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Potential of fluorescence spectroscopy to predict fatty acid composition of beef

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ABSTRACT

The present study aimed to evaluate and compare the ability of front face (FFFS) and synchronous fluorescence spectroscopy (SFS) to predict total fat and FA composition of beef LT muscles coming from 36 animals of 3 breeds (Angus, Limousin and Blond d'Aquitaine). The regression models were performed by using Partial Least Square (PLS) method. In spite of the low number of samples used, the results of this preliminary study demonstrated the ability of fluorescence spectroscopy to predict meat lipids. Nonetheless, the results suggested that the fluorescence spectroscopy is more suited to measure SFA ($R^2p \geq 0.66$; RPD ≥ 2.29) and MUFA ($R^2p \geq 0.48$; RPD ≥ 1.49) than PUFA ($R^2p \leq 0.48$; RPD ≤ 1.63). Moreover, R^2 and RPD factors obtained with FFFS were greater compared to the ones obtained with SFS suggesting that FFFS is more adapted to measure lipid composition of beef meat.

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1. Introduction

Fat and fatty acids (FAs) are major contributors of meat nutritional value. Indeed, numerous studies have highlighted the link between lipid intake and potential health effects in humans (Hooper, Abdelhamid, Moore, Douthwaite, & Murray, 2012; Riccardi, Giacco, & Rivellese, 2004; Wahrburg, 2004). Even if the issue remains controversial (McAfee et al., 2010), most of the dietary recommendations advise to lower the contribution of fat and saturated FA (SFA) to daily energy intakes while increasing the intake of omega-3 polyunsaturated FA (PUFA) to reduce the risk of cardiovascular disease (Gidding et al., 2005; Siri-tarino, Sun, Hu, & Krauss, 2010a, 2010b). Furthermore, some *trans*-fatty acids (C18:1 *trans*-11) present within meat were shown to have no cholesterol-raising effect (Chardigny et al., 2008). There is also emerging evidence that conjugated linoleic acid (CLA) isomer *cis*-9 *trans*-11 is associated with prevailing beneficial health effects for humans (Benjamin & Spener, 2009).

Thus, several strategies, mainly modulating genetics and dietary factors, are currently investigated to enhance meat FA composition (Scollan et al., 2014; Wood et al., 2008). To support this effort, fluorescence analysis was suggested to be a rapid and efficient tool useful in the quality estimation of meat products (Karoui & Blecker, 2010; Swatland, 1987). Front-face fluorescence spectroscopy (FFFS) notably allows the investigation of solid food samples in the wavelength range of 200–750 nm. In this case, excitation and emission spectra are acquired at fixed wavelength determined as a function of the fluorophores present in the analyzed food matrix, namely tryptophan, vitamin A, riboflavin, lipid oxidation products and NADH in meat products. In a variant acquisition mode, synchronous fluorescence spectroscopy (SFS), spectra results from simultaneously scanning both the excitation and emission monochromators keeping a fixed wavelength interval between them (Karoui & Blecker, 2010). Firstly initiated by Lloyd (1971), SFS allows the consideration of the whole fluorescence landscape meaning it retains information related to several fluorophores of the food matrix. Therefore, it could be more suitable for the analysis of complex multi-component samples as compared to conventional FFFS. Fluorescence spectroscopy has been mainly used to characterize quality traits of meat products, such as sensory properties (Dufour & Frencia, 2001; Olsen et al., 2005; Swatland, Gullett, Hore, & Buttenham, 1995). Extensive works using fluorescence spectroscopy associated with chemometrics were also carried out to authenticate and classify meat products as a function of their rearing conditions (Gatellier et al., 2007), manufacturing process, storage and cooking conditions (Gatellier, Santé-Lhoutellier, Portanguen, & Kondjoyan, 2009; Møller, Parolari, Gabba, Christensen, & Skibsted, 2003) or microbial spoilage (Aït-Kaddour, Boubellouta, & Chevallier, 2011). Quantitative evaluations of some meat components, namely collagen and heterocyclic aromatic amine contents, were developed using multivariate regression techniques in meat products (Egelandsdal, Dingstad, Tøgersen, Lundby, & Langsrud, 2005; Sahar, Portanguen, Kondjoyan, & Dufour, 2010; Wold, Kvaal, & Egelandsdal, 1999). However, very few data is currently available regarding the feasibility of using fluorescence spectroscopy to evaluate total fat content in meat. Wold and collaborators investigated the ability of FFFS

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to determine fat content in beef and exhibited fair models of cross validated prediction (RMSECV = 1.89%, R^2 = 0.71 and RMSECV = 1.10%, R^2 = 0.71, respectively) (Wold, Kvaal et al., 1999; Wold, Lundby, & Egelandsdal, 1999). Another work reported poor prediction models (R^2 = 0.57) when testing the potential of fluorescence spectroscopy to evaluate intramuscular fat content in porcine meat (Brondum et al., 2000). Surprisingly, to our knowledge, no work investigating the potential interest of fluorescence spectroscopy in assessing fatty acid composition of meat was reported so far. Yet, an advantage of fluorescence detection compared to absorption measurements (e.g., IR) is the greater sensitivity (100–1000 times) achievable, meaning that fluorescent compounds can be investigated at very low concentration levels (in the parts per billion range).

It would be therefore interesting to determine in what extent fluorescence spectroscopy would be useful to predict the specific FA composition of meat. Similar approaches were carried out using near infrared reflectance spectroscopy. Globally, this tool appeared to accurately predict the concentration of some prominent FA and FA groups such as saturated, branched and monounsaturated FA in beef, poultry and lamb meat (Guy, Prache, Thomas, Bauchart, & Andueza, 2011; Prieto et al., 2014; Sierra et al., 2008).

Thus, the purpose of this study is to evaluate and compare the ability of front face and synchronous fluorescence spectroscopy to predict total fat and FA composition of beef.

2. Materials and methods

2.1. Animals and meat samples

The muscle samples came from 36 bulls of 3 genotypes with varying lipogenesis capacities (Angus > Limousin > Blond d'Aquitaine). All the details on the experimental design and diets for the experiment were previously described by Gruffat et al. (2013). At the end of the experimental period, the bulls were slaughtered in the experimental abattoir of INRA (Saint-Genès-Champanelle, France). Samples (~100 g) of the *Longissimus thoracis* (LT) muscle of the 36 animals were collected at 24 h post mortem from the 10th thoracic rib on the right side of the ribbed carcass. Muscle samples were cut into small cubes (1 cm^3), immediately frozen in liquid nitrogen and stored at −80 °C. Just before analysis, the frozen samples of the LT muscle were ground into fine and homogeneous powders in liquid nitrogen with a mixer mill (Retch MM 301, Hann Germany).

2.2. Fatty acid analysis by gas liquid chromatography

Total lipids were extracted according to the method of Folch, Lees, and Sloane (1957) by mixing the LT muscle powder with a 2/1 chloroform/methanol mixture (vol/vol) and quantified by gravimetry. Fatty acid extraction and transmethylation into fatty acid methyl esters (FAME) were subsequently performed according to the method of Bauchart, Gladine, Gruffat, Leloutre, and Durand (2005). Fatty acid methyl ester analysis was performed with Gas Liquid Chromatography (GLC) using a Peri 2100-chromatography system (Perichrom Society, Saulx-les-Chartreux, France) fitted with a CP-Sil 88 glass capillary column (Varian, Palo Alto, CA; length = 100 m; diam. = 0.25 mm). The carrier gas was H_2 and the oven and flame ionization detector temperatures described by Scislowski, Durand, Gruffat, and Bauchart (2004) were used. Total FAs were quantified using C19:0 as an internal standard. The identification of each individual FAME and the calculation of the response coefficients for each individual FAME were performed using the quantitative mix C4-C24 FAME (Supelco, Bellafonte, PA).

2.3. Fluorescence spectroscopy

Samples fluorescence came from the same powder as that used for fatty acid analysis (i.e. LT muscle collected at 24 h post mortem, immediately frozen in liquid nitrogen and stored at −80 °C). Frozen samples were ground into fine and homogeneous powders in liquid nitrogen and stored at −80 °C until analyses. Before acquisitions, the homogeneous powder of ground LT muscles was thawed during 1 h00 at 20 °C. Then a proportion 3 g of meat powder was placed between a powder sample holder and a quartz cell and mounted in a solid sample holder. Before fluorescence acquisition the sample was visually controlled to ensure that all the measurement window was totally covered with meat sample. Fluorescence spectra were recorded in two excitation modes successively on the same sample, classical excitation mode (FFFS: Front Face Fluorescence Spectra) and synchronous excitation mode (SFS: Synchronous Fluorescence Spectra) by using a FluoroMax-4 spectrofluorometer (Jobin–Yvon, Longjumeau, France) equipped with a solid sample holder with an incidence angle of the excitation radiation set at 60° in order to minimize reflected light, scattered radiation and depolarization phenomena. For SFS, the excitation wavelength (λ_{ex}) and emission wavelength (λ_{em}) are scanned simultaneously (synchronously), usually maintaining a constant wavelength interval, named offset or $\Delta\lambda$, between λ_{ex} and λ_{em}. In this study, six $\Delta\lambda$ (20, 40, 60, 80, 100, and 120 nm) were used. All the excitation fluorescence spectra were recorded between 250 and 550 nm.

For FFFS, the emission spectra were recorded at 305–400, 340–540, 360–570, 400–650 and 410–700 nm after excitation at 290, 322, 335, 350 and 382 nm respectively. Those excitation spectra mainly addressed the fluorescence of tryptophan, collagen/pyridinolin/riboflavin, NADH, vitamin A, and vitamin E, respectively. The two first fluorophores were chosen because they are clearly liposoluble while the other fluorophores were chosen because their fluorescence properties can be influenced by their environment (Mazerolles, Devaux, Dufour, Qannari, & Courcoux, 2002). SFS and FFFS were recorded in duplicates for each sample giving a total of 792 spectra recorded.

2.4. Data pre-processing

The proper choice of pre-processing is difficult to assess prior to model validation but pre-processing should maintain or decrease the effective model complexity. So, in order to improve the calibration models different pre-processing methods were investigated. Firstly, noise of the spectral datasets was reduced by using the Savitzky–Golay method with a polynomial order and a filter width of 2 and 12 respectively. Secondly, the scattering effects were minimized by using Standard Normal Variate (SNV), Multiple Scattering Correction (MSC) or by area normalization (reducing the area under each spectrum to a value of 1: AREA). These pre-processing techniques are desired to reduce the (physical) variability between samples due to scatter. Data preprocessing were performed by using the PLS-Toolbox v.7.5 (Eigenvector Research) for MATLAB R2013b.

2.5. Partial least square regression

Partial Least Square Regression (PLS-R) models were validated by independent datasets. The initial data sets were divided into two sets, 70% of the samples were used for the calibration and, 30% for the validation by using the Nearest Neighbor Thinning method proposed in the PLS-Toolbox v.7.5 (Eigenvector Research) that permits to select validation samples which best fill out all covariance space. The models performance can be evaluated by different factors. Williams (2003) defines 7 levels of model accuracy based on the R^2 values obtained for prediction. Nonetheless, considering the R^2 can conduct to over estimation of the model accuracy. In order to have a clear idea of model performance other statistics such as the Ratio of Performance to Deviation (RPD = SD/RMSEP) factor was considered. Five levels of model accuracy based on the RPD values obtained for prediction were considered (Table 1).

PLS-R analyses were performed by using the PLS-Toolbox v.7.5 (Eigenvector Research) for MATLAB R2013b.

Table 1
Interpretation of RPD (Ratio of Performance to Deviation) value (Williams, 2003).

RPD value	Classification	Application
0.00–1.50	Not recommended	No application
1.50 and 2.00	Distinguish between high and low values	Rough screening
2.00 and 2.50	Approximate quantitative prediction	Quality control
2.50 and 3.00	Good	Process control
>3.0	Excellent	Any application

3. Results

3.1. Fatty acid composition measured using the GLC reference method

The means, ranges, SD (standard deviation) and CV (coefficient of variation) of LT intramuscular lipid and FA concentrations are presented in Table 2. Mean SFA and MUFA were the most abundant FA groups in LT muscle and their contents were similar (600.05 mg/100 g and 571.25 mg/100 g respectively). On the other hand, mean PUFA contents were 3 times lower (202.95 mg/100 g). Palmitic (16:0) and oleic (18:1 Δ9 *cis*) acids were on average the most abundant individual FAs (299.68 mg/100 g of fresh muscle, ranging from 50.64 to 1215.19 mg/100 g and 388.47 mg/100 g of fresh muscle, ranging from 41.26 to 1512.13 mg/100 g, respectively). Stearic acid (18:0) averaged 216.1 mg/100 g, ranging from 41.26 to 631.1 mg/100 g. Among PUFA, n-6 PUFA were the most represented in LT muscle (159.95 mg/100 g) with linoleic acid (18:2 n-6) as major FA (101.84 mg/100 g). In contrast, n-3 PUFA contents were low (35.2 mg/100 g), mainly represented by linolenic acid (18:3 n-3, 20.6 mg/100 g). All total PUFAs categories (except *trans* PUFA n-6 and total CLA) and individual 20:3 n-3, 20:4 n-3 and 22:6 n-3 had a lower CV (under 40%) than individual and total SFAs and MUFAs.

Table 2
Total lipid and total fatty acid (g/100 g fresh meat) contents and fatty acid composition (mg/100 g fresh meat) of LT muscle samples.

Component	Mean	Min	Max	SD	CV
Total lipids	2.07	0.78	6.06	1.22	59%
Total FA	1.39	0.36	4.35	0.98	71%
Total FA/total lipids	0.63	0.38	0.90	0.11	17%
Fatty acid families					
Total SFA	600.05	106.53	2121.08	471.60	79%
Total linear SFA	578.06	100.15	2067.75	457.83	79%
Total non-linear SFA	21.99	6.37	58.48	14.18	64%
Total MUFA	571.25	96.20	1975.96	466.32	82%
Total cis MUFA	483.09	77.27	1796.27	406.62	84%
Total trans MUFA	88.16	15.33	326.59	70.39	80%
Total PUFA	202.95	128.38	358.24	49.27	24%
Total n-3 PUFA	35.20	17.88	65.45	9.94	28%
Total n-3 LC PUFA	14.60	7.41	26.31	3.53	24%
Total n-6 PUFA	159.95	99.86	274.96	37.91	24%
Total n-6 cis PUFA	135.01	80.38	230.58	28.41	21%
Total n-6 trans PUFA	24.94	2.89	94.58	21.00	84%
Total n-6 LC PUFA	30.74	17.09	57.62	8.05	26%
Total CLA	6.64	0.51	23.03	5.84	88%
Individual fatty acids					
14:0	30.26	2.38	114.69	29.88	99%
16:0	299.68	50.64	1215.99	256.94	86%
18:0	216.10	41.26	631.10	149.60	69%
18:1 Δ 9 cis	388.47	41.72	1512.13	343.22	88%
18:1 Δ 9 trans	5.43	0.57	22.29	4.63	85%
18:1 Δ 10–11 trans	66.37	8.85	268.60	55.75	84%
18:2 n-6 cis cis	101.84	61.97	165.82	21.25	21%
18:3 n-3	20.60	6.31	46.39	7.80	38%
20:3 n-3	0.14	0.01	1.19	0.26	186%
20:4 n-3	0.80	0.01	2.75	0.62	78%
20:4 n-6	21.73	11.92	39.31	5.52	25%
20:5 n-3	3.90	2.00	6.10	1.03	26%
22:5 n-3	9.26	5.15	16.47	2.23	24%
22:6 n-3	0.49	0.01	1.67	0.45	92%

SFA, saturated FA; Total SFA: linear + non-linear SFA; Linear SFA: 12:0 up to 24:0; Non-linear SFA: iso [14 up to 18] + anteiso [15 + 17], FA mainly synthesized by rumen microorganisms; MUFA, monounsaturated FA; Total MUFA: *cis* + *trans* MUFA; Total cis MUFA: 14:1 Δ9*cis* + 15:1 Δ9*cis* + 16:1 Δ9*cis* + 17:1 Δ8 and Δ9*cis* + 18:1 Δ6*cis* up to Δ15*cis* + 20:1 Δ9*cis* + 22:1 Δ9cis; Total trans MUFA: 16:1 Δ9trans + 18:1 Δ6*trans* up to Δ16*trans*; PUFA, polyunsaturated FA; Total PUFA: n-6 PUFA + n-3PUFA + CLA; Total n-3 PUFA: 18:3 n-3 + 20:3 n-3 + 20:4 n- 3 + 20:5 n-3 + 22:3 n-3 + 22:4 n-3 + 22:5 n-3 + 22:6 n-3; Total n-6 PUFA: 18:2 n-6 + 18:3 n-6 + 20:2 n-6 + 20:3 n-6 + 20:4 n-6 + 22:2 n-6 + 22:4 n-6 + 22:5 n-6; LC, long chain; CLA, conjugated linoleic acid; Total CLA: 9*cis*,11*trans*-CLA + 11*cis*,13*trans*-CLA + total *cis*,*cis* CLA + total *trans*,*trans* CLA.
[a]Abbreviations: SD: standard deviation; Min: minimum; Max: maximum.

3.2. Description of fluorescence spectra of meat samples

Average spectra recorded in the two modes (FFFS and SFS) are presented in Figs. 1 and 2. The Fig. 1 presented fluorescence emission spectra obtained at 5 excitation wavelengths 290, 322, 335, 350, and 382 nm.

The fluorescence spectra obtained after excitation at 290 nm exhibited a peak at 334 nm. Emission fluorescence spectra obtained after excitation at 322 nm presented peaks at 353, 379, 527 nm and a large band from 418 to 524 nm with a maximum at 466 nm. The emission spectra obtained after excitation at 335 nm presented different bands, a high one at 369 nm, a large one between 420 and 543 nm with a maximum at 458 nm and small ones at 378 and 551 nm. Excitation of meat samples at 350 nm resulted in an emission fluorescence spectrum presenting a broad band between 420 and 543 nm with a maximum at 466 nm and two small peaks at 573 and 596 nm. Finally, the fluorescence spectra obtained after excitation at 382 nm exhibited peaks at 419, 596, 628 nm and a broad band between 428 and 546 nm with a maximum at 468 nm.

Fig. 2 presented average front face synchronous spectra recorded at the different Δλ (20, 40, 60, 80, 100 and 120 nm). The spectra recorded with an offset of 20 nm presented one prominent peak at 327 nm (emission 347 nm) and smaller one between 449 and 525 nm (emission between 469 and 545 nm) with a maximum at 491 nm (511 nm). This band shifted to lower wavelength 294, and 293 nm when the spectra are recorded respectively with offsets 80 and 60 nm. The synchronous spectra of 100 nm offset presents one large band cantered at 279 nm. The offset of 120 nm permits to obtain spectra with bands at 271 nm (emission 391 nm), 325 (emission 445 nm) and 356 nm (emission 476 nm). Here, the band around 290 nm, which was prominent with the previous offsets (20 to 80 nm), disappears. The band located between 450 and 525 nm seems to disappear when the offset value increases.

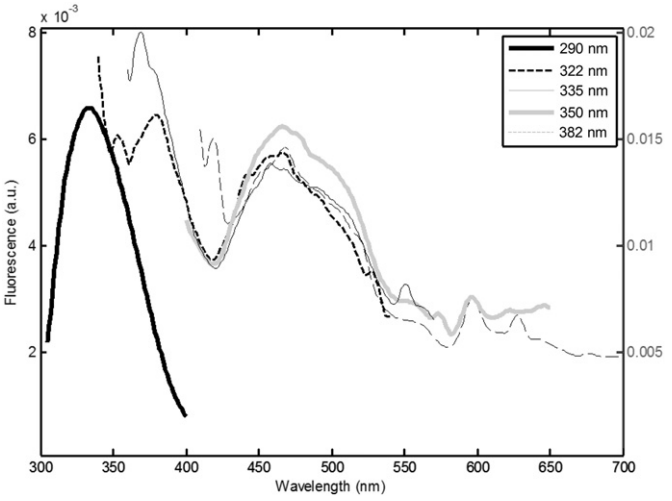

Fig. 1. Average Front Face Fluorescence emission spectra recorded after excitation at 290, 322, 335, 350, and 382 nm on meat samples (a.u. arbitrary unit).

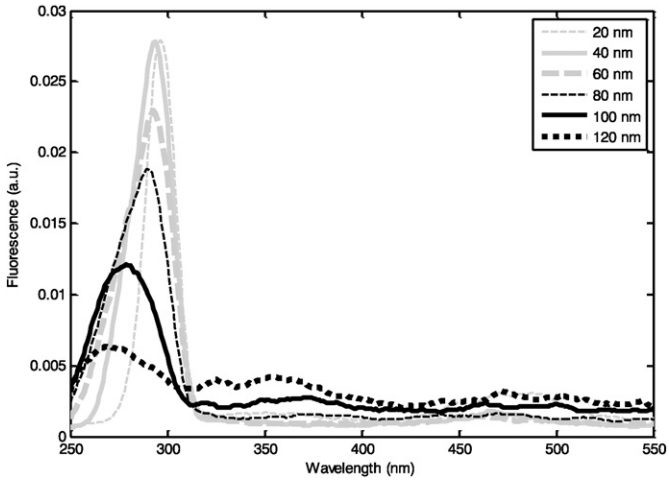

Fig. 2. Average Synchronous Fluorescence spectra recorded at $\Delta\lambda = 60$, 80 and 120 nm on meat samples (a.u. arbitrary unit).

3.3. Prediction of lipid contents with FFFS

The PLS regression statistics corresponding to the selected equations of the best models developed from FFFS are summarized in Table 3. With FFFS, the best predictive models were obtained for total lipid content (RPD = 2.67), total FA (RPD = 2.61), SFA (RPD = 2.65), *linear* SFA (RPD = 2.65), MUFA (RPD = 2.62), *cis* MUFA (RPD = 2.77) and for individual unsaturated and mono-unsaturated FA, C14:0 (RPD = 2.88), C16:0 (RPD = 2.61), C18:0 (RPD = 2.50), Δ9 *cis* C18:1 (RPD = 2.80), and Δ9 *trans* C18:1 (RPD = 2.51). All these models were obtained with the 322 nm excitation band after applying the normalization preprocessing method except for the C14:0 and C16:0 models that were obtained without preprocessing (i.e. RAW).

Seven other models, that can be used to distinguish between high and low values and applied for rough screening (Williams, 2003), were identified for the total *trans* MUFA (λex: 335 nm, RPD = 1.54), PUFA (λex: 350 nm, RPD = 1.62), n-3 PUFA (λex: 322 nm, RPD = 1.54), LC n-3 PUFA (λex: 322 nm, RPD = 1.57), n-6 *trans* PUFA (λex: 322 nm, RPD = 1.50), and CLA (λex: 350 nm, RPD = 1.53). These models were calculated after spectral data normalization except for the sum of n-3 PUFA model that was calculated after applying MSC preprocessing to spectra. For the other components, the model accuracies were poor and cannot be used for prediction ($0.89 < RPD < 1.49$; $R^2_p < 0.35$).

3.4. Prediction of lipid contents with SFS

The PLS regression statistics of models developed with SFS are summarized in Table 4. Considering SFS, the best predictive models

Table 3
Performance of the best PLS regression models to predict lipids and fatty acid contents of minced beef meat obtained with classical Front Face Fluorescence excitation method[a].

Component	λex (nm)	PPS	LV	R^2_C	R^2_{CV}	R^2_P	RMSEC	RMSEP	RPD_p
Total lipids	322	AREA	5	0.67	0.60	0.68	0.78	0.46	2.67
Total FA	322	AREA	6	0.72	0.63	0.68	0.58	0.37	2.61
Total FA/total lipids	322	AREA	5	0.44	0.17	0.42	0.09	0.08	1.42
Fatty acid families									
Total SFA	322	AREA	5	0.69	0.63	0.66	291.72	178.19	2.65
Total linear SFA	322	AREA	5	0.69	0.62	0.66	283.93	172.57	2.65
Total non-linear SFA	322	AREA	8	0.75	0.61	0.68	7.76	6.04	2.35
Total MUFA	322	AREA	6	0.71	0.61	0.68	279.80	177.76	2.62
Total cis MUFA	322	AREA	5	0.69	0.61	0.67	256.07	146.68	2.77
Total trans MUFA	335	AREA	9	0.65	0.43	0.51	41.78	46.07	1.54
Total PUFA	350	AREA	7	0.40	0.06	0.45	40.33	32.37	1.62
Total n-3 PUFA	322	MSC	2	0.13	0.04	0.23	9.90	6.95	1.54
Total n-3 LC PUFA	322	AREA	3	0.14	0.01	0.18	3.41	2.34	1.57
Total n-6 PUFA	350	MSC	7	0.47	0.07	0.44	29.04	24.61	1.63
Total n-6 cis PUFA	335	SNV	1	0.00	0.01	0.06	31.44	24.21	1.22
Total n-6 trans PUFA	322	AREA	6	0.46	0.34	0.33	16.22	14.80	1.50
Total n-6 LC PUFA	335	SNV	5	0.05	0.05	0.14	8.27	6.21	1.37
Total CLA	350	AREA	10	0.79	0.44	0.58	2.75	3.90	1.53
Individual fatty acids									
14:0	322	RAW	7	0.77	0.66	0.75	15.27	11.23	2.88
16:0	322	RAW	10	0.83	0.71	0.65	119.98	98.54	2.61
18:0	322	AREA	5	0.73	0.67	0.66	85.50	59.91	2.50
18:1 Δ 9 cis	322	AREA	5	0.69	0.61	0.68	214.88	122.79	2.80
18:1 Δ 9 trans	322	AREA	6	0.64	0.50	0.63	3.12	1.85	2.51
18:1 Δ 10–11 trans	335	AREA	9	0.60	0.35	0.48	34.813	37.244	1.49
18:2 n-6 cis cis	350	SNV	1	0.01	0.06	0.09	23.34	18.11	1.26
18:3 n-3	322	MSC	2	0.13	0.05	0.17	7.67	6.05	1.37
20:3 n-3	290	MSC	10	0.15	0.00	0.33	0.43	0.36	0.89
20:4 n-3	322	AREA	6	0.12	0.00	0.16	0.62	0.48	1.39
20:4 n-6	290	AREA	3	0.05	0.00	0.00	5.65	4.02	1.45
20:5 n-3	322	MSC	2	0.07	0.00	0.10	1.02	0.88	1.21
22:5 n-3	322	AREA	4	0.21	0.05	0.15	2.00	1.65	1.37
22:6 n-3	290	MSC	3	0.09	0.04	0.28	0.43	0.37	1.24

SFA, saturated FA; Total SFA: linear + non-linear SFA; Linear SFA: 12:0 up to 24:0; Non-linear SFA: iso [14 up to 18] + anteiso [15 + 17]; MUFA, monounsaturated FA; Total MUFA: *cis* + *trans* MUFA; Total *cis* MUFA: 14:1 Δ9cis + 15:1 Δ9*cis* + 16:1 Δ9*cis* + 17:1 Δ8 and Δ9*cis* + 18:1 Δ6*cis* up to Δ15*cis* + 20:1 Δ9*cis* + 22:1 Δ9cis; Total *trans* MUFA: 16:1 Δ9*trans* + 18:1Δ6*trans* up to Δ16*trans*; PUFA, polyunsaturated FA; Total PUFA: n-6 PUFA + n-3PUFA + CLA; Total n-3 PUFA: 18:3 n-3 + 20:3 n-3 + 20:4 n-3 + 20:5 n-3 + 22:3 n-3 + 22:4 n-3 + 22:5 n-3 + 22:6 n-3; Total n-6 PUFA: 18:2 n-6 + 18:3 n-6 + 20:2 n-6 + 20:3 n-6 + 20:4 n-6 + 22:2 n-6 + 22:4 n-6 + 22:5 n-6; LC, long chain; CLA, conjugated linoleic acid; Total CLA: 9cis,11*trans*-CLA + 11cis,13*trans*-CLA + Total *cis*,*cis* CLA + Total *trans*,*trans* CLA.

[a] Abbreviations: λex: excitation wavelength; PPS: preprocess; RAW: without preprocessing; SNV: Standard Normal Variate; AREA: normalization; LV: loading vector; MSC: Multiple Scattering Correction; R^2_C: coefficient of determination for calibration; R^2_p: coefficient of determination for prediction; RMSEC: Root mean square error of calibration; RMSEP: Root mean square error of prediction; RPDp: Ratio of Performance to Deviation for prediction.

were obtained for the total lipid content (RPD = 2.76), MUFA (RPD = 2.55), *cis* MUFA (RPD = 2.77), C14:0 (RPD = 2.76), Δ9 *cis* C18:1, (RPD = 2.63), and Δ9 *trans* C18:1 (RPD = 2.68). Based on the RPD and R^2_p, those models can be used for process control (Williams, 2003). The different models were calculated with an offset value of 60 nm (maximum emission 353 nm) except for the PLS-R model of Δ9 *trans* C18:1 obtained with an offset value of 120 nm (maximum emission 391 nm). All these predictive models were obtained with the data corrected by SNV preprocessing except for the predictive model of the C14:0 that was calculated after correcting spectra with the AREA preprocessing method.

The regression models of total amount of FA (RPD = 2.46), SFA (RPD = 2.40), *linear* SFA (RPD = 2.39), CLA (RPD = 2.25), C16:0 (RPD = 2.29), C18:0 (RPD = 2.39) can be used for approximate quantitative prediction. Those models were obtained with an offset of 120 nm except for total amount of FA and C16:0 calculated with two offsets 60 and 80 nm respectively. These models were obtained after applying the SNV preprocessing method to spectral data except for total FA and CLA that were calculated after performing the normalization preprocessing method.

Seven other models, that can be used to distinguish between high and low values and applied for rough screening (Williams, 2003), were identified for the sum of MUFA *trans* (RPD = 1.95), PUFA (RPD = 1.76), n-3 PUFA (RPD = 1.85), n-6 *trans* PUFA (RPD = 1.82), Δ10–11 *trans* C18:1 (RPD = 1.71), n-3 C18:3 (RPD = 1.83), and n-3 C20:4 (RPD = 1.64). These different predictive models were calculated with the normalization preprocessing method except for the sum of n-3 PUFA predictive model that was obtained after applying the MSC method to spectral data.

The accuracy of the other predictive models was low based on their RPD values ($1.06 < RPD < 1.47$ and $R^2_p < 0.58$).

Table 4
Performance of the best PLS regression models to predict lipids and fatty acid contents of minced beef obtained with Synchronous Fluorescence excitation method[a].

Component	Δλ (nm)	PPS	LV	R^2_C	R^2_P	RMSEC	RMSEP	RPD_p
Total lipids	60	AREA	8	0.87	0.77	0.46	0.46	2.76
Total FA	60	AREA	8	0.87	0.80	0.35	0.40	2.46
Total FA/total lipids	80	AREA	4	0.26	0.58	0.10	0.08	1.47
Fatty acid families								
Total SFA	120	SNV	7	0.93	0.80	122.32	195.24	2.40
Total linear SFA	120	SNV	7	0.93	0.80	118.82	189.68	2.39
Total non-linear SFA	60	AREA	7	0.84	0.76	5.80	6.09	2.37
Total MUFA	60	SNV	7	0.86	0.79	175.83	182.27	2.55
Total cis MUFA	60	SNV	7	0.87	0.81	145.76	153.48	2.62
Total trans MUFA	60	AREA	5	0.52	0.54	51.39	38.17	1.95
Total PUFA	120	AREA	4	0.48	0.48	36.93	29.36	1.76
Total n-3 PUFA	40	MSC	6	0.46	0.37	7.80	5.78	1.85
Total n-3 LC PUFA	60	SNV	3	0.25	0.03	3.29	2.88	1.32
Total n-6 PUFA	120	AREA	4	0.44	0.39	29.12	26.45	1.47
Total n-6 cis PUFA	20	MSC	5	0.17	0.13	25.33	24.78	1.17
Total n-6 trans PUFA	60	AREA	6	0.56	0.43	15.08	12.51	1.82
Total n-6 LC PUFA	40	AREA	10	0.25	0.16	6.65	8.02	1.07
Total CLA	120	AREA	5	0.72	0.71	3.01	2.52	2.25
Individual fatty acids								
14:0	60	AREA	8	0.86	0.83	11.38	10.95	2.76
16:0	80	SNV	8	0.92	0.77	73.36	115.14	2.29
18:0	120	SNV	5	0.81	0.78	64.60	62.16	2.39
18:1 Δ 9 cis	60	SNV	7	0.87	0.81	121.59	128.85	2.63
18:1 Δ 9 trans	120	SNV	4	0.70	0.74	2.37	1.63	2.68
18:1 Δ 10–11 trans	20	AREA	7	0.69	0.50	30.75	32.46	1.71
18:2 n-6 cis cis	20	MSC	5	0.16	0.09	19.33	18.50	1.20
18:3 n-3	40	AREA	6	0.38	0.39	6.59	4.61	1.83
20:3 n-3	80	MSC	10	0.15	0.00	0.24	0.26	1.09
20:4 n-3	60	AREA	2	0.08	0.11	0.66	0.42	1.64
20:4 n-6	100	AREA	10	0.29	0.05	4.48	5.89	1.06
20:5 n-3	40	SNV	5	0.29	0.11	0.92	0.76	1.44
22:5 n-3	120	AREA	4	0.33	0.16	1.86	1.76	1.30
22:6 n-3	120	RAW	6	0.51	0.34	0.32	0.32	1.41

SFA, saturated FA; Total SFA: linear + non-linear SFA; Linear SFA: 12:0 up to 24:0; Non-linear SFA: iso [14 up to 18] + anteiso [15 + 17]; MUFA, monounsaturated FA; Total MUFA: cis + trans MUFA; Total cis MUFA: 14:1 Δ9cis + 15:1 Δ9cis + 16:1 Δ9cis + 17:1 Δ8 and Δ9cis + 18:1 Δ6cis up to Δ15cis + 20:1 Δ9cis + 22:1 Δ9cis; Total trans MUFA: 16:1 Δ9trans + 18:1Δ6transup to Δ16trans; PUFA, polyunsaturated FA; Total PUFA: n-6 PUFA + n-3PUFA + CLA; Total n-3 PUFA: 18:3 n-3 + 20:3 n-3 + 20:4 n- 3 + 20:5 n-3 + 22:3 n-3 + 22:4 n-3 + 22:5 n-3 + 22:6 n-3; Total n-6 PUFA: 18:2 n-6 + 18:3 n-6 + 20:2 n-6 + 20:3 n-6 + 20:4 n-6 + 22:2 n-6 + 22:4 n-6 + 22:5 n-6; LC, long chain; CLA, conjugated linoleic acid; Total CLA: 9cis,11trans-CLA + 11cis,13trans-CLA + Total cis,cis CLA + Total trans,trans CLA.

[a] Abbreviations: Δλ: offset in nm; PPS: preprocess; RAW: without preprocessing; SNV: Standard Normal Variate; AREA: normalization; LV: loading vector; MSC: Multiple Scattering Correction; R^2_C: coefficient of determination for calibration; R^2_P: coefficient of determination for prediction; RMSEC: root mean square error of calibration; RMSEP: root mean square error of prediction; RPDp: residual predictive deviation for prediction.

4. Discussion

4.1. Total lipids and fatty acids prediction in meat

A few studies have attempted to confirm the ability of fluorescence spectroscopy to predict total lipids of meat (Brondum et al., 2000; Wold, Kvaal et al., 1999; Wold, Lundby et al., 1999). Wold, Lundby et al. (1999) reported a R^2 of 0.71 and a RPD of 1.86 for intact beef *Longissimus dorsi* muscle and Brondum et al. (2000) found lower R^2 (0.57) and RPD (1.52) on pork intact muscles. The higher predictive accuracy of the models reported in the present work (FFFS: $R^2 = 0.68$ and RPD = 2.61; SFS: $R^2 = 0.80$, RPD = 2.46) are probably associated with two factors: (i) a higher variability in the data base: increasing variability by the use of several breeds fed, several diets, and muscle type in sampling had a positive effect on beef FA prediction (Brondum et al., 2000; Mourot et al., 2015); (ii) the preparation of the samples: in this study, meat samples were transformed into fine powder under liquid nitrogen whereas in other studies, samples were intact. The fluorescence measurement can be considered as a surface measurement and the grinding of the sample can permit to record a fluorescence signal that is more representative of the sample. NIR spectroscopy is undoubtedly the most used method for predicting total lipids in beef, some studies after cross-validation reported a R^2_{cv} and RPD higher than 0.9 and 3.5 respectively (De Marchi, Berzaghi, Boukha,Mirisola, & Gallo, 2007; Mourot et al., 2015; Tøgersen, Arnesen, Nilsen, & Hildrum, 2003) suggesting that this spectral method presented probably a higher accuracy to predict total lipids in beef.

As far as we know, this study brings new insight regarding the ability of FFFS to predict FA composition of beef because no study reported equivalent investigation. According to Williams (2003), the regression models calculated for total SFA, linear SFA, MUFA, *cis* MUFA and for some individual unsaturated and mono-unsaturated FA (C14:0, C16:0, C18:0, Δ9 *cis* C18:1, and Δ9 *trans* C18:1) could be used for process control ($R^2_p \geq 0.63$ and $RPD_p \geq 2.50$) and the one obtained for nonlinear SFA would be suited for approximate quantitative prediction.

The poor predictive models calculated for the other FAs (i.e. total or individual MUFA and PUFA) except the oleic acid Δ9 *cis* C18:1, could be attributed either to a narrow variation range of the reference values (giving small CV). Nonetheless, this hypothesis was not verified for total CLA, total *trans* MUFA, 18:1 Δ 10-11 *trans*, 20:3 n-3, 20:4 n-3, and 22:6 n-3 that presented high CV and low RPD values. We suggested that those components are not well predicted due both to a large RMSEP compared to SD of the reference values (Prevolnik, Skrlep, Skorjanc, & Candek-Potokar, 2010) and to their lower concentration in meat (Tables 2 and 3). Indeed, the concentration of MUFA and PUFA are lower compared to the other FA except for the oleic acid. Moreover, we assumed the presence of a different minimum threshold of detection between the SFA and MUFA. Indeed, the total MUFA and cis MUFA are correctly predicted compared to individual MUFA except for oleic acid which is the most abundant FA.

The best predictive models discussed above were obtained by using three excitation wavelengths (322, 335 or 350 nm) that were previously

reported as being sensitive to meat fat. The 322 nm was assigned to vitamin A (Skjervold et al., 2003). The 335 nm excitation wavelength was used to predict intramuscular fat content on beef (Wold, Kvaal et al., 1999; Wold, Lundby et al., 1999). Nonetheless, this excitation band was also reported as sensitive to NADH (Karoui, Thomas, & Dufour, 2006). Even if its concentration is small in meat sample (Chan et al., 1996; Gatellier, Hamelin, Durand, & Renerre, 2001), we considered the 350 nm excitation wavelength associated to the fluorescence of vitamin E. Nonetheless, some inconsistencies concerning the assignment of this band to vitamin E have to be pointed out in such a complex product (Sikorska, Khmelinskii, & Sikorski, 2011).

No interesting models were obtained with the 290 and 382 nm excitation bands probably due to their low relation with meat fat. Indeed, the 290 nm band was mainly assigned to tryptophan residues in proteins and usually applied as a fingerprint to discriminate muscles (Dufour & Frencia, 2001; Sahar, Boubellouta, Portanguen, Kondjoyan, & Dufour, 2009), to predict microbial spoilage of meat (Aït-Kaddour et al., 2011) and chicken breast filet (Sahar, Boubellouta, & Dufour, 2011). For the 382 nm band, Wold, Kvaal et al. (1999), Egelandsdal, Wold, Sponnich, Neegård, and Hildrum (2002) and Skjervold et al. (2003) have shown that this excitation wavelength is more adapted to extract information related to connective tissue. This was confirmed by Sahar et al. (2009), nonetheless they reported that riboflavin could also be excited.

The SFS mode used in this study demonstrates that spectra recorded on meat samples exhibit peaks corresponding to different fluorophores as reported by Sahar et al. (2009). The results demonstrated that the influence of $\Delta\lambda$ can be substantial on the shape, location, and signal intensity of the fluorescence peaks. Indeed, depending of the $\Delta\lambda$ value, synchronous fluorescence spectra are substantially modified. As previously noted (Boubellouta & Dufour, 2008), SFS makes it possible to narrow the spectral bands and, compared to emission or excitation spectra, to have information on the fluorescence properties of several intrinsic fluorophores on a given spectrum. This suggests the potential of synchronous fluorescence spectra to be suited for the prediction of different lipid components in beef.

As far as we know, this study brings also new insight regarding the ability of SFS to predict total FA and other lipids of beef because no study reported equivalent investigation. As discussed for FFFS, the regression models calculated for total lipids, MUFA, cis MUFA and individual unsaturated and mono-unsaturated FA (C14:0, C18:1, and Δ9 trans C18:1) could be used for process control and the one obtained for total FA, linear SFA, C16:0, and C18:0 would be suited for approximate quantitative prediction (Williams, 2003). The lower accuracy of predictive models calculated for the other FAs can be attributed, as for FFFS, either to a narrow variation range of the reference values, to large RMSEP compared to SD of the reference values and to their lower concentration in meat. Moreover, a higher ability of SFS to predict SFA can be noted compared to FFFS.

In the present study, three offsets can be identified as the most suited for meat lipid prediction, 60, 80, and 120 nm. The synchronous fluorescence excitation spectra obtained with those three offsets presented different peaks that can be related to specific fluorophores. The spectral band identified around 296 nm seems to originate from the fluorescence of the protein tryptophan residues (Boubellouta & Dufour, 2008; Sahar et al., 2009). While, the assignment of the band, between 450 and 525 nm, to a specific fluorophore remains unclear. For example, it was previously assigned to riboflavin and to Maillard reaction products (Sahar et al., 2009; Yaacoub et al., 2009).

As previously mentioned, no studies have investigated the potential of fluorescence spectroscopy to predict individual or total FA composition of ground LT muscles. Nonetheless, few studies have used NIR spectroscopy to predict those components in ground LT muscles (Cecchinato et al., 2012; Mourot et al., 2015; Sierra et al., 2008). The models reported in those studies for 14:0, 16:0, 18:0, 18:1 Δ9, 20:3 n-3, 22:6 n-3 were more accurate than those reported in the present study. Nonetheless, the models for 18:1 Δ9 *trans*, 20:4 n-6, 20:5 n-3, and 22:5 n-3 presented higher RPD values whatever the fluorescence spectroscopy method (SFS or FFFS) compared to the NIR spectroscopy (0.40 in average).

For total SFA, linear SFS, MUFA, *cis* MUFA higher predictive statistics were reported for NIR spectroscopy. In the study of Mourot et al. (2015), that reported the best statistics for predictive models, the RPD_{cv} was improved by an average value of 0.98. Total PUFA, n-3 PUFA, n-3 LC PUFA, n-6 PUFA, n-6 *trans* PUFA, n-6 LC PUFA were more accurately predicted by fluorescence spectroscopy (SFF and FFFS) compared to NIR spectroscopy (the RPD is improved in our study by a value of 0.32). For total *trans* MUFA, total CLA, 22:6 n-3, and 18:3 n-3 higher RPD value (+ 0.12 in average) was obtained only by SFS excitation method compared to NIR spectroscopy. Those observations could suggest that fluorescence spectroscopy is more suited for predicting the PUFA than NIR spectroscopy. Nonetheless, it seems whatever the method used that the major FAs (representing over 1% of the total FAs identified such as 14:0, 16:0, 18:0, 18:1 Δ9,…) were more accurately predicted than minor FAs (Mourot et al., 2015). Also, total and many individual SFAs and MUFAs were better predicted than total and individual PUFAs.

The best regression models for total fat, lipids and each FAs were obtained using different mathematical preprocessing. In the present study, nearly all-preprocessed models are simpler or more parsimonious (i.e. use fewer PLS factors) than the non-preprocessed models (i.e. RAW models), independent of the fluorescence spectra used, FFFS or SFS. The best preprocessing method differs between components considered suggesting that it is impossible to know beforehand which preprocessing would lead to the most accurate model previously reported (Fernández-Cabanás, Garrido-Varo, Olmo, Pedro, & Dardenne, 2007). Nonetheless, it is interesting to note both that the models created from normalized (i.e. AREA) spectra are generally the simplest models and the best preprocessing method. This is more obvious for the models calculated with FFFS that gave 20 components among 33 compared to the models calculated with SFS that presented 16 components among 33 presenting the normalization as the best preprocessing method (Tables 3 and 4). Those results suggested that the normalization approach appears as a good preprocessing strategy for the prediction of fat and fatty acid contents from fluorescence spectra. This study shows, and recalled, that a normalization is not at all a neutral operation and has to be carefully chosen.

4.2. Comparison of the ability of FFFS and SFS to predict fatty acid composition

The comparison between the two fluorescence excitation modes was conducted whenever a predictive model was usable for process control (RPD ≥ 2.50). Compared to the SFS, the models obtained with FFFS presented generally lower values of R^2_p, loading vectors, RMSEP and higher values of RPD factors. The b-coefficient obtained with the SFS presented a noisy shape (results not shown) indicating an over fitting of models calculated. This implies that the models obtained with FFFS are generally more accurate and simplest or more parsimonious than the models obtained with SFS data. Considering that meat is a complex and anisotropic matrix presenting different physicochemical properties, the use of only some excitation or emission wavelengths for the excitation of its intrinsic fluorophores would have limited the ability of the FFFS to predict efficiently some lipids in meat compared to the SFS excitation mode. Nonetheless, the present study clearly shows that the FFFS is the best method for prediction of fat and fatty acid contents in meat.

Those results can be assigned to the fact that a synchronous fluorescence spectrum exhibits peaks corresponding to several different fluorophores. These compounds are present at the sample surface resulting in an increase of spectral loss and interferences. This induces instability in models compared to the FFFS that allows exciting a

fluorophore more specifically. This observation is in agreement with the observation of Patra and Mishra (2002) who reported that in spite of the sensitivity, selectivity, for multi-component analysis, SFS fails for very complex mixtures.

5. Conclusion

This study can be considered as a first step to demonstrate the ability of fluorescence spectroscopy to predict FA contents of the LT muscle coming from three breeds of cattles. This method globally appears as a promising tool to support the current effort of research carried out to improve the nutritional quality of meat products. The results suggested that the fluorescence spectroscopy is more suited to measure SFA and MUFA because this method failed when the prediction of PUFA was investigated. It seems that a higher minimum threshold was necessary to predict correctly the PUFA compared to the SFA. Indeed, the total MUFA and cis MUFA are correctly predicted compared to individual MUFA except for oleic acid which is the most abundant FA. However more effort should be done to validate those hypotheses. Taking into account the number of samples used in the present study and experimental conditions, the models obtained are not ready to be used in practical conditions. A more complete calibration and validation phases, with different muscle categories, feeding regimes and higher number of breeds will be necessary to build robust predictive models and to ensure that fluorescence spectroscopy is adapted for prediction of meat fat composition and for industrial applications.

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