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Nir Spectroscopy and Electronic Nose Evaluation on Live Rabbits and on the Meat of Rabbits Fed Increasing Levels of Chia (S*alvia Hispanica* L.) Seeds

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Abstract: Three groups of ten young rabbits each received a diet enriched with Chia (*Salvia hispanica* L.) seeds at 0% (C0), 10% (C10) and 15% (C15), respectively. At the end of the experimental period, which lasted 35 days, all the rabbits were slaughtered. The Longissimus Dorsi (LD) muscle and perirenal fat samples were collected 24 h post mortem from each carcass and analysed with a GC method for the Fatty Acid (FA) profiles and their indexes. *In vivo* Spectroscopy, was conducted using a portable UV-Vis. NIR spectrophotometer (Model LSP LabSpec-Pro; 350-2500 nm). The LD muscle specimens (2×2 cm long) were fixed in 95% ethanol, stored for 3 days and finally scanned after the tissues had been exposed for 2 h. LD muscle samples were also analysed raw and cooked using a ten-MOS Electronic Nose (EN) device (AIRSENSE). Discrimination of the individuals between couple of groups, fitted with 1 or 2 dummy values, was performed through a Partial Least Square Discriminant Analysis using WinISI II software. The cross-validated R^2 coefficients were retained to compare the matrix distances that were clustered in a Hierarchical Analysis. The *in vivo* and ethanol LD specimen NIR spectra and EN profiles were calibrated with a set of 81 variables. The average cluster, based on the 81 variables, clearly separated the two treated groups from the control group (av.ge R^2 of the matrix = 0.854). The *in vivo* NIR evaluation was similar to the previous one, but at a low level (R^2 = 0.316), while the ethanol muscle specimen highlighted the same pattern, in particular at a higher level (R^2 = 0.374). The EN evaluation confirmed the differences for the raw muscle (R^2 = 0.443), which were then reduced after cooking (R^2 = 0.137). The NIRS applied to the live experimental rabbits showed that this feed experiment produced real differences between the groups. The NIRS applied to the muscle tissues prepared with ethanol showed meat quality traits that were also evaluated by a panel test. In conclusion, EN offer significant knowledge, which normally can only be achieved by a trained panel. The digital spectra can be linked to lipo-oxidation of the intramuscular fat and to a wide set of laboratory analyses, but only for very useful indications and not for purely analytical purposes ($0.40<R^2<0.70$).

Key words: NIRS, electronic nose, meat panel, rabbit, chia, PUFA, TBARS

INTRODUCTION

Progress in Near Infra Red Spectroscopy (NIRS), using recent miniaturized devices, has made it possible to transfer from the laboratory bench to portable applications, for herds or farms. NIR light penetrates the body for some cm (Egawa *et al*., 2003) and the interacting reflected rays are displayed with specific patterns relative to the penetrated tissues and organs. The composition of the carcass of pigs of very different ages was estimated through NIR scans (Mitchell *et al*., 2005) the measurements made on the carcass predicted better carcass fat percents (R = 0.71) than the measurements made on the live animals (R = 0.66), however, both could be improved substantially by including live body weight in the prediction equation (R = 0.93 and 0.91, respectively). The spectral information indicated that the depth of tissue penetration and reflectance may be the main limitations at the present state of this technology. A preliminary report by Masoero *et al.* (2006) concerning live rabbits described a systematic co-variation linked to body weight (R^2 = 0.83) and to herd-genetic factors (R^2 = 0.84), while the hair from dead rabbits appeared very different from that of live rabbits (R^2 = 0.67).

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The NIRS examination of soft animal tissue could be easy if the specimen is immerged in ethanol (Masoero *et al*., 2007a). With firm tissues, such as the perirenal fat of broiler rabbits fed seeds rich in n-3 polyunsaturated fatty acids (PUFA), NIRS analysis successfully discriminated the groups and the Fatty Acid (FA) composition (Masoero *et al*., 2007b).

Rabbits are able to directly incorporate dietary FA into adipose and muscle tissue lipids, making it possible to modify their FA profile through the strategic use of unsaturated dietary fat sources (Dalle Zotte, 2002). Enriched n-3 PUFA rabbit meat obtained by feeding Chia (*Salvia hispanica* L.) could be an alternative to fish to help consumers meet health recommendations, without having to change dietary habits (Peiretti and Meineri, 2008).

The aims of this research were to assess: whether NIRS applied to live experimental rabbits, can show whether feed experiments can produce real differences between groups; whether NIRS applied to muscle tissues prepared with ethanol, can show meat quality traits as an alternative to a panel test; whether Electronic Nose (EN) can discriminative between raw or cooked rabbit meat as an alternative to a panel test; to what extent these digital spectra could be linked to a wide set of laboratory analyses.

MATERIALS AND METHODS

Animals, diets and analytical determinations: Thirty weaned crossbred coloured rabbits aged 50 days and weighing, on average, 1433±28 g were randomly assigned to three groups of ten (Peiretti and Meineri, 2008). The animals were assigned three isocaloric and isonitrogenous dietary treatments containing 0% (C0), 10% (C10) and 15% (C15) of Chia seeds. The experimental period lasted 35 days. The Longissimus Dorsi (LD) muscle and perirenal fat samples were collected 24 h post mortem from the carcass and immediately frozen at -20°C until analysed. The specimens of LD muscle specimen (2 ×2 cm long) and perirenal fat were fixed in 95% ethanol, stored for 3 days and finally scanned after the tissues were exposed for 2 h (Masoero *et al*., 2007a). The proximate composition of the meat and the gas chromatography of the perirenal fat and intramuscular fat were carried out as reported by Peiretti *et al*. (2007). The thiobarbituric acid reactive substance (TBARS) assay was performed as described by Sun *et al*. (2001) on the meat samples after 1, 8 and 14 days of storage at 4°C and after 2 months of refrigeration at 20°C. The results were expressed as mmoli Malonildialdehyde/g dry matter. A set of 81 laboratory variables was used for each observation (Table 1).

Sensory traits: The sensory analysis was performed on thawed samples roasted in a hot air oven at 165°C until an internal temperature of 70°C was reached without salt or spices. The cooked samples were immediately sliced into eight pieces and randomly offered to a trained panel. The trial consisted of six sessions and the assessed traits were: Odour of the raw and cooked longissimus dorsi and of the cooked meat: Flavour, tenderness, juiciness, fibrousness and acceptability. A five-point scale was used: 1 referring to very disagreeable, very tough, very dry, very fibrous and 5 to very agreeable, extremely tender, very juicy, without fibre (Cross *et al*., 1986).

NIRS scan: A spectroscopy scan of the back of live rabbits was conducted using a Model LSP 350-2500P LabSpec-Pro portable spectrophotometer (ASD, Analytical Spectral Devices Inc., Boulder, CO), which was equipped to collect spectra from 350-2500 nm. The probe was an A122100 ASD Model high-intensity reflectance probe that served as an external light source (2900 K colour temperature quartz halogen light) to illuminate the object of interest. This probe can be used to collect reflectance spectra on an area as large as 25 mm in diameter. The reflected light was collected through a 04-14766 ASD Model 1 m long fiber optic jump cable that consisted of a bundle of forty-four, 200 lm fibers. A set of 20 spectra, was collected on the shoulder and then averaged per rabbit. The ethanol specimens of the LD muscle were scanned after 2 h of aeration.

EN analyses: Raw and cooked meat samples were also analysed using a ten-MOS electronic device (AIRSENSE, Analytics GmbH, Schwerin, Germany), as described by Barbera *et al*. (2006). This method utilizes the aromatic and volatile characteristic compounds related to some chemical parameters of the sample. The presence of volatile compounds induces a variation in the Sn-oxide MOS sensor conductivity with respect to the value in standard conditions (zerogas). This variation is due to the reaction between the oxygen species adsorbed on the sensor surface and the gas mix, which comes in contact. The air flux method was used in this trial. The fluxed aroma was obtained using an output needle inserted into a teflon cover of a 50 mL vial, containing 1 g of meat. The meat was then thawed for 24 h and put into a vial 3 h before analysis. A second needle allowed aspiration of the charcoal-filtered air at a 400 mL min^{-1} flux rate. The sample run lasted 30 sec and this was followed by a 120 sec flush time. Each measurement, carried out in duplicate, was controlled and recorded on a text file using WinMunster v.1.6 software.

Table 1: Laboratory variables of the whole data set

Rabbit characteristics	FA of LD muscle	FA of perirenal fat	Meat characteristics
Initial weight	C14:0	C10:0	pH at 0h
Final weight	C15:0	C12:0	pH at 24h
Total feed consumption	C16:0	C14:0	Water
Total weight gain	C16:1	C15:0	Protein
Daily feed	C17:0	C16:0	Lipid
Daily weight gain	C18:0	C16:1	Ash
Feed/gain ratio	C18:1	C17:0	Drip losses
Slaughter weight	C18:2n-6	C18:0	Cooking losses
Commercial carcass weight	C18:3n-3	C18:1	L*
Carcass yield %	C20:4n-6	18:2n-6	a*
Head weight	SFA[1]	C18:3n-3	b*
Liver weight	MUFA[2]	SFA[1]	Chrome
Kidney weight	PUFA[3]	MUFA[2]	Hue
Heart and lung weight	PUFA n-3[4]	PUFA[3]	TBARS at 1 days
Skin and limb weight	PUFA n-6[5]	PUFA n-3[4]	TBARS at 8 days
Hind leg weight	n-6/n-3[6]	PUFA n-6[5]	TBARS at 14 days
Fore leg weight	S/P[7]	n-6/n-3[6]	TBARS at 2 months
Breast and rib weight	Atherogenic Index	S/P[7]	Fish Odour
Loin weight	Thrombogenic Index	Atherogenic Index	Fish taste
		Thrombogenic Index	Tenderness
			Juiciness
			Fibrousnesses
			Acceptability

[1]SFA: Saturated fatty acid; [2]MUFA: Monounsaturated fatty acid; [3]PUFA: Polyunsaturated fatty acid; [4]PUFA n-3: Polyunsaturated fatty acid series n-3, [5]PUFA n-6: Polyunsaturated fatty acid series n-6; [6] n-6/n-3: PUFA n-6/ PUFA n-3 ratio; [7]S/P: Saturated fatty acid/Unsaturated fatty acid

Chemometric analyses: The spectra consisted of 2151 points in the UV-Vis-NIR radiation. Some mathematical pre-treatment was applied case-by-case. Chemometrics was performed with the Modified Partial Least Squares (MPLS) method using WinISI II software, supplied by Infrasoft International (ISI, State College, PA, USA). A cross-validation system was used to assess the optimal number of latent variables that had to be included in the equations and one passage was sometimes allowed for the elimination of any outliers (t>2; H>10). The statistics used for the equation development and evaluation was the coefficient of determination in cross validation (1-VR) reported as R^2 in the tables. The olfactometric measurements of the 10 MOS sensors registered during the first 30 sec were elaborated as concatenated vectors, in a kind of 300 digit spectrum, then calibrated as above. Multivariate analysis of the 81 determinations was performed with the MPLS method using the same software as above, without mathematical pre-treatment. Finally, Hierarchical Ascending Clustering (Stabox V6.5, Grimmersoft, Paris, France) was applied to the matrix distances with (1-VR) coefficients, to build the patterns of the specific comparative sets.

RESULTS AND DISCUSSION

Multivariate analysis of the laboratory variables: The reference pattern based on the 81 variables (Table 1) showed very high values for the discriminant function of the groups, on average 0.854 for the elements in the matrix of the distances (Table 2).

Table 2: Between-group distances (1-VR) in cross-validation mode, of the whole set of 81 variables

	C0	C10	C15	Average
C 0	0	0.984	0.979	
C10	0.984	0	0.598	
C15	0.979	0.598	0	0.854

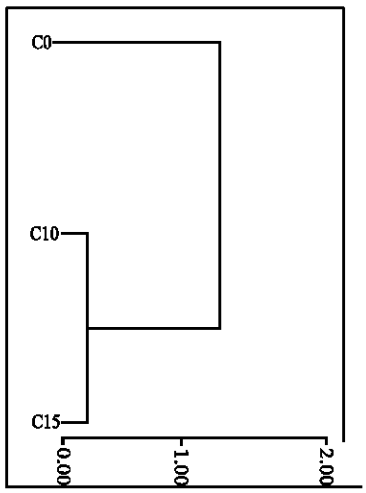

Fig. 1: Average cluster of the groups of the whole set

At the end of the experiment, after a long series of analyses had been carried out, the relative pattern showed a remarkable difference between the control groups and both the 2 groups fed with Chia (Fig. 1).

As far as the instability to lipid peroxidation of intramuscular lipids of rabbits fed Chia seeds is concerned, the four TBARS determinations were elaborated as multivariate. The relative position of the

groups (Fig. 2) appeared to be similar to the previous figure, but the between-group distances were lower than the *in vivo* NIRS (0.264; Table 3). A weak relationships between the *in vivo* NIR spectra and muscle TBARS was apparent for the 2nd and 3rd determinations (0.51 and 0.47, respectively).

NIRS *in vivo*: Before slaughtering, the elaborated *in vivo* NIRS scan predicted the same relative positions as the three groups with a cluster, which appeared quite similar to the final one (Fig. 3), but the between-group distances were low, resulting in an average coefficient of 0.316 (Table 4), a value, which however represents 1/3 of the global achievement with the 81 variables. A number of traits appeared to be linked to the *in vivo* NIR spectra.

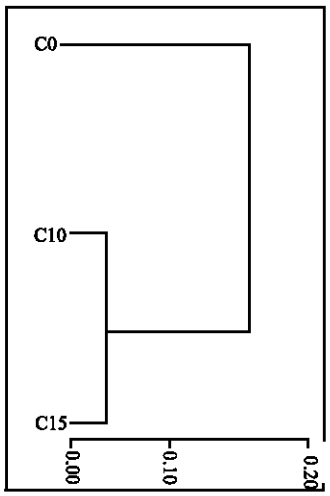

Fig. 2: Average cluster of the groups on the four TBARS determinations

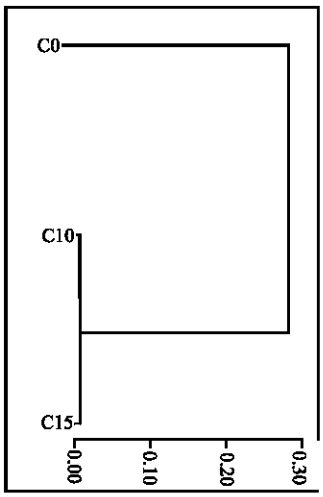

Fig. 3: Average cluster of the groups of *in vivo* NIRS

The livebody weight (0.63) could explain the individual variations, which were confirmed by previous results (Masoero *et al*., 2006). The experimental differences between the groups appeared to be more linked to: C15:0 in the muscle (0.64), C17:0 in the perirenal fat (0.54), drip losses (0.42) and TBARS measured at 8 days (0.51) and at 14 days (0.47).

NIRS of the ethanol muscle specimens: When the ethanol LD specimens were scanned by NIRS and elaborated, the groups were clustered as shown in Fig. 4; nevertheless, the distances were at a level similar to the *in vivo* NIRS, resulting in an average coefficient of 0.374 for (1-VR; Table 5), a value, which represents 44% of the global achievement. A set of special relationships linked these spectra to the panel scores, that is, tenderness (0.56), fibrousnesses (0.65) and acceptability (0.64). These results, concerning the mechanical properties of the cooked meat as testyed by the panellists, corroborated previous results carried out on beef (Masoero *et al*., 2007a).

Table 3: Between-group distances based on the four TBARS determinations

	C0	C10	C15	Average
C 0	0	0.198	0.431	
C10	0.198	0	0.164	
C15	0.431	0.164	0	0.264

Table 4: Between-group distances based on *in vivo* NIRS

	C0	C10	C15	Average
C 0	0	0.271	0.539	
C10	0.271	0	0.136	
C15	0.539	0.136	0	0.316

Table 5: Between-group distances based on the NIRS of the 95% ethanol muscle specimen

	C0	C10	C15	Average
C 0	0	0.470	0.468	
C10	0.470	0	0.184	
C15	0.468	0.184	0	0.374

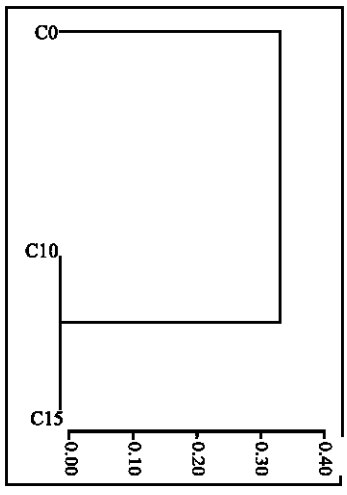

Fig. 4: Average cluster of the groups of the NIRS of the 95% ethanol muscle

Table 6: Between-group distances based on the Electronic Nose (EN) evaluation of raw and cooked muscle

EN raw	C0	C10	C15	Average	EN cooked	C0	C10	C15	Average
C 0	0	0.735	0.550		C 0	0	0.242	0.185	
C 10	0.735	0	0.045		C10	0.242	0	-0.016	
C 15	0.550	0.045	0	0.443	C15	0.185	-0.016	0	0.137

Table 7: Calibration and cross-validation of NIRS and Electronic Nose (EN) digits on the 81 laboratory variables: significant results (1-VR >0.40)

Variables	Type	RSQ	SECV	1-VR
TBARS at 2 months	EN cook[1]	0.92	9.79	0.70
Juiciness	EN raw[2]	0.95	0.27	0.67
Fibrousnesses	EtOH NIRS[3]	0.96	0.30	0.65
C15:0 of LD muscle	*In vivo* NIRS[4]	0.71	0.02	0.64
Acceptability	EtOH NIRS	0.92	0.44	0.64
Live weight	*In vivo* NIRS	0.87	114.00	0.63
Tenderness	EtOH NIRS	0.64	0.40	0.56
C17:0 of perirenal fat	*In vivo* NIRS	0.82	0.03	0.54
TBARS at 8 days	EN cook	0.55	2.82	0.53
TBARS at 8 days	*In vivo* NIRS	0.92	2.70	0.51
Juiciness	EN cook	0.98	0.34	0.50
TBARS at 14 days	EN cook	0.78	3.39	0.48
TBARS at 14 days	*In vivo* NIRS	0.79	2.62	0.47
Tenderness	EN cook	0.54	0.42	0.45
TBARS at 2 months	EN raw	0.70	13.10	0.45
Drip losses	*In vivo* NIRS	0.64	0.59	0.42
Tenderness	EN raw	0.57	0.43	0.41

[1]EN cook: electronic nose on the cooked meat sample; [2] EN raw: electronic nose on the raw meat sample.[3] EtOH NIRS: NIRS on the raw meat sample in 95% ethanol; [4] *in vivo* NIRS: NIRS on the back of the live rabbit

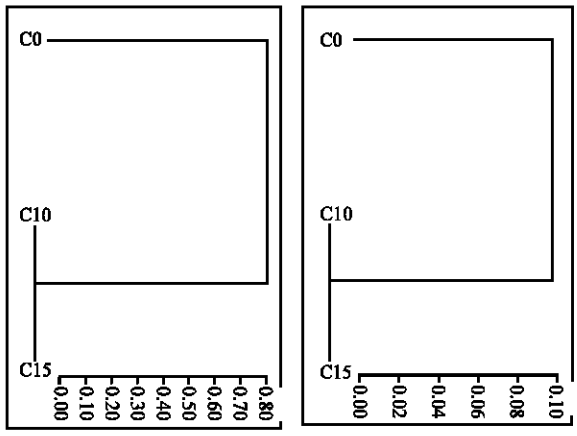

Fig. 5: Average cluster of the groups of the electronic nose of raw (left) and cooked (right) muscle

Electronic nose evaluation: The inclusion of Chia seeds clearly modified the aroma of the raw muscle. When the EN digits were elaborated, the R^2 average values were much higher for the raw muscle and lower for the cooked muscle (0.443 vs 0.137; Table 6), but the patterns were very close to the general one (Fig. 5).

Variables related to NIRS and electronic nose analysis: NIRS is in general reputed as a rapid chemical instrument. According this meaning, none of the selected variables given in Table 7 could be discussed here, because their cross-validation coefficients were in a range between 0.40 and 0.70. When looking at the variables linked to spectra or to MOS sensors it can be observed that the panel scores and to a lesser extent the TBARS, were depended by different tissues and devices.

CONCLUSION

NIRS, applied to the back of live experimental rabbits, can show that a feed experiment can produce real differences between groups. Moreover, the NIRS technique can examine muscle tissues prepared with ethanol to show meat quality traits, especially the rheological properties, which are usually evaluated in a panel test. EN can obtain significant knowledge which is usually only achieved by a trained panel.

In short, some digital spectra can be closely linked to lipid peroxidation of intramuscular fat as well as to a wide set of laboratory analyses, but limited to very useful indications about the clustering of the groups in the trial and not for rigorous, individual, short analytical purposes.

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