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Handheld near-infrared spectrometer allows *on-line* prediction of beef quality traits

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ABSTRACT

The aim of this study was to evaluate the ability of a miniaturized near-infrared spectrometer to predict chemical parameters, technological and quality traits, fatty acids and minerals in intact *Longissimus thoracis* and *Trapezius* obtained from the ribs of 40 Charolais cattle. Modified partial least squares regression analysis to correlate spectra information to reference values, and several scatter correction and mathematical treatments have been tested. Leave-one-out cross-validation results showed that the handheld instrument could be used to obtain a good prediction of moisture and an approximate quantitative prediction of fat or protein contents, a*, b*, shear force and purge loss with coefficients of determination above 0.66. Moreover, prediction models were satisfactory for proportions of MUFA, PUFA, oleic and palmitic acids, for Fe and Cu contents. Overall, results exhibited the usefulness of the on-line miniaturized tool to predict some beef quality traits and the possibility to use it with commercial cuts without sampling, carcass deterioration nor grinding and consequent meat products' loss.

1. Introduction

Quality characteristics such as color and drip loss or exudation along with nutritional value, convenient price and authenticity are the main criteria consumers rely on when they buy meat (ElMasry, Sun, & Allen, 2011; Grunert, Bredahl, & Brunsø, 2004; Monin, 1998). Since consumer perception itself impacts on meat industry profitability (Troy & Kerry, 2010), its efforts are focused on meeting consumer requirements of consistent quality and thus deliver high eating quality (Liu et al., 2020) and healthy products (Scollan et al., 2014). A more widespread knowledge of health risks (McAfee et al., 2010) and a greater interest in the healthiness of foods made it essential to inform consumers of the fatty acid (FA) and mineral profile. To reduce a possible negative impact on health, a higher intake of polyunsaturated FAs and a lower intake of saturated FAs are recommended (Scollan et al., 2014); furthermore, red meat is a source of iron, essential for many cellular processes and for carrying oxygen in the blood as constituent of hemoglobin (McAfee et al., 2010), zinc biologically important as catalyst, structural, and regulatory ion (Chasapis, Spiliopoulou, Loutsidou, & Stefanidou, 2012), and other minerals in smaller amount and their presence need to be guaranteed avoiding deficiencies.

The reference procedures carried out to assess meat quality traits consist of chemical and instrumental methods which are time consuming, expensive and sometimes destructive (Prieto et al., 2014). Therefore, there is a constant need for reliable quality evaluation techniques that can be useful and more easily applicable on a large-scale. A reliable method, which is also rapid, cheap, non-destructive, and suitable for industrial application to obtain prediction of quality and nutritional parameters is near-infrared spectroscopy (NIRS). Due to its efficiency and simplicity, it is the most widely used spectroscopic technique for foodstuff analysis at- and in-line; however, nowadays the development of miniaturized tools has led to an increasing use of handheld instruments (Kademi, Ulusoy, & Hecer, 2018) which also offer an advantage in terms of on-line large-scale application.

Several studies demonstrated the ability of NIRS technology to predict moisture, protein and fat contents using benchtop equipment (Prevolnik, Škrlep, Škorjanc, & Čandek-Potokar, 2010; Prieto et al., 2014; Su et al., 2014) and its increasing use as food analytical tool in meat industries for routine controls (Alomar, Gallo, Castañeda, & Fuchslocher, 2003; Balage, da Luz e Silva, Gomide, de Bonin, & Figueira, 2015; Kademi et al., 2018). However, unsatisfactory results to be used for screening purposes in the meat industry have been obtained for the

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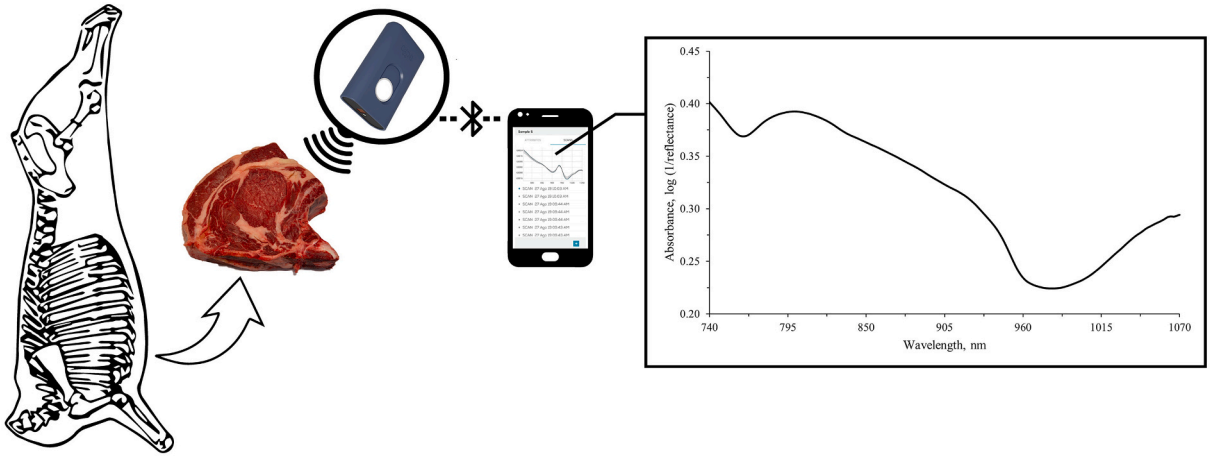

Fig. 1. Graphic representation of the average NIR raw spectra collection procedure through Bluetooth connection to smartphone application.

prediction of quality attributes such as color, pH, and tenderness (Andrés et al., 2008; Prieto, Andrés, Giráldez, Mantecón, & Lavín, 2008). In fact, according to Williams (2014) residual predictive deviation (RPD) should be above 2.4 to indicate the ability of NIRS prediction models to quantify the parameters or at least discriminate between high and low concentrations, even if the equation is not accurate enough to be used as a substitute of the reference method. Moreover, in the literature there is a number of studies in which FAs are predicted in beef muscles using NIR benchtop spectrometers (Andueza et al., 2019; Giaretta et al., 2019; Realini, Duckett, & Windham, 2004; Sierra et al., 2008). Whereas, NIRS can predict mineral contents with difficulty, since these minerals have no specific absorption bands in the infrared region unless they are part of organic complexes (Büning-Pfaue, 2003; Goi, Manuelian, Currò, & De Marchi, 2019). However, a limitation to the use of instruments which work on-line is that this approach requires the samples to be transported to a laboratory and processed before the acquisition of spectra. On the other hand, in the last decade several portable tools that work on-line became widely used; in fact, their utilization in the abattoir avoids the transportation and processing of samples, whereas the application of portable NIR spectrometers at market level can allow for the classification of food products and their authentication (Nolasco Perez et al., 2018). However, the limitations are their size, which does not allow an easy spectra collection along the production chain (De Marchi, 2013), and that spectra may be highly variable compared to the benchtop systems, likely caused by a lack of standardization in the spectra collection procedure. The purpose of the present study was to assess the reliability of NIRS prediction models for protein, fat, FA and mineral contents, pH, color, purge loss, and shear force (SF), from spectra collected through a cheap and web-based wireless handheld instrument designed for consumer use which could ensure a quicker and more practical use of this technology on a large scale.

2. Materials and methods

2.1. Samples

This study was approved by the Ethical Committee for the Care and Use of Experimental Animals of the University of Padova, Italy (approval no. 74/2018) and was conducted in accordance with Italian law (Decreto legislativo no. 26/2014) and EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

Samples used in the present study were a part of a study carried out on more than 1200 Charolais cattle that entered five commercial specialized beef fattening farms associated to a cooperative of beef producers (AZoVe) located in the Veneto region (Cittadella, Italy) between July 2018 and August 2019. A total of 40 rib cuts taken at the level of the 5th rib were collected from 40 Charolais beef cattle that were born between October 2017 and December 2018 and started the fattening cycle between October 2018 and August 2019. All the animals

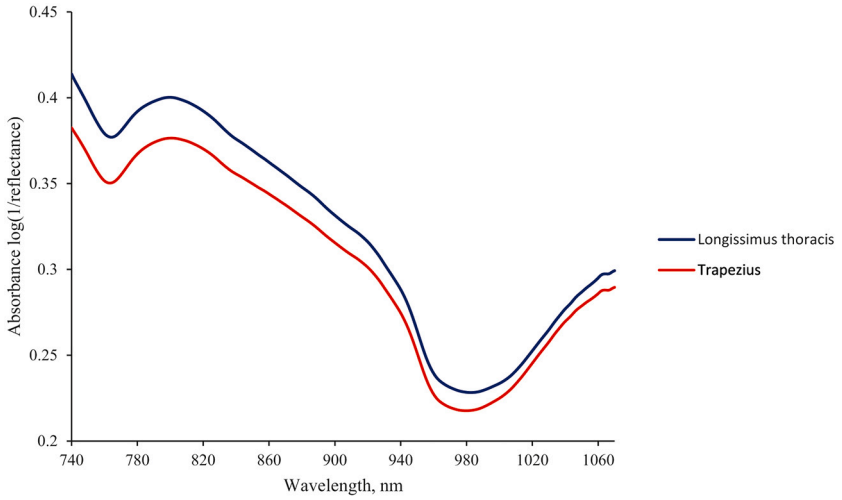

Fig. 2. Average raw spectra of beef collected using near-infrared spectroscopy according to the muscle type: *Longissimus thoracis* ($n = 80$) and *Trapezius* ($n = 40$).

were slaughtered between June and October 2019 at an average live body weight of 650.85 ± 101.91 kg, age at slaughter of 531 ± 46.2 days, and average carcass weight of 388.04 ± 67.86 kg. Immediately after the cutting phase (24 h post-mortem), the ribs were vacuum-packed and transported to the food laboratory of the Department of Agronomy, Food, Natural resources, Animals and Environment of the University of Padova (Legnaro, Italy) where they were weighed and stored at 4°C until the analyzes were carried out 48 h after slaughter.

For each sample, a second weight was recorded following the removal of the packaging and the exudates from the surface in order to calculate the purge losses as weight loss during the storage for 24 h due to the release of fluid from tissues (James & James, 2010). The weight loss was computed as the difference between the weight of the rib at arrival and the weight after 24 h of storage at 4°C and then expressed as percentage loss based on the initial weight. The cutting was then carried out with the aim of isolating the *Longissimus thoracis* (LT) and *Trapezius* muscles.

2.2. Near-infrared spectroscopy analysis

After the isolation of the muscles, 120 spectra, 40 LT at day 1, the same 40 LT after 7 days of aging, and 40 Trapezius at day 1 from the same animals, were collected with SCiO (Consumer Physics Inc., Tel Aviv, Israel), the handheld wireless instrument that operates in reflectance mode in the NIR region between 740 and 1070 nm of wavelength at intervals of 1 nm (Figs. 1 and 2). The decision to include both types of muscles and different aging times was due to the attempt to detect and involve greater variability of the data in order to obtain more robust calibrations. To reduce the occurrence of abnormal values, each spectrum was calculated as the average of 5 sub-spectra recorded applying the scanning head of the instrument at 1 cm over the surface of the muscle at different points. Subsequently, spectra were collected using Mosaic software (FOSS, Hillerød, Denmark) and converted to absorbance (log(1/reflectance)) to develop the prediction models.

2.3. Laboratory analyses

A total of 80 muscles sampled at day 1, LT ($n = 40$) and *Trapezius* ($n = 40$), were sliced perpendicularly to muscle fiber direction to obtain a total of 120 samples (2 cm thick, 2 slices of LT and a single slice of *Trapezius*), and measurements of pH, color traits expressed as lightness (L*), redness (a*) and yellowness (b*), and Allo-Kramer SF were recorded on all the samples the first day of analysis. A single slice from each LT was stored at 4°C and analyzed for the same traits after 7 days of aging. The pH was detected using a model HD2107.2 Delta Ohm (Delta Ohm, Padova, Italy) pH-meter with a high precision (± 0.002 pH units) and the final value was obtained as the average of 5 measurements taken at different points on the muscles' surface.

The L*, a*, and b* color indexes (Commission International de l'Eclairage, 1976) were measured after the muscles had been exposed to air for an hour using a Minolta colorimeter (CM-600d, Konica-Minolta Sensing Inc. Ramsey, NJ). Five measurements were performed at different points on the muscles' surface and averaged to obtain a single value for each sample.

Shear force (N/g of raw meat) was measured cutting across the fiber axis of each muscle slice (1 × 7 × 3 cm) in single for Trapezius and in duplicate for LT using a LS5 Single Column Bench Mounted (AMETEK Lloyd Instruments Ltd., West Sussex, UK) equipped with a 10-blade (8 × 7 cm) Allo-Kramer shear compression cell using a 500-kg load cell with a cutting speed of 500 mm/min and blade thickness of 22 mm; data was integrated using NEXYGEN PLUS 3 software (AMETEK Lloyd Instruments Ltd., West Sussex, UK).

Only LT were ground with a Grindomix mill (Retsch Grindomix GM200; Retsch GmbH & Co, Haan, Germany) and 10 g of each sample was freeze-dried to perform the quantification of mineral and FA contents. Major mineral (Ca, P, Mg, Na, K, and S) and trace mineral (Al, B, Ba, Cr, Cu, Fe, Li, Mn, Ni, Pb, Si, Sn, Sr, Ti, and Zn) analysis was performed by mineralization of 0.30 g of tissue in closed vessel with hydrogen peroxide and nitric acid (Merck Chemicals GmbH, Darmstadt, Germany) in a microwave digestion system (Ethos 1600 Milestone S.r.l., Sorisole, Bergamo, Italy). Dissolved samples were diluted in ultrapure water to obtain a final volume of 25 mL, and then concentrations were quantified with inductively coupled plasma optical emission spectrometry (ICP-OES) Ciros Vision EOP (Spectro Analytical Instruments GmbH, Kleve, Germany). All instrument operating parameters were optimized for nitric acid solution and the conditions were 2 mL/min of sample uptake rate, 1400 W plasma power, coolant gas flow 12 L/min, auxiliary flow 0.80 L/min, nebulizer flow 0.90 L/min, and integration time of 28 s. Calibration standards for each mineral were prepared from mono-element solutions (Inorganic Ventures, Christiansburg, VA, USA) with 5% nitric acid and 65% Suprapur at concentration of 0, 1, 2, 5, 10, 20, 50, and 100 mg/L. The ICP-OES determined Ca at 315.887 nm, P at 177.495 nm, Mg at 285.213 nm, Na at 589.592 nm, K at 766.941 nm, S at 182.034 nm, Al at 167.078 nm, B at 208.959 nm, Ba at 455.404 nm, Cr at 205.618 nm, Cu at 324.754 nm, Fe at 259.941 nm, Li at 670.780 nm, Mn at 257.611 nm, Ni at 231.604 nm, Pb at 220.353 nm, Si at 251.612 nm, Sn at 189.991 nm, Sr at 407.771 nm, Ti at 334.941 nm, and Zn at 213.856 nm.

Accelerated solvent extraction method was performed following the Dionex Application Note n.334 using ASE 200 (Dionex Corporation, Sunnyvale. CA, USA) with 22 mL stainless steel extraction cells. Freeze-dried samples of 2.5 g and petroleum ether as solvent were used for lipid extraction. Subsequently, 40 mg of the obtained extract was methylated following the procedure described by Christie (1982): fatty acid methyl ester solution was centrifuged at 693 ×*g* for 10 min at 10°C, and transferred to a 1.5 mL vial for gas chromatographic analysis. An Agilent 7820A GC System (Agilent Technologies, Santa Clara, CA, USA) was equipped with an automatic Omegawax® capillary GC column (24136 Supelco; Sigma Aldrich, Castle Hill, Australia) of 30 m length, 0.25 mm of inner diameter, and 0.25 mm film thickness. The working conditions were: hydrogen flow rate of 1.4184 mL/min, detector temperature set at 250°C, oven temperature initially held at 50°C for 2 min, and then increased at a rate of 4°C/min to 220°C held for 17.5 min. The individual FAs were identified by comparing the retention time of each with that of a standard FA mix. Finally, FAs were expressed as absolute concentration (g/100 g fresh meat) through the following formula: FA (% of total FA) × total lipids × 0.916 (Greenfield & Southgate, 2003).

To calculate chemical parameters (i.e., moisture, fat, and protein contents), the same meat slices were ground, and 100 g samples were analyzed using the NIR spectrometer FoodScan (FOSS, Hillerød, Denmark) as reference method since this NIR equipment has been approved by AOAC (2007) for the commercial analysis of moisture, fat and protein in meat and meat products using FOSS prediction models (Prieto et al., 2009). The determination was carried out using commercial global FOSS calibrations pre-installed in the instrument, which have been validated by Anderson (2007) against chemical analysis methods officially approved such as AOAC Official Methods for fat (960.39) and protein (992.15).

2.4. Chemometric analysis

Spectral and reference data were used to develop prediction equations for moisture, protein, fat, FA and mineral contents, pH, color, purge loss, and SF. Calibration models were performed using WinISI 4 software (Infrasoft International, Port Matilda, PA, USA) through modified partial least squares (mPLS) regression analysis to correlate spectral information to reference values using the complete dataset. The applied scatter correction to the raw spectra were: no correction (None), detrending (D), standard normal variate (SNV), standard normal variate and detrending (SNV + D), and multiplicative scatter correction (MSC) to reduce noise effect. Each pre-processing technique was combined with derivative mathematical treatments: 0,0,1,1; 1,4,4,1; 1,8,8,1;

Table 1
Descriptive statistics of chemical composition and technological and quality traits in beef samples ($n = 40$ LT at day 1; $n = 40$ LT at day 7; $n = 40$ Trapezius at day 1).

Traits	Mean	SD	Minimum	Maximum	CV[a]
Moisture content, %	68.67	1.47	65.32	71.36	2.1
Fat content, %	4.10	1.75	1.24	8.97	42.6
Protein content, %	22.01	1.01	19.81	24.30	4.6
pH	5.61	0.08	5.46	5.81	1.5
L*	40.26	2.73	33.29	46.94	6.8
a*	18.81	3.14	12.97	28.40	16.7
b*	12.60	2.89	7.85	21.70	23.0
Shear force, N/g	52.66	35.96	12.55	182.18	68.3
Purge loss, %	2.27	0.81	0.58	3.47	35.5

[a] CV = coefficient of variation.

2,5,5,1; and 2,10,10,1. In particular, the first digit is the number of the derivative, the second is the gap over which the derivative is calculated, the third is the number of data points in the first smoothing, and the fourth is the number of data points in the second smoothing (Shenk, Westerhaus, & Abrams, 1989).

To increase the accuracy of the calibration, spectral outliers were eliminated using the Mahalanobis distance (Global H > 3.0) and subsequently, after the mPLS regression analysis, data underwent three passes of outliers' elimination to build the final prediction model setting the critical T-statistic value to ±2.5 standard error, therefore removing samples for whose predicted values exceeded ±2.5 standard error of the reference values. Prediction models were then tested performing a leave-one-out cross-validation, therefore a training set was created excluding randomly a single spectrum from the entire dataset, calibration calculations were performed, and then prediction equations were tested to the excluded sample. The procedure was repeated until all the samples have been left out once from the training test and used as validation set. The best models were assessed based on the number of latent factors (LF) which minimized the root-mean-square error (RMSE) of cross-validation (Simoni, Goi, De Marchi, & Righi, 2021), the lower standard error of calibration (SE_C) and of cross-validation (SE_{CV}), the greater the coefficient of determination of calibration (R^2_C) and of cross-validation (R^2_{CV}) and the greater the residual predictive deviation of cross-validation (RPD), calculated as the ratio of SD to SE_{CV} (Williams & Sobering, 1993). The interpretation of R^2_{CV} was based on Karoui et al. (2006) and that of RPD was based on Williams (2014).

3. Results and discussion

3.1. Chemical composition

Summary statistics of meat quality traits are shown in Table 1. The coefficient of variation (CV) varied from 16.7% to 68.3% for fat, color parameters a* and b*, SF, and purge loss, whereas it was lower for moisture (CV = 2.1%), protein (CV = 4.6%), pH (CV = 1.5%) and L* (CV = 6.8%). Purge loss' CV was found to be high because of the presence in each rib of different muscle types. In fact, *post-mortem* proteolysis, intramuscular fat or marbling, connective tissue, and the contractile state of the muscle could contribute to the difference in tenderness between different muscles within the same beef carcass (Belew, Brooks, McKenna, & Savell, 2003). Moreover, differences in collagen characteristics, sarcomere length of the myofiber, fiber size, and fiber type composition could affect the water loss (Abdullah, Qudsieh, & Nusairat, 2011). In general, the high variability of the data is a key point to develop robust calibration models (Alomar et al., 2006). However, low variability has been already confirmed by other authors for pH (Andrés et al., 2008; De Marchi, Penasa, Cecchinato, & Bittante, 2013), protein content (Leroy et al., 2003), and L* (Page, Wulf, & Schwotzer, 2001). The most variable technological characteristic was the Allo-Kramer SF with the LT (30.65 N/g), as expected, more tender than the Trapezius (96.90 N/g) (Sullivan & Calkins, 2011), most likely due to the different location and function within the animal which induces differences in the proportions of the fiber types (Realini et al., 2013).

Descriptive statistics of fatty acids are reported in Table 2. Saturated

Table 2
Descriptive statistics of individual fatty acids, groups, and ratios in beef samples ($n = 40$ LT at day 1; $n = 40$ LT at day 7).

Fatty acids, mg/100 g of fresh tissue muscle	Mean	SD	Min	Max	CV[a]
Groups					
SFA[b]	2213.29	740.55	767.06	4536.41	33.5
MUFA[c]	1670.58	729.99	433.88	3677.14	43.7
PUFA[d]	123.24	43.10	39.22	227.98	35.0
BFA[e]	72.64	21.65	33.11	137.30	29.8
CLA[f]	18.80	10.34	4.98	73.62	55.0
n-6	89.80	37.05	13.53	171.04	41.3
n-3	17.31	10.28	4.53	92.12	59.4
Ratios					
PUFA/SFA	0.06	0.02	0.02	0.09	32.4
n-6/*n*-3	5.95	2.72	0.81	15.65	45.8
Major fatty acids					
C14:0 (myristic)	124.17	50.57	35.07	275.84	40.7
C16:0 (palmitic)	1186.59	457.28	331.76	2382.73	38.5
C16:1 *n*-9	97.20	68.07	3.63	290.00	70.0
C17:0	40.12	19.71	0	101.20	49.1
C18:0 (stearic)	828.38	237.54	385.51	1729.05	28.7
C18:1 *n*-7c (vaccenic)	94.87	57.26	4.82	222.10	60.4
C18:1 *n*-9 (oleic)	1558.20	692.55	427.88	3501.69	44.4
C18:2 *n*-6 (linoleic)	82.66	37.55	7.64	168.08	45.4
Minor fatty acids					
C6:0	0.84	1.22	0	10.62	144.7
C8:0	1.18	2.19	0	17.99	185.4
C10:0	3.02	1.62	0.50	13.29	53.8
C12:0	2.64	2.73	0	19.36	103.4
C13:0	1.15	2.55	0	21.99	222.5
*iso*C14:0	2.57	0.93	0	6.22	36.3
C15:0	17.09	4.66	7.75	32.36	27.3
*iso*C15:0	6.63	2.24	2.84	18.38	33.8
*anteiso*C15:0	8.05	3.37	0	25.69	41.9
*iso*C16:0	8.76	3.78	0	20.55	43.2
C16:1 *n*-7 (palmitoleic)	15.41	21.80	0.71	80.53	141.4
C16:1 t	0.99	0.73	0	4.44	74.0
C16:2	1.10	0.72	0	4.47	65.9
*iso*C17:0	14.49	4.76	0.46	27.72	32.9
*anteiso*C17:0	24.92	8.41	0	47.65	33.7
C17:1 *n*-7	2.92	6.07	0	52.70	207.9
C17:1 *n*-8	22.20	9.36	5.13	46.07	42.2
*iso*C18:0	7.21	2.96	0	15.56	41.1
C18:1 *n*-5	10.38	4.08	2.56	21.92	39.3
C18:3 *n*-6 (γ-linolenic)	2.71	3.27	0.27	23.51	120.9
C18:3 *n*-3 (α-linolenic)	8.71	4.38	0	27.83	50.2
C18:4 *n*-3	5.43	4.36	0	20.35	80.4
C19:0	1.66	1.48	0	7.29	89.2
C20:0 (arachidic)	5.16	2.83	0.62	21.26	54.9
C20:1 *n*-9	2.45	2.77	0	14.46	113.1
C20:2 *n*-6 (eicosadienoic)	1.09	1.61	0	8.29	147.8
C20:3 *n*-6	2.14	2.03	0	13.95	95.1
C20:4 *n*-6 (arachidonic)	1.21	2.77	0	24.77	228.6
C20:3 *n*-3	0.50	1.27	0	11.04	254.8
C20:5 *n*-3	0.17	0.51	0	3.33	309.4
C21:0	1.30	2.94	0	19.57	225.8
C22:0	2.26	8.81	0	77.75	390.6
C22:6 *n*-3	2.51	5.65	0	47.07	224.9
C24:1 *n*-9	2.10	2.98	0	20.74	142.0
CLA c9t11	16.39	7.73	1.34	38.01	47.1
tt-CLA	2.41	4.54	0	35.60	188.5

[a] CV = coefficient of variation; [b]SFA = saturated fatty acids (C6:0 + C8:0 + C10:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C19:0 + C20:0 + C21:0 + C22:0); [c]MUFA = monounsaturated fatty acids (C16:1 *n*-7 + C18:1 *n*-9 oleic + C18:1 *n*-7c + C24:1 *n*-9); [d]PUFA = polyunsaturated fatty acids (C18:2 *n*-6 + C18:3 *n*-6 + C18:3 *n*-3 + C18:4 *n*-3? + C20:2 *n*-6 + C20:3 *n*-3 + C20:3 *n*-6 + C20:4 *n*-6 + CLA c9t11 + *tt*-CLA); [e]BFA = branched fatty acids (*iso*C14:0 + *iso*C15:0 + *anteiso*C15:0 + *iso*C16:0 + *iso*C17:0 + *anteiso*C17:0 + *iso*C18:0); [f]CLA = conjugated linoleic acids (CLA c9t11 + *tt*-CLA).

Table 3
Descriptive statistics of major and trace minerals in beef samples ($n = 40$ LT at day 1; $n = 40$ LT at day 7).

Minerals	Mean	SD	Min	Max	CV[a]
Major minerals, mg/kg					
Ca	69.80	20.08	41.70	149.48	28.8
K	4351.80	223.51	3804.53	4781.14	5.1
Mg	230.96	10.33	201.15	258.10	4.5
Na	466.60	37.34	402.44	598.85	8.0
P	2078.01	89.94	1816.27	2314.87	4.3
S	2200.52	85.41	2031.38	2412.82	3.9
Trace minerals, μg/kg					
Cr	67.66	18.17	35.34	128.37	26.9
Cu	4504.44	8424.08	442.51	51,696.77	187.0
Fe	14,990.22	2150.27	10,497.25	19,151.25	14.3
Mn	74.70	15.28	45.82	115.11	20.5
Zn	53,244.15	5594.80	43,412.82	67,914.82	10.5
B	84.20	31.21	24.24	178.25	37.1
Al	1608.13	3068.92	246.18	11,080.00	190.8
Ti	91.36	92.42	20.49	440.55	101.2
Si	2632.49	813.62	1423.05	5167.00	30.9
Sr	39.15	9.97	20.08	56.53	25.5
Ba	51.15	67.14	7.78	496.38	131.3

[a] CV = coefficient of variation.

fatty acid was the most abundant group, including palmitic (C16:0) and stearic (C18:0) acids as the most abundant, followed by MUFA, in particular oleic (C18:1 *n*-9) acid among them which was the FA present in the greatest quantity overall. The same result has been previously described by other authors in different beef muscle types (Andueza et al., 2019; Sierra et al., 2008). The average conjugated linolenic acid (CLA) content was an intermediate value between those reported in the cited studies, whereas branched fatty acid (BFA) content was greater but also more variable among samples. The CV ranged from 29.8% (BFA) to 59.4% (*n*-3), assuring a good variability to perform predictions. Both the *n*-6 and *n*-3 contents were lower than that reported by Nuernberg et al. (2005). However, their ratio was similar to the average value measured in *Longissimus* muscle of German Simmental cattle fed with concentrate (Nuernberg et al., 2005). Moreover, PUFA and SFA contents expressed as a percentage of total FAs, 55.24% and 3.08%, respectively, were slightly higher but with the same ratio (0.06) compared to the results of Realini et al. (2004) in grain-fed beef. Overall, the major FAs were present in the same order of quantity described in the literature, with the exception of a study that reported a higher content of palmitic acid than oleic acid in *Longissimus thoracis* of yearling bulls (Sierra et al., 2008). Coefficients of variation of FAs ranged from 27.3% (C15:0) to 222.5% (C13:0) which is much more than other studies have shown (Andueza et al., 2019; Sierra et al., 2008).

A total of 17 minerals, 6 major minerals and 11 trace minerals, were present in the samples in amount above the LOD and their descriptive statistics are shown in Table 3. All the major minerals quantified (Ca, P, Mg, Na, K, and S) and Cu, Fe, Mn, Si, and Zn among trace minerals were above the limit of detection (LOD) of the instrument expressed on fresh samples. On the other hand, the quantification of Al, B, Ba, Cr, Sr, and Ti was not possible for some samples in which the elements were below the LOD and for the same reason in almost all the samples Li, Ni, Pb, and Sn were not detectable. Thus, from this list of trace minerals, Al ($n = 24$), Li, Ni, Pb and Sn has been excluded from the calibration procedure because 50 samples was considered a minimum for calibration selection (Windham & Coleman, 1989). The average mineral content was similar to that reported by Flowers et al. (2018) and Czerwonka and Szterk (2015) in beef *Longissimus thoracis*, with the exception of Fe which had a greater value in the latter study that may be due to a different feed administered and the different breed. However, the most abundant minerals were K and Zn among major and trace minerals, respectively, as previously reported in literature (Czerwonka & Szterk, 2015; Domaradzki, Florek, Staszowska, & Litwińczuk, 2016; Flowers et al., 2018).

3.2. Near-infrared predictions

Statistics for prediction models of meat technological and quality traits are reported in Table 4. The number of latent factors considered ranged between 3 (L*) and 9 (moisture, fat and SF), and outliers were below 5.8% for all the parameters analyzed. The most used scatter correction was detrending (D) and second derivative as mathematical treatment, followed by first derivative. According to the interpretation of R^2_{CV} of Karoui et al. (2006), the best prediction equation was developed for moisture ($R^2_{CV} = 0.84$; RPD = 2.48), which could give a good estimation of the reference value, followed by fat and SF, which both reached a R^2_{CV} of 0.79 and RPD above 2 (Table 4). This result is in accordance to those obtained by Ripoll, Albertí, Panea, Olleta, and Sañudo (2008) in homogenized samples, who determined the tenderness using the Warner–Bratzler method, and could be due to the high variability of the traits. The calibration model for protein had a lower R^2_{CV} (0.66) than the other chemical variables and difficulties in predicting meat protein content have been previously stated by Ripoll et al. (2008), most likely because of the narrow protein range in bovine meat (Prieto, Andrés, Giráldez, Mantecón, & Lavín, 2006). Moreover, prediction equations for a*, b*, and purge loss had a lower accuracy but R^2_{CV} was above 0.66 thus indicating the possibility of using these models for a rough screening although, because the R^2_{CV} was below 0.82, they are not sufficiently accurate for a good prediction (Karoui et al., 2006). Similar results were obtained by De Marchi et al. (2013) for a* using visible/near-infrared spectroscopy in intact meat samples, but lower values for b* and purge loss, which was also reported by Cecchinato, De Marchi, Penasa, Albera, and Bittante (2011) and De Marchi, Berzaghi,

Table 4
Goodness of fit statistics of modified partial least squares regression models in leave-one-out cross-validation for chemical and technological beef traits developed using pocket-size handheld NIR spectrometer on intact samples ($n = 120$).

Trait	Scatter correction[a]	Math[b]	LF[c]	outliers	SE_C[d]	R^2_C[e]	SE_{CV}[f]	R^2_{CV}[g]	RPD[h]
Moisture	SNV + D	0,0,1,1	9	4	0.53	0.87	0.60	0.84	2.48
Fat	MSC[i]	0,0,1,1	9	6	0.71	0.84	0.80	0.79	2.19
Protein	SNV[j]	2,10,10,1	6	3	0.50	0.76	0.59	0.66	1.72
pH	D[k]	2,5,5,1	5	4	0.05	0.65	0.06	0.52	1.46
L*	None	1,4,4,1	3	3	1.78	0.56	1.88	0.51	1.43
a*	D	1,4,4,1	7	3	1.50	0.78	1.74	0.70	1.82
b*	D	2,5,5,1	4	3	1.31	0.80	1.47	0.74	1.98
Shear force	D	1,8,8,1	9	7	12.82	0.84	14.95	0.79	2.17
Purge loss	MSC	2,10,10,1	8	0	0.38	0.78	0.45	0.67	1.76

[a] Scatter correction = pre-processing technique to reduce noise; [b]Math = mathematical treatment (first digit indicates the derivative treatment); [c]LF = number of modified partial least squares latent factors used to develop the calibration model; [d]SEC = standard error of calibration; [e]R2C = coefficient of determination of calibration; [f]SECV = standard error of cross-validation; [g]R2CV = coefficient of determination of cross-validation; [h]RPD = residual prediction deviation calculated as SD after outliers' removal/SECV; [i]MSC = multiplicative scatter correction; [j]SNV = standard normal variate; [k]D = detrending.

Table 5
Goodness of fit statistics of modified partial least squares regression models in leave-one-out cross-validation for beef individual fatty acids, groups, and ratios developed using pocket-size handheld NIR spectrometer on intact samples ($n = 80$).

Fatty acids	Scatter correction[a]	Math[b]	LF[c]	outliers	SE_C[d]	R^2_C[e]	SE_{CV}[f]	R^2_{CV}[g]	RPD[h]
Groups									
SFA	MSC[i]	0,0,1,1	8	5	0.27	0.87	0.32	0.82	2.35
MUFA	SNV[j]	2,5,5,1	3	4	0.31	0.81	0.35	0.75	2.03
PUFA	SNV	0,0,1,1	1	2	0.04	0.15	0.04	0.12	1.07
BFA	SNV	1,4,4,1	4	4	11.53	0.69	12.71	0.62	1.63
CLA	D[k]	2,5,5,1	3	4	5.01	0.64	5.52	0.56	1.51
n-6	SNV	0,0,1,1	1	2	34.95	0.12	35.37	0.08	1.05
n-3	SNV + D	2,5,5,1	2	3	4.40	0.45	4.82	0.33	1.23
Ratios									
PUFA/SFA	None	2,5,5,1	2	2	0.02	0.30	0.02	0.18	1.11
n-6/*n*-3	SNV	1,4,4,1	8	3	1.85	0.47	2.35	0.13	1.08
Major fatty acids									
C14:0	SNV + D	1,8,8,1	5	3	26.17	0.70	29.37	0.62	1.64
C16:0	SNV	0,0,1,1	9	4	155.90	0.88	193.95	0.82	2.34
C16:1 *n*-9	MSC	2,5,5,1	2	4	31.08	0.74	33.62	0.69	1.81
C17:0	SNV + D	1,8,8,1	5	11	6.84	0.75	7.98	0.65	1.71
C18:0	SNV + D	1,4,4,1	7	2	118.66	0.75	150.42	0.60	1.59
C18:1 *n*-7c	D	1,4,4,1	9	2	27.71	0.77	37.35	0.57	1.54
C18:1 *n*-9	SNV	2,5,5,1	3	4	282.97	0.82	318.92	0.77	2.10
C18:2 *n*-6	SNV	0,0,1,1	1	2	35.41	0.12	35.81	0.09	1.05
Minor fatty acids									
C6:0	MSC	2,5,5,1	2	13	0.20	0.47	0.22	0.36	1.26
C8:0	SNV	0,0,1,1	8	6	0.27	0.60	0.30	0.49	1.41
C10:0	SNV	2,10,10,1	2	3	0.85	0.43	0.92	0.32	1.22
C12:0	D	2,10,10,1	2	26	0.87	0.45	0.96	0.32	1.23
C13:0	MSC	2,10,10,1	5	21	0.22	0.64	0.29	0.38	1.28
*iso*C14:0	MSC	1,4,4,1	3	5	0.64	0.21	0.68	0.11	1.07
C15:0	MSC	1,8,8,1	5	3	3.07	0.57	3.53	0.42	1.32
*iso*C15:0	SNV	0,0,1,1	6	5	1.09	0.55	1.19	0.47	1.38
*anteiso*C15:0	D	0,0,1,1	8	8	1.55	0.33	1.75	0.13	1.08
*iso*C16:0	MSC	1,4,4,1	3	8	2.14	0.48	2.33	0.38	1.28
C16:1 *n*-7	None	2,5,5,1	5	7	6.85	0.89	8.62	0.82	2.36
C16:1 t	None	2,5,5,1	2	67	0.41	0.30	0.48	0.04	1.03
C16:2	SNV + D	2,10,10,1	2	11	0.32	0.57	0.35	0.47	1.39
*iso*C17:0	SNV	1,4,4,1	3	2	2.61	0.70	2.88	0.63	1.66
*anteiso*C17:0	SNV + D	0,0,1,1	8	4	3.99	0.73	4.48	0.65	1.70
C17:1 *n*-7	D	0,0,1,1	7	13	1.28	0.35	1.43	0.18	1.11
C17:1 *n*-8	SNV + D	2,10,10,1	2	3	4.77	0.71	5.11	0.67	1.75
*iso*C18:0	MSC	2,5,5,1	3	5	1.58	0.66	1.79	0.56	1.52
C18:1 *n*-5	D	2,5,5,1	2	2	2.78	0.55	2.96	0.48	1.40
C18:3 *n*-3	None	1,8,8,1	7	13	1.80	0.42	2.18	0.14	1.09
C18:3 *n*-6	SNV	0,0,1,1	6	4	1.82	0.24	1.95	0.12	1.07
C18:4 *n*-3	None	2,10,10,1	4	67	1.97	0.75	2.34	0.64	1.68
C19:0	SNV	1,4,4,1	8	66	0.83	0.57	1.10	0.23	1.15
C20:0	MSC	1,8,8,1	7	75	1.29	0.66	1.50	0.53	1.48
C20:1 *n*-9	SNV + D	2,5,5,1	3	59	1.30	0.59	1.57	0.39	1.30
C20:2 *n*-6	None	2,5,5,1	2	59	0.69	0.39	0.77	0.23	1.15
C20:3 *n*-3	SNV	2,5,5,1	2	47	0.17	0.50	0.20	0.31	1.22
C20:3 *n*-6	SNV	2,5,5,1	1	68	1.25	0.25	1.32	0.15	1.09
C20:4 *n*-6	D	2,5,5,1	4	69	0.45	0.57	0.57	0.30	1.20
C21:0	D	2,5,5,1	2	33	1.58	0.64	1.88	0.47	1.39
C24:1 *n*-9	SNV	2,10,10,1	8	68	0.73	0.79	1.01	0.60	1.59
CLA c9t11	SNV + D	2,10,10,1	2	76	4.81	0.58	5.17	0.51	1.44
tt-CLA	MSC	0,0,1,1	6	76	2.02	0.40	2.19	0.28	1.18

[a] Scatter correction = pre-processing technique to reduce noise; [b]Math = mathematical treatment (first digit indicates the derivative treatment); [c]LF = number of modified partial least squares latent factors used to develop the calibration model; [d]SE_C = standard error of calibration; [e]R^2_C = coefficient of determination of calibration; [f]SE_{CV} = standard error of cross-validation; [g]R^2_{CV} = coefficient of determination of cross-validation; [h]RPD = residual prediction deviation calculated as SD after outliers' removal/SECV; [i]MSC = multiplicative scatter correction; [j]SNV = standard normal variate; [k]D = detrending.

Boukha, Mirisola, and Gallo (2007) in minced samples. Protein calibration was found to have intermediate accuracy compared to other studies on ground sample (Cozzolino, De Mattos, & Martins, 2002; Cozzolino & Murray, 2002). Unsatisfactory predictions ($R^2_{CV} < 0.66$; Karoui et al., 2006) were obtained for pH and L*, the less variable parameters, and this result is confirmed by De Marchi (2013) for L*, but in contrast to those showed by Cozzolino and Murray (2002) for pH, whose samples were more variable and thus provided a greater range of values to develop a prediction model.

The performance of NIRS prediction models for FAs, reported in Table 5, were obtained using the amount of FA instead of percentage basis, which was expected to correlate better to NIR data since the absorbance is based on the amount of molecular bonds (Prieto et al., 2011). Outliers detected for FA groups were < 6.3% and LF ranged between 1 (PUFA and *n*-6) and 8 (SFA); the best prediction equations were developed for SFA ($R^2_{CV} = 0.82$; RPD = 2.35) indicating that the model could provide a fairly good estimation of the parameter, and for MUFA ($R^2_{CV} = 0.75$; RPD = 2.03) which reach an accuracy good for a rough screening in meat samples. In general, SFA and MUFA are better predicted than PUFA, in agreement with Mourot et al. (2015) and prediction models of the other groups have not provided satisfactory results. Among major FAs, the best prediction models were developed for the most abundant palmitic ($R^2_{CV} = 0.82$; RPD = 2.34) and oleic ($R^2_{CV} = 0.77$; RPD = 2.10) acids followed by C16:1 *n*-9 ($R^2_{CV} = 0.69$; RPD =

Table 6
Goodness of fit statistics of modified partial least squares regression models in leave-one-out cross-validation for beef major and trace minerals developed using pocket-size handheld NIR spectrometer on intact samples ($n = 80$).

Minerals	Scatter correction[a]	Math[b]	LF[c]	n^d	outliers	SE_C[e]	R^2_C[f]	SE_{CV}[g]	R^2_{CV}[h]	RPD[i]
Major minerals, mg/kg										
Ca	D[j]	1,8,8,1	8	80	7	11.79	0.47	15.48	0.06	1.04
K	MSC[k]	1,4,4,1	8	80	13	107.85	0.76	141.40	0.58	1.56
Mg	D	0,0,1,1	3	80	3	7.22	0.47	7.79	0.38	1.28
Na	None	1,4,4,1	7	80	3	23.91	0.53	29.18	0.29	1.19
P	MSC	1,4,4,1	3	80	4	61.38	0.45	66.77	0.35	1.24
S	SNV + D	1,8,8,1	8	80	2	65.12	0.43	76.52	0.20	1.13
Trace minerals, μg/kg										
B	MSC	2,10,10,1	3	73	2	24.32	0.39	28.19	0.17	1.10
Ba	SNV + D	2,5,5,1	8	52	2	10.68	0.79	21.39	0.12	1.08
Cr	SNV + D	2,10,10,1	1	59	4	13.71	0.24	14.55	0.13	1.08
Cu	D	2,10,10,1	6	80	5	2408.75	0.85	3060.25	0.76	2.04
Fe	SNV[l]	2,5,5,1	5	80	4	635.88	0.91	830.31	0.85	2.58
Mn	SNV + D	1,4,4,1	6	80	4	9.83	0.56	11.95	0.34	1.24
Zn	D	2,5,5,1	3	80	2	4434.62	0.37	5084.69	0.16	1.10
Si	None	2,10,10,1	6	80	7	414.01	0.54	528.84	0.25	1.16
Sr	None	2,5,5,1	3	60	2	7.89	0.39	9.48	0.11	1.07
Ti	None	1,4,4,1	8	65	7	30.79	0.52	39.42	0.20	1.13

[a] Scatter correction = pre-processing technique to reduce noise; [b]Math = mathematical treatment (first digit indicates the derivative treatment); [c]LF = number of modified partial least squares latent factors used to develop the calibration model; [d]n = number of samples above the limit of detection; [e]SE_C = standard error of calibration; [f]R^2_C = coefficient of determination of calibration; [g]SE_{CV} = standard error of cross-validation; [h]R^2_{CV} = coefficient of determination of cross-validation; [i]RPD = residual prediction deviation calculated as SD after outliers' removal/SECV; [j]MSC = multiplicative scatter correction; [k]SNV = standard normal variate; [l]D = detrending.

1.81), in accordance with Sierra et al. (2008) who used NIR transmittance spectroscopy, and Andueza et al. (2019) that obtain good results also for stearic acid with visible/NIRS in reflectance mode. The best calibrations of minor FAs were obtained for C16:1 *n*-7 ($R^2_{CV} = 0.82$; RPD = 2.36) and C17:1 *n*-8 ($R^2_{CV} = 0.67$; RPD = 1.75). For all other FAs quantified in the study, including ratios calculated between groups, predictions were not considered satisfactory, which may be due to the low quantities or the moderate variability which makes the prediction in beef of minor FAs difficult (Andueza et al., 2019). In fact, this conclusion was also confirmed by other authors (De Marchi, Riovanto, Penasa, & Cassandro, 2012; Riovanto, De Marchi, Cassandro, & Penasa, 2012) using reflectance and transmittance NIRS, respectively. Some minor FAs were not predicted by NIRS because many samples did not contain them and therefore the dataset was too small to allow the development of calibrations. However, they were not excluded but used in groups or ratios. The low accuracy in predicting FAs may be also due to the narrow range of wavelength in which the instrument works (740–1070 nm), which does not include absorption bands related to C—H bonds (1100 nm) linked to the presence of fat (Cecchinato et al., 2012). Moreover, since spectra were collected on intact muscles which could lack homogeneity, as concluded by several studies whose results have been reported by Prieto, Pawluczyk, Russell Dugan, and Aalhus (2017), the prediction ability of the instrument might have been affected. Overall, among FAs' groups and ratios, the most used scatter correction was SNV and second or no derivative were the most selected options (Table 5).

Cross-validation statistics for determination of minerals are shown in Table 6. Latent factors ranged from 3 (Mg and P) to 8 (Ca, K, and S) for major minerals, whereas from 1 (Cr) to 8 (Ba and Ti) for minor minerals; outliers were ≤ 11% for all the parameters, with exception for K (16.3%). The calibrations which allowed the most accurate predictions (greatest R^2_{CV}) were developed mainly using the D scatter correction as reported by De Marchi et al. (2017) predicting Na content in processed meat, and SNV + D, and the second derivative as mathematical treatment. Prediction models for major minerals showed a R^2_{CV} below the threshold of 0.66 to be considered good even just for carrying out a screening (Karoui et al., 2006) and thus they were not recommended for any kind of practical application. On the other hand, among minor minerals Cu ($R^2_{CV} = 0.76$; RPD = 2.04) and Fe ($R^2_{CV} = 0.85$; RPD = 2.58) were the parameters that reached the greatest accuracy, allowing for the former an approximate quantitative prediction and for the latter a good estimation of its content in any sample. A similar result for Fe content was obtained by González-Martín, González-Pérez, Hernández-Méndez, and Alvarez-García (2002) in pork meat and the good accuracy could be due to the binding of the mineral to proteins that, as organic molecules, are detected by NIRS. Viljoen, Hoffman, and Brand (2007) predicted Cu content in mutton beef but did not observed a similar accuracy, with a coefficient of determination <0.66 and therefore below the value to be assessed as adequate (Karoui et al., 2006), probably because of the lower variability of the content for this element compared to this study.

For Al, Li, Ni, and Pb contents, it was not possible to develop calibration equations since there were few or no samples in which the elements were present above the LOD. Overall, the unsatisfactory performances of NIRS prediction models for these minerals were most likely due to the low contents of these components, the lack of specific absorption bands in the near-infrared region (Goi et al., 2019), and the narrow range of wavelengths in which the instrument works (Goi, Simoni, Righi, Visentin, & De Marchi, 2020). In fact, since the absorbance depends on the number of molecular bonds, and a low amount of the element is reflected in fewer molecular bonds that can be excited, it is difficult to predict minerals that are generally present in low quantities. Moreover, minerals can be detected only if they are linked to organic complexes and chelates, or if they interfere with water shifting its absorption band (Begley, Lanza, Norris, & Hruschka, 1984), which likely means that not all the amount can be detected and predicted.

Overall, RPD is more restrictive than R^2_{CV} and imposes stricter limits to define the goodness of a prediction model, thus according to Williams (2014) our results indicated that calibrations were not accurate enough for quality control in industry even if they could be applicable for a rough screening and several models (moisture, fat, SF, SFA, MUFA, palmitic, oleic, and palmitoleic acids, Cu, and Fe) could be used for a quantitative estimation, which may be useful for consumers. The linear regression of measured versus predicted values for the main traits with the best prediction eqs. (2 < RPD < 2.4) are represented in Fig. 3 and may be applicable for rough consumer screening (Williams, 2014). Therefore, an increase of the number of samples may result in more robust and accurate calibrations.

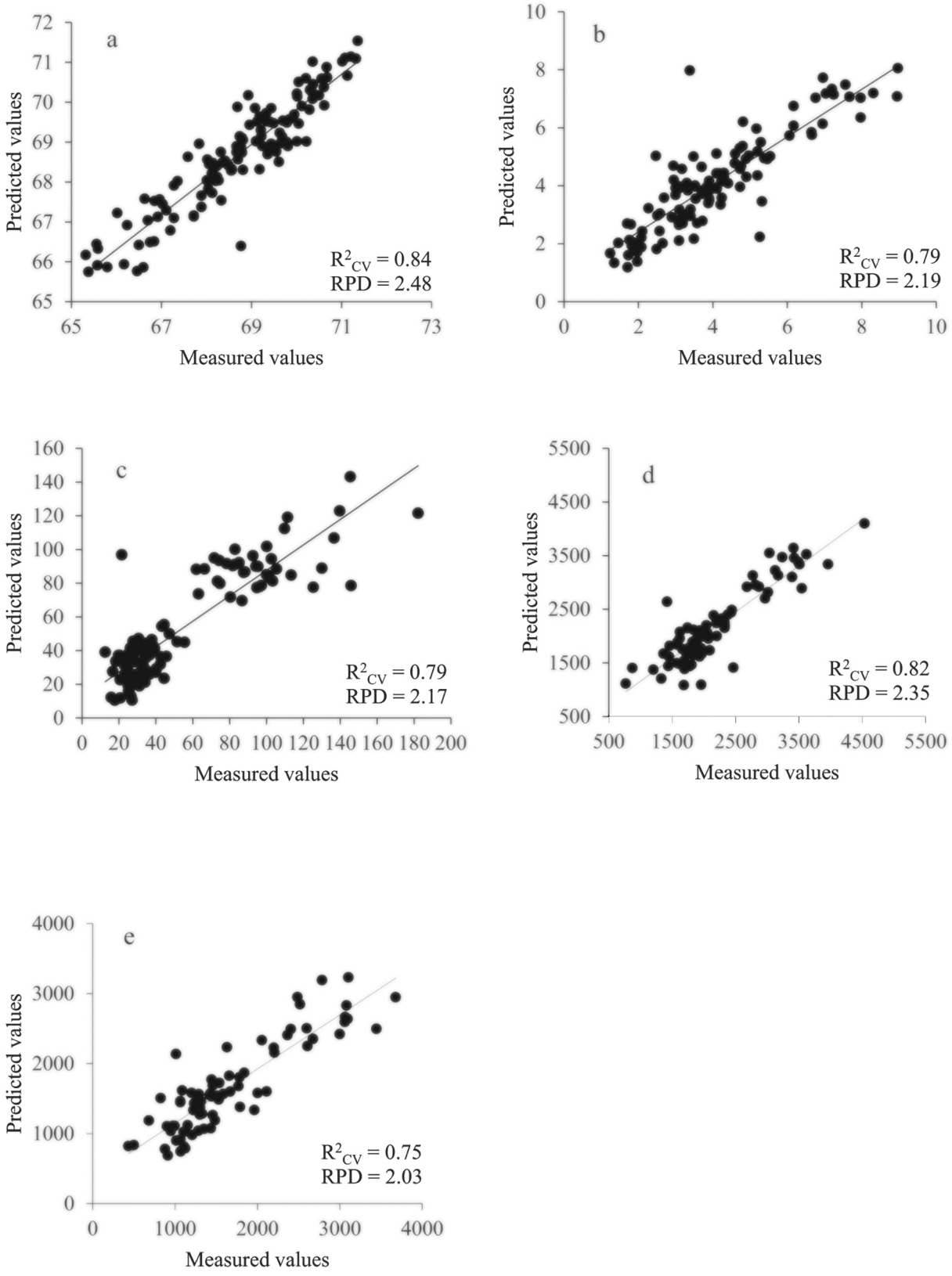

Fig. 3. Linear regression plots of measured versus predicted values ('as is') for (**a**) moisture, %; (**b**) fat, %; (**c**) shear force, N/g; (**d**) SFA, mg/100 g; (**e**) MUFA, mg/100 g.

4. Conclusion

The present study suggests that the pocket-size handheld NIR instrument can somewhat successfully predict chemical parameters such as moisture and fat and some of the quality traits of intact beef muscles such as a*, b*, SF, and purge loss. This ability could be useful to predict beef quality using a rapid on-line system at the retail level to assess traits that could influence the acceptability of the product by the consumer, and at consumer level allowing them to influence their dietary choices. Among FAs, the results showed the applicability of the miniaturized device to make predictions with good accuracy and thus perform a quantitative estimation of SFA, MUFA, palmitic and oleic acids proportions. This ability could be used to evaluate the levels of nutritional parameters which are strictly connected to cardiovascular risk or health benefits and therefore this might allow to obtain quickly and at low cost additional information to be reported on the label while keeping the product intact and avoiding sampling and loss of products. Iron and Cu were the only minerals for which it was possible to develop adequate prediction models to be used in practice although in general the accuracy was not adequate for a secure quality control. Lastly, due to the low content of many considered parameters, the narrow working range of the instrument in the near-infrared region, and the inherent difficulty of the technology used in predicting inorganic elements such as minerals, the instrument could be considered as a consumer-grade tool although at this stage it is not applicable at industry level because of insufficient accuracy, and in any case, it would be advisable to carry out further studies expanding the number of analyzed samples.

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CRediT authorship contribution statement

Arianna Goi: Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft. **Jean-François Hocquette:** Validation, Writing – review & editing. **Erika Pellattiero:** Data curation. **Massimo De Marchi:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Declaration of Competing Interest

None.

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