramuscular fat content of pectoralis profundus muscle. The other reason may be the high balance of the chicken strain, which resulted low sample to sample difference, thus fixing the calibration was relatively hard. It seems, that by such a low value and varience of constituent, NIR calibration needs higher sample number.

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