Current Research in Food Science 8 (2024) 100675

Contents lists available at ScienceDirect

Current Research in Food Science

journal homepage: www.sciencedirect.com/journal/current-research-in-food-science

Performance of benchtop and portable spectroscopy equipment for discriminating Iberian ham according to breed

Miriam Hernández-Jiménez [a], Isabel Revilla [a,*], Ana M. Vivar-Quintana [a], Justyna Grabska [b], Krzysztof B. Beć [b], Christian W. Huck [b]

[a] Food Technology Area, Universidad de Salamanca, Escuela Politécnica Superior de Zamora, Avenida Requejo 33, Zamora, 49022, Spain
[b] Institute of Analytical Chemistry and Radiochemistry, Leopold-Franzens University of Innsbruck, 6020, Innsbruck, Austria

ARTICLE INFO

Handling Editor: Professor Aiqian Ye

Keywords:
Iberian breed
Crossbreed
Near infrared (NIR) spectroscopy
Raman spectroscopy
Handheld
Desktop

ABSTRACT

Iberian ham is a highly appreciated product and according to Spanish legislation different labels identify different products depending on the genetic purity. Consequently, "100% Iberian" ham from purebred Iberian animals is more expensive than "Iberian" ham from Iberian x Duroc crosses. The hypothesis of this study was that to avoid labelling fraud it is possible to distinguish the breed (Iberian or Iberian x Duroc) of acorn-fed pigs of Iberian ham without any prior preparation of the sample by using spectroscopy that is a rapid and reliable technology. Moreover, portable devices which can be used *in situ* could provide similar results to those of benchtop equipment. Therefore, the spectra of the 60 samples (24 samples of 100% Iberian ham and 36 samples of Iberian x Duroc crossbreed ham) were recorded only for the fat, only for the muscle, or for the whole slice with two benchtop near-infrared (NIR) spectrometers (Büchi NIRFlex N-500 and Foss NIRSystem 5000) and five portable spectrometers including four portable NIR devices (VIAVI MicroNIR 1700 ES, TellSpec Enterprise Sensor, Thermo Fischer Scientific microPHAZIR, and Consumer Physics SCiO Sensor), and one RAMAN device (BRAVO handheld). The results showed that, in general, the whole slice recording produced the best results for classification purposes. The SCiO device showed the highest percentages of correctly classified samples (97% in calibration and 92% in validation) followed by TellSpec (100% and 81%). The SCiO sensor also showed the highest percentages of success when the analyses were performed only on lean meat (97% in calibration and 83% in validation) followed by microPHAZIR (84% and 81%), while in the case of the fat tissue. Raman technology showed the best discrimination capacity (96% and 78%) followed by microPHAZIR (89% and 81%). Therefore, spectroscopy has proved to be a suitable technology for discriminating ham samples according to breed purity; portable devices have been shown to give even better results than benchtop spectrometers.

1. Introduction

Iberian ham is highly appreciated by consumers for its organoleptic and gastronomic quality. This quality depends on the genetics of the pig, on the technology used in production, and on the pigs' diet, which may be based on fodder or on the *montanera* system (the consumption of acorns and grass by free grazing) (Cava et al., 1997; Ruiz et al., 1998; Fuentes et al., 2014). Therefore, Iberian pork products are officially classified in different commercial categories depending on the feeding system and racial purity according to Spanish legislation RD 4/2014, which approves a quality standard for Iberian meat, ham, shoulder, and loin. A black label thus identifies pieces from 100% purebred Iberian pigs fed on acorns free-range which are known as "Bellota 100%". A red label identifies pieces from acorn-fed animals which are 99%–50% Iberian (crosses with Duroc); these are known as "Bellota Ibérica". The price depends on the racial purity and varies greatly (Tejeda et al., 2002; García et al., 2006), which encourages labelling fraud and misleads the consumer.

To avoid this, several techniques has been explored in order to classify Iberian ham according to the breed (100% Iberian vs Iberian x Duroc crosses) such as genetic markers (García et al., 2006), the triacylglycerol and fatty acid composition (Tejeda et al., 2002; Petrón et al., 2004), quality traits (Fuentes et al., 2014; Ventanas et al., 2006), the instrumental color (Carrapiso and García, 2005), the volatile profile (Antequera et al., 1996; Carrapiso et al., 2003b), or the sensory characteristics (Carrapiso, Bonilla, et al., 2003a). More recently, 1H NMR

* Corresponding author.
E-mail addresses: miriamhj@usal.es (M. Hernández-Jiménez), irevilla@usal.es (I. Revilla).

https://doi.org/10.1016/j.crfs.2024.100675
Received 11 July 2023; Received in revised form 25 December 2023; Accepted 4 January 2024
Available online 6 January 2024
2665-9271/© 2024 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

spectroscopy has been used to analyze the lipid profile of Iberian hams, obtaining satisfactory separation regarding the breed with minimal sample handling (Pajuelo et al., 2022). However, as these analyses are time-consuming and imply the destruction of the samples, a non-destructive system not requiring sample preparation and a reliable analytical method would be more suitable. The analysis of samples taken with needles by Gas Chromatography coupled with Ion Mobility Spectrometry (GC-IMS) (Martín-Gómez et al., 2019) has recently provided validated classification rates of 100% regarding the breed, although this technique has to be performed in a laboratory.

In this context, spectroscopy has not only been shown to be a reliable, green, and rapid analytical method with minimal or non-sample preparation (Valand et al., 2020; Hernández-Jiménez et al., 2021a; McGrath et al., 2021); in addition, the recent development of portable equipment, mainly for near-infrared (NIR) spectroscopy measuring, means that it has great potential as an *in situ* control tool and for routine monitoring along value chains in real time (Beć et al., 2020, 2021a).

Nowadays a large number of miniaturized portable spectrometers are available, such as microPHAZIR (Thermo Fischer Scientific, USA); NIRONE sensors (Spectral Engines, Finland); Neo Spectra (Si-Ware Systems, Egypt); nanoFTIR NIR (SouthNest Technology, China); MicroNir ProEs (VIAVI solutions, USA); SCiO (Consumer Physics, Israel), and Enterprise Sensor NIR (TellSpec, UK) (Beć et al., 2020, 2021a; Huck et al., 2022). However, miniaturized devices implement different technology solutions from benchtop equipment. The Hadamard-transform principle, the Fabry–Perot filter, the micro-optoelectro-mechanical system (MOEMS), and the linear variable filter (LVF) coupled with an array detector or dispersive grating combined with a digital micro-mirror device (DMD) can be mentioned for the optical configurations employing single-element detectors, which lead to different operational wavelength regions, spectral resolution, and signal to-noise ratios (Beć et al., 2021a, 2021b). These drawbacks may affect the analytical performance or the applicability to a specific matrix (Plans et al., 2013; Pu et al., 2021; Sun et al., 2020; Yu et al., 2020, 2020b; Kappacher et al., 2022). In fact, although portable NIR spectroscopy has great potential in the meat sector (Kademi et al., 2019) and this equipment has been used on pork meat and carcasses in order to classify (Zamora-Rojas et al., 2012, 2013; Horcada et al., 2020; Pérez-Marín et al., 2022) and predict certain quality parameters such as the basic composition, color, texture, fatty acids, the iodine value, thiobarbituric reactive substances (TBARS), or stable isotopes (Garrido-Varo et al., 2016; Prieto et al., 2018; Piotrowski et al., 2019; Kucha and Ngadi, 2020; González-Martín et al., 2021), only a few portable equipment devices have been studied. In addition, when comparing the calibration and prediction accuracy obtained by benchtop and portable equipment, the results do not always agree (Dixit et al., 2020; Piotrowski et al., 2019; Savoia et al., 2020; Patel et al., 2020, 2021; González-Martín et al., 2021).

As far as the application of spectroscopy for Iberian ham classification is concerned, although some studies have addressed the identification of the breed of pork fat or carcasses using NIR spectroscopy equipment (benchtop or portable) (Horcada et al., 2020; Hernández-Jiménez, 2021b; Pérez-Marín et al., 2022), few methods have been proposed to authenticate the final product. Therefore, Martín-Gómez et al. (2021) proposed a method based on Raman spectroscopy as a rapid *in situ* screening tool for Iberian ham samples which classifies the breed correctly in 87% of cases. However, no studies have been made of the feasibility of discriminating Iberian breeds, i.e. distinguishing "black label" from "red label" dry-cured ham by using NIR Spectroscopy.

Taking all this into account, the aim of this study was to compare the ability of different spectroscopy devices to discriminate the breed (Iberian or Iberian x Duroc) of acorn-fed pigs and therefore the commercial category, using the final product (Iberian ham) without any prior preparation of the sample To do so, spectra of the samples were recorded only from the fat, only from the muscle, or from the whole slice with two benchtop NIR spectrometers (Büchi NIRFlex N-500 and Foss NIRSystem 5000) and five portable spectrometers including four portable NIR devices (MicroNIR 1700 ES, TellSpec Enterprise Sensor. microPHAZIR, and SCiO Sensor) and one RAMAN device (BRAVO handheld). The spectra obtained were evaluated and optimized by testing different mathematical pre-treatments and using RMS-x residual as the discriminating algorithm.

2. Material and methods

2.1. Samples

A total of 60 samples of ham were analyzed: 24 samples belonged to the 100% Iberian (100% Bellota) category, which are hams from 100% Iberian breed animals fed on acorns and grass in *montanera*. The other 36 samples were from the Iberian (Ibérico bellota) category, which are hams from crossbred animals (Iberian x Duroc) fed on acorns and grass in *montanera*. All the animals were reared in the same geographical area under the RD October 2014 certificate (RD 4/2014), and during the last part of the fattening period they were fed exclusively on pasture and acorns. The production and curing of the Iberian hams over 36 months was carried out by the industry itself (Guijuelo, Salamanca) in the traditional manner.

Dry-cured ham cuts including *Biceps femoris*, *Semimembranosus,* and *Semitendinosus* muscles were sliced 1 mm thick and all the slices were taken at approximately the same depth from the front of each ham.

100 g of dry-cured Iberian ham slices were vacuum-packed and kept at 6–8 °C until analysis without temperature fluctuations and in darkness to avoid alterations in the product, until NIR recording. Once the sample bag was opened on the same day and consecutively, the same sample was measured with the different spectrometer. The samples analyzed each day were chosen at random. Before recording, the ham packages were tempered for 2 h until the samples reached room temperature of 20 ± 2 °C. Four slices of each package were separated and placed one on top of each other in the same direction. The slices analyzed by Raman spectroscopy were placed on aluminum foil and the samples analyzed by NIR spectroscopy were placed on a black background and plastic foil.

2.2. Spectroscopic measurements

All samples were analyzed by NIR spectroscopy using two types of desktop equipment: Foss NIRSystem 5000 (Foss NIRSystems, Silver Spring MD) and Buchi NIRFlex N-500 (Büchi, Flawil, Switzerland); and four portable devices: MicroNIR 1700 ES (VIAVI, Milpitas, CA, USA); Enterprise Sensor (TellSpec, Toronto, Canada); SCiO Sensor (Consumer Physics, Tel Aviv, Israel) and microPHAZIR, (Thermo Fisher Scientific, Waltham, USA). Consecutively, the samples were analyzed by Raman spectroscopy using a portable device (BRAVO handheld, Bruker Optik GmbH; Ettlingen, Germany). The main characteristics and technical parameters of the devices were summarized in the supplementary material (S1).

The NIR spectra of the samples obtained using a Foss NIRSystem 5000 (Hillerod, Denmark) were recorded using a fiber-optic probe (1.5 m 210/210, Ref. n° R6539-A) coupled to a 5 cm × 5 cm window quartz. The spectra of the sample were recorded in the 9090-5000 cm^{-1} (1100–2000 nm) range at intervals of 9.06 cm^{-1} (2 nm), which means that a total of 451 reflectance datapoints were obtained for each sample, and 32 scans were performed for each recording. The window was applied to the surface of the ham directly at three different points of the slice (corresponding to the three muscles *Biceps femoris*, *Semimembranosus,* and *Semitendinosus)* to include both lean muscle and fat; the whole slice spectra were therefore obtained by averaging the three. The reference tests were carried out before starting the sample measurements.

The spectral measurements were taken by the benchtop spectrometer NIRFlex N-500 in the region of 10,000–4000 cm^{-1}, with 32 scans

collected per spectrum at a spectral resolution of 4 cm^{-1}. The spectra were measured using the fiber probe accessory 'Fiber Optic Solids' (Büchi, Flawil, Switzerland), which was directly applied to the surface of the slice. The measurement of the references was repeated after every 10 sample measurements.

The measurements with MicroNIR 1700 were taken with a default configuration setup; each spectrum constituted 125 spectral points between 11,013 and 5967 cm^{-1}, with average data spacing of 37 cm^{-1}. Black and white references were taken every 10 min, which is the instrument manufacturer's recommended parameter.

As previously reported by Beć et al. (2021a) the control software suites for both TellSpec Enterprise Sensor and SCiO Sensor do not allow any adjustment of the operational parameters of the instruments. Therefore, the spectra recorded with the TellSpec Enterprise Sensor were taken in the region of 11,111–5882 cm^{-1} with an average data spacing of 13 cm^{-1}, which resulted in 256 data points. In the case of the SCiO Sensor, the spectra were recorded in the 13,514–9346 cm^{-1} range and the spectra consisted of 331 spectral points. The reference tests were carried out before starting the sample measurements for both devices.

The NIR-spectra recorded using the miniaturized ThermoScientific microPHAZIR device were taken in the region of 6266 cm^{-1} to 4173 cm^{-1} with 10 scans collected per spectrum and. an average resolution of 21 cm^{-1}. This equipment collects an internal reference for each measurement.

For NIRFlex N-500 and all the portable devices, the window of the equipment was directly applied to the surface of the slice and six spectra on the lean muscle (two points from each of the three muscles *Biceps femoris*, *Semimembranosus,* and *Semitendinosus)* and four spectra on the fat (two sampling points in the internal fat with a surface area large enough to cover the recording window and another two spectra in the perimeter fat area) were taken for each slice. Subsequently the means of all the spectra were calculated to obtain the whole slice spectra.

The NIR measurements were performed in diffuse reflectance and as a first step the spectra were converted from reflectance (R) into log $1/R$.

Regarding Raman spectroscopy, the measurements were taken by the Bruker BRAVO Handheld Raman Spectrometer with 2000 ms integration time and 30 scans accumulated per spectrum; the measurement range is between 3200 and 400 cm^{-1} with average data spacing of 10–12 cm^{-1}. In this case, the spectra were recorded in five points of the fatty area of the slice.

2.3. Statistical analyses

Spectral pre-treatments were carried out and a discrimination method applied to the NIR and RAMAN data using WinISI 4.10 software (Infrasoft International, State Collee, PA, USA). The RMS-X residual method was carried out for classifying the samples according to breed using the whole or the split spectrum. The RMS-X residual method is a supervised pattern recognition method, as a member of a certain group or class is known in advance. The RMS(C) statistic is defined by the following formula (Shenk and Westerhaus, 1995), and consists in corroborating classes by using residual data.

In which y_{ij} is the log (1/R) of the subsample j for the wavelength I (λ_i), y_{ik} is the (log 1/R) of the subsample k or the wavelength I (λ_i), and n is the number of used wavelengths. This measuring is useful to detect a spectral variation dissimilar to the spectral variation in the set of data of the products of reference (Nørgaard et al., 2014).

The spectra had previously been subjected to the following spectral pre-treatments which were applied one by one and to each spectrum individually: Standard Normal Variate (SNV), Detrend Only (DT), or None to correct scattering phenomena. The aim pursued was thus to eliminate or reduce the effects which hinder the appropriate signal and to find the best discrimination between the samples (Nørgaard et al., 2014; Norris and Williams, 1984). The various mathematical treatments were encoded with 4 digits (a, b, c, d) (ISI, 2000), which means: a (the order of the derivative), b (the number of points at which the derivative is carried out), c (the number of points at which the first smoothing is carried out), and d (the number of points at which the second smoothing is carried out). The application of derivatives is one of the most widely used pre-treatments in NIR spectroscopy because of its ability to overcome the characteristic problems of this technique: band overlap and baseline shifts. The first derivative eliminates constant baseline shifts and the second derivative eliminates shifts which vary linearly with wavelength.

In order to study the classification capacity according to the breed (100% Iberian vs Iberian), data were divided into a training set containing 80% of the samples of each category to build the calibration models and a validation set including the remaining 20%. This procedure was randomly performed 3 times in order to perform an internal validation, and then, the mean and the standard deviation of the models were calculated. The best mathematical treatment for distinguishing between the samples was selected taking into account the highest mean percentage of correctly classified samples.

3. Results and discussion

3.1. Spectral characteristics of the samples

Fig. 1 shows the mean absorbance value of the untreated spectra registered with all NIR devices (Foss NIRSystem 5000, NIRFlex N-500, MicroNIR 1700 ES, TellSpec Enterprise Sensor, microPHAZIR, and SCiO Sensor) from muscle, fat and the mean which would reflect the whole slice spectra. Visual differences were observed between the spectra recorded from muscle, fat and the calculation for the whole spectra with the absorbance being higher in the former. This was more clearly observed for the NIRFlex N-500 and the TellSpec Sensor spectra in the bands observed at 1460 and 1960 nm and for the SCiO spectra in the band observed at 980 nm. According to Cozzolino and Murray (2004), this fact is related to the higher water content of the lean muscle, as absorption at these spectral bands is related to –OH third, second and first stretch overtones. However, the differences between registering points were lower at the maximums observed at 1200, 1740 and 2300 nm because these bands are associated with the fat content of the sample. Therefore, at around 1200 nm absorption bands are related to the –CH second overtone, at 1738 nm they are related to the $–CH_2$ stretch first overtone of both fat and fatty acids, and at 2310 nm with –CH combinations associated with the fat content and with saturated and unsaturated fatty acids (Murray, 1986). These peaks were more clearly observed in the fat spectra.

3.2. Spectra pre-treatment and chemometrics

The first step was to systematically evaluate different mathematical pre-treatments of the spectra so as to develop the best performance approach for each of the spectrometers and sample types: fat, muscle, or the whole slice. As mentioned above, derivatives (first and second derivatives) and smoothing were applied with a varying number of points, together with the Standard Normal Variate (SNV) or Detrend Only (DT) pre-treatments. In order to do so, the set of samples were divided into a calibration set (48 samples) and a validation set (12 samples) and the percentage of samples correctly classified according to the breed (100% Iberian vs Iberian) was calculated. This process was repeated three times with different calibration and validation sets and the means were then calculated. Fig. 2 shows the results of the optimization process for each of the devices tested and for the three types of spectral acquisition methods; muscle, fat, and the whole slice.

Fig. 2a shows the results for the two types of benchtop equipment used, i.e. NIRFlex N-500 and Foss NIRSystem 5000; the latter equipment is only able to record the spectra of the whole slice. It can be observed that both devices gave similar results and that the differences in the discrimination capability depending on the acquisition place were not significant. In general, better percentages of samples correctly classified

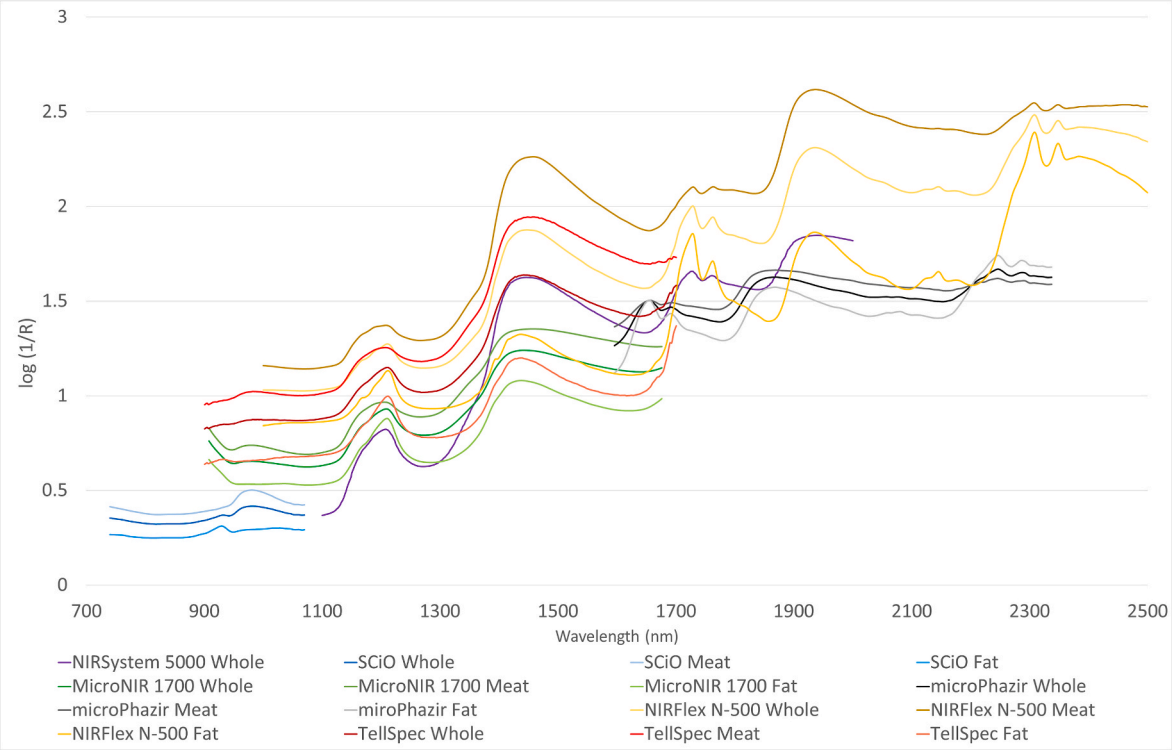

Fig. 1. Mean of the raw near-infrared spectra of all the samples measured on the three areas studied (lean meat, fat and whole slices) by the spectrometers used in this study.

in calibration were obtained when derivatives were applied to reach values of between 95 and 100%. This can be related to the fact that derivatives are a tool which allows the reduction of band overlap and baseline shift owing to the physical characteristics of the samples (Williams, 1987). However, as far as the validation set is concerned, it is noteworthy that the best results for NIRFlex N-500 were obtained when no derivative was applied, giving some specific treatment values of between 75 and 78%, while for the remainder of the treatments the discrimination rates were lower than 70%.

Regarding microPHAZIR (Fig. 2b), in general very high percentages (>97%) of samples correctly classified in the calibration group for fat, muscle, and the whole slice were obtained. However, the results for the validation group were very different depending on the registration point and there was not a clear trend regarding pre-treatment; the best results were between 80 and 70%. The analysis of the TellSpec Enterprise Sensor results (Fig. 2c) revealed that in contrast to the devices previously mentioned there were strong differences both in calibration and validation depending on the point of spectra registration. The best classification rates were therefore obtained with spectra from the whole slice showing percentages of correct classifications of 100% and 80% in calibration and validation respectively.

Fig. 2d shows the results of the optimization process for MicroNIR 1700; it can be noticed that the percentage of samples correctly classified were the lowest and no clear trend regarding the place of recording or the pre-treatment applied for satisfactory discrimination was observed. Finally, the results obtained with the SCiO device are shown in Fig. 2e. The application of the first and second derivatives significantly increased the number of samples correctly classified in both calibration and validation, especially the second derivative case. In general the best results were obtained from the whole slice with values of between 94 and 100% in calibration and between 80 and 92% in validation being reached. On the other hand, when the spectra were recorded from the fat the percentage of samples correctly classified in validation was less than 70%. These results pointed out that portable devices showed strong differences in the discrimination capability depending on the sample spot from which the spectra were recorded. Moreover, it is crucial to essay different sampling combinations to find the best one, especially for this type of equipment.

In the case of Raman, only the SNV and SNV Detrend treatment showed the ability to discriminate the sample type in the scope of this study (>90% in classification and between 60 and 80% in validation). If a pre-treatment was not applied or only the baseline correction was used (Detrend only) the discrimination was not possible as the samples were considered as unknown instead of being allocating to one of the two groups. Other studies have sought methods capable of eliminating the fluorescence problem inherent to the Raman technology for biological applications. In this context, the SNV treatment has also been successfully applied in biological samples to eliminate interferences (Afseth et al., 2006; Liland et al., 2016), while other studies used curve fitting of the broadband variation with a high-order polynomial based on the Savitzky-Golay method (Lieber and Mahadevan-Jansen, 2003; Chen et al., 2014; Wei et al., 2015). However, Martín-Gómez et al. (2021) were able to discriminate Iberian ham samples using Raman spectra without any mathematical pre-treatment by optimizing their own classification algorithm.

3.3. Discrimination of samples according to breed

Table 1 shows the results of the best predictive performance obtained for each of the spectrometers, indicating the best spectral pre-treatment based on the percentage of correctly classified samples in the calibration and validation steps (the mean of three repetitions). Well-fit models were achieved in calibration for all instruments and sampling points, with MicroNIR 1700 showing the lowest values while the test-set validation indicated lower predictive performance.

As for those devices which allow the recording of the NIR spectra in different tissues, the best one for acquiring spectra depended very much on the device. In general, the best results were obtained for the whole slice; in the case of the NIRSystem 5000 it was the only way to acquire spectra. For this type of sampling the SCiO and TellSpec tended to

Fig. 2. Percentage of the samples correctly classified in calibration (CAL) and validation (VAL) for the different devices a) NIRFlex N-500 and NIRSystem 5000, b) microPHAZIR, c) Tellspec, d) MicroNIR 1700 e) ScIO, and for the three types of recording: whole slices, lean meat or fat using different pre-treatments.

Table 1
Percentage of samples correctly classified in calibration (CAL) and validation (VAL) according to the breed for the different devices and the three type of recording: meat, fat or whole.

	Meat			Fat			Whole		
	Pre-treatment	CAL	VAL	Pre-treatment	CAL	VAL	Pre-treatment	CAL	VAL
NIRFlex N-500	SNV (0,0,1,1)	81%	78%	SNV Detrend (0,0,1,1)	90%	78%	Detrend (0,0,1,1)	83%	72%
NIRSystem 5000							SNV (2,4,4,1)	97%	67%
microPHAZIR	SNV Detrend (2,10,10,1)	89%	81%	SNV Detrend (1,4,4,1)	97%	75%	SNV Detrend (0,0,1,1)	99%	69%
ScIO	None (1,4,4,1)	97%	83%	Detrend (2,4,4,1)	97%	69%	Detrend (2,10,10,1)	97%	92%
Tellspec Enterprise	None (2,8,6,1)	85%	61%	Detrend (2,8,6,1)	80%	72%	None (2,4,4,1)	100%	81%
MicroNIR 1700	Detrend (2,8,6,1)	78%	61%	SNV (0,0,1,1)	72%	64%	Detrend (2,4,4,1)	77%	61%
Bravo				SNV Detrend (1,4,4,1)	96%	78%			

perform better. On the other hand, microPHAZIR showed better results for lean meat part, with this device and mainly the SCiO equipment giving the best results.

Regarding the fat tissue, in general the performance was lower but also less disproportionate between the instruments compared. The BRAVO device, which uses RAMAN spectroscopy, was the equipment which gave the best results followed by microPHAZIR, while NIRFlex N-500, in contrast to the remainder of the NIRS devices, showed a slightly better prediction capacity when the spectra were recorded from fat rather than from lean meat or the whole slice.

Previous results have shown that microPHAZIR showed a similar or even better discrimination capability than benchtop devices

(Zamora-Rojas et al., 2012), which coincides with the results obtained. On the other hand, these results differ from those previously reported by Kappacher et al. (2022) who found that better discrimination results from intact samples were obtained with NIRFlex N-500 and MicroNIR 1700. Other studies also indicated that MicroNIR 1700 showed a similar discrimination ability to benchtop devices (de Lima et al., 2018; Liu et al., 2018), which does not agree with the results of this study in which the worst results were obtained with this device. On the other hand, some studies also highlight the satisfactory discrimination percentages obtained with SCiO, while a lower discrimination capability was observed for the Enterprise Sensor (Kappacher et al., 2022). Therefore, this study agrees with this previous result for SCiO but not for TellSpec, which showed a satisfactory discrimination capability for fat tissue and for the whole slice registering area.

3.4. Optimization of the spectral range

Fig. 3 shows the mean spectra of 100% Iberian and Iberian breed ham samples after the mathematical pretreatment which allowed the best discrimination results according to breed purity for two of the devices used in this study (Foss NIRSystem 5000 and NIRFlex N-500, spectra of the whole slice). The figure allows us to appreciate that for some spectral ranges the differences between the two groups were clearer (1144–1752 nm for Foss5000; 1700–2019 and 2086–2500 nm for NIRFlex N-500), while for other zones of the spectra there were no visible differences. Taking this into account, these zones were discarded and the RMSX-residuals were applied again to the selected spectral ranges showing greater differences between groups. This process was carried out for all spectrometers and recording zones and the results of the discrimination capability in both the calibration and validation groups are shown in Table 2.

The results showed that the spectral range selected varied among devices. It was also observed that the predictive performance improved mainly when the whole slice spectra were considered. Therefore, NIRFlex N-500 and Foss NIRSystem 5000 showed a significant increase in the discrimination capability in the validation group followed by microPhazir, while MicroNIR 1700 only showed higher values for the calibration group. This result indicates that some intervals could provide "noise" for the spectral information and lowers the percentage of correctly classified samples as previously observed for Iberian fat analysis (Hernández-Jiménez et al., 2021b).

However, for lean meat the increase in the percentage of samples

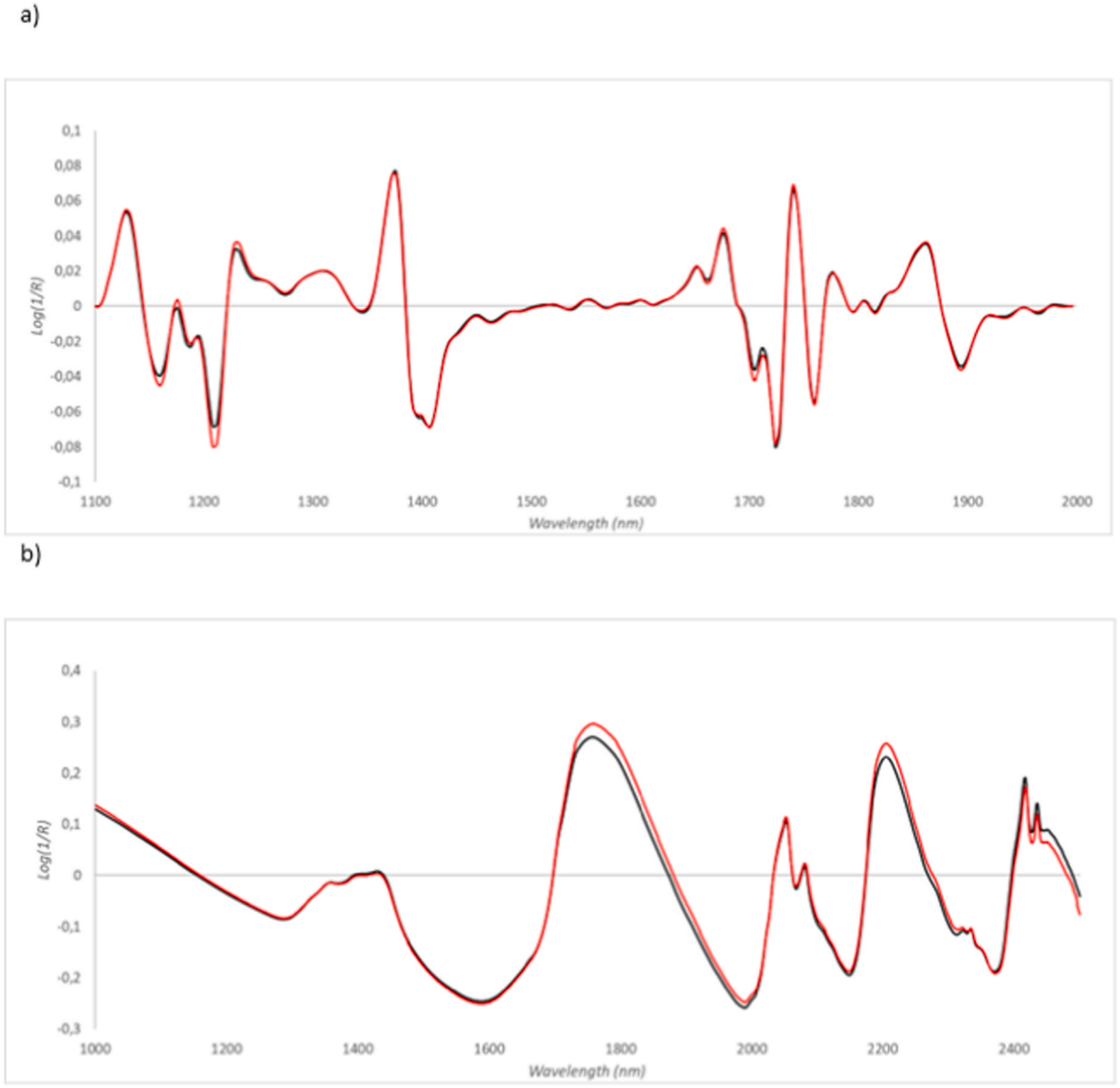

Fig. 3. Mean spectra of whole slices of 100% Iberian (black) and Iberian ham (red) samples after pre-treatment a) NIRSystem 5000 (SNV (2,4,4,1)) b) NIRFlex N-5000 (Detrend, 0,0,1,1). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Percentage of samples correctly classified after spectral range selection in calibration (CAL) and validation (VAL) according to the breed for the different devices and the three type of recording: meat, fat or whole.

	Meat			Fat			Whole		
	Spectral range	CAL	VAL	Spectral range	CAL	VAL	Spectral range	CAL	VAL
NIRFlex N-500	1000-1689; 2248-2500	82%	67%	1380-2027; 2145-2495	84%	75%	1700-2019; 2056-2500	83%	80%
NIRSystem 5000							1144–1752	95%	75%
microPHAZIR	1639–2238	89%	81%	1526-2001; 2169-2314	95%	72%	1673–2337	100%	72%
ScIO	844–1046	95%	79%	869–1040	88%	64%	850–1020	96%	92%
Tellspec Enterprise	1113-1265; 1331–1480; 1635-1673	74%	50%	1123–1559	71%	53%	1146–1683	99%	67%
MicroNIR 1700	1081–1434	74%	72%	1155–1614	72%	64%	932-1064; 1137–1378; 1428-1657	81%	61%
Bravo				1440–1896	94%	75%			

correctly classified was only remarkable for MicroNIR-1700 while there were no differences when the spectra were recorded from a fat surface; this shows that noise interference may vary between devices and sampling points.

4. Conclusions

This study indicates that it was possible to discriminate ham samples according to breed purity using NIR and Raman spectroscopy which are rapid, non-invasive and inexpensive techniques. Portable devices showed a suitable performance, specially SCiO equipment when the recording was made on lean meat and microPHAZIR when the whole slice was considered, while for the recording of the spectra on fat tissue the Raman technology showed the best results followed by microPHAZIR, pointing out that handheld spectrometers showed the ability to measure characteristic absorption regions of the chemical constituent relevant for the discrimination. In fact, the selection of the spectral range used for discrimination was only useful for whole slice recording and especially for benchtop equipment. Moreover, the discrimination capacity depended on the spectra recording area being in general the whole slice spectra, calculated as the mean value of the spectra obtained for fat and lean meat, the best one. The results point out that portable equipment may be an alternative to benchtop devices for the classification of Iberian pork products, however more samples should be analyzed in order to have more robust and reliable results.

Funding

This research was funded by Salamanca County Council (Spain) under grant number 18VEUH 463AC06.

CRediT authorship contribution statement

Miriam Hernández-Jiménez: Formal analysis, Investigation, Visualization. **Isabel Revilla:** Conceptualization, Supervision, Project administration, Funding acquisition, Writing – original draft. **Ana M. Vivar-Quintana:** Funding acquisition, Writing – review & editing. **Justyna Grabska:** Software, Validation, Writing – review & editing. **Krzysztof B. Beć:** Methodology, Software, Validation, Writing – review & editing. **Christian W. Huck:** Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

We are grateful to “Carrasco Ibéricos” Guijuelo (Salamanca) for their collaboration and Hernández-Jiménez M. is grateful to the Own Program III: Grants for Pre-doctoral Contracts of the University of Salamanca co-funded by Banco Santander and the E-Cost Grant Short-Term Scientific Mission (Ref.: CA-19145).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2024.100675.

References

Afseth, N.K., Segtnan, V.H., Wold, J.P., 2006. Raman spectra of biological samples: a study of preprocessing methods. Appl. Spectrosc. 60 (12), 1358–1367.
Antequera, T., Martín, L., Ruiz, J., Cava, R., Timón, M., Ventanas, J., 1996. Differentiation of Iberian Hams from Iberian and Iberian Duroc Pigs by Analysis of Volatile Aldehydes, 42nd. ICoMST. Matforsk, Norway, pp. 491–492.
Beć, K.B., Grabska, J., Huck, C.W., 2021a. Principles and applications of miniaturized near-infrared (NIR) spectrometers. Chem. Eur J. 27 (5), 1514–1532. https://doi.org/10.1002/chem.202002838.
Beć, K.B., Grabska, J., Plewka, N., Huck, C.W., 2021b. Insect protein content analysis in handcrafted fitness bars by NIR spectroscopy. Gaussian process regression and data fusion for performance enhancement of miniaturized cost-effective consumer-grade sensors. Molecules 26 (21). https://doi.org/10.3390/molecules26216390. Article 21.
Beć, K.B., Grabska, J., Siesler, H.W., Huck, C.W., 2020. Handheld near-infrared spectrometers: where are we heading? NIR News 31 (3–4), 28–35. https://doi.org/10.1177/0960336020916815.
Carrapiso, A.I., García, C., 2005. Instrumental colour of Iberian ham subcutaneous fat and lean (biceps femoris): influence of crossbreeding and rearing system. Meat Sci. 71 (2), 284–290. https://doi.org/10.1016/j.meatsci.2005.03.024.
Carrapiso, A.I., Bonilla, F., García, C., 2003a. Effect of crossbreeding and rearing system on sensory characteristics of Iberian ham. Meat Sci. 65 (1), 623–629. https://doi.org/10.1016/S0309-1740(02)00256-5.
Carrapiso, A.I., Jurado, A., García, C., 2003b. Note: effect of crossbreeding and rearing system on Iberian ham volatile compounds. Food Sci. Technol. Int. 9 (6), 421–426. https://doi.org/10.1177/1082013203040396.
Cava, R., Ruiz, J., López-Bote, C., Martín, L., García, C., Ventanas, J., Antequera, T., 1997. Influence of finishing diet on fatty acid profiles of intramuscular lipids, triglycerides and phospholipids in muscles of the Iberian pig. Meat Sci. 45 (2), 263–270. https://doi.org/10.1016/S0309-1740(96)00102-7.
Chen, K., Zhang, H., Wei, H., Li, Y., 2014. Improved Savitzky–Golay-method-based fluorescence subtraction algorithm for rapid recovery of Raman spectra. Appl. Opt. 53 (24), 5559–5569. https://doi.org/10.1364/AO.53.005559.
Cozzolino, D., Murray, I., 2004. Identification of animal meat muscles by visible and near infrared reflectance spectroscopy. LWT–Food Sci. Technol. 37 (4), 447–452. https://doi.org/10.1016/j.lwt.2003.10.013.
de Lima, G.F., Andrade, S.A.C., da Silva, V.H., Honorato, F.A., 2018. Multivariate classification of UHT milk as to the presence of lactose using benchtop and portable NIR spectrometers. Food Anal. Methods 11 (10), 2699–2706. https://doi.org/10.1007/s12161-018-1253-7.
Dixit, Y., Pham, H.Q., Realini, C.E., Agnew, M.P., Craigie, C.R., Reis, M.M., 2020. Evaluating the performance of a miniaturized NIR spectrophotometer for predicting intramuscular fat in lamb: a comparison with benchtop and hand-held Vis-NIR spectrophotometers. Meat Sci. 162, 108026 https://doi.org/10.1016/j.meatsci.2019.108026.
Fuentes, V., Ventanas, S., Ventanas, J., Estévez, M., 2014. The genetic background affects composition, oxidative stability and quality traits of Iberian dry-cured hams: purebred Iberian versus reciprocal Iberian×Duroc crossbred pigs. Meat Sci. 96 (2), 737–743. https://doi.org/10.1016/j.meatsci.2013.10.010. Part A).

García, D., Martínez, A., Dunner, S., Vega-Pla, J.L., Fernández, C., Delgado, J.V., Cañón, J., 2006. Estimation of the genetic admixture composition of Iberian dry-cured ham samples using DNA multilocus genotypes. Meat Sci. 72 (3), 560–566. https://doi.org/10.1016/j.meatsci.2005.09.005.

Garrido-Varo, A., Vega, S., Maroto-Molina, F., Haba, M. J. D. la, Pérez-Marín, D., 2016. On-site quality control of processed land animal proteins using a portable micro-electro-mechanical-systems near infrared spectrometer. J. Near Infrared Spectrosc. 24 (1), 47–58. https://opg.optica.org/jnirs/abstract.cfm?URI=jnirs-24-1-47.

González-Martín, M.I., Escuredo, O., Hernández-Jiménez, M., Revilla, I., Vivar-Quintana, A.M.A., Martínez-Martín, I., Hernández-Ramos, P., 2021. Prediction of stable isotopes and fatty acids in subcutaneous fat of Iberian pigs by means of NIR: a comparison between benchtop and portable systems. Talanta 224, 121817. https://doi.org/10.1016/j.talanta.2020.121817.

Hernández-Jiménez, M., González-Martín, M.I., Martínez-Martín, I., Revilla, I., Vivar-Quintana, A.M., 2021a. Carbon stable isotopes, fatty acids and the use of NIRS to differentiate Iberian pigs. Meat Sci. 182, 108619 https://doi.org/10.1016/j.meatsci.2021.108619.

Hernández-Jiménez, M., Revilla, I., Arce, L., Cardador, M.J., Ríos-Reina, R., González-Martín, I., Vivar-Quintana, A.M., 2021b. Authentication of the montanera period on carcasses of Iberian pigs by using analytical techniques and chemometric analyses. Animals 11 (9). https://doi.org/10.3390/ani11092671. Article 9.

Horcada, A., Valera, M., Juárez, M., Fernández-Cabanás, V.M., 2020. Authentication of Iberian pork official quality categories using a portable near infrared spectroscopy (NIRS) instrument. Food Chem. 318, 126–133. https://doi.org/10.1016/j.foodchem.2020.126471.

Huck, C.W., Bec, K.B., Grabska, J., 2022. Current status and future trends in sensor miniaturization. In: Chu, X., Guo, L., Huang, Y., Yuan, H. (Eds.), Sense the Real Change: Proceedings of the 20th International Conference on Near Infrared Spectroscopy Springer Nature, pp. 59–72. https://doi.org/10.1007/978-981-19-4884-8_5.

ISI, 2000. Manual FOSS NIRSystems. In the Complete Software Solution Using a Single Screen for Routine Analysis, Robust Calibrations, and Networking (Infrasoft International:Silver Spring).

Kademi, H.I., Ulusoy, B.H., Hecer, C., 2019. Applications of miniaturized and portable near infrared spectroscopy (NIRS) for inspection and control of meat and meat products. Food Rev. Int. 35 (3), 201–220. https://doi.org/10.1080/87559129.2018.1514624.

Kappacher, C., Trübenbacher, B., Losso, K., Rainer, M., Bonn, G.K., Huck, C.W., 2022. Portable vs. Benchtop NIR-sensor technology for classification and quality evaluation of black truffle. Molecules 27 (3). https://doi.org/10.3390/molecules27030589. Article 3.

Kucha, C.T., Ngadi, M.O., 2020. Rapid assessment of pork freshness using miniaturized NIR spectroscopy. J. Food Meas. Char. 14 (2), 1105–1115. https://doi.org/10.1007/s11694-019-00360-9.

Lieber, C.A., Mahadevan-Jansen, A., 2003. Automated method for subtraction of fluorescence from biological Raman spectra. Appl. Spectrosc. 57 (11), 1363–1367.

Liland, K.H., Kohler, A., Afseth, N.K., 2016. Model-based pre-processing in Raman spectroscopy of biological samples. J. Raman Spectrosc. 47 (6), 643–650. https://doi.org/10.1002/jrs.4886.

Liu, N., Parra, H.A., Pustjens, A., Hettinga, K., Mongondry, P., van Ruth, S.M., 2018. Evaluation of portable near-infrared spectroscopy for organic milk authentication. Talanta 184, 128–135. https://doi.org/10.1016/j.talanta.2018.02.097.

Martín-Gómez, A., Arroyo-Manzanares, N., García-Nicolás, M., López-Lorente, Á.I., Cárdenas, S., López-García, I., Viñas, P., Hernández-Córdoba, M., Arce, L., 2021. Portable Raman spectrometer as a screening tool for characterization of Iberian dry-cured ham. Foods 10 (6). https://doi.org/10.3390/foods10061177. Article 6.

Martín-Gómez, A., Arroyo-Manzanares, N., Rodríguez-Estévez, V., Arce, L., 2019. Use of a non-destructive sampling method for characterization of Iberian cured ham breed and feeding regime using GC-IMS. Meat Sci. 152, 146–154. https://doi.org/10.1016/j.meatsci.2019.02.018.

McGrath, T.F., Haughey, S.A., Islam, M., Elliott, C.T., Kelly, S.D., Suman, M., Rindy, T., Taous, F., García-González, D.L., Singh, D., Nan Wai, S., Zitek, A., Jandric, Z., Almirall, J., Acosta, A., Thao, C., Islam, M., Hoo Fung, L., Kim, J.-K., Handagiripathira, H.M.N.L., 2021. The potential of handheld near infrared spectroscopy to detect food adulteration: results of a global, multi-instrument inter-laboratory study. Food Chem. 353, 128718 https://doi.org/10.1016/j.foodchem.2020.128718.

Murray, I., 1986. The NIR spectra of homologous series of organic compounds. Proceedings of the International Near Infrared Diffuse Reflectance/Transmittance Spectroscopy Conference, pp. 13–28.

Nørgaard, L., Westerhaus, M., Kjeldahl, K., Haunstrup, I., 2014. Qualification: adulteration screening with NIR- a case on skim milk powder. P/N, 1026716. Issue 1.

Norris, K., Williams, P., 1984. Optimization of mathematical treatments of raw near-infrared signal in the. Cereal Chem. 61 (2), 158–165.

Pajuelo, A., Sánchez, S., Pérez-Palacios, T., Caballero, D., Díaz, J., Antequera, T., Marcos, C.F., 2022. 1H NMR to analyse the lipid profile in the glyceride fraction of different categories of Iberian dry-cured hams. Food Chem. 383, 132371 https://doi.org/10.1016/j.foodchem.2022.132371.

Patel, N., Toledo-Alvarado, H., Bittante, G., 2021. Performance of different portable and hand-held near-infrared spectrometers for predicting beef composition and quality characteristics in the abattoir without meat sampling. Meat Sci. 178, 108518 https://doi.org/10.1016/j.meatsci.2021.108518.

Patel, N., Toledo-Alvarado, H., Cecchinato, A., Bittante, G., 2020. Predicting the content of 20 minerals in beef by different portable near-infrared (NIR) spectrometers. Foods 9 (10). https://doi.org/10.3390/foods9101389. Article 10.

Pérez-Marín, D.C., Torres, I., Entrenas, J.A., Garrido-Varo, A., 2022. Breed authentication in Iberian pork meat analysed in situ using Near Infrared Spectroscopy. Proc. Sensing for Agriculture and Food Quality and Safety XIV. https://doi.org/10.1117/12.2619802. PC1212003.

Petrón, M.J., Muriel, E., Timón, M.L., Martín, L., Antequera, T., 2004. Fatty acids and triacylglycerols profiles from different types of Iberian dry-cured hams. Meat Sci. 68 (1), 71–77. https://doi.org/10.1016/j.meatsci.2004.01.012.

Piotrowski, C., Garcia, R., Garrido-Varo, A., Pérez-Marín, D., Riccioli, C., Fearn, T., 2019. Short Communication: the potential of portable near infrared spectroscopy for assuring quality and authenticity in the food chain, using Iberian hams as an example. Animal 13 (12), 3018–3021. https://doi.org/10.1017/S1751731119002003.

Plans, M., Simó, J., Casañas, F., Sabaté, J., Rodriguez-Saona, L., 2013. Characterization of common beans (Phaseolus vulgaris L.) by infrared spectroscopy: comparison of MIR, FT-NIR and dispersive NIR using portable and benchtop instruments. Food Res. Int. 54 (2), 1643–1651. https://doi.org/10.1016/j.foodres.2013.09.003.

Prieto, N., Dugan, M.E.R., Juárez, M., López-Campos, Ó., Zijlstra, R.T., Aalhus, J.L., 2018. Using portable near-infrared spectroscopy to predict pig subcutaneous fat composition and iodine value. Can. J. Anim. Sci. 98 (2), 221–229. https://doi.org/10.1139/cjas-2017-0033.

Pu, Y., Pérez-Marín, D., O'Shea, N., Garrido-Varo, A., 2021. Recent advances in portable and handheld NIR spectrometers and applications in milk, cheese and dairy powders. Foods 10 (10). https://doi.org/10.3390/foods10102377. Article 10.

RD 4/2014, de 10 de enero, por el que se aprueba la norma de calidad para la carne, el jamón, la paleta y la caña de lomo ibérico.

Ruiz, J., Cava, R., Antequera, T., Martín, L., Ventanas, J., López-Bote, C.J., 1998. Prediction of the feeding background of Iberian pigs using the fatty acid profile of subcutaneous, muscle and hepatic fat. Meat Sci. 49 (2), 155–163. https://doi.org/10.1016/S0309-1740(97)00136-8.

Savoia, S., Albera, A., Brugiapaglia, A., Di Stasio, L., Ferragina, A., Cecchinato, A., Bittante, G., 2020. Prediction of meat quality traits in the abattoir using portable and hand-held near-infrared spectrometers. Meat Sci. 161, 108–117. https://doi.org/10.1016/j.meatsci.2019.108017.

Shenk, J., Westerhaus, M., 1995. Routine operation, calibration development and network system management manual for near infrared instruments version 3. 1. Infrasoft International.

Sun, Y., Wang, Y., Huang, J., Ren, G., Ning, J., Deng, W., Li, L., Zhang, Z., 2020. Quality assessment of instant green tea using portable NIR spectrometer. Spectrochim. Acta Mol. Biomol. Spectrosc. 240, 118576 https://doi.org/10.1016/j.saa.2020.118576.

Tejeda, J.F., Gandemer, G., Antequera, T., Viau, M., García, C., 2002. Lipid traits of muscles as related to genotype and fattening diet in Iberian pigs: total intramuscular lipids and triacylglycerols. Meat Sci. 60 (4), 357–363. https://doi.org/10.1016/S0309-1740(01)00143-7.

Valand, R., Tanna, S., Lawson, G., Bengtström, L., 2020. A review of Fourier Transform Infrared (FTIR) spectroscopy used in food adulteration and authenticity investigations. Food Addit. Contam. 37 (1), 19–38. https://doi.org/10.1080/19440049.2019.1675909.

Ventanas, S., Ventanas, J., Jurado, Á., Estévez, M., 2006. Quality traits in muscle biceps femoris and back-fat from purebred Iberian and reciprocal Iberian×Duroc crossbred pigs. Meat Sci. 73 (4), 651–659. https://doi.org/10.1016/j.meatsci.2006.03.009.

Wei, D., Chen, S., Liu, Q., 2015. Review of fluorescence suppression techniques in Raman spectroscopy. Appl. Spectrosc. Rev. 50 (5), 387–406. https://doi.org/10.1080/05704928.2014.999936.

Williams, P., 1987. Variables affecting near-infrared reflectance spectroscopic analysis. Near-Infrared Technology in the Agricultural and Food Industries 143–167.

Yu, H., Liu, H., Wang, Q., van Ruth, S., 2020. Evaluation of portable and benchtop NIR for classification of high oleic acid peanuts and fatty acid quantitation. LWT–Food Sci. Technol. 128, 109398 https://doi.org/10.1016/j.lwt.2020.109398.

Zamora-Rojas, E., Garrido-Varo, A., De Pedro-Sanz, E., Guerrero-Ginel, J.E., Pérez-Marín, D., 2013. Prediction of fatty acids content in pig adipose tissue by near infrared spectroscopy: at-line versus in-situ analysis. Meat Sci. 95 (3), 503–511. https://doi.org/10.1016/j.meatsci.2013.05.020.

Zamora-Rojas, E., Pérez-Marín, D., De Pedro-Sanz, E., Guerrero-Ginel, J.E., Garrido-Varo, A., 2012. In-situ Iberian pig carcass classification using a micro-electro-mechanical system (MEMS)-based near infrared (NIR) spectrometer. Meat Sci. 90 (3), 636–642. https://doi.org/10.1016/j.meatsci.2011.10.006.