DISCRIMINATION OF HOUSING SYSTEM EFFECTS IN THE MUSCLE OF FATTENING RABBITS BY NIRS USING ETHANOL OR FREEZE-DRIED SPECIMENS

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Introduction

Meat researchers have long sought after non-destructive and objective techniques to predict meat quality (Leroy *et al*., 2002). Several studies have stated that near-infrared reflectance spectroscopy (NIRS) can be used to predict extrinsic meat traits, such beef tenderness and texture traits. Cozzolino and Murray (2004), capitalized on the advances in instrumentation, that now allow a single instrument to scan the entire electromagnetic spectrum (350–2500 nm, UV-Vis-NIRS), and developed a repeatable, non-destructive technique for the spectroscopic evaluation of meat quality. Many authors have investigated off-line experimental procedures, which are destructive in that they require excision of a muscle sample for spectroscopy. In the present work, we are interested in obtaining easy and stable samples from animal muscle. Ethanol preparation of specimens for NIRS analysis was first utilized in rabbits (Masoero *et al*., 2004), and was then replicated in buffalo (Masoero *et al*., 2005) and in cattle (Masoero *et al*., 2006) allowing significant, easy and rapid NIRS discrimination results of the systematic main factors to be obtained. The aim of this research, where NIRS methodology has gained a prominent role, was to establish the consequences on the meat quality traits of two main factors concerning the intensive housing of fattening rabbits, i.e. the rabbit density and the floor type.

Materials and Methods

Twelve subgroups, derived from three Housing Density Ratios (HDR: 10, 13 and 16 rabbits/m^2) and four combinations of floor types (Wire/Straw Floor: WSF) were tested. The 4 WSF combinations were wire5-11wks; Straw5-11wks; Wire5-9wks+ Straw10-11wks; Wire5-7wks+ Straw8-11wks. The rabbits were slaughtered at 11wks in a randomized order on the same morning. After 6 days of chilling, a slice of approximately 30 mm height and 20 mm diameter taken from the *Longissimus dorsi* (LD) muscle was plugged into a tube of 50 ml containing 30 ml of ethanol 95% and stored in the dark. The specimens extracted from the tube were tested using a penetrometer (Schiltknecht, Ing., Zurich) with repeated needle perforation (n=10). Spectroscopy was conducted on the 2-hr air-exposed samples 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 to 2500 nm. The probe was an ASD Model A122100 high-intensity reflectance probe that served as an external light source (2900 K color 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. Reflected light was collected through an ASD Model 04-14766 1-m long fiber optic jumper cable. A spectrum was collected for each specimen, on a surface of the freshly exposed LD muscle. Spectra were collected with the LabSpec Pro software ''sample spectrum count'' option set to 20 scans averaged per located observation. A second scan was conducted on the freeze-dried powdered meat obtained from the whole hindleg (HL) after anatomic dissection and mincing. A whole set of 16 laboratory analyses, reported in Table 1, were performed according to Masoero *et al*. (2004) and to Berzaghi *et al.* (2005). All the 2151 spectra digits (350-2500 nm) were statistically analyzed by using the multivariate chemometric method using the Modified Partial Least Squares (MPLS) software of WINISI-II from Infrasoft International (ISI, State College, PA, USA). A cross-validation system assessed the optimal number of latent variables to be included in the equations and permitting one passage for elimination of the outlier samples. Pretreatment of the spectra (normalization, detrend and derivation) was tailored on each calibration. The two main factors of the experiment design, HDR and WSF were separately fitted to their scalar values (HDR = 10/13/16 rabbits/m^2; WSF = 1/2/1.33 and 1.67, respectively. The 4 WSF classes were fitted according to the time spent on Wire (1) or Straw (2). Furthermore, three binary contrasts were calculated, according Barker and Rayens (2003), for the internal discrimination of the HDR effects. The conventional 16 laboratory variables were elaborated by the same MPLS software, without pretreatment, and this served as a basis of comparison for reference discrimination. A simple regression analysis was run to locate the most significant variable in the models.

Results and Discussion

Table 1 reports the variability concerning the laboratory variables, normally distributed with an elongated tail on the left for cooking loss and cholesterol or on the right tail for the pH6d and strength measurements. The pertinent group (1-12) was discriminated by the HL water content (R^2=0.63, Table 2) as well as by using the whole set of variables (R^2=0.62) and similarly by the NIRS of the HL-FD (R^2=0.57) and slightly lower than LT-ETH (0.47). The housing density was best discriminated by the NIRS of HL-FD (R^2=0.70), but was worse in the LD-ETH specimens (R^2=0.54).

The maximum explication was obtained by cooking loss (R^2=0.69) which appeared to be negatively correlated to the rabbits/m^2 (r=-0.83). In fact, only the higher density (16 rabbits/m^2) was really discriminated from the two other densities, except for the NIRS of the HL-FD (R^2=0.36). The correlation coefficients of the number of rabbits per m^2 with the cholesterol content and the cooking loss were also negative at the highest density (r= –0.95 and –0.86, respectively) and both the ETH and the FD specimens gave efficacious discriminations. The floor effect was inconsistent as far as to the laboratory variables and NIRS of the ETH specimens are concerned but only the FD preparations gave a weak discrimination of the four classes (R^2=0.23).

Table 1. Laboratory variables

N=112	Unit	Mean	SD	Skewness
1. LD pH 6d		6.09	0.24	1.22
2. LD L* 6d		52.82	4.65	-0.47
3. LD a* 6d		4.64	1.39	-0.02
4. LD b* 6d		1.92	1.59	-0.20
5. LD Cooking loss	%	29.52	2.67	-0.98
6. LD W-B shear force	kg/cm^2	6.20	0.89	0.80
7. Penetrometer	mm	21.54	7.99	0.90
8. HL Protein	%DM	82.79	2.72	0.01
9. HL Ash	%DM	4.43	0.20	-0.38
10.HL Ether extract	%DM	12.70	2.93	0.04
11. HL Cholesterol	mg/100g DM	247.6	16.3	-0.97
12. HL Water	%	74.11	0.97	-0.22
13. HL Protein	%	21.41	0.40	-0.15
14. HL Ash	%	1.15	0.03	-0.35
15. HL Ether extract	%	3.31	0.87	0.25
16. HL Cholesterol	mg/100g	64.0	3.3	-1.02

Table 2. Fitting the spectra and variables to the models

Models	R^2cv 16 Laboratory Variables	R^2cvNIRS LD-ETH Ethanol	R^2cvNIRS HL-FD freeze-dried
Group (1-12)	0.62	0.47	0.57
# 12 r^2 0.63			
Wire Straw Floor – WSF	0.02	0	0.23
0			
Housing Density Ratio – HDR	0.67	0.54	0.70
# 5 r^2 0.69			
HDR 10-13	0.06	0.17	0.36
#2 r^2 0.05			
HDR 10-16	0.89	0.66	0.89
#11 r^2 0.90			
HDR 13-16	0.71	0.66	0.59
#11 r^2 0.74			
Average R^2cv	0.49	0.42	0.56

R^2cvNIRS= R^2 cross validation; LD-ETH = *Longissimus dorsi* in ethanol; HL-FD= Hindleg Meat freeze-dried. DM=dry matter; # = the variable with higher r^2

Conclusions

With the purpose of discriminating specific groups of rabbits, affected by special intrinsic characteristics, whose partial extrinsic traits were low cholesterol content and weak cooking losses, the NIRS evaluation of the freeze-dried muscle tissue specimens appeared to be superior to a complete set of laboratory analyses for meat quality determination. When the fixation of the muscle mass was obtained by an easy and rapid immersion in ethanol, the subsequent NIRS evaluation demonstrated that the discrimination ability preserved almost 85% of its effectiveness, but resulted in an immeasurable great efficiency.

References

1. Barker M., Rayens W. (2003). Partial least squares for discrimination. Journal of Chemometrics. 17, 166-173.
2. Berzaghi P., Dalle Zotte A., Jansson L.M., Andrighetto I. (2005). Near-infrared reflectance spectroscopy as a method to predict chemical composition of breast meat and discriminate between different n-3 feeding sources. Poultry Science. 84, 128-136.
3. Cozzolino D., Murray I. (2004). Identification of animal muscles by visible and near infrared reflectance spectroscopy. Lebensmittel - Wissenschaft Technologie. 37, 447–452.
4. Leroy B., Lambotte S., Dotreppe O., Lecocq H., Istasse L., Clinquart A. (2002). Prediction of technological and organoleptic properties of beef *Longissimus thoracis* from near-infrared reflectance and transmission spectra. Meat Science. 66, 45-54.
5. Masoero G., Brugiapaglia A., Bergoglio G., Chicco R. (2004). FT-NIR spectroscopy of fresh and treated muscle tissue in young female rabbits. Proc. "8th World Rabbit Congress", Puebla, Mexico, 7-10 September. 1409-1415.
6. Masoero G., Bergoglio G., Vincenti F., Destefanis G., Brugiapaglia, A. (2005). A rapid new ethanol preparation for early muscle sampling submitted to NIR analysis: preliminary results for cattle and buffalo. Italian Journal of Animal Science. 4(2), 299.
7. Masoero G., Iacurto M., Sala G. (2006). Ethanol specimen of beef muscles samples for NIRS is rapid but efficient as the freeze-dried. Proc. "EAAP Congress". Anthalya, Turkey, 60.