

INVESTIGATION OF RABBIT CARCASS COMPOSITION AND EXPERIMENTAL EFFECTS BY NIRS OF DRIED MUSCLE

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SUMMARY

The NIR spectra of the meat products are representative of a widerange of substances. The aims of this experiment were to investigate the ontogenetic relationships of the NIR spectrum of the muscular tissue with meatiness and fatness of the carcass and also to perceive chemical modifications caused by experimental effects, i.e. after clenbuterol treatment. In 55 meat rabbits aged 86.6 ± 3.7 days and weighing 2440 ± 336 grams the NIR spectra of freeze-dried integer and homogenized longissimus dorsi lumborum (LD) and homogenized hindleg muscles (HL) dried by two freeze-dryers, were calibrated and cross-validated to meatiness traits by removing few outliers. The meat/bone ratio of the hindleg was calibrated with R^2c 0.77 and 0.60, and cross-validated with R^2cv 0.40 and 0.33 respectively from LD and HL, while the bone percentage was fitted with R^2c 0.62 and 0.58, and R^2cv 0.23 and 0.40. Other tissues and correlative traits were fitted with similar or less accuracy, except the fatness (R^2c 0.7-0.9). The ability of the NIRS to discriminate individual clenbuterol treated (1 ppm) vs untreated was demonstrated by a R^2c 0.91 and R^2cv 0.75 from LD and 0.86 and 0.72 from HL. The muscle origin was fitted with R^2cv 0.97. The tool had power to investigate together chemical and ontogenetic relationships.

Introduction

The Near Infrared Reflected or Transmitted Spectra of the meat products are representative of a wide range of substances: minerals, moisture, fat, protein and collagen (Chevalier et al., 1990). After a first direct endeavour to calibrate many rabbit's traits from the skin of living rabbits (Masoero et al. 1992) it was attempted an unconventional use of the NIRS: not as direct analytical instrument but as indirect tool to perceive some fixed experimental effects and body composition. A quick development of cooperative researches involving 458 freeze-dried muscles from 338 animals (preliminarily reported in Masoero et al., 1994) advanced knowledge in rabbits experiments. This trial was conducted to evaluate the correlativity of NIR spectra with: i) the ontogenetic relationships of meatiness and fatness, and: ii) the discrimination of beta-adrenergic agonists (BAA) treated from untreated individuals, by perceiving chemical as well as induced ontogenetic modifications.

Materials and methods

Fifty-five albino rabbits were reared in single wired cages since 43 to 81 days being fed with a commercial diet (156 g/kg crude protein, 156 g/kg fiber). Five groups of rabbits were constituted and fed respectively: 1) ad libitum untreated; 2) restricted untreated; 3) restricted and treated with Clenbuterol (SIGMA C-5423) at 1 ppm; 4) restricted and treated with genystein at 20 ppm; 5) restricted and treated with 100 ppm of Trifolium mediterraneum extract: the withdrawal lasted 5-6 days. The groups 1, 2, 4, 5 were pooled as untreated (UNT) vs the group 3 (BAA). On the 43th day of the study, animals were submitted to ultrasound scan by a Dynamic mod. Concept 500 device over the hair cut loin to measure the depth of LD at the points 10, 35 and 60 mm from the hip's tip; then they were stunned and killed by cervical dislocation and exsanguination. The carcass was refrigerated at $+4^\circ C$ for 20 hours, then submitted to hindleg dissection (Blasco et al., 1993); two longitudinal samples of 20 g each of LD alternatively freeze-dried intact or previously homogenized for 6" were obtained. Carcass analysis involved separation of the humerus and dissection of the hindleg as muscle, bone (femur and tibia) and other tissues. The muscles of the hindleg were homogenized for 30", then two samples of each were submitted to two different freeze-dryers, working respectively from -47 to $+45^\circ C$ during 72 hours, as for LD, and from -40 to $+20^\circ C$ overnight. The freeze-dried muscles (LD and HL) were ground for 20" in a domestic

coffee-mill. The weight of water in the muscles was estimated by loss after the longest freeze drying. After stabilization of residual moisture oven drying overnight at +40 °C the samples were then submitted to a NIR Systems 4500 Model in a rotating cup. The spectra from 1300 to 2400 nm were mathematically transformed (as $1, 4, 4, 1$ of $\log 1/R$), then fitted to the laboratory values or to a conventional dummy variables (1 or 2 for BAA treatment discrimination) by the standard NIRS-2 (ver. 2.05) software. The chief chemometric method used was the Modified Partial Least Squares (PLS), while Multilinear Regression (MLR) was also adapted to identify some relevant wavelengths. The limits for rejection of extraneous observations were fixed at $t > 2.4$ (regression) and H of Mahalanobis > 7 (spectral distance from gravity centre). Only one passage was allowed in the datasets for removing few outliers. The internal cross-validation was performed by six groups.

Statistical Analysis of the data was performed by the SAS (1985) GLM procedure with a monofactorial multivariate linear full model including 29 variables, fitted to BAA (as dummy value 2) or UNT (=1) values. By a stepwise multilinear regression model the most significant variables ($P < 0.15$) were identified.

The i.m. fat and the protein contents of LD, on d.m. basis, were predicted by NIRS using two previous unpublished PLS equations (R^2_{cv} 0.97 and 0.96; SEP 0.63 and 0.93%), obtained in similar 40 samples of LD removed of aponeurosis, homogenized, then freeze-dried 72 hours.

Results and discussion

The table 1 reports the clear effects of the BAA treatment on the body composition. The dressing percentage was largely increased, mainly because a strong reduction of the skin, which included the subcutaneous fat layer. The skeleton was insignificantly affected, according to a general rule well assessed on a between species basis, while major modifications involved muscles and other tissues (fat plus tendons): the net result was a significant raising of 8% in the muscle/bone ratio of the hindleg. A strong fat reduction (-40 to -44%) occurred in the hindleg region (intermuscular fat plus tendons) and in the carcass depots; the i.m. fat contents of LD was also reduced (-19%). Water contents of LD was significantly increased according to a parallel raising of the protein while in the HL the water increase was more pronounced: this fact can explain observed higher cooling losses.

Ability of the NIRS to be related with ontogeny (table 1) was verified for meatiness and fatness. Muscle/bone ratio of the hindleg was predicted with R^2_c 0.77 and 0.60 from LD and HL, but decreased to R^2_{cv} 0.40 and 0.33; one reason was an appreciable NIRS evaluation of the strongly correlated bone percentage ($r = -0.976$). The muscle percentage was already moderately calibrated only in LD, but not in HL. This correlativity of the NIRS with some skeleton components also recurred in previous experiments with 338 rabbits (Masero et al., 1994). The fatness of the carcass was clearly identified by NIRS method, especially from HL, but it did not appear to be responsible of the possibility of estimation of meatiness because independency of the two traits.

The table 2 evidences the covariation of all the characters in the discrimination of the individual BAA treated. When all the 29 variables were fitted to 52 complete objects, 85% of the variance was explained, but a selected set of 12 variables (the most representative being skin, muscle percentage and water concentration in HL) accounted for 83%. The NIRS direct discrimination of the treatment was maximum from LD ($R^2_c = 0.91$) and it was also high from HL (0.86); these chemometric relationships were quite stable, having cross-validation R^2_{cv} values of 0.75 or 0.72. The role of specific wavelengths involved in the BAA treatment discrimination did not show any accounting for more than 38% (1728 nm in HL), but the pattern between muscles was different. Furthermore, the first vibration disappeared after the overtones: this is typical of NIR analyzed by ML Regression, but PLS method can linearly capitalize all significant spectral variations avoiding overfitting. Nevertheless we tried useful information about some specified wavelengths which were able to resume a significant part of spectral covariance with traits, i.e.: the 1848 in LD and the 1708 in HL which explained respectively 30% and 48% of the variation in the skin percentage, and 48% and 75% of the variation in water contents of hindleg muscles. This last finding is interesting because the original moisture was almost entirely removed before NIRS processing and disappeared from spectra, thus it supports the hypothesis of an ontogenetic relationship. Other components of the fibres (for LD), or of the whole region of muscles (from epimysium, aponeurosis and tendons for HL) not analyzed in this experiment could have been determined in a down to up investigation which limits are a priori fixed at a high level because strong discriminability of different ontogenetic status.

Previous (unpublished) knowledge about a strong spectral distinction of muscle types were dramatically confirmed in this experiment because the PLS distinction of LD from HL muscles raised a probability of 98% by

PLS method, and the most implicated wavelengths were the 1818 nm, which on itself resumed 84%, and the jointed 2038 and 1338 nm which explained 92% of the differences.

Conclusions

The NIR spectroscopy of dried muscles may be interesting not only for scientific, but also for production purposes. Discrimination of drastic ontogenetic and chemical modifications may be an indirect tool to guarantee the stability of sure meats, while future (and also past "freeze-stocked") experiments ought to include spectroscopic NIR study, which can be very easily expanded at low cost. If the NIR spectroscopy is of informative or of really predictive value it will be recognized by other combined researches.

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