DRYING MEAT SAMPLES FOR THE NIRS ANALYSIS: APPLICATION TO APPRAISE HOUSING EFFECTS IN RABBITS

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Background

Near Infrared Reflectance Spectroscopy (NIRS) is a physical not destructive method appreciated for fast chemical analyses, when operating in a quantitative manner on fresh (Naes et al., 1996) or freeze-dried meat (Masoero et al., 1994). By this technique we also obtained approximate estimation of macro and micro-mineral content of beef freeze-dried meat samples (Masoero et al., 1996).
Nevertheless the NIRS technique can appreciate global differences among tissues or products (Masoero, 2000).

Objectives

The aims of this trial were to compare freeze-drying *vs* other methods to dry meat samples for off-line NIRS analysis and to estimate effects of the environment on rabbits housed in different conditions.

Methods

Thirty-six New Zealand White rabbit females aged 90 d were fed *ad libitum* till to 120 d in three housing systems: 1-(Small) - Small space in wired cage (cm 31 * 41 * h 31 / 1 rabbit), 2-(Large) - Large space in wired cage (cm 64*60* h 44 / 1 rabbit), 3-(Triplet) - rearing of three females in a Large wired cage (cm 64 * 60* h 44 / 3 rabbits). After slaughtering, the carcasses were refrigerated 24 h, then dissected according to harmonised methods (Blasco et al., 1993). In the framework of collecting sampling for genetic and NIRS investigations (Masoero et al., 2000) hind-leg minced meat samples of about 70 g each were recovered in duplicated for this study. After storage at –20°C the samples were submitted to the four drying systems: a- Freeze-drying (FD); b- Vacuum-Micro-Wave (VMW) using a Milestone HETHOS-900 device (300 W for 15 min, pressure starting from 60 then raising at max 180 mb after 10-13 min); c- Conventional-Micro-Waved (CMW) (Moulinex *compact*, 240 W for 24 min); d- ventilated conventional Oven (O) (3d at 60 °C). After having ground the samples, NIR spectra recorded by a NIRSystem 4500 Foss-electric monochromator were mathematically treated as 1,4,4,1 and submitted to stepwise regression to calibrate equations in order to: i- point out the most important wavelengths discriminating the drying systems (a-d), and, ii – fit the housing system by a qualitative binary discrimination, calibrated on dummy values (1,2) according to Fearn (1997) for the three couples of comparisons (1-2, 1-3 and 2-3). The scores resulting from chemometrics, which was performed by NIRS 2 v. 3.0 standard software, were statistically analysed, to obtain correlation of the reference drying system (FD=a) with the alternative ones (b, c and d), to test the interaction Drying system * Housing group, to fit to expected values, and to discriminate the housing groups. This last discrimination was carried on even on the individuals, using the averages of the estimated scores relative to the four drying systems.

Results and discussion

Each system of drying the muscles produced original and very strong effects, which were amplified by the NIR Spectroscopy and capitalised by chemometrics in R-squares coefficient, which, with one only wavelength reached values from 0.898 to 0.995 (Tab. 1). These wavelengths are very interesting because they are linked to the strong dehydration systems: the 2190 nm perfectly differentiated the 3-d long warming processing of the muscle in a oven at 60 °C (O system) and also the Conventional-Micro-Waved (CMW) samples from the freeze dried tissues: a thermal effect can be hypothesised. The main differences in Conventional-Micro-Wave system *vs* Vacuum-Micro-Wave were found out in the 2062 nm region; the applied electric power was higher, while the occurrence time was shorter in Vacuum-Micro-Wave, but the denaturative effect of the 2450 MHz (12.25 cm) radiation was *a priori* reduced, because at 180 mb water evaporates near 65 °C.
The effect of housing system (Tab. 2) was strongly perceived by the NIR Spectroscopy of the hind-leg minced muscles. The large availability of space (2- Large) produced rabbits with different characteristics in their muscles as compared to the muscles obtained in the other two housing systems where the rabbits grown with a limited amount of available space, both as single (1- Small) and as group (3-Triplet). Thus the general scheme emerging from the freeze-dried samples (FD), elected as the reference method showed that the 1-3 samples were nearer (R^2c=0.40) than 1-2 and 2-3 (R^2c=0.63 and 0.62 respectively, even stable when cross-validated). For the most distant groups, the two Micro-Wave dryings and Oven gave the same figures at a somewhat lower level, while the narrower differences between 1-3 samples were only appreciated by Conventional-Micro-Wave (correlation r=0.45) and not with other systems, because a significant interaction of Drying * Housing systems occurred. In spite of the examined R^2 values, a confirmation of equivalence of the methods came from the comparison of the wavelengths involved into differentiating the groups. Two bands, the first around 2200-2300 nm and the second around 1700 nm, appeared responsible of the discrimination between the groups 1-2 and 2-3. In a companion paper on beef cattle housed as tied or free-stalled (Masoero et al., 2002) the most discriminant wavelength was set at 2220 nm. With Conventional-Micro-Wave preparation these bands appeared to be anticipated in the regions at 2100 and at 1300 nm. The correlation coefficient of scores with the FD reference method ranged from 0.86 to 0.53 in the comparisons 1-2 and 2-3, but it was reduced from 0.45 to –0.25 in the more restraint domain of variation into the comparison 1-3. When the scores of the four drying systems were averaged, a perfect discrimination of individuals was possible between the groups 1-2 and between the groups 1-3 (correct comparisons between individuals = 144/144, Fig.1) and to a lesser extent also between the animals reared in small cages or by triplets (comparison 2-3 = 141/144).
As conclusion, when a short-cut would be envisaged to speed up NIRS analyses of meat, avoiding refrigerated transportation and long-time freeze-drying operation, a local immediate preparation of samples by a Conventional-Micro-Wave dehydration could maintain potential effects (and related substances) to be analysed by NIR and eventually by IR Spectroscopy. The other essayed preparations, i.e. the Vacuum-Micro-Wave and the normal prolonged Oven treatment did not evidence significant advantages. A confirmation of these inferences, however, should derive from an object-oriented investigation about specific goal (i.e. collagen content, lipids, mineral composition .etc...) and not only to ontogenetic, environmental and generic effects. This trial is however important because it confirms NIRS ability to enhance housing effects in experimental comparative operations.

Pertinent literature

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Table 1. NIRS discrimination ability of the Drying system. Simple regression on the most relevant wavelength

	FD	VMW		CMW		O	
		R^2c	nm	R^2c	nm	R^2c	nm
FD- Freeze-Drying	1	0.983	2198	0.975	2190	0.995	2190
VMW – Vacuum-MicroWave		1		0.923	2062	0.898	2190
CMW – Conventional MicroWave				1		0.924	2190
O – Oven 60°C						1	

$R^2c = R^2$ coefficient in calibration mode of the NIR spectra; nm = the most relevant wavelength

Table 2. Discrimination of NIRS to separate individuals between the three groups and parameters of the stepwise regression equations

Comparison	R^2SAS average	Freeze-Drying FD				Vacuum-Micro-Wave VMW				Conventional Micro Wave CMW			Oven 60°C O		
Discrimination	R^2SAS individ. *P(int)*	R^2c R^2cv r(ref)	nm1	nm2	nm3	R^2c R^2cv r(ref)	nm1	nm2	nm3	R^2c R^2cv r(ref)	nm1	nm2	R^2c R^2cv r(ref)	nm1	nm2
1 Small – 2 Large	0.85	0.63				0.44				0.64			0.40		
144/144	0.59	0.56	2294	1720	2270	0.21	2294	1720	2286	0.59	2102	1308	0.33	2166	
	0.24	1				0.66				0.53			0.66		
1 Small – 3 Triplet	0.59	0.40				0.09				0.15			0.02		
141/144	0.31	0.30	2054	1744		0.01	1608			0.10	2078		0.00	2078	
	0.004	1				0.37	208			0.45	132		-0.25		
2 Large – 3 Triplet	0.80	0.62				0.65				0.56			0.50		
144/144	0.62	0.54	2334	1720		0.56	1728	2286	1696	0.51	1348		0.46	1712	1720
	0.73	1				0.86				0.64			0.79		

Comparison = Binary contrast of individuals between the three Housing systems (1,2 and 3); Discrimination = Number of correct comparisons (in total 12*12=144) by the two groups based on the average of the four binary estimates with corresponding R^2 SAS average= R-square coefficient of average's ANOVA by comparisons. R^2 SAS individ. = R-square coefficient of individual's ANOVA by comparisons; P(int)= Probability of the interaction Drying * Housing systems; R^2c= R-square coefficient in calibration mode; R^2cv= R-square coefficient in cross-validation mode; r(ref)=correlation with the reference method (FD); nm1-3= wavelength selected by stepwise regression

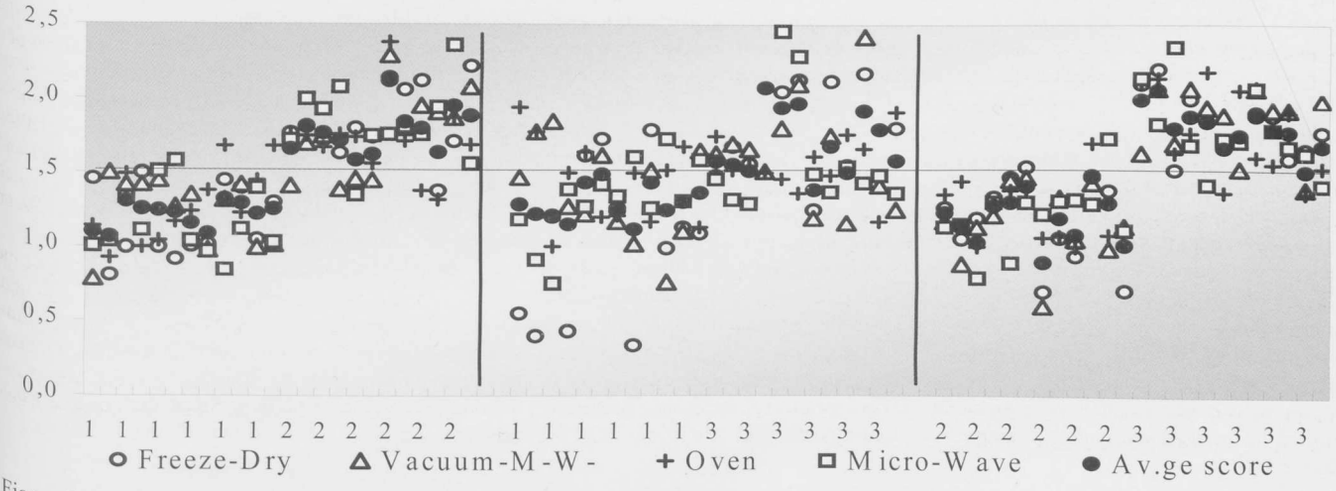

Figure 1. Plot of individual estimates from the binary discrimination of groups 1-2, 1-3 (significant interaction) and 2-3