Use of near infrared spectroscopy for the prediction of intramuscular fat and fatty acid content in rabbit meat

Zomeño C., Juste V., and Hernández P.

Institute for Animal Science and Technology, Universidad Politécnica de Valencia, Valencia, Spain

Abstract—**The objective of this study was to evaluate the use of near infrared spectroscopy (NIRS) for the prediction of intramuscular fat (IMF) and fatty acid (FA) content of rabbit *Longissimus* muscle (LM). 142 rabbits slaughtered between 5 and 61 weeks of age were used. Freeze-dried LM were scanned by NIRS reflectance between 1100 and 2498 nm with a monochromator (model 5000, NIRSystem). IMF of LM was determined by ether extraction with a previous acid hydrolysis. FA content of LM was analysed by gas chromatography. Prediction equations were obtained using modified partial least squares method and the best equation was selected attending to determination coefficient of cross validation (R^2) and RPD=SD/SECV (SD=standard deviation and SECV=standard error of cross-validation). Equations reported accurate predictions for IMF (r^2=0.98; RPD=7.57), SFA and MUFA (r^2=0.96 and 0.98, RPD=5.09 and 6.69, respectively). Lower accuracy was obtained for PUFA (r^2=0.83 and RPD=2.40). Several individual FA were also accurately predicted such as C14:0, C15:0, C16:0, C16:1, C17:0, C18:0, C18:1 n-9, C18:2 n-6 and C18:3 n-3 with r^2 between 0.91 and 0.97 and RPD between 3.28 and 6.10. Other minor FA as C18:1 n-7, C20:2 n-6, C20:5 n-3 and C22:5 n-3, presented less accurate statistics (R^2 between 0.73 and 0.82 and RPD lower than 3). Finally, C20:3 n-6 and C20:4 were more difficult to predict by NIRS (R^2 of 0.57 and 0.61 and RPD of 1.52 and 1.60, respectively). Results confirmed the potential of NIRS for the determination of IMF and FA content in rabbit meat.**

Keywords—**intramuscular fat, fatty acid, NIRS**

I. INTRODUCTION

Rabbit meat offers good nutritive and dietetic properties. It has a lower fat and higher polyunsaturated fatty acid content than other meats [1]. The most ubiquitous fatty acids (FA) are palmitic (C16:0), oleic (C18:1 n-9) and linoleic (C18:2 n-6) acids, showing percentages higher than 20% of total FA [1].

Chemical conventional methods used to determine intramuscular fat (IMF) and FA content are laborious, expensive, time-consuming and destructive. Near infrared reflectance spectroscopy (NIRS) is a fast, accurate and cheap analytical technique, therefore it could be a suitable alternative to these conventional methods. Previous studies have demonstrated NIRS as a good predictor of IMF content in meat [2]. FA content has also been predicted by NIRS; nevertheless, this technique has a limited ability for estimating some individual FA due to the similarities in their NIR absorption pattern [3]. In rabbit, NIRS has been used for estimate IMF of *Longissimus* muscle [4, 5], whereas FA content has been only studied in hind leg meat [6].

The objective of this study was to evaluate the potential use of NIRS for the prediction of IMF and FA content of rabbit *Longissimus* muscle.

II. MATERIAL AND METHODS

A. Animals and meat samples

A total of 142 rabbits from three different synthetic lines were used for NIRS calibration. Animals were slaughtered between 5 and 61 weeks of age by electrical stunning and exsanguination. After the slaughter, the carcasses were stored at 3-5ºC during 24 hours and then *Longissimus* muscles (LM) were excised from the carcass. Meat obtained from LM was ground, freeze-dried, vacuum-packed and stored at -80ºC until analyses.

B. Intramuscular fat analyses

Total lipids were determined by ether extraction (Soxtec 1043, Tecator, Höganäs, Sweden) with a previous acid hydrolysis (Soxcap 2047, Tecator

Höganäs, Sweden) in triplicate from freeze-dried LM in 142 samples. Lipid content was expressed as grams per 100 g of fresh tissue, this value was obtained taking into account the dry matter content determined from the weight of minced LM before and after freeze-drying.

C. Fatty acid analyses

Fatty acid methyl esters (Fame) of LM were prepared as described by O´Fallon et al. [7] in 123 samples. Fame were analyzed in a Focus Gas Chromatograph (Thermo, Milan, Italy) equipped with a split/splitless injector and a flame ionization detector. The separation of methyl esters was performed in a fused silica capillary column SPTM 2560 (Supelco, PA, USA) (100 m x 0.25 mm x 0.2 μm film thickness). The carrier gas was Helium at a linear velocity of 20 cm/sec. The samples were injected with a split ratio of 1/100. The initial oven temperature was set at 140ºC held for 5 min and increased to 240 at 4ºC/min and finally maintained at that temperature for 30 min. Both detector and injector temperatures were set at 260ºC. The individual FA were identified by comparing their retention times with standards of Fame supplied by Supelco (PA, USA) and quantified by using C21:0 as internal standard.

D. NIR analyses

Samples were scanned between 1100 and 2498 nm with a monochromator (model 5000, NIRSystem INC., Silver Spring, MD, USA) equipped with a transport module. Sample measurements were taken in round sample cups with quartz windows of 3.8 cm diameter. A sample cup was filled two times and rotated 90° for each sample. The four reflectance spectra obtained for each sample were averaged.

Calibration development was performed using the chemometric software WINISI-4 ver. 1.60 (Infrasoft International, LLC and FOSS). Prediction equations were obtained using Modified Partial Least Squares as regression method [8]. Cross-validation was performed in order to select the optimal number of factors and avoid overfitting. The cross-validation operated with 5 groups. Regression equations were obtained using several mathematical treatments. The best equation was selected attending to determination coefficient of cross validation (R^2) and RPD= SD/SECV, SD being the standard deviation and SECV the standard error of cross-validation.

III. RESULTS AND DISCUSSION

Descriptive statistics for and IMF and FA content of LM are summarized in Tables 1 and 2. LM lipid content was 1.32 g/100g muscle. Similar values were found by Pla et al. (2004) in this muscle. LM has a low IMF since it is the leanest muscle of the carcass. IMF showed a wide range of variability (CV=40.1).

Table 1. Descriptive statistics for intramuscular fat (IMF) (g/100 g muscle) (n=139) and fatty acids groups (mg/100 g muscle) (n=119) in rabbit *Longissimus* muscle.

	Mean	SD	Range	CVx100
IMF	1.32	0.53	0.75-3.25	40.1
SFA	352	164	162-858	46.6
MUFA	266	162	91.7-778	60.9
PUFA	319	89.1	143-568	27.9
n-6	264	79.7	110-493	30.2
n-3	54.3	11.4	23.6-82.2	21.0

SD: standard deviation, CV: coefficient of variation, SFA: saturated fatty acids= C14:0+C15:0+C16:0+C17:0+C18:0, MUFA: monounsaturated fatty acids= C16:1+C18:1 n-9+C18:1 n-7, PUFA: polyunsaturated fatty acids= C18:2 n-6+C18:3 n-3+C20:2 n-6+C20:3 n-6+C20:4 n-6+C20:5 n-3+C22:4 n-6+C22:5 n-3+C22:6 n-3, n-6= C18:2 n-6+C20:2 n-6+C20:3 n-6+C20:4 n-6+C22:4 n-6; n-3=C18:3 n-3+C20:5 n-3+C22:5 n-3+C22:6 n-3.

The main FA of *Longissimus* muscle were saturated (SFA) and polyunsaturated (PUFA), with percentages around 37% and 36% of total FA, respectively. Monounsaturated (MUFA) FA represented lower percentage (27%). Among PUFA, n-6 FA were the most abundant with percentages of 30% while n-3 FA were less represented (6%). PUFA/SFA and n-6/n-3 ratios, used to evaluate the nutritional quality of fat, showed values of 0.98 and 4.87, respectively. These values were close to the nutritional recommendations (higher than 0.45 for PUFA/SFA and lower than 4 for n-6/n-3) [9]. SFA and MUFA content had a high variability; however PUFA, n-6 and n-3 showed a lower variability.

The most ubiquitous FA in *Longissimus* muscle were palmitic (C16:0), oleic (C18:1 n-9) and linoleic (C18:2 n-6) acids (Table 2), showing percentages of 26%, 23% and 21%, respectively. Stearic (C18:0) and arachidonic acids (C20:4 n-6) were also important

with percentages around 8% and 6%. Linolenic acid (C18:3 n-3) and some long chain PUFA (i.e. C20:5 n-3, C22:4 n-6 and C22:6 n-3) were also present in rabbit meat although at a lower content. Most individual FA showed a wide range of variation, mainly C14:0, C16:1, C18:1 n-9 and C18:3 n-3.

Table 2. Descriptive statistics for individual fatty acids (mg/100 g muscle) in rabbit *Longissimus* muscle (n=119).

	Mean	SD	Range	CVx100
C14:0	18.3	13.4	3.71-62.5	73.2
C15:0	4.43	2.18	0.31-10.8	49.2
C16:0	251	122	113-621	48.6
C16:1	28.2	27.1	3.41-120	96.1
C17:0	6.18	2.70	2.08-15.0	43.7
C18:0	72.7	24.8	39.4-153.1	34.1
C18:1 n-7	13.8	6.83	3.79-38.1	49.5
C18:1 n-9	224	130	78.1- 619.6	58.0
C18:2 n-6	194	73.4	52.9- 418.5	37.8
C18:3 n-3	14.8	8.85	1.37-41.8	59.8
C20:2 n-6	2.33	0.80	0.45-4.80	34.3
C20:3 n-6	4.05	0.81	2.21-6.47	20.0
C20:4 n-6	48.1	9.21	32.4-71.5	19.1
C20:5 n-3	11.9	4.20	0.79-22.2	35.3
C22:4 n-6	15.9	2.57	10.4-23.3	16.2
C22:5 n-3	7.11	1.86	4.39-12.3	26.2
C22:6 n-3	20.5	6.60	8.52-42.3	32.2

SD: standard deviation, CV: coefficient of variation.

Calibration equation results for IMF and FA groups are shown in Table 3. The parameters corresponding to IMF calibration indicated a good prediction ability (R^2=0.98 and RPD=7.57). Accurate NIRS calibrations for IMF have also been reported in rabbit [4, 5], poultry, beef and pork meat [2].

Table 3. Statistical parameters of equations for near infrared reflectance spectroscopy calibrations of intramuscular fat content and fatty acids groups in rabbit *Longissimus* muscle.

Trait	N	Mean	SD	R^2	SECV	RPD
IMF	137	1.32	0.53	0.98	0.07	7.57
SFA	119	352	164	0.96	32.2	5.09
MUFA	116	263	162	0.98	24.2	6.69
PUFA	119	319	89.1	0.83	37.2	2.40
n-6	117	262	78.3	0.87	27.8	2.82
n-3	117	54.4	11.1	0.50	7.87	1.41

N: number of samples, SD: standard deviation, R^2: coefficient of determination of cross-validation, SECV: standard error of cross validation, RPD: SD/SECV.

Equations for SFA and MUFA content (Table 3) showed good accuracy (R^2 of 0.96 and 0.98 and RPD of 5.09 and 6.69, respectively). However, statistics for PUFA and n-6 FA were less accurate (R^2=0.83 and 0.87, RPD=2.40 and 2.82, respectively), but adequate enough to predict according to Shenk and Westerhaus [8]. Results obtained for n-3 FA were insufficient for accurate predictions (R^2=0.50 and RPD=1.41). The higher accuracies for SFA and MUFA compared to PUFA found in this study are in line with findings of other authors [11, 12] and might be related to the narrow range of variability in PUFA content (Table 1) and a less ability of NIRS to detect the higher double bonds presents in PUFA. Prediction for n-6 FA was similar to those observed by Pla et al. [6] and Guy et al. [11]. A low accuracy for n-3 FA prediction was also reported by Pla et al. [6] and might be due to the lower variability of n-3 FA in rabbit meat.

Table 4. Statistical parameters of equations for near infrared reflectance spectroscopy calibrations of individual fatty acid content in rabbit *Longissimus* muscle.

Fatty acids	N	Mean	SD	R^2	SECV	RPD
C14:0	116	17.5	12.5	0.94	2.96	4.22
C15:0	115	4.40	2.12	0.92	0.60	3.53
C16:0	118	249	121	0.96	24.5	4.94
C16:1	115	27.1	26.6	0.92	7.42	3.58
C17:0	112	5.88	2.43	0.91	0.74	3.28
C18:0	115	71.9	24.7	0.92	6.95	3.55
C18:1 n-7	117	13.8	6.87	0.82	2.90	2.37
C18:1 n-9	116	221	130	0.97	21.3	6.10
C18:2 n-6	115	190	70.9	0.91	21.3	3.33
C18:3 n-3	112	14.1	8.51	0.95	1.99	4.28
C20:2 n-6	112	2.29	0.75	0.78	0.35	2.14
C20:3 n-6	115	3.99	0.76	0.57	0.50	1.52
C20:4 n-6	117	47.8	8.98	0.61	5.60	1.60
C20:5 n-3	115	12.0	3.91	0.73	2.01	1.95
C22:4 n-6	118	15.8	2.49	0.12	2.34	1.06
C22:5 n-3	113	7.05	1.85	0.77	0.89	2.08
C22:6 n-3	117	20.2	6.25	0.38	4.95	1.26

N: number of samples, SD: standard deviation, R^2: coefficient of determination of cross-validation, SECV: standard error of cross validation, RPD: SD/SECV.

Calibration equation results for individual FA are shown in Table 4. The best calibration equations were found for C18:1 n-9, C16:0 and C18:3 n-3 with R^2 higher than 0.95. Satisfactory statistics were also obtained for C14:0, C15:0, C16:1, C17:0, C18:0 and C18:2 n-6 with R^2 between 0.91 and 0.94. RPD

statistics of these equations showed values higher than those recommended in literature [13]. Other minor FA as C18:1 n-7, C20:2 n-6, C20:5 n-3 and C22:5 n-3, presented less accurate statistics (R^2 between 0.73 and 0.82 and RPD lower than 3) but were adequate to predict [8]. Equations for C20:3 n-6 and C20:4 n-6 (R^2 of 0.57 and 0.61 and RPD of 1.52 and 1.60, respectively) were insufficient for accurate predictions but may provide a good separation of samples into high, medium and low groups [8]. Finally, C22:4 n-6 and C22:6 n-3 were problematic to predict (R^2 of 0.12 and 0.38 and RPD of 1.06 and 1.26, respectively).

Results of calibration for most individual FA were similar to those obtained in lamb *Longissimus* muscle [12]. Pla et al. [6] found lower predictions in hind leg meat of rabbit for most individual FA except for C18:2 n-6, C20:4 n-6 and 20:3 n-6, which showed similar accuracies. In our case, this is the first analysis of the FA content of IMF in rabbits, whereas Pla et al [6] analyzed the FA content of the whole hind leg meat, including inter and intramuscular fat. Our results were better than those reported in beef [11, 14] and pig [10] *Longissimus* muscle for most individual FA.

IV. CONCLUSIONS

Results of the present experiment confirmed the potential of NIRS for the determination of IMF and FA content in rabbit meat. Accurate predictions were obtained for IMF as well as for the FA present at medium-high content, and lower predictions were obtained for the FA present at low content.

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