NIR Spectroscopy of muscle ethanol prepared specimens to differentiate rearing mode and genetic type of Italian heavy pigs

Cenci Goga B.[1], Masoero G.[2] and Barbera S.[3]

[1] Dipartimento Scienze Biopatologiche Veterinarie - Università degli Studi di Perugia
[2] CRA – PCM – Torino – Italy.
[3] Dipartimento di Scienze Zootecniche – Università degli studi di Torino – Italy

Abstract — **Road map to traceability in animal meat production may include non-destructive and objective techniques to fingerprint a typical product. Optic observation of muscle specimens by the NIRS radiation, after easy preparation in ethanol, may retrospect some intrinsic effects in a simple way. The present work aims to evaluate the characterization ability of the method applied to a rare typical heavy Italian pig production.**

Italian heavy pigs from *Mora Romagnola* breed (M, n=14) and commercial Hybrids (H, n=16) were reared in three farm conditions: Intensive (I), Confined (C), Pasture (P). Small specimens of *longissimus thoracis* muscle were fully immersed in ethanol 95%. After 2h aeration the samples were scanned by a LabSpec-Pro(ASD) portable (UV-Vis-NIRS:350-2500nm). Chemometrics MPLS was performed to contrast the two genetic types and the three rearing methods, then clustered.

According UV-Vis-NIRS features P and C were differentiated from the I pigs. R^2cv (cross-validation) for P-C, P-I and C-I were 0.32, 0.95 and 0.84 respectively, while multivariate R^2cv of 10-Lab were 0.05, 0.47 and 0.48. The spectra from I system were less rich and 13% lower. Genetic type was discriminated by NIRS with R^2cv 0.59 a value corresponding to a concrete 90% recovery of the *Mora Romagnola* or *Hybrids* subjects while from 5-Meat-Lab the R^2cv was 0.17.

In conclusions NIRS examine of ethanol preparations may reveal intrinsic properties of typical products mainly if they will be considered as lot and not as single "*forensic*" specimen.

Keywords— **NIRS, ethanol-muscle, pig.**

I. INTRODUCTION

Road map to traceability in animal meat production may include non-destructive and objective techniques to fingerprint a typical product. Optic observation of muscle specimens by the NIRS radiation, after easy preparation in ethanol, may retrospect some intrinsic effects in a simple way. Meat researchers have long sought after non-destructive and objective techniques to predict meat quality [1]**.** Several studies have stated that near-infrared reflectance spectroscopy (NIRS) can be used to predict extrinsic meat traits, such beef tenderness and texture traits. [2], 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 repeatedly utilized in rabbits [3] [4] [5] and was also utilized in buffalo [6] and in cattle [7] [8] [9] 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 management of pig fattening, i.e. the genetic type of the animal, more or less improved, and the housing-feeding conditions, intensive *vs* confined *vs* pasture. The present work aims to evaluate the characterization ability of the method applied to a rare typical heavy Italian pig production in comparison to a modern hybrid.

II. MATERIALS AND METHODS

Italian heavy pigs from *Mora Romagnola* breed (M, n=14) and commercial Hybrids (H, n=16) were reared in three farm conditions: Intensive (I), Confined (C), Pasture (P). Rearing data where collected at farm and

at slaughterhouse. Samples of m. *longissimus thoracis* were taken to evaluate: pH and temperature to 45' and 24h, meat colour (CIE L*, a*, b*). Laboratory analyses were carried out according to the protocol developed at the "Scienze Zootecniche" Department [10]. Small carrots (15 g, ½ inch) specimens of *longissimus thoracis* muscle were fully immersed in ethanol 95% and preserved at +5° in dark for a prolonged time. After 2h aeration in ambient temperature, the samples were then scanned by a LabSpec-Pro(ASD) portable (UV-Vis-NIRS: 350-2500nm). All the 2151 spectra digits (350-2500 nm) were statistically analyzed by using the multivariate chemometric method using the Modified Partial Least Squares (MPLS) in the 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. Pre-treatment of the spectra (normalization, detrend and derivation) was tailored on each calibration. The contrasts regarded to the two genetic types and the to three rearing methods, whose distance matrix was then clustered by CHA methods (StatBox, v6.5, *Grimmer Logiciel, Paris*).

III. RESULTS AND DISCUSSIONS

The rearing system displayed significant statistical effect in the living animals, with maximum growth allowed in the Confined system (Table 1). The initial pH was high in the Pastured pigs, but it became uniform between groups at 24h. A prominent fitting of the NIR spectra to the living and to the carcass traits was only achieved for the Live-Weights and the average growth rate, while the carcass and meat traits were only medium related, or nothing.

When looking at the absorbance curve, reported in Figure 1, the full radiation of the ethanol treated specimens appeared to be different between the rearing systems; in fact the I-Intensive group was clearly less intense, and on avg. 13% lower than the other two.

The chemometrics applied at the entire radiation (Table 2) confirmed that P and C were strongly differentiated from the pigs reared in the

Table 1. Effect of the rearing type on the performances of the animals and on the meat quality traits and fitting of the UV-Vis-NIR spectra (350-2500nm).

	P	C	I	NIRS R^2cv
L.W. Initial, kg	47.9b	51.03b	73.1a	.95
L.W. Final, kg	156.9a	175.8a	151.1b	.57
ADG, g*d-1	498.2ab	597.6a	438.3b	.57
Cold Carcass, kg	122	139	119	.35
Dressing, %	77.8	79.2	78.5	.37
pH_1h	6.34a	5.93b	5.93b	.37
pH_24	5.58	5.52	5.49	.00
Colour, L*	55.8	56.4	53.3	.39
Colour, a*	18.8	17.9	18.6	.19
Colour, b*	8.3	7.7	7.2	.28

a>b>c, P<0.05; R^2cv= R^2 in cross-validation mode.

I system: the R^2cv (cross-validation) for P-C, P-I and C-I were 0.32, 0.95 and 0.84, respectively.

The differential relationships appeared to be quite constant in the Visible (350-800 nm) and in the NIR (801-2500 nm) regions, except for the P *vs.* C contrast. Since univariate analysis did not signified differences in colour properties (Table 1) other meat traits could be supposed to intervene about.

Multivariate PLS elaboration of the 5-live traits attained an avg. R^2cv value of 0.28, comparable to the previous consistent results that have been emerged from univariate analyses. Conversely, the multivariate

Figure 1. UV-Vis-NIR radiation of ethanol muscle of pig in Pasture, Confined or Intensive rearing.

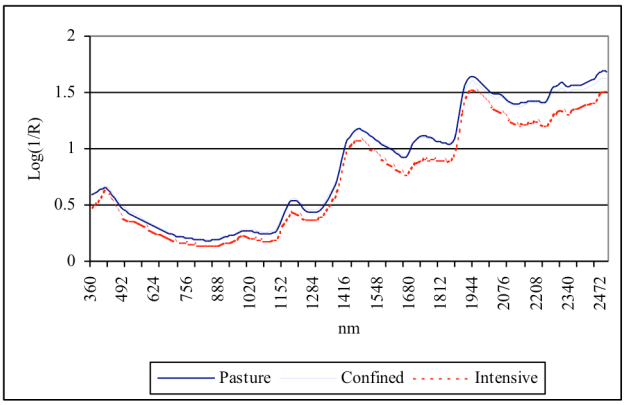


Table 2. Differentiation of the 3 Systems and of the 2 Genetic effects by MPLS analysis of 10 variables or by NIRS of ethanol specimens of *longissimus thoracis*.

System	Live - 5	Meat -5	All -10	UV-Vis 350-800 nm	NIR 801-2500 nm	Full 350-2500 nm
P *vs.* C	.08	.11	.05	.00	.45	.32
P *vs.* I	.44	.26	.47	.89	.85	.95
C *vs.* I	.34	.12	.48	.97	.72	.84
Avg.	*.28*	*.16*	*.33*	*.62*	*.68*	*.70*
Genetic H *vs.* M	.72	.23	.67	.31	.40	.59

of the 5-meat traits (avg. R^2cv= 0.16) confirmed the inexistence of rearing System effects, almost as measured by these limited set of laboratory analyses of the meat.

In a recent study on veals [8, 9] NIRS of the ethanol was highly related to panel results. Also in rabbit meat studies the NIRS of muscle in ethanol was able to synthesize significant effects induced from nutritional effects [5] or from rearing systems [4] where the AA observed that 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 as compared to a set of 16 laboratory analyses, but resulted in an immeasurable great efficiency.

The homogeneity of the patterns of the contrasts between the three groups obtained from the Variables and from the NIRS, as reported in Table 2, was demonstrated by the clusters carried on by CHA calculation and represented in Figures 2 and 3: in that scheme the I-Intensive group was discarded from the other two neighboured groups, but the branches of the clusters were amplified in the NIRS methods.

The genetic levels of the growth featured very different in the two compared strains. The *Mora Romagnola* recorded an ADG of 449 g*d^{-1}, i.e. the 22% less than the 574 g*d^{-1} recorded by the Hybrids animals (P<0.02). The recorded meat quality traits, analysed as separate variables, did not differ between genetic origins. However, because of covariance effects, when the multivariate PLS was applied to the 5-meat variables, the R^2cv raised to 0.23, a featureless level as compared to the results from the 5-live variables (0.72). The level of discrimination for the genetic origin of the animals which was attained by UV-Vis radiation of the muscle samples was 0.31, then 0.40 from NIR region, and 0.59 for the two regions combined. Results from this last function corresponded to a concrete 90% recovery of the *Mora Romagnola* or Hybrids subjects from the spectra (Figure 4) while from the 5-Meat Lab the R^2cv, which was 0.23, corresponding to a 77% recovery rate. In a study [11] about 18 Friesian cattle partially treated by desame-thazone, the illegal treatment was deceived in the *Longissimus thoracis* with R^2cv 0.84 and in *Semitendinosus* with 0.67.

Figure 2. CHA average cluster of the three rearing types by 10 Variables

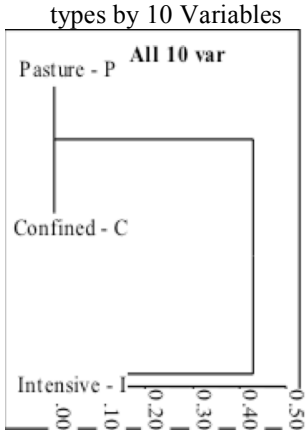
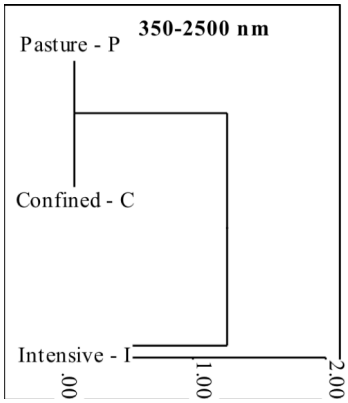


Figure 3. CHA average cluster of the three rearing types by UV-Vis-NIRS radiation.



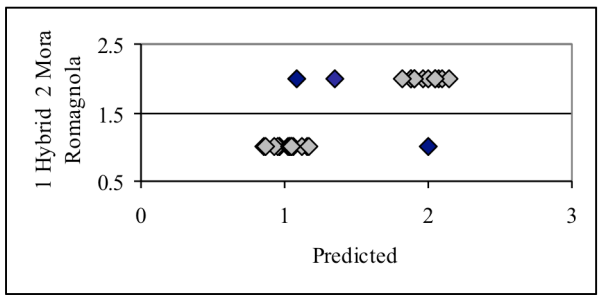
IV. CONCLUSIONS

For the NIRS preparation of specimens it could be recommend to place a maximum of 15g/30ml 95% ethanol into a tube, either in single or multiple specimens, even from different tissues, but of similar size.

Examine of ethanol preparations by UV-Vis-NIRS radiation in the actual experimental conditions had revealed intrinsic properties of a typical product.

The challenge now would be to consider the ethanol-NIRS method to certify each "lot of production" complying a rule, and not as single "forensic" specimen.

Figure 4. Discrimination of genetic origin by UV-Vis-NIR spectra of muscle, reclassification error rate10%.



REFERENCES.

1. Prieto N, Roehe R, et al. (2009) Application of near infrared reflectance spectroscopy to predict meat and meat products quality: a review. Meat Sci 83:175-186
2. Cozzolino D, Murray I (2004) Identification of animal muscles by visible and near infrared reflectance spectroscopy. Lebensmittel - Wissenschaft Technologie 37:447-452
3. Masoero G, Brugiapaglia A, et al. (2004) FT-NIR spectroscopy of fresh and treated muscle tissue in young female rabbits. 8th World Rabbit Congress, Puebla, Mexico, pp 1409-1415
4. Dalle Zotte A, Masoero G, et al. (2007) Effects of housing system on the meat quality of fattening rabbits by NIRS using ethanol or freeze-dried specimens. Proc. 53rd International Congress of Meat Science and Technology, Beijing, China, pp 339-340.
5. Masoero G, Sala G, et al. (2008) NIR spectroscopy and electronic nose evaluation on live and in the meat of rabbits fed with increasing levels of Chia (Salvia hispanica L.) seeds. J. Anim Vet. Adv 7:1394-1399
6. Masoero G, Bergoglio G, et al. (2005) A rapid new ethanol preparation for early muscle sampling submitted to NIR analysis: preliminary results for cattle and buffalo. Italian J Anim Sci 4(2):299
7. Masoero G, Iacurto M, Sala G (2007) Ethanol specimen of beef muscle samples for NIRS is rapid but as efficient as the freeze-dried sample preparations. "New developments in evaluation of carcass and meat quality in cattle and sheep" C. Lazzaroni (Ed.) EAAP Scientific Series N 123, Wageningen Academic Publishers Wageningen, The Netherlands, pp 215-219
8. Brugiapaglia A, Destefanis G, et al. (2011) NIRS analysis of different meat sample preparations to discriminate feeding plan and genetic type effects in veal calves. Italian J Anim Sci, Book of Abstract, vol. 10(1):84
9. Brugiapaglia A, Destefanis G, et al. (2011) NIRS analysis of different meat sample preparations from veal calves and panel test prediction. Proc. ICOMST 2011 In press
10. Isoppo S, Sala G, Barbera S (2009) A Protocol for the assessment of physical and sensorial characteristics of meat. ICoMST 55th International Congress of Meat Science and Technology. Copenhagen, Denmark, 2009, ICOMST_PS4_Part2_ver2_Final.pdf. PE4.75 pp 736-739
11. Masoero G, Sala G, et al. (2008) Impiego della spettroscopia NIR per l'individuazione di trattamenti illeciti con corticosteroidi nel bovino da carne. NIR ITALIA 08, 3°Simposio italiano di Spettroscopia nel Vicino Infrarosso, "Un raggio di luce a difesa della qualità", Lazise (VR), 22-23 Maggio 2008, p-20, 1-6. ISBN 9788890406447