PE3.06 Potential use of near infrared spectroscopy for the on-line prediction of fatty acid composition in limousin and Aberdeen angus beef samples 308.00

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***Abstract*—The objective of this study was to test the on-line estimation of the concentration of individual fatty acids (FA) and groups of FA in crossbred Limousin (LIM) and Aberdeen Angus (AA) beef samples using NIR spectroscopy (NIRS), immediately after exposing the meat surface in the abattoir at 48 h post mortem. Samples from 106 LIM and 88 AA *M. longissimus thoracis* were scanned over the NIR spectral range from 1100-1800 nm and samples of the *M. longissimus lumborum* were analyzed for fatty acid composition. NIR calibrations, tested by cross-validation, showed high predictability in LIM meat samples for the C16:0, C16:1, C18:0, C18:1, cis9, trans11 C18:2, C20:1, saturated (SFA) and monounsaturated (MUFA) fatty acids, with R^2 of 0.689, 0.692, 0.714, 0.755, 0.714, 0.713, 0.676 and 0.753 and SE_{CV} (mg.100 g^{-1} muscle) of 146.2, 28.4, 62.4, 192.5, 2.87, 0.92, 235.5 and 239.8, respectively. Fatty acids such as C18:2 n-6 and polyunsaturated (PUFA) were more difficult to predict by NIRS in those samples (R^2 = 0.653 and 0.643, SE_{CV} = 13.1 and 17.3 mg.100 g^{-1} muscle, respectively). The accuracy of prediction for individual and groups of FA concentration in AA beef samples was lower than that found in LIM ones, probably due to a less homogeneous distribution of meat sample population. The correlation of NIR measurements and several FA in the range from 0.82 to 0.87 indicated that NIRS is a useful on-line technique for the early, fast and relatively inexpensive estimation of FA composition in LIM beef samples in the abattoir.**

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***Index Terms*— Near infrared spectroscopy, Fibre-optic probe, Fatty acid, Beef.**

I. INTRODUCTION

The amount and proportions of fatty acids (FA) in beef intramuscular fat are key factors that influence technological and sensory meat quality, especially shelf-life (lipid and pigment oxidation) and flavour [1]. Additionally, consumers are interested in fat composition of meat since nutritional guidelines recommend less saturated FA (SFA) in the diet. Scientific evidence suggests that diets high in saturated fat are associated with increased levels of blood total and LDL-cholesterol, which results in increased risk of cardiovascular diseases. In contrast, the consumption of beef in human diets also supplies conjugated linoleic acids (CLA), which are a group of positional and geometrical isomers that are associated with important health-related benefits [2]. In addition, the amount and composition of ruminant intramuscular fat, which depends on factors such as the genetic origin of the animals [3], influences the final quality of the product. This also explains the increasing interest in defining the FA profile of beef from different breeds.

Quantitative chemical techniques for the determination of FA involve extraction of total lipids with diethyl ether followed by conversion of the fatty acids to their methyl esters and analysis by capillary gas chromatography, a costly and time-consuming process. Near-infrared spectroscopy (NIRS) is a rapid and non-destructive method, neither requiring reagents nor producing waste [4]. Because of these advantages, it has been widely used in research for large-scale meat quality evaluation to predict the chemical composition [5, 6] and physical and sensory characteristics of meat [7, 8]. Moreover, the structure of FA can produce individual spectral characteristics and they are, therefore, very accessible for detection by NIRS [9]. Hence, this technology has been applied to study the FA content in Iberian pig fat [10], intact pork loins [11] and ground beef [12].

The aim of this study was to test the on-line estimation of FA (individual and groups) composition in crossbred Limousin and Aberdeen

Angus beef samples using NIR spectroscopy, by direct application of a fibre-optic probe to the *M. longissimus thoracis* immediately after exposing the meat surface in the abattoir.

II. MATERIALS AND METHODS

2.1. Animals and meat samples

Data were collected on 194 Aberdeen Angus (AA) or Limousin (LIM) crossbred steers and heifers, whereby 106 and 88 were sired by either LIM or AA high genetic merit sires, respectively. A total of 144 animals were selected from Scottish Agricultural College farms and were finished using mixed forage:concentrate diets. A further 50 were selected from commercial farms. Animals were slaughtered in 11 batches from autumn 2006 until spring 2008 (batch 1 to 3, 4 to 8, and 9 to 11 in 2006, 2007 and 2008, respectively) at an average live weight of 609 and 582 kg and age at slaughter of 544 and 546 days for LIM and AA sired beef cattle, respectively.

The left sides of the carcasses were cut at the 13th rib at 48 h post mortem. NIR measurements were taken on the caudal cut surface of the *M. longissimus thoracis*. After removing a 125mm section, the next 25mm of the *M. longissimus lumborum* was taken, vacuum packed and frozen for subsequent analysis of FA composition.

2.2. Fatty acid analysis

Fatty acids analysis was carried out by direct saponification as described in detail by Teye et al. [13]. Samples were hydrolysed with 2M KOH in water:methanol (1:1) and the FA extracted into petroleum spirit, methylated using diazomethane and analysed by gas liquid chromatography. Samples were injected in the split mode, 70:1, onto a CP Sil 88, 50m x 0.25mm fatty acid methyl esters column (Chrompack UK Ltd, London) with helium as the carrier gas.

2.3. NIR data

NIR measurements were taken by placing the scanning head, 63.5 mm in diameter active area, over the surface of the exposed *M. longissimus thoracis* and recording a spectrum from 1100 to 1800 nm, by means a NIR spectrophotometer (Qualityspec Pro, ASD Inc., Boulder, Colorado). Twenty replicate measurements were taken by moving and rotating the scanning head around the muscle surface and then averaged. The scanning head incorporated a broad-band light source for tissue illumination and a sampling fibre optic probe that passed the reflected light back to the spectrometer, which interpolated the data to produce measurements in one nm steps, resulting in a diffuse reflectance spectrum of 801 data points. Absorbance data were stored as log (1/*R*), where *R* being the reflectance.

Calibration and validation were performed using The UNSCRAMBLER program (version 8.5.0, Camo, Trondheim, Norway). Two passes of elimination of outliers (*H* and *T*) were allowed. Partial least square regression type I (PLSR1) was used for predicting FA concentration using NIR spectra as independent variables. Internal full cross-validation was performed in order to avoid over-fitting the PLSR equations.

III. RESULTS AND DISCUSSION

Ranges, means and standard deviations (SD) of the FA profile (individual and groups) of LIM and AA muscle samples are summarized in Table 1. In general terms, the values of concentration for individual and groups of FA in both LIM and AA beef samples were within the normal range of variation reported by Sierra et al. [12]. Most individual FA showed a wide range of variability in both LIM and AA sample populations, mainly the palmitic, oleic and CLA acids. Those FA with a higher range of concentration were those with a higher presence in meat.

Regarding FA groups (Table 1), SFA and MUFA showed a high variability of concentration, probably due to the high heterogeneity of the total fat content in the meat samples included in this study (ranging from 0.77 to 5.41 and 1.19 to 6.62 g FA.100 g^{-1} muscle for LIM and AA beef samples, respectively). However, PUFA showed lower variability among samples. Generally, FA concentration was higher in AA than in LIM beef samples, statically significant differences ($P < 0.001$) being observed in most FA between the two breeds studied (data not shown).

The calibration equation results for each FA in LIM and AA beef samples are shown in Table 2. The prediction equations for most individual and groups of FA studied in AA beef samples presented R^2 and RPD lower than 0.481 and 1.12, respectively. Only for the linoleic and icosaenoic acids, the percentage of variance explained by the model was over 60% (R^2 = 0.701 and 0.618, respectively). Nevertheless, for these FA, the SE_{CV} (SE_{CV} = 12.7 and 1.55 mg.100 g^{-1} muscle, respectively) were still high and the RPD statistics (ratio of SD respect to the SE_{CV}) were low (RPD = 1.41 and 1.27, respectively), regarding that considered in the literature as suitable for screening purposes [14, 15]. When plotting the FA concentration predicted by NIRS against that obtained by chemical analysis for AA beef loin samples (graphs not shown), it was observed that

the samples for FA such as linoleic, CLA and PUFA were not uniformly distributed along the regression line, but mostly located closely together with a few samples as extreme values. The fact that the sample population was not homogeneously distributed along the regression line could have provided a high variability among samples in terms of SD and relatively high R^2 for the linoleic and icosaenoic acids, but their predictability by NIRS was still low. Thus, including more samples of AA into the NIR calibration would probably improve the NIRS predictability. Furthermore, most of the tested animals were from an experimental farm using sires of high genetic potential, which may not spread homogenously over the range of FA content in AA. In agreement with our results, other studies have shown that determining FA is quantitatively difficult [16]. According to Windham and Morrison [17], the failure to accurately determine some individual FA is probably due to the similarities in their NIR absorption pattern, because different FA have the same absorbing molecular group ($-CH_2-$).

The accuracy of prediction for individual and groups of FA content in LIM beef samples was higher than that found in AA ones. Accurate predictions were found for most major FA in LIM beef samples (Table 2), the best predictions being obtained for the palmitic, palmitoleic, stearic and oleic FA (R^2 = 0.689, 0.692, 0.714 and 0.755; SE_{CV} = 146.2, 28.4, 62.4 and 192.5 mg.100 g^{-1} muscle, respectively). Consequently, RPD statistics ranged from 1.70 to 1.95. On the contrary, the linoleic FA was less predictable by NIRS (R^2 = 0.653, RPD = 1.52). It is well known that the success of this procedure relies partially on the variability present in the samples analyzed, thus a low variability among samples for that FA (Table 1) could have reduced the NIRS predictability. Minor FA in LIM samples were accurately predicted, with R^2 of 0.714 and 0.713 and RPD of 1.83 and 1.85, for CLA and icosaenoic FA, respectively.

The results found in this study for LIM beef samples are in accordance with those reported by González-Martín et al. [9] in subcutaneous fat of swine for palmitic and oleic acids. However, Sierra et al. [12], González-Martín et al. [18] and Pla et al. [19] showed better predictions for the FA content in beef, swine subcutaneous fat and rabbit meat, respectively. This lack of agreement within studies could be due to the fact that Sierra et al. [12] and Pla et al. [19] used beef and rabbit meat samples from different genetic origins and fed different diets to perform NIR calibrations, in order to ensure a reasonable variety of samples that represent the different sources of variation during analysis. Additionally, in our study the samples of muscle were directly scanned (without previous fat extraction) to assess the on-line implementation of NIRS, whereas González-Martín et al. [18] scanned subcutaneous fat of swine, which is expected to provide more spectral information directly on FA content. For stearic acid, our results agree with those reported by Sierra et al. [12], González-Martín et al. [18] and they are better than those showed by González-Martín et al. [9, 11] and Pla et al. [19], who presented lower predictabilities (RPD < 1.41). With regard to minor FA, the icosaenoic acid was estimated in our study with much more accuracy than in the study carried out by González-Martín et al. [9, 11, 18] and Pla et al. [19]. Only a few authors have estimated CLA content by NIRS despite its increasing importance for human health. In the current study, CLA was more accurately predicted than in the study carried out by Sierra et al. [12], who achieved a R^2 of 0.59 and a RPD of 1.56.

When FA were grouped (Table 2), the calibration equation for SFA and MUFA for LIM beef samples showed accurate predictions (R^2 = 0.676 and 0.753, SE_{CV} = 235.5 and 239.8 mg.100 g^{-1} muscle, RPD = 1.72 and 1.86, respectively), whereas the predictability of NIRS for PUFA content was less reliable (R^2 = 0.643, SE_{CV} = 17.3 mg.100 g^{-1} muscle, RPD = 1.50), probably due to a narrower range of variability (Table 1). In general, most researchers have described accurate NIR calibrations for SFA and MUFA in meat [9, 12, 18, 19]. With regard to PUFA, González-Martín et al. [18] and Pla et al. [19] reported better predictions than these obtained in the current study; however, our results are in accordance with those reported by González-Martín et al. [9, 11] and are better than those reported by Sierra et al. [12].

IV. CONCLUSION

The results of this research show that NIR spectroscopy could be used on-line in the abattoir as an early predictor for the main individual FA, SFA and MUFA content in LIM meat samples. In AA beef samples the accuracy of prediction was lower than that found in LIM ones, probably due to a less homogeneous distribution of meat sample population; thus further studies including a larger number of samples are required. Under practical conditions, much more variation in FA profile is expected than for these high genetic merits, mostly

experimental animals and so these correlations may be at the lower end of those possible for predicting FA content.

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Table 1. Descriptive statistics (mg.100 g^{-1} muscle) for individual and groups of fatty acids in crossbred Limousin (n = 106) and Aberdeen Angus (n = 88) beef samples.

	Limousin			Aberdeen Angus		
Fatty acid	Range	Mean	SD	Range	Mean	SD
FA						
C16:0 (palmitic)	148-1520	654	259.2	248-1784	856	307.8
C16:1 (palmitoleic)	19-282	111	48.4	36-299	141	55.5
C18:0 (stearic)	93-673	330	114.7	166-937	421	141.1
C18:1 (oleic)	194-1978	926	374.6	346-2638	1219	443.4
C18:2 n – 6 (linoleic)	57-166	90	19.8	61-182	88	18.5
*cis*9, *trans*11 C18:2 (CLA)	1.8-26.7	10.0	5.25	4.1-55.6	14.5	7.93
C20:1 (icosaenoic)	0.7-9.3	2.9	1.70	0.9-9.4	3.9	1.94
Groups of FA						
SFA (saturated)	251-2339	1059	405.1	442-2805	1393	478.6
MUFA (monounsaturated)	238-2376	1117	445.3	415-3096	1485	520.5
PUFA (polyunsaturated)	120-276	176	26.0	141-409	180	33.4

n: number of samples, SD: standard deviation, SFA: C12:0 + C14:0 + C16:0 + C18:0, MUFA: C16:1 + Ct18:1 + C9c18:1 + C11c18:1 + C20:1, PUFA: C18:2n-6 + C18:3n-3 + C20:3n-6 + C20:4n-6 + C20:5n-3 + C22:4n-6 + C22:5n-3 + C22:6n-3.

Table 2. Prediction of individual and groups of fatty acids in crossbred Limousin and Aberdeen Angus beef samples using NIR spectra.

	Limousin					Aberdeen Angus				
	n	p	R^2	SE_{CV}	RPD	n	p	R^2	SE_{CV}	RPD
FA										
C16:0 (palmitic)	103	7	0.689	146.2	1.77	85	3	0.448	283.4	1.10
C16:1 (palmitoleic)	103	7	0.692	28.4	1.70	85	3	0.481	50.3	1.12
C18:0 (stearic)	103	9	0.714	62.4	1.85	85	2	0.343	140.4	1.02
C18:1 (oleic)	103	8	0.755	192.5	1.95	85	3	0.435	406.1	1.09
C18:2 n – 6 (linoleic)	104	7	0.653	13.1	1.52	85	7	0.701	12.7	1.41
cis9, trans11 C18:2 (CLA)	103	8	0.714	2.87	1.83	85	2	0.374	6.86	1.17
C20:1 (icosaenoic)	103	8	0.713	0.92	1.85	85	7	0.618	1.55	1.27
Groups of FA										
SFA (saturated)	103	7	0.676	235.5	1.72	85	2	0.369	452.4	1.07
MUFA (monounsaturated)	103	8	0.753	239.8	1.86	85	3	0.419	482.9	1.08
PUFA (polyunsaturated)	103	9	0.643	17.3	1.50	85	2	0.215	32.2	1.03

n: number of samples after eliminating outliers, p: number of PLS terms utilized in the calibration equation, R^2: coefficient of determination of calibration, SE_{CV}: standard error of cross-validation, RPD: ratio performance deviation calculated as SD/ SE_{CV}.