

# Prediction of fatty acid content in rabbit meat and discrimination between conventional and organic production systems by NIRS methodology

M. Pla <sup>a,\*</sup>, P. Hernández <sup>a</sup>, B. Ariño <sup>a</sup>, J.A. Ramírez <sup>b</sup>, Isabel Díaz <sup>c</sup>

<sup>a</sup> *Departamento de Ciencia Animal, Universidad Politécnica de Valencia, Camino de Vera 14, 46022 Valencia, Spain*

<sup>b</sup> *Facultad de Ciencias Químicas/Universidad Autónoma de San Luis Potosí, Av. Dr. Manuel Nava No. 6, Zona Universitaria, C.P. 78210, San Luis Potosí, S.L.P. México, Mexico*

<sup>c</sup> *IRTA Institut de Recerca i Tecnologia Agroalimentàries, Centre de Tecnologia de la Carn, Granja Camps i Armet, 17121 Monells, Girona, Spain*

Received 13 June 2005; received in revised form 20 September 2005; accepted 20 September 2005

## Abstract

To investigate the feasibility of using the NIRS methodology to analyse the fatty acid content of rabbit meat and to discriminate between conventional and organic production, the meat of a hind leg of 119 rabbits was scanned between 1100 and 2498 nm and 104 samples were sent to the laboratory for reference analysis of fatty acids by gas chromatography. A commercial spectral analysis program (WINISI-2, v. 1.04) was used to process the data and to develop chemometric models. The better calibration equation for each fatty acid, leading to a higher determination coefficient of cross-validation ( $r^2$ ) and low standard error of cross-validation (SECV) was retained. Prediction of linoleic, palmitic, palmitoleic and oleic acid content was excellent or good ( $r^2$  between 0.90 and 0.70); prediction of arachidonic, stearic,  $\alpha$ -linolenic and eicosatrienoic FA has  $r^2$  between 0.69 and 0.50. However, miristic, vaccenic, icosanoic and eicosadienoic FA are problematic to predict. When fatty acids were grouped, the  $r^2$  of the calibration equations were: 0.85 for saturated FA, 0.83 for MUFA, 0.92 for PUFA and 0.91 for  $n - 6$  FA, indicating excellent or good prediction. Prediction of  $\alpha$ -linolenic FA ( $r^2 = 0.59$ ) needs more precision. The obtained equations have been applied for predicting meat fatty acid composition of both groups of production systems, conventional and organic, for an other 52 rabbit meat samples ( $2 \times 26$ ). Meat of the organic source had lower ( $p = 0.000$ ) mono-unsaturated FA (30.54% vs. 34.64%) and higher ( $p = 0.019$ ) polyunsaturated FA (27.28% vs. 23.66%) than rabbit meat from the conventional system, while the saturated FA content was similar (42%) in both groups. The discriminant model correctly classified (98%) between conventional or organic produced rabbit meat.

© 2005 Elsevier Ltd. All rights reserved.

**Keywords:** Near-infrared spectroscopy; Prediction; Fatty acids; Organic rabbit meat

## 1. Introduction

The scientific evidence establishes that diets high in saturated fat and cholesterol are associated with increased levels of blood total and LDL-cholesterol, with increased levels of cardiovascular diseases (Hu & Willett, 2002). The ratio polyunsaturated:saturated fatty acids and the ratio  $(n - 6):(n - 3)$  fatty acids have served also to evaluate

the nutritional quality of fat, and the recommendation of the Department of Health and Social Security UK (1994) is 0.45 or higher for the ratio P:S and a maximum of 4.0 for the ratio  $n - 6:n - 3$ . In monogastrics, the quantity and composition of the fatty acids is directly influenced by the composition of the diet (see in rabbits Bernardini, Dal Bosco, & Castellini, 1999; Oliver et al., 1997), and by the selection for growth rate (in pigs Cameron et al., 2000; in rabbits Ramírez et al., 2005) and it is increasing the interest in organic production as a supply of healthy foods (Kouba, 2003).

\* Corresponding author. Tel.: +34 963 879438; fax: +34 963 877439.  
E-mail address: [mpla@dca.upv.es](mailto:mpla@dca.upv.es) (M. Pla).

Recent years have seen a sharp rise in demand for organic animal products because consumers perceive that this food is safer. But there is no evidence of consistent differences in nutritional qualities between organic products and conventional ones (Kouba, 2003). Besides, consumers of organic products are disposed to pay a higher price for those products, but they want to be sure of the organic origin; a simple and rapid method to distinguish one meat from another is useful for this purpose.

Quantitative chemical techniques for the determination of fatty acids involve the extraction of fat with diethyl ether followed by conversion of the fatty acids to their methyl esters and analysis by capillary gas chromatography. This procedure is most time-consuming and generates hazardous waste, so physical methods also have been applied to the determination of total fat and fatty acids in foods without prior fat extraction. Near-infrared spectroscopy (NIRS) is a rapid and non destructive method requiring little or no sample preparation and its precision can be high. In contrast to traditional chemical analysis, no reagents are required and no waste is produced. In simple terms, NIRS has revolutionised analysis (Büning-Pfaue, 2004).

NIRS has been used to predict the composition of loin pork (Solis et al., 2001), rabbit meat (Pla, Pascual, & Ariño, 2004) or breast meat (Berzaghi, Dalle Zotte, Jansson, & Andrighetto, 2005). Park, Chen, Hruschka, Shackelford, and Koochmaraie (1998) showed that NIRS might be able to predict tenderness of beef steaks. NIRS has also been applied to study the fatty acid content of beef cuts (Mitsumoto, Maeda, Mitsuhashi, & Ozawa, 1991), in Iberian pig carcasses (Pedro, Garrido, Barnes, Casillas, & Murray, 1992; Pedro, Garrido, Lobo, Dardenne, & Murray, 1995) or in bovine neck muscle (Windham & Morrison, 1998). Furthermore, discriminant analysis makes it possible to use NIRS for identification and control of sample quality (Murray, Aucott, & Pike, 2001) and has been applied to the authentication to meat speciation (McElhinney, Downey, & Fearn, 1999), cut classification (Hervás, Garrido, Lucena, García, & de Pedro, 1994) or feeding sources (Berzaghi et al., 2005).

The primary objective of this study was to investigate the feasibility of using NIRS to analyse fatty acid content of rabbit meat and, if the response is positive, to study the effect of the organic production system on fatty acid composition of rabbit meat. The second objective was to investigate the use of NIRS to discriminate between rabbit meats produced in conventional or organic systems.

## 2. Materials and methods

### 2.1. Animals

In this experiment 171 rabbits were used. For NIR calibration and to ensure a reasonable variety of samples that represent the different sources of variation expected during analysis, 119 rabbits from different genetic origins,

fed different diets and produced in conventional or organic systems were used. Another 52 rabbits ( $2 \times 26$ ) were used for studying the effect of the organic production system, one group of 26 rabbits (sex balanced) of a three-way cross of A, V and R lines (Baselga, 2002) fed with a commercial and pelleted diet (18.5% crude protein, 14.7% fibre, 3.5% fat) were slaughtered at 63 d and constituted the Conventional group. The Organic group was constituted by 26 rabbits (sex balanced) of the same genetic origin but fed with a mixture of organic products (16.8% crude protein, 18% fibre, 2.3% fat) and maintained in regulatory organic conditions; these rabbits were slaughtered at 90 d, that is the age determined by Spanish regulation of rabbit organic production (complementary norms of R (CEE) 1804/1999).

### 2.2. NIR calibration samples and spectra

After slaughtering with humane care in a experimental slaughterhouse, the carcasses were stored at 3–5 °C during 24 h and a whole hind leg of each one was entirely deboned to separate bone (that includes bone and cartilages) from edible meat (that includes different muscles, intermuscular and intramuscular fat and tendons).

The different samples of meat were ground using a domestic mincing machine and 10 g of each one were weighed, vacuum sealed, packed in aluminium bags, and stored at –80 °C until needed for analysis. The samples were scanned between 1100 and 2498 nm with a monochromator (model 5000, NIRSystem Inc., Silver Spring, MD, USA) equipped with a transport module. Two round sample cups with quartz windows of 3.8 cm diameter were filled with each sample and two spectra, rotating 90° each cup were recorded. The four reflectance spectra of each sample were visually examined for consistency and then averaged.

From 119 calibration samples, 15 were deleted based on their spectral properties using the program CENTER of the WINISI-2 v. 1.04 software (Intrasoft International, LLC) and the remaining 104 samples were sent to laboratory for reference analysis of fatty acids.

### 2.3. Fatty acid composition

Lipids were extracted from 5 g of minced muscle with chloroform–methanol (2:1 v/v) according to the method of Folch, Lees, and Stanley (1957).

Fatty acid methyl esters of total lipids were prepared as described by Berry, Cervallos, and Wade (1965). The analysis was carried out in a Fison 8160 gas chromatograph equipped with a split injector and a flame ionisation detector. The capillary column (30 m long, 0.25 mm i.d., 0.25 µm film thickness) was a DB-FFAP (ChromLab). The carrier gas was nitrogen at flow rate of 1 ml/min. The oven temperature was held at 160 °C for 10 min, increased to 220 °C at 4 °C min<sup>-1</sup> and maintained for 25 min. The injector and detector temperature were 250

and 240 °C, respectively. The individual fatty acids were identified by comparing their retention times with those of standard fatty acids. Peaks areas were calculated using Chrom-Card Software (version 1.17) (Fisons Instruments). The results were expressed as a percentage of the amount of present methyl esters.

#### 2.4. Calibration

A commercial spectral analysis program (WINISI-2, v. 1.04, Infrasoft International Inc.) was used to process the data and develop chemometric models. The calibration was made with the modified partial least square (MPLS) method (Shenk and Westerhaus, 1996) and cross-validation was used as statistical tool because a small sample test was used (Williams & Sobering, 1996). The number of times required for outlier elimination was 2. Critical values for removing outliers were  $t$  (actual versus predicted) = 2.5;  $H$  de Mahalanobis (distance from the spectral mean) = 10, and  $X$  (spectra that are poorly modelled) = 10. The cross-validation operates with 10 groups.

Different regression equations were obtained using many mathematical treatments that included four orders of derivatives, different number (0–10) of data points in the segment used to calculate the derivative, different number (1–10) of data points over which running average smoothing was conducted and with or without scatter correction. For each FA or FA group the best equation was selected with respect to: determination coefficient of cross-validation ( $r^2$ ),  $RPD = SD/SECV$  and  $RER = \text{range}/SECV$ ,  $SD$  being the standard deviation and  $SECV$  the standard error of cross-validation.

Comparing the selected equations with the spectra, the composition on fatty acids of fat (intermuscular and intramuscular) on the meat was quantified.

#### 2.5. Statistical analysis

Least squares means were calculated to compare the FA composition of meat of conventional and organic produced rabbits using the GLM program of the SAS statistical package (Statistical Analysis Systems Institute, SAS, 2000). The model was

$$y_{ij} = \mu + S_i + e_{ij},$$

where  $y_{ij}$  is the dependent variable (the fatty acid),  $\mu$  is the general mean,  $S$  is the production system ( $i$  = conventional and organic), and  $e_{ij}$  is the residual term.

To discriminate between conventional or organic production, a discriminant analysis was performed using partial least square regression as the calibration method using the procedure PLS 2 of the WINISI-2 program. The spectral files from the two different groups were entered and cross-validation was used to test the accuracy of the model. The cross-validation operates with 10 groups and a detection level of 2.

### 3. Results and discussion

#### 3.1. Analysis

In rabbit hind leg meat samples the values of total saturated, monounsaturated and polyunsaturated fatty acids determined by the reference method ranged from 30.3% to 46.3%, 20.8% to 37.2% and 19.3% to 48.9%, and the means were 38.1%, 29.51% and 32.4%, respectively (Table 1). The more abundant fatty acids were palmitic, linoleic and oleic as is characteristic in rabbit meat (Raimondi, de Maria, Auxilia, & Masoero, 1975), and our results are in the same range as in other works (Cambero, de la Hoz, Sanz, & Ordóñez, 1991; Ramírez et al., 2005). Although oleic acid is the most abundant fatty acid in the majority of meats, such as beef, lamb and pork (Enser, Hallet, Fursey, & Wood, 1996), the fat of rabbit meat is richer in palmitic acid.

#### 3.2. NIR calibration

After elimination of outliers and testing different mathematical treatments, with or without scatter and detrend correction, the better calibration equation for each fatty acid, with respect to higher determination coefficient of cross-validation ( $r^2$ ) and lower standard error of cross-validation (SECV) are shown in Table 2. The higher outlier elimination was in icosanoic, eicosatrienoic and stearic acids and as a consequence the ranges and means of these

Table 1

Interval, range, mean and standard deviation (SD) of relative percentage of fatty acids, determined by gas chromatography analysis, of the hind leg meat of rabbits (intermuscular and intramuscular fat) used for NIRS calibration ( $n = 104$ )

Fatty acids	Interval	Range	Mean	SD
C14:0 (myristic)	1.66–3.70	2.04	2.46	0.32
C16:0 (palmitic)	22.85–34.76	11.91	28.12	3.02
C16:1 <i>cis</i> $n - 7$ (palmitoleic)	0.91–6.82	5.91	3.61	1.37
C18:0 (stearic)	4.96–11.22	6.26	7.50	1.12
C18:1 $n - 9$ (oleic)	18.52–30.18	11.66	24.72	3.16
C18:1 $n - 7$ (vaccenic)	0.96–1.73	0.77	1.28	0.19
C18:2 $n - 6$ (linoleic)	14.99–41.19	26.20	26.88	6.88
C18:3 $n - 3$ ( $\alpha$ -linolenic)	1.82–4.71	2.89	3.03	0.74
C20:1 (icosanoic)	0.00–0.66	0.66	0.37	0.14
C20:2 $n - 6$ (eicosadienoic)	0.23–1.17	0.94	0.44	0.14
C20:3 $n - 6$ (eicosatrienoic)	0.00–0.97	0.97	0.26	0.12
C20:4 $n - 6$ (arachidonic)	0.65–3.38	2.73	1.81	0.53
Saturated	30.26–46.03	15.77	38.06	3.81
Monounsaturated	20.81–37.21	16.40	29.53	4.44
Polyunsaturated	19.34–48.93	29.59	32.41	7.67
$n - 6$	17.17–44.22	27.05	29.38	7.16

Saturated = C14:0 (myristic) + C16:0 (palmitic) + C18:0 (stearic).

Monounsaturated = C16:1  $n - 7$  (palmitoleic) + C18:1  $n - 9$  (oleic) + C18:1  $n - 7$  (vaccenic) + C20:1 (icosanoic).

Polyunsaturated = C18:2  $n - 6$  (linoleic) + C18:3  $n - 3$  ( $\alpha$ -linolenic) + C20:2  $n - 6$  (eicosadienoic) + C20:3  $n - 6$  (eicosatrienoic) + C20:4  $n - 6$  (arachidonic).

$n - 6$  = C18:2  $n - 6$  (linoleic) + C20:2  $n - 6$  (eicosadienoic) + C20:3  $n - 6$  (eicosatrienoic) + C20:4  $n - 6$  (arachidonic).

Table 2  
Statistical parameters of the equations of NIR calibration corresponding to fatty acids (% weight) of intramuscular and intermuscular fat on the hind leg meat of rabbit

Fatty acids	<i>n</i>	Interval	Range	Mean	SD	$r^2$	SECV	RPD	RER
C14:0 (myristic)	103	1.66–3.12	1.46	2.45	0.30	0.21	0.26	1.12	5.51
C16:0 (palmitic)	102	22.85–34.76	11.91	28.10	2.98	0.83	1.21	2.46	9.85
C16:1 <i>cis</i> <i>n</i> – 7 (palmitoleic)	100	0.91–6.83	5.92	3.10	1.34	0.77	0.64	2.08	9.19
C18:0 (stearic)	96	5.03–9.74	44.71	7.42	0.89	0.50	0.63	1.41	7.49
C18:1 <i>n</i> – 9 (oleic)	99	18.52–30.18	11.66	24.72	3.14	0.84	1.26	2.49	9.24
C18:1 <i>n</i> – 7 (vaccenic)	102	0.96–1.73	0.77	1.28	0.18	0.33	0.15	1.21	5.03
C18:2 <i>n</i> – 6 (linoleic)	100	14.99–41.19	26.20	26.60	6.85	0.91	2.08	3.29	12.60
C18:3 <i>n</i> – 3 ( $\alpha$ -linolenic)	101	1.82–4.72	2.90	3.01	0.74	0.59	0.47	1.55	6.12
C20:1 (icosaenoic)	92	0.19–0.53	0.34	0.40	0.07	0.08	0.07	1.04	4.86
C20:2 <i>n</i> – 6 (eicosadienoic)	97	0.23–0.63	0.40	0.41	0.09	0.23	0.08	1.14	5.13
C20:3 <i>n</i> – 6 (eicosatrienoic)	93	0.15–0.47	0.32	0.26	0.06	0.54	0.04	1.49	7.44
C20:4 <i>n</i> – 6 (arachidonic)	101	0.65–3.17	2.52	1.79	0.50	0.63	0.31	1.63	8.21
Saturated	99	30.26–46.03	15.77	38.04	3.73	0.85	1.43	2.60	11.00
Monounsaturated	99	20.81–37.21	16.4	29.53	4.45	0.83	1.81	2.46	9.04
Polyunsaturated	98	20.11–46.78	26.67	32.20	7.42	0.93	2.03	3.65	13.11
<i>n</i> – 6	100	17.17–42.26	25.09	29.26	7.08	0.91	2.17	3.27	11.58

Saturated = C14:0 (myristic) + C16:0 (palmitic) + C18:0 (stearic).

Monounsaturated = C16:1 *n* – 7 (palmitoleic) + C18:1 *n* – 9 (oleic) + C18:1 *n* – 7 (vaccenic) + C20:1 (icosaenoic).

Polyunsaturated = C18:2 *n* – 6 (linoleic) + C18:3 *n* – 3 ( $\alpha$ -linolenic) + C20:2 *n* – 6 (eicosadienoic) + C20:3 *n* – 6 (eicosatrienoic) + C20:4 *n* – 6 (arachidonic).

*n* – 6 = C18:2 *n* – 6 (linoleic) + C20:2 *n* – 6 (eicosadienoic) + C20:3 *n* – 6 (eicosatrienoic) + C20:4 *n* – 6 (arachidonic).

$r^2$  = coefficient of determination of cross-validation.

SECV, standard error of cross-validation.

RPD = SD/SECV.

RER = range/SECV.

FA varied as previously indicated in Table 1 for all the samples. The lesser variation was in the myristic that had only one outlier elimination.

The  $r^2$  of the cross-validation was greater than 0.90 in linoleic acid, indicating that the correspondent calibration equations reports excellent quantitative information (Shenk & Westerhaus, 1996). The  $r^2$  was between 0.70 and 0.89 in palmitic, palmitoleic and oleic acids, indicating good quantitative information, and near to this group is arachidonic acid with a  $r^2$  of 0.63, while the  $r^2$  of stearic,  $\alpha$ -linolenic, and eicosadienoic acids were between 0.50 and 0.69 that indicates a good separation of samples into high, medium and low groups (Shenk & Westerhaus, 1996). Only four of the individual fatty acids are excellent or well predicted; another four of them have a prediction near to the good form and the myristic, vaccenic, icosaenoic and eicosadienoic fatty acids are problematic to predict.

There are some references about NIR prediction of meat composition (King-Brink, DeFreitas, & Debranek, 1996; Solis et al., 2001) and also chemical composition of rabbit meat can be well predicted (Masoero, Xiccato, Zotte, Parigi-Bini, & Bergoglio, 1994; Pla et al., 2004) but the number of references with respect to the fatty acid prediction on meat by NIRS is smaller. In beef neck lean meat, Windham and Morrison (1998) considered NIR prediction of individual fatty acids as problematic with an  $r^2$  between 0.01 and 0.78 corresponding to linoleic and oleic acid, respectively. Büning-Pfaue, Hartmann, Kehraus, and Urban (1998) consider that the different fatty acids, including trans fatty

acids in 'consumable meals' can be predicted by NIR spectrometry. Perez Marin, Pedro, Garcia-Olmo, and Garrido (2001) for the fat of the Iberian breed pig showed an  $r^2$  of 0.65 for myristic acid and  $r^2$  from 0.92 to 0.99 for palmitoleic and oleic acid, respectively. Nevertheless no references about NIRS prediction of fatty acid in rabbit meat have been found in the bibliography, and therefore no comparisons can be made.

When fatty acids are grouped, the  $r^2$  of the calibration equations (Table 2) was 0.85 for saturated fatty acid, 0.83 for monounsaturated, 0.92 for polyunsaturated, 0.91 for *n* – 6 FA that indicates excellent or good quantitative information. Only the  $r^2$  of the *n* – 3 fatty acid that corresponds to  $\alpha$ -linolenic acid was less than 0.70 (0.59). The RPD (ratio of standard deviation respect to the standard error of cross-validation) ranged from 2.46 to 3.65 in monounsaturated and polyunsaturated acids respectively and only the RPD of *n* – 3 acid was less than 2 (1.55). The RER, defined as the range divided by SECV, varied between 9.04 and 13.11 in monounsaturated and polyunsaturated acids, respectively, and the RER of the *n* – 3 acid was 6.12. Williams and Sobering (1996) consider that the RPD should ideally be at least three and the RER at least 10. This is the case of polyunsaturated and *n* – 6 fatty acids, while saturated and unsaturated acids are near to these conditions; the correspondent equations are useful for prediction. In case of the *n* – 3 fatty acid, more samples should be included in the calibration exercise but the correspondent equation could be used as a first approximation.

Table 3

Least square means and standard error of relative percentage of fatty acids, determined by NIRS, of the hind leg meat of rabbits (intermuscular and intramuscular fat) produced in conventional or organic production systems

Fatty acids	Mean		SE	Significance
	Conventional (n = 26)	Organic (n = 26)		
Saturated	42.05	42.27	0.40	ns
Monounsaturated	34.64	30.54	0.61	***
Polyunsaturated	23.66	27.28	1.05	*
n – 6	21.11	23.39	0.93	**
n – 3	2.47	2.64	0.06	**

Saturated = C14:0 (myristic) + C16:0 (palmitic) + C18:0 (stearic).

Monounsaturated = C16:1 n – 7 (palmitoleic) + C18:1 n – 9 (oleic) + C18:1 n – 7 (vaccenic) + C20:1 (icosaenoic).

Polyunsaturated = C18:2 n – 6 (linoleic) + C18:3 n – 3 ( $\alpha$ -linolenic) + C20:2 n – 6 (eicosadienoic) + C20:3 n – 6 (eicosatrienoic) + C20:4 n – 6 (arachidonic).

n – 6 = C18:2 n – 6 (linoleic) + C20:2 n – 6 (eicosadienoic) + C20:3 n – 6 (eicosatrienoic) + C20:4 n – 6 (arachidonic).

n – 3 = C18:3 n – 3 ( $\alpha$ -linolenic).

\*  $p < 0.05$ .

\*\*  $p < 0.10$ .

\*\*\*  $p < 0.001$ .

### 3.3. Effects of organic production system

Hind-leg meat of the organic source rabbits is poorer in monounsaturated and richer in polyunsaturated FA than that of the conventional rabbits (Table 3) when FA are determined by NIRS methodology. As the content of saturated FA is similar in both groups, meat of the organic system produced rabbits is better from a nutritional point of view, because the P:S ratio is higher (Department of Health & Social Security UK, 1994) although the weakness on n – 3 FA content imposes caution in this respect. Usually a real comparison between organic and conventionally produced meat is difficult because different breeds are used (Kouba, 2003); in our case the genetic origin is the same and the detected differences can be explained by the production system, mainly due to the diet and age at slaughter. Cambero et al. (1991) comparing rabbits fed different diets and slaughtered at different age found that it is difficult to establish a clear relationship between the fatty acid content in rabbit meat and age, sex, diet or breed. The conclusion may be that organic production seems responsible for a more nutritious rabbit meat.

### 3.4. Discriminant analysis

The calibration method applied to discriminant analysis was partial least squares (PLS) and spectra files were entered for each group to be discriminated: conventional (C) and organic system (O) (26 samples in each group). Seven mathematical treatments with or without scatter and detrend (SNV-DT) correction were used and the SNV-DT applied to 1,4,4,1 math using nine PLS terms had the best results. The SEC was 0.20, SECV = 0.23 and  $r^2 = 0.78$ . Only in one case was organic group misclassified

and PLS discriminant analysis of the NIRS spectra successfully (98%) distinguished between the conventional and organic meat samples.

## References

- Baselga, M. (2002). Line A, line V, line H and line R (Spain). In M. H. Khalil & M. Baselga (Eds.), *Rabbit genetic resources in Mediterranean countries* (pp. 221–262). Zaragoza, Spain: Options Méditerranéennes CIHEAM.
- Bernardini, M., Dal Bosco, A., & Castellini, C. (1999). Effect of dietary n – 3/n – 6 ratio on fatty acids composition of liver, meat and perirenal fat in rabbits. *Animal Science*, 68, 647–654.
- Berry, J. F., Cervillos, W. H., & Wade, R. R. (1965). Lipid class and fatty acid composition of intact peripheral nerve and during wallerian degeneration. *Journal of American Oil Chemistry Society*, 42, 492–495.
- 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.
- Büning-Pfaue, H. (2004). A revolution in analysis. *New Food*, 7(3), 41–47.
- Büning-Pfaue, H., Hartmann, R., Kehraus, S., & Urban, C. (1998). Near infrared spectrometric analysis of food and its achievable performance. *Journal of Near Infrared Spectroscopy*, 6, 27–33.
- Cambero, M. I., de la Hoz, L., Sanz, B., & Ordóñez, J. A. (1991). Lipid and fatty acid composition of rabbit meat: 1 – apolar fraction. *Meat Science*, 29, 153–166.
- Cameron, N. D., Enser, M., Nute, G. R., Whittington, F. M., Penman, J. C., Fiske, A. C., et al. (2000). Genotype with nutrition interaction on fatty acid composition of intramuscular fat and the relationship with flavour on pig meat. *Meat Science*, 55, 187–195.
- Department of Health and Social Security (1994). Nutritional aspects of cardiovascular disease (Report on health and social subjects no. 46). London: H.M. Stationery Office.
- Enser, M., Hallet, B., Fursey, G. A. J., & Wood, J. D. (1996). Fatty acid content and composition of English beef, lamb and pig at retail. *Meat Science*, 42(4), 443–456.
- Folch, J., Lees, M., & Stanley, G. H. S. (1957). A simple method for the isolation and purification of lipids from animal tissues. *Journal of Biological Chemistry*, 226, 497–509.
- Hervás, C., Garrido, A., Lucena, B., García, N., & de Pedro, E. (1994). Near infrared spectroscopy for classification of Iberian pig carcasses using an artificial neural network. *Journal of Near Infrared Spectroscopy*, 2, 177–184.
- Hu, F. B., & Willett, W. C. (2002). Optimal diets for prevention of coronary heart disease. *Journal of the American Medical Association*, 288, 2569–2578.
- King-Brink, M., DeFreitas, M., & Debrank, J. G. (1996). Use of near infrared transmission for rapid analysis of meat composition. In A. M. C. Davies & P. Williams (Eds.), *Near infrared spectroscopy: The future waves* (pp. 142–148). Chichester, UK: NIR Publications.
- Kouba, M. (2003). Quality of organic animal products. *Livestock Production Science*, 80, 33–40.
- McElhinney, J., Downey, G., & Fearn, T. (1999). Chemometric processing of visible and near infrared reflectance spectra for species identification in selected raw homogenized meats. *Journal of Near Infrared Spectroscopy*, 7, 145–154.
- Masoero, G., Xiccato, G., Zotte, A., Parigi-Bini, R., & Bergoglio, G. (1994). Dalle analysis of freeze-dried rabbit meat by NIRS. *Zootecnica Nutrizione Animale*, 20, 319–329.
- Mitsumoto, M., Maeda, S., Mitsuhashi, T., & Ozawa, S. (1991). Near-infrared spectroscopy determination of physical and chemical characteristics in beef cuts. *Journal of Food Science*, 56(6), 1493–1496.
- Murray, I., Aucott, L. S., & Pike, I. H. (2001). Use of discriminant analysis on visible and near infrared reflectance spectra to detect adulteration of fishmeal with meat and bone meal. *Journal of Near Infrared Spectroscopy*, 9, 297–311.

- Oliver, M. A., Guerrero, L., Díaz, I., Gispert, M., Pla, M., & Blasco, A. (1997). The effect of fat-enriched diets on the perirenal fat quality and sensory characteristics of meat from rabbits. *Meat Science*, *47*(1/2), 95–103.
- Park, B., Chen, Y. R., Hruschka, W. R., Shackelford, S. D., & Koohmaraie, M. (1998). Near-infrared reflectance analysis for predicting beef longissimus tenderness. *Journal of Animal Science*, *76*, 2115–2120.
- Pedro, E., Garrido, A., Barnes, I., Casillas, M., & Murray, I. (1992). In T. Hildrum, T. Isaksson, T. Naes, & A. Tandberg (Eds.), *Near infra-red spectroscopy: Bridging the gap between data analysis and NIR applications* (pp. 345–348). Chichester, UK: Ellis Horwood.
- Pedro, E., Garrido, A., Lobo, A., Dardenne, P., & Murray, I. (1995). In G. D. Batten, P. C. Flinn, L. A. Welsh, & A. B. Blakeney (Eds.), *Leaping ahead with near infrared spectroscopy* (pp. 291–295). Vic., Australia: Royal Australian Chemical Institute.
- Perez Marin, M. D., Pedro, E., Garcia-Olmo, J., & Garrido, A. (2001). Análisis NIRS de grasa de cerdo ibérico: Efect del fichero de repetibilidad. *ITEA Extra*, *22*, 610–612.
- Pla, M., Pascual, M., & Ariño, B. (2004). Protein, fat and moisture content of retail cuts of rabbit meat evaluated with the NIRS methodology. *World Rabbit Science*, *12*, 149–158.
- Raimondi, R., de Maria, C., Auxilia, M. T., & Masoero, G. (1975). Effetto della grasatura dei mangimi sulla produzione della carne di coniglio. III. Contenuto in acidi grassi delle carni e del grasso perirenale. *Annali dell'Istituto Sperimentale per la Zootecnia*, *8*(2), 167–181.
- Ramírez, J. A., Díaz, I., Pla, M., Gil, M., Blasco, A., & Oliver, M. A. (2005). Fatty acid composition of leg meat and perirenal fat of rabbits selected by growth rate. *Food Chemistry*, *90*, 251–256.
- SAS (2000). *SAS/STAT user's guide*. Release 8.1. Statistical analysis system. Cary, NC, USA: SAS Institute Inc.
- Shenk, J. S., & Westerhaus, M. O. (1996). Calibration the ISI way. In A. M. C. Davis & P. Williams (Eds.), *Near infrared spectroscopy: The future waves* (pp. 198–202). Chichester, UK: NIR Publications.
- Solis, M., Pedro, E., Garrido, A., García-Olmo, J., Sileo, I., Rodríguez, M. C., et al. (2001). Evaluación de la composición del lomo de cerdo ibérico mediante la tecnología NIRS. *ITEA Extra*, *22*, 613–615.
- Williams, P., & Sobering, D. (1996). How do we do it: brief summary of the methods we use in developing near infrared calibrations. In A. M. C. Davis & P. Williams (Eds.), *Near infrared spectroscopy: The future waves* (pp. 185–188). Chichester, UK: NIR Publications.
- Windham, W. R., & Morrison, W. H. (1998). Prediction of fatty acid content in beef neck lean by near infrared reflectance analysis. *Journal of Near Infrared Spectroscopy*, *6*, 229–234.