FT-NIR SPECTROSCOPY OF TREATED BLOOD PLASMA TO PREDICT CARCASS AND MEAT QUALITY OF YOUNG FEMALE RABBITS

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ABSTRACT

In two replications, involving a total of 32 young females (slaughter-weight 3648 g), half of the rabbits were restricted from solid feeding for 36 hours. The applied fasting treatment resulted in minor global effects (multivariate R^2 = 0.35) in comparison to the replication effects (R^2 = 0.81), which caused remarkable differences in some conventional variables of meat quality. The blood samples were centrifuged and the plasma (1 ml) was submitted to a rapid reaction with ethanol (ETA: 0.5 ml) or to a freeze-drying (FD) process before being examined by FT-NIR spectroscopy (1000-2500 nm). The spectra were correlated and cross-validated to fixed experimental effects: fasting and replication, as binary data (1, 2). Some biological and quality traits of the meat and carcass were also fitted by ISI-2 software chemometrics. In short, 1-VR cross-validated values, which resulted after a double passage for outlier elimination using a liberal t-level of 2, are reported. Replication effects were clearly present in the NIR spectra and appeared to be higher for the FD samples (0.87) than for the ETA ones (0.59). Intramuscular fat in *Obliquus abdominis* and hindleg muscles were more represented in the ETA (0.55; 0.63 respectively) than in the FD spectra (0.26; 0.26), as were the interscapular fat percentage (0.44; 0), the water content of *Longissimus thoracis et lumborum* (0.41; 0) and of *Obliquus abdominis* muscles (0.52; 0.32), and the meat-to-bone ratio (0.39; 0.25), but the liver percentage (0.25; 0.40) was not. The colour and rheological meat quality traits did not generally appear to be correlated to the FT-NIR spectra. The first conclusion that was made was that the freeze-drying of plasma samples does not improve the method, which can function rapidly after a very simple alcohol reaction. The confirmation of several significant relationships with the FT-NIR spectra of plasma will encourage this kind of study, starting from live animals.

Key words: rabbit, plasma, FT-NIR, fasting, meat quality.

INTRODUCTION

The first attempts in a multispecies approach to correlate the FT-NIR spectra of freeze-dried blood plasma of domestic animals to the physiological traits, performances and experimental treatments were made in 2003. Plasma samples in poultry (MASOERO *et al.,* 2003a) collected in two experiments with organic hens (N=25) and broilers (N=22) were analysed with the Radical Oxygen Molecules (ROMs) test. One ml of intact

plasma, freeze-dried in a vial, was transreflected and the spectra were calibrated *vs.* clinical measurements or experimental effects, then the chemometrics were evaluated after cross-validation (1-VR). The FT-NIR enhanced the capacity of separating the kinds of animals (laying hens *vs*. broilers, 0.85) and treatments in laying hens (cage *vs*. bioplus, 0.59), with the involved genetic types on trial (0.68), or in broilers (bio_movement *vs*. bio_resting, 0.68). The equivalent figures, based only on the ROMs analyses were lower: 0.43; 0.51; 0.25 and 0.55. In general, the ROMs values appeared to be correlated to FT-NIR (1-VR= 0.49). A total of 327 samples of *Piemontese* beef cow plasma, stored at –20 °C for 9-44 months (MASOERO *et al.,* 2003b), were thawed then freeze-dried in vials. The effect of the storage duration was clearly perceived by the FT-NIR (0.67). The gestation duration, calving facility and parity instead appeared to be barely linked (1-VR= 0.21; 0.11 and 0.27 respectively) as were some clinical analyses (Packed Cell Volume, 0.39; creatinine, 0.24; insulin, 0.14 and glucose, 0.18). All the effects of the nutritional treatments on 130 cows during late gestation in 5 experiments were only appreciated in the calibration mode. All these preliminary findings dealt with plasma samples that were previously freeze-dried in glass vials.

The aim of this paper was to test the rapid coagulation of liquid plasma, according to its reaction with ethanol, compared to the preparation based on the 3-d long freeze-drying process. The experiment was carried out on rabbits that had been submitted or not submitted to a mild preslaughter fasting, which had been replicated twice. The fresh *vs* freeze-drying *vs.* ethanol fixation procedures of three rabbit muscles for FT-NIR reflectance spectroscopy have also been compared in a companion paper (MASOERO *et al.,* 2004).

MATERIAL AND METHODS

The experiment was carried out in two replications using a total of 32 hybrid females, at around 80% of their adult body weight, 16 rabbits for each repetition, the half of which was made to fast. Before the final fasting began, the rabbits were weighed then made to fast for a 36-hour period. The other animals were fed 150 g/d of a commercial diet (crude protein 16.5%, crude fibre 14.0%) until slaughtering occurred. At the bleeding, a sample of the mixed blood was collected in a 10-mL Li-heparin Venoject tube (Terumo Europe, Leuven, Belgium). It was refrigerated, then centrifuged (2.850 x g for 20 min) within two hours from bleeding. After further two hours, 1 ml of plasma was placed in a glass vial, then 0.5 ml of ethanol 95% (ETA) was added and a scan was made after mild and stable coagulation. A second vial containing 1 ml of intact plasma was stored at –18 °C for 45 days before freeze-drying (FD). Carcasses chilled for 24 hours at 4 °C were examined according to harmonised procedures (BLASCO *et al.,* 1996). The right *Longissimus thoracis et lumborum* (LL) muscle and two strips of muscles cut around the *linea alba* of *Obliquus abdominis* (OA) were sampled from each carcass. Both these LL and OA muscles were divided into three portions. The first share was used to determine the moisture. The second was placed in a plastic tube, immersed in ethanol 95% and analysed for dry matter content after two days of storage. The third one was stored at -18 °C, and was then weighed and freeze-dried, to calculate the sample water by freeze-drying. After dissection and separation of the hindleg (HL) into meat and bone, a sample of the meat was minced and stored at -18 °C until freeze-drying was carried out, and the

water loss was determined after freeze-drying. The pH - using a Crison 507 pH-meter - and L* a* b* colour, chroma (C*) and hue (H*) indexes - using a Minolta CR331C Chromameter - were determined on the raw left LL. The cooking losses of the LL - 30' at 70 °C - and the peak shear force (kg/cm^2) were measured – using an Instron with a Warner-Bratzler device – both on the LL cooked sample and on the LL share stored with ethanol for two days.
The vials containing the liquid (ETA) or intact freeze-dried (FD) plasma were directly submitted to FT-NIR spectroscopy in transreflectance mode using a Spectrum IdentiCheck FTNIR System (Perkin-Elmer, Beaconsfield, England; 1000-2500 nm, 3001 points). The spectra were imported in the NIRS-2 software, then mathematically treated as 1,5,5,1 with SNV and Detrend, and calibrated with an MPLS method set to 2 passages for outlier elimination with a liberal t-level (2.00), standard X (10) and H values (10). Cross-validation was performed on several subgroups (25) and the retained statistic parameter from this software was the 1-VR, corresponding to an R^2 in validation mode. The fitted values were binary data, replication and fasting, and quantitative for the biological variates. Statistical analyses of laboratory data were performed using an analysis of variance with a two-factor model and also using a stepwise regression (SAS), which ranked the most relevant variables while accounting for the main effects of fasting and replication.

RESULTS AND DISCUSSION

The loss of live weight in animals that have been made to fast for the final 36 hours rose from 2.2% to 3% in the first and second replications respectively. Therefore, the slaughter weight of the fasted rabbits appeared to be 1.4% lower in comparison with the fed ones because a reduction in gross (-12.1%) and urine-net (-6.2%) gut % and in liver (-16.7%) (Table 1). According to the stepwise discrimination, the drip loss percentage of the carcass explained the maximum of the fasting effect (+23%; R^2=0.45), followed by the net-urine gut incidence (R^2=0.065). The occurrence of the perirenal fat (0.037) and of the hindleg percentage (0.039) appeared to be quite spurious, because they were not significant as individual variates (Table 1). The final pH increase (Table 2; P<0.03) and the consequent reduction in Lightness (L*: P<0.01) agree with the previous findings (OUHAYOUN and LEBAS, 1994; MASOERO *et al.,* 2003c). The shear force, which appeared to be quite high because of the relative advanced age of the animals, both the cooked (-6%) and the ethanol treated LL (-10%), was reduced though not significantly from solid fasting, and this was in contrast with previous findings (MASOERO *et al.*, 2003c). The general hydration status of the fasted animals was apparently increased, as denoted by the higher drip losses (a key-variable), but after refrigeration there was no difference in absolute water, between the LL and the OA. Nevertheless, the water holding capacity of the muscles was markedly lowered, because after the freeze-drying process the sublimated water was higher in HL (P<0.01), LL (P<0.09) and OA (P<0.19) (Table 2). Furthemore, during the ethanol fixation process, the substitution of water was easier in the muscles of the fasted animals, thus the final apparent water (ethanol-water mixture) tended to be increased in OA (P<0.17).
As far as the multivariate discrimination is concerned, the general trend of the conventional analyses showed a global discrimination level that was clearly higher for

the replication effect (R^2=0.81) than for the fasting effect (R^2=0.35), when the variables in Table 2 -excluding body composition traits- were regressed. Some not singularly significant variables became discriminative when they were covariated. This was the case for the shear force of cooked LL for the fasted animals, while the pH lost significance. When the replication effects were examined, many minor variables reached discriminative ability. The variables linked to the water content appeared to be the most discriminant of the replication experimental conditions: the R^2 coefficient 0.81 mainly depended on the three water-linked measures of LL (variables #12-14) and the water of OA (#15) (R^2= 0.55). These last findings emphasize the difficulty of respecting all the operational conditions exactly.

Table 1. Slaughter results and carcass measurements

	Fed	Fasted	(Fasted-Fed)/mean	*P*	RSD	Stepwise analysis R^2
Slaughter weight (g) (SW)	3674	3623	-1.4%	*0.71*	376	
Skin (% SW)	15.52	15.85	2.1%	*0.23*	0.75	
Gross Gut incidence (% SW)	16.62	14.73	-12.1%	*0.01*	2.24	
Net-urine Gut incidence (% SW)	17.02	16.00	-6.2%	*0.001*	1.55	0.065
Chilled carcass weight (g)	2275	2274	0.0%	*0.99*	249	
Drip loss percentage (HCW)	1.44	1.81	22.8%	*0.0001*	0.21	0.450
Cold dressing percentage (% SW)	61.87	62.81	1.5%	*0.19*	1.96	
Reference carcass (g) (RC)	1881	1885	0.2%	*0.96*	249	
Perirenal fat (% RC)	2.89	2.51	-14.1%	*0.59*	1.99	0.037
Interscapular fat (% RC)	0.61	0.61	0.0%	*0.99*	0.58	
Head (% RC)	10.34	10.63	2.8%	*0.62*	1.62	
Liver (% RC)	4.34	3.67	-16.7%	*0.07*	0.98	
Kidneys (% RC)	1.44	1.24	-14.9%	*0.33*	0.55	
Hindlegs (% RC)	31.08	30.77	-1.0%	*0.68*	2.06	0.039
Hindleg bone (g)	38.16	38.16	0.0%	*1.00*	3.56	
Hindleg muscle/bone ratio	7.66	7.62	-0.5%	*0.90*	0.80	
Femur weight (g)	16.27	16.21	-0.4%	*0.93*	1.73	

[1] HCW = Hot carcass weight.

The conclusions from the NIR evaluations in general agree with the conclusions from conventional laboratory analyses examined as multivariate. According to the FT-NIR chemometrics in cross-validation mode (1-VR), the replication effects were obviously present and higher for the FD (0.87) than for the ETA spectra (0.59). Only the ETA spectra showed fasting effects, but at a very low level (0.10). Therefore, the preparation-by-ethanol reaction could appear to be more stable and less sensitive to the replication noising effects. Some conventional chemical variables appeared to be linked to the FT-NIR spectra in cross-validation mode. In particular, the estimated intramuscular lipids of OA and HL were more represented in the ETA (1-VR = 0.55 and 0.63) than in the FD spectra (0.26 and 0.26) as were for many water content measurements of the three muscles. It was no surprise to observe that the FT-NIR spectra can be linked to the tissue composition traits. The most relevant variables were the meat / bone ratio of the hindleg (1-VR = 0.39 for ETA *vs* 0.25 for FD), regarding meatiness, and the liver

percentage (0.25; 0.40), while fatness only appeared to be linked to the ETA plasma (0.44).

Table 2. Meat quality and selected body traits: uni-multivariate analysis and chemometrics by FT-NIR of ethanol-treated and freeze-dried plasma

					Fasting		Replication			1-VR	1-VR
#		Mean (M)	RSD	(Fasted-Fed)/M	Prob.	R^2 Stepwise [1]	(Rep2-Rep1)/M	Prob.	R^2 Stepwise [1]	PLASMA ETA [2]	PLASMA FD [3]
1	Replication								0.81	0.59	0.87
2	Fasting					0.35				0.10	0
3	pH	5.73	0.11	2%	*0.03*		-1%	*0.50*	0.03	0.03	0.06
4	L*	61.05	1.87	-3%	*0.01*	0.18	1%	*0.24*	0.02	0.04	0
5	a*	8.78	2.02	-6%	*0.49*		4%	*0.61*		0	0.15
6	b*	6.33	2.1	-4%	*0.75*		-9%	*0.41*		0	0
7	C*	10.68	1.73	-9%	*0.12*	0.12	*4%*	*0.51*		0	0.23
8	H*	35.04	7.14	-7%	*0.37*		0%	*0.99*	0.02	0	0
9	Cooking losses (%)	21.58	2.05	-2%	*0.58*		-7%	*0.05*	0.05	0	0.34
10	Peak shear force in cooked LL (kg/cm^2)	4.15	1.06	-6%	*0.47*	0.05	5%	*0.60*	0.07	0	0
11	Peak shear force in ETA LL (kg/cm^2)	4.70	1.04	-10%	*0.21*		13%	*0.10*		0	0.40
12	Water LL (%)	74.44	1.17	0%	*0.44*		0%	*0.42*	0.08	0.41	0
13	Dry matter LL in ETA	31.84	1.54	2%	*0.43*		-4%	*0.01*	0.22	0.11	0.37
14	Water LL by FD	72.02	0.9	1%	*0.09*		0%	*0.75*	0.07	0.30	0.16
15	Water Obliquus abdominis (OA)(%)	64.08	5.96	1%	*0.77*		5%	*0.11*	0.18	0.52	0.32
16	Dry matter OA in ETA	50.42	7.51	8%	*0.17*		14%	*0.01*		0.54	0.26
17	Water OA by FD	64.01	6.48	5%	*0.19*		7%	*0.07*		0.47	0.21
18	Water Hindleg (HL) by FD	73.95	1.25	2%	*0.01*		1%	*0.37*		0.48	0.17
19	Estimated LL lipids [4] (% d.m.)	3.62	2.3	-13%	*0.58*		33%	*0.15*	0.07	0.12	0
20	Estimated OA lipids [4] (% d.m.)	44.02	19.7	-2%	*0.87*		-3%	*0.83*		0.55	0.26
21	Estimated HL lipids [4] (% d.m.)	2.10	2.73	-54%	*0.24*		-54%	*0.24*		0.63	0.26
22	Muscle/bone of Hindleg	7.6	0.8	-1%	*0.90*		7%	*0.10*		0.39	0.25
23	Perirenal fat %	2.7	1.99	-14%	*0.59*		-38%	*0.16*		0.11	0
24	Interscapular fat %	0.6	0.58	0%	*0.99*		-16%	*0.66*		0.44	0
25	Liver %	4.0	0.98	-17%	*0.07*		16%	*0.08*		0.25	0.40

# = item; ETA = ethanol 95%; FD = freeze-drying; [1] = R^2 Stepwise regression excluding items # 22-25;
[2] = Number of outliers: mean±sd: 2.7±1.6; [3] = Number of outliers: mean±sd: 3.0±2.4; [4] the lipids were estimated from our previous NIR equations (MASOERO *et al*., 2004); the negative values of 1-VR were set to zero.

This work, therefore, confirms our previous recent findings. In literature the authors have found no other similar reference except to the work by DOORNENBALL *et al.,* (1986) concerning the creatinine and other nitrogen metabolites with meatiness in pigs.

CONCLUSIONS

In this preliminary study the calibration process excluded about 10% of the samples as outliers. The cross-validation of several subgroups, however, offered a guarantee about the reliability of the NIR relationships, which should be intended, according to the aim of the paper, as being an exploratory method, and not strictly analytical.
The freeze-drying of plasma samples did not improve the method, which can function rapidly after a very simple alcohol reaction to scan a trans-reflectance spectrum.
The confirmation of many significant relationships with the FT-NIR spectra of plasma will encourage this kind of study, starting from live animals.

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