NEAR INFRARED SPECTROSCOPY (NIR) TO DISCRIMINATE MEAT OF LINSEED-FED RABBITS

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I. INTRODUCTION

Rabbit meat, thanks to its low cholesterol and fat content with a high percentage of polyunsaturated fatty acids, more than 30%, could be meet healthy lifestyle of modern consumers [1]. The relationship between the dietary fatty acids profile and rabbit meat fat composition has been widely evaluated. Usually, lipid supplementation in the rabbits' diet is a common practise and linseed is could be used due to its high content in α-linolenic acid (C18:3n-3) that reduces the n-6/n-3 ratio and increases healthiness of meat [1]. The authenticity of n-3 enriched products must be guaranteed to the consumer. Therefore, it is necessary to find a fast and not destructive method to discriminate and trace the rabbit meat obtained with linseed enriched feeding. For this purpose, the Near Infrared Spectroscopy (NIR), could be an appropriate tool [2]. The aim of this work was to calibrate models able to discriminate n-3-enriched rabbit meat through chemometric techniques applied to NIR spectrometry.

II. MATERIALS AND METHODS

In this trial, 168 weaned (35d) rabbits (white hybrid from Italian Martini group) were divided into two homogeneous groups according to the diet: control group (Cg), fed with commercial pelleted feed, and linseed group (Lg), fed with the same diet as Cg, but supplemented with 3% of linseed. At slaughter, 85 days, the two groups reach similar weight in live and carcass (2.67 ± 0.09 kg and 1.52 ± 0.15 kg, respectively). Fatty acids (FA) were analysed as reported in Cifuni et al. [3] on rabbit thigh muscles finely grounded and expressed as percentage of total fatty acid methyl ester (FAME). The NIR analysis was performed using a NIRFlex Solid N-500 (BÜCHI) at a wavelength range of 1000–2500 nm. Spectral data were converted in absorbance units (log 1/R) and were mathematically pre-treated by standard normal variate (SNV) to correct light scattering effects.
The data was randomly divided in calibration (112 samples) and validation (56 samples) set. Partial Least Square (PLS) regression was performed for predict FA content using NIR spectra. PLS Discriminant Analysis (PLS-DA) and Linear Support Vector Machine (L-SVM) models were used to discriminate the groups [2] with and without linseed supplementation (Cg vs Lg used as dummy variables). Multivariate analysis was performed with Unscrambler 10X software.

III. RESULTS AND DISCUSSION

Means and standard deviations (SD) of principal FA in rabbit meat, were summarized in Table 1. The linoleic (C18:2n-6) and α-linolenic acids (C18:3n-3) were the principal polyunsaturated fatty acids (PUFAs) modified by linseed supplementation, in fact the last one showed the major variability due to feed (1.8% and 5.9% $P<0.001$ for Cg and Lg, respectively). The PLS models were able to estimate some fatty acids using NIR spectrometric data, with exception for monounsaturated fatty acids (MUFA). The others FA showed R^2_p ranging from 0.63 to 0.75 for validation sets and low SEP (standard error in prediction), particularly for C18:3n3 and n6/n3 ratio with RPD >2 (Ratio of Predicted

to Deviation=SD/SEP) for validation set [2]. The number of latent variables (LV) were between 3 and 7, therefore the models did not show overfitting.

Table 1 Statistical results for calibration and validation of partial least squared models used to estimate fatty acids in rabbit meat using NIR spectra.

	Calibration set n=112		Validation set n=56			Calibration set		Calibration set		
	Mean	SD	Mean	SD	LV	SEC	R^2_C	SEP	R^2_P	RPD
ΣSFA	35.4	2.04	35.5	2.09	5	0.94	0.74	1.12	0.68	1.87
ΣMUFA	23.8	1.98	23.5	2.19	3	1.33	0.53	1.67	0.45	1.31
C18:2n6	29.7	1.61	29.7	1.80	6	0.78	0.71	0.92	0.66	1.95
C18:3n3	3.8	2.09	3.8	2.27	5	0.86	0.80	1.04	0.74	2.18
ΣPUFA	40.7	2.45	41.0	2.50	7	1.14	0.70	1.45	0.63	1.72
n6/n3	8.6	3.75	8.9	4.08	5	1.88	0.81	2.01	0.75	2.03

SD = Standard Deviation; LV = Latent Variables; SEC = Standard Error in Calibration, SEP = Standard Error Prediction; R^2_C, R^2_P = coefficients of determination in calibration and prediction; RPD = Ratio of Predicted to Deviation (SD/SEP).

The NIR was therefore able to adequately predict the FA composition as confirmed also by Prieto et al. [2], this constitutes the assumption of a correct discrimination for the n3 enriched meat. In fact, considering the PLS-DA model (Table 2) we obtained a correct classification of 87.5% of studied samples in the validation group, contrary to what was reported by Pla et al. [4]. The model obtained with L-SVM was even better, obtaining a correct meat classification equal to 92.8%. Unfortunately, the C value, which estimates the distance between the two groups, was high (C=1), showing an excessive proximity of the two groups in a multidimensional plane [2]. However, with a larger group of data, it could be possible to decrease this value, obtaining a clear separation of the two groups.

Table 2 Discriminant analysis models from control (Cg) and linseed (Lg) fed rabbit.

							N non classified		Correctly classified
	Pre-treatment	LV*	R^2_c*	SEC*	R^2_P*	SEP*	Cg_p	Lg_p	Validation set
PLS-DA	No treat.	6	0.83	0.208	0.79	0.230	3	4	87.5%
		C value		Accuracy	Cg_c	Lg_c	Cg_p	Lg_p	Validation set
L-SVM	SVN	1		99.1%	0	1	1	3	92.8%

*See table 1; C value= Cost or regularization parameter.

IV. CONCLUSIONS

This study highlighted the potential of NIR and chemometric analysis as rapid and non-destructive tool to distinguish rabbit meat rich in n-3 fatty acids. Chemometric models using NIR data were able to assign to the correct group in a good percentage of cases. This technique could find application in the future to improve the traceability of rabbit meat.

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