PREDICTION OF FATTY ACID COMPOSITION OF BEEF USING NEAR INFRARED SPECTROSCOPY: TISSUES AND SAMPLES PREPARATION EFFECTS

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Abstract – The aims of the study were to determine the best site of bovine carcass for predicting fatty acid (FA) composition using a NIRS (near infrared spectroscopy) portable equipment and to study the effect of different methods of sample preparation. 78 animals were sampled from different types and rearing systems. Seven tissues (*Longissimus thoracis*, *Infraspinatus*, *Diaphragma*, *Rectus abdominis*, shoulder subcutaneous adipose tissue (SAT), intercostal SAT and intermuscular fat at the 5th rib) were measured after sampling and grinding in liquid nitrogen. The effect of samples preparation were measured on carcass (C0), muscle without grinding (B0), ground with a meat chopper (B1), ground with a knife mill (B2) on RA muscle. FA composition was assessed using gas chromatograph and the spectra were measured at wavelengths between 350 and 2500 nm. For adipose tissue, FA were not correctly predicted from NIRS. However, predictions were more satisfactory for the major FA (C16:0, C18:0, C18:1d9c), total saturated and monounsaturated FA of muscles. The results show a better prediction of FA composition concomitant with an increased gradient of sample homogenization. For other FA and especially polyunsaturated fatty acids, the performances were not satisfactory for quantitative purposes whatever the grinding method.

Key Words –Bovine muscles, Fat tissue, Fatty acid, Ground samples, Intact tissue, NIRS.

I. INTRODUCTION

The use in slaughterhouses of near-infrared spectroscopy (NIRS) in the meat industry is developing. Among the measured parameters, rapid quantification and at lower cost of fatty acids (FA) is of particular interest for meat differentiation pathways on their lipid quality. In the end, this tool should allow industries in the sector to guide early carcasses to the most appropriate marketing channels based on their FA composition. The reference method for measuring the fatty acid composition, the gas liquid chromatography (GLC), is destructive, time-consuming and costly. The pioneering work showed that NIRS is an alternative method of measurement, both in pigs [1], lamb [2] and even in bovine [3] and has the advantage of being easy, fast, non-destructive, cost-effective and frequently used in line. The study reported here was initially designed to evaluate the performance of FA composition prediction of different cattle tissues using a portable NIRS. Secondly, the objective is to study the effect of different sample preparation methods on the prediction performance of the FA composition, for use in a slaughterhouse.

II. MATERIALS AND METHODS

Seven tissues of which 4 muscles i) *Longissimus thoracis* (LT), ii) *Infraspinatus* (In), iii) *Diaphragma* (Di), iv) *Rectus Abdominis* (RA), and 3 adipose tissues i) shoulder subcutaneous adipose tissue (SSAT) ii) intercostal SAT (ISAT), iii)

intermuscular fat of the 5th rib (IMF) were collected from 78 carcasses of cattle from different genetic types (milk, meat), different types of animal (young cattle , cows, oxen) fed diets based on grass or corn silage with or without extruded linseed supplementation. Spectral measurements were carried out on the target tissues on the carcass before sampling and then on finely ground tissues in liquid nitrogen (B3). Infrared spectra measurements were performed under different conditions i) directly on the carcass (C0), ii) taken muscles without grinding (B0), iii) minced with a meat grinder (B1), iv) ground with a knife mill (B2) and v) finely ground in liquid nitrogen (B3).

The spectra were acquired using a portable spectrometer ASD LabSpec 4 (range of wavelength: 350-2500 nm). The FA profiles (g/100g of tissue) were determined by GLC, on the samples ground in liquid nitrogen, after total lipid extraction and transmethylation [2].

Spectra were processed mathematically with different treatments (pretreatment and dispersion correction of the spectra: smoothing, first derivative and second). The models were developed by PLS and validated by cross validation, with the R software for the whole spectrum. Predictions of model performance for different FA are evaluated on the basis of the following criteria: standard error of cross-validation (SE_{CV}) and coefficient of determination of cross-validation (R^2_{CV}). A model with a R^2_{CV} between 0.66 and 0.81 and between 0.82 and 0.91, respectively, is considered average or good. Finally when a model has a R^2_{CV}> 0.91, then the prediction is considered excellent. [4]

The "tissue's effect" on prediction performance is evaluated on B3 tissue preparation (the highest homogeneity gradient allowing optimal performance predictions [5]), while the effect of "sample preparation" was tested on RA muscle.

III. RESULTS AND DISCUSSION

The results of the FA prediction performance of beef of the 7 tissues are presented in Table 1. Les résultats des performances de prédiction des AG de la viande bovine des 7 tissus finement broyés (B3) sont présentés dans le Tableau 1. In adipose tissue, regardless of FA considered, they are not correctly predicted by NIRS (R^2_{CV} <0.55), contrary to [1] observed on pork. We have no explanation for these unsatisfactory results especially as [6] obtained superior prediction performance in subcutaneous beef fat.

Table 1: Results of prediction models by NIRS of FA (g/100g of tissue) for the 7 ground tissues in liquid nitrogen (B3)

	Di		**LT**		**RA**		**In**		**SSAT**		**ISAT**		**IMF**	
	SE_{CV}	R^2_{CV}	SE_{CV}	R^2_{CV}	SE_{CV}	R^2_{CV}	SE_{CV}	R^2_{CV}	SE_{CV}	R^2_{CV}	SE_{CV}	R^2_{CV}	SE_{CV}	R^2_{CV}
Total FA	1.24	**0.83**	1.37	**0.80**	0.93	**0.89**	0.91	**0.92**	10.70	0.48	8.87	0.05	10.23	0.03
Total lipids	1.23	**0.85**	1.47	**0.81**	0.83	**0.93**	0.99	**0.92**	8.84	0.59	8.96	0.00	8.27	0.16
C16:0	0.41	**0.78**	0.40	**0.80**	0.26	**0.90**	0.27	**0.91**	3.25	0.51	3.29	0.13	5.15	0.00
C18:0	0.32	**0.79**	0.25	**0.76**	0.16	**0.84**	0.18	**0.85**	2.24	0.49	2.07	0.36	2.43	0.23
C18:1 d9c	0.54	**0.79**	0.55	**0.81**	0.41	**0.87**	0.33	**0.94**	4.65	0.41	4.26	0.20	3.96	0.12
C18:2 n-6	0.07	0.00	0.04	0.10	0.04	0.33	0.04	0.17	0.26	0.37	0.25	0.48	0.23	0.57
C18:3 n-3	0.04	0.00	0.02	0.20	0.01	0.48	0.02	0.31	0.11	0.36	0.17	0.00	0.15	0.06
SFA	0.76	**0.80**	0.68	**0.80**	0.44	**0.89**	0.48	**0.90**	5.29	0.55	5.06	0.19	5.70	0.12
MUFA	0.61	**0.80**	0.64	**0.82**	0.46	**0.89**	0.44	**0.93**	5.45	0.48	5.25	0.27	4.70	0.14
PUFA n-6	0.09	0.00	0.05	0.28	0.05	0.43	0.06	0.25	0.38	0.26	0.40	0.22	0.39	0.01
PUFA n-3	0.07	0.09	0.04	0.02	0.03	0.32	0.04	0.19	0.11	0.31	0.18	0.00	0.15	0.07
PUFA	0.12	0.03	0.08	0.26	0.07	0.53	0.08	0.21	0.53	0.32	0.61	0.09	0.55	0.05

Di: Diaphragma; LT: Longissimus thoracis; RA: Rectus Abdominis; In: Infraspinatus; SSAT: shoulder subcutaneous adipose tissue; ISAT: intercostal subcutaneous adipose tissue; IMF: intermuscular fat

Table 2: Results of prediction models by NIRS of FA (g/100g of tissue) of RA muscle depending on the grinding mode

	C0		B0		B1		B2	
	SE_{CV}	R^2_{CV}	SE_{CV}	R^2_{CV}	SE_{CV}	R^2_{CV}	SE_{CV}	R^2_{CV}
Total FA	1.71	0.64	1.63	**0.66**	1.89	0.55	1.19	**0.82**
Total lipids	1.85	0.65	1.71	**0.69**	1.94	0.61	1.17	**0.86**
C16:0	0.58	0.53	0.49	0.66	0.55	0.57	0.37	**0.81**
C18:0	0.26	0.60	0.25	0.64	0.31	0.44	0.22	**0.73**
C18:1 d9c	0.72	0.60	0.66	0.65	0.80	0.49	0.50	**0.80**
C18:2 n-6	0.04	0.35	0.04	0.20	0.05	0.02	0.04	0.33
C18:3 n-3	0.01	0.47	0.01	0.43	0.01	0.54	0.01	0.52
SFA	0.87	0.59	0.77	**0.67**	0.89	0.56	0.57	**0.82**
MUFA	0.84	0.64	0.83	0.64	0.96	0.53	0.56	**0.84**
PUFA n-6	0.05	0.45	0.05	0.32	0.06	0.09	0.05	0.47
PUFA n-3	0.04	0.22	0.03	0.54	0.03	0.47	0.04	0.26
PUFA	0.08	0.38	0.08	0.39	0.08	0.34	0.08	0.45

The predictions look better in this study, for the most lean tissue: RA, LT, and In The predictions are quite satisfactory for major FA. (C16:0, C18:0, C18:1 d9), total saturated FA (SFA) and total monounsaturated (MUFA) of muscles. (R^2_{CV}> 0.76), according to the literature [2], [4].

Also, as expected, the polyunsaturated FA (PUFA) appear to be poorly predicted by portable NIRS (R^2_{CV} <0.48). Currently, it is assumed that the unsatisfactory predictions of PUFA are due to either a lack of variability in the data (probably because of their structural function in membrane phospholipids) or as [7] have mentioned, a threshold minimum amount of FA is required especially when the accuracy of a portable NIRS device is less than a laboratory device [8].

Diaphragma muscle, fatter than the other 3 (10.2% of lipids *vs*. 5.9% on average for the other), records the lowest calibration performance of muscles, those of three other muscles are very close. This is explained by a lower variability of the major FA of Di muscle than the other muscles. The coefficient of variation (CV) of SFA and MUFA for Di muscle does not exceed 40% and the CV of LT, RA and In muscles for the same FA is greater than 55%. The success of the NIRS partially based on the variability present in the analyzed samples. Therefore, a narrow range of variability in baseline data could have a negative impact on NIRS prediction performance [9].

The best prediction performances are with In muscle. However, taking into account all the AG including PUFAs, and to optimize the prediction of the FA composition, it seems preferable to perform measurements for routine analysis on lean muscles as RA, especially as this muscle is easily accessible by slaughterhouse.

However, the grinding of the samples in liquid nitrogen is binding and expensive. Thus, different meat preparation gradients were tested (Table 2). Predictions on C0 and B0 samples are essentially the same and are unsatisfactory for most FA (R^2_{CV} <0.69). Instead, the grinding mode B2 and B3 show a superior prediction quality. Indeed, NIRS provides a good measure of lipids content (R^2_{CV} = 0.86 and 0.93, respectively) and total AG (R^2_{CV} = 0.82 and 0.89, respectively). The major fatty acids C16:0, C18:0, C18:1 d9 and total SFA and MUFA are well predicted (R^2_{CV}> 0.73) and are all the better for the B3 grinding. For other FA in particular for n-6 and n-3 PUFA, performances are not satisfactory for quantitative purposes and this irrespective of the grinding mode.

The sample preparation method appears to be an important factor. More the sample is homogeneous, the higher the prediction is good, in accordance with the work of [2]. However, this preparation is not the most optimal for an industrial environment. The integration of additional samples with more variability could better determine major FA by NIRS directly on the carcass or the intact muscle.

The results of these studies showed that the prediction of PUFA is not satisfactory as in most previous studies. Thus, an alternative strategy was

put in place to indirectly predict PUFA from the SFA and MUFA using multiple linear regression [10]. New prediction equations were developed on the same principle as the methodology presented by [10], but using this time values of major FA directly predicted by NIRS on samples ground in liquid nitrogen. These equations predict total PUFA, n-6 PUFA, n-3 PUFA and C18:2 n-6 with adjusted R^2 of 0.87, 0.87, 0.67 and 0.89 respectively.

IV. CONCLUSION

The portable NIRS appears to have significant potential for use to classify and orient carcasses on the basis of their FA composition. The tissue that is most appropriate for the determination of all FA including PUFAs is the RA muscle in its ground form (knife mill type) by an approach combining both NIRS and prediction models of minor FA (PUFAs) from FA predicted by NIRS. The integration of new RA muscle samples is currently underway and is expected to strengthen the current results.

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