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# Comparison of Miniaturized and Benchtop NIR Spectrophotometers for Quantifying the Fatty Acid Profile of Iberian Ham

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**Featured Application: This work shows that it is possible to predict the fatty acid profile of Iberian ham, which is one of the most important quality characteristics of these premium products, by using miniaturized devices. This article also identifies the most suitable equipment and the optimal area for spectral recording, which depends on the device, to achieve optimal analytical performance.**

**Abstract:** Iberian ham is a highly valued product, and considerable efforts have been made to characterize it quickly and accurately. In this scenario, portable NIR devices could provide an effective solution for the assessment of its attributes. However, the calibration quality of NIR equipment is directly influenced by the relevance of the used spectral region. Therefore, this study aims to evaluate the suitability of different NIR spectrometers, including four portable and one benchtop instrument, with varying spectral working ranges for quantifying the fatty acid composition of Iberian ham. Spectral measurements were carried out on both the muscle and the fat of the ham slices. The results showed that 24 equations with an RSQ > 0.5 were obtained for both the muscle and fat for the NIRFlex N-500 benchtop instrument, while 19 and 14 equations were obtained in the muscle and 16 and 10 equations in the fat for the Enterprise Sensor and MicroNIR, respectively. In general, more fatty acids could be calibrated when the spectra were taken from lean meat, except with the SCiO Sensor. Measurements performed in the lean and fat zones delivered complementary information. These initial findings indicate the suitability of using miniaturized NIR sensors, which are faster, are less expensive, and enable on-site measurements, for analyzing fatty acids in Iberian ham.

**Keywords:** Near Infrared spectroscopy; benchtop device; portable device; fat spectra; meat spectra; optimizing forecast; fast analysis



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## 1. Introduction

Near Infrared (NIR) spectroscopy has emerged as an extremely useful tool in the meat and meat product industry, equally for the analysis of raw meat and the products derived from it [1]. Factors contributing to this include its high speed of analysis, minimal or no sample preparation, and its non-destructive, non-invasive methodology [2]. Its ability to perform non-destructive measurements means less product waste, which not only reduces costs but also contributes to more sustainable practices in the food industry [3]. Another important aspect is the versatility of this equipment, which can be adapted to various types of meat, meat derivatives, and processing conditions [4]. NIR spectroscopy equipment has been successfully applied to the quantitative determination of the main constituents of meat and meat products such as moisture, fat, and protein [5–7], being approved by the

Association of Analytical Chemists (AOAC) as a method for the commercial analysis of these parameters in meat and meat products [8].

Recently developed portable NIR spectroscopy equipment plays a crucial role in offering an efficient and versatile solution for the rapid and accurate assessment of various meat attributes [9]. However, as reported by Kademi et al. [10], the results obtained with these instruments in meat and meat products are unrealistic in some applications and exhibit higher detection limits and lower sensitivity compared to benchtop equipment. Difficulties in using portable equipment may be related to the significant influence of the spectral region on the predictive ability of this technology [11]. In addition, some spectral regions may be particularly subject to interference due to the presence of other substances in the sample [12]; therefore, choosing the correct spectral region can minimize interference and improve the selectivity of the analysis. For this reason, the quality of calibration and modeling of NIR equipment is directly related to the spectral region used.

As part of the meat industry, the pork meat sector is of significant importance worldwide, both from an economic and food point of view. Pork is an essential source of protein and nutrients, and its production and consumption have grown steadily in recent decades [13]. In the specific case of the pork sector, NIR spectroscopy has been successfully applied for the prediction of meat composition parameters such as protein, fat, and moisture [14]; intramuscular fat [15] and Warner–Bratzler shear force [16]; and pH and color values [17]. However, it has not proved useful for the prediction of drip loss on intact carcasses [15]. In pork meat derivatives, this technology has been applied to predict fat, moisture, and protein in chopped pork sausage mixes [18] and sodium content in commercial meat products [19] among other applications.

In the Spanish meat sector, Iberian pork is known for its distinctive quality and flavor, influenced by factors such as genetics, rearing systems, and especially feed, which may be based on fodder or the *montanera* system, i.e., free grazing with the consumption of acorns and grass [20]. This combination makes Iberian ham a product highly appreciated by consumers, 100% Iberian acorn-fed being the most preferred [21]. Portable NIR instruments have been used in Iberian pork to classify individual carcasses by feeding regime [2], to discriminate animals by fat content and fatty acid profile [22], to classify animals according to legal standards and requirements [23] and for the analysis of the fatty acid content of individual carcasses of Iberian pigs [24,25]. Among the meat derivatives of the Iberian pig, Iberian ham is highly valued both nationally and internationally. The portable equipment has already been applied to classify Iberian ham according to commercial categories [26], pig breed [27], and NaCl content [28]. The determination of the fatty acid composition is commonly used to assess both the quality and authenticity of Iberian ham [29]. This determination is usually carried out by gas chromatography, which is a slow method and involves the use of chemical reagents. The application of NIR technology in the determination of this parameter would be particularly interesting to reduce the time and cost of analysis.

The present work aims to evaluate the suitability of different portable NIR spectrometers for the quantification of fatty acid composition in Iberian pork hams. For this purpose, four different portable devices and one benchtop instrument, differentiated by the implemented optical solutions and distinct spectral working ranges, have been evaluated and compared based on their analytical performance in this application. The spectral measurements have been carried out on both the muscle and fat of the ham, with both used separately for the development of predictive models. The hypothesis of this study is that miniaturized NIR devices can perform comparably to benchtop models in fatty acid prediction. The identification of the most suitable equipment for this analysis is of great importance not only from a research point of view, pushing the frontier of the application of modern miniaturized sensors combined with artificial intelligence, but also for the production sector itself. On-site NIR technology is already being adapted as an in-line analysis tool; the method for the determination of fatty acids as proposed in this work is

readily suited to be easily implemented to deliver additional important quality parameters in routine industrial analysis.

## 2. Materials and Methods

### 2.1. Samples

A total of 60 Iberian ham samples, consisting of 24 acorn-fed 100% Iberian purebred and 36 acorn-fed crossbred (Iberian × Duroc) hams, were analyzed. All animals were raised in the same geographical area, adhering to the RD 10/2014 certificate [30]. During the latter part of the fattening period, their diet exclusively consisted of pasture and acorns. The industry itself, situated in Guijuelo, Salamanca, undertook the production and curing of the Iberian hams following traditional practices [31].

After 36 months of maturation, the sampling process was carried out as follows: once the bone had been removed, a total of 100 g of sample was taken (in slices 1 mm thick) from each of the 60 hams. The samples were cut in the central area of the ham piece along its entire transverse profile. The cut was taken perpendicular to the ham bone at the same depth from the front of each ham. The cuts included the *Biceps femoris*, *Semimembranosus,* and *Semitendinosus* muscles and had a similar lean/fat muscle ratio. The ham slices were vacuum-packed and kept at 6–8 °C until analysis for a maximum of 4–6 months.

### 2.2. Reference Analysis

The analysis of the lipid profile was carried out by extracting the intramuscular fat of the *Biceps femoris* muscle after removing the adjacent intermuscular fat. The extraction was carried out on 25 g of the muscle using the method of Folch et al. (1957) [32], and the quantification of fatty acids (FAs) was carried out on 0.1 g of extracted fat. Afterwards, methylation and gas chromatography analysis were carried out according to the method described by Hernández-Jiménez et al. [33]. Briefly, methylation using methanolic KOH was performed on 0.1 g of extracted fat. A GC 6890 N (Agilent Technologies, Santa Clara, CA, USA) chromatograph equipped with an automatic injector 7683 (Agilent Technologies) and a fused silica capillary column (100 m × 0.25 mm; 0.20 µm) (SP-2560, Supelco, Inc., Bellefonte, PA, USA) were used. The injector and detector were maintained at 250 °C. The column oven temperature was 150 °C, and the temperature was increased 1 °C/min to 165 °C, then 0.20 °C/min to 167 °C, and finally increased 1.50 °C/min to 225 °C over 15 min. The carrier gas was helium, supplied at a flow rate of 1 mL/min. The injection volume was 1 µL in splitless mode. The different fatty acids were identified by their retention times using a mixture of fatty acid standards (47885-U Supelco, Sigma-Aldrich, Steinheim, Germany). Fatty acid content was calculated using the peak areas of the chromatograms and expressed as grams per 100 g of total fatty acid methyl esters.

### 2.3. Spectroscopy Measurements

The measurement protocol involved pre-conditioning the samples for two hours prior to analysis, ensuring they reached a room temperature of 20 ± 2 °C, to guarantee the quality and repeatability of the recorded spectra [1]. Subsequently, four slices from each package were segregated and stacked in alignment. The samples subjected to NIR spectroscopy were placed on a black cardboard covered with a PVC plastic film to make the background uniform for analysis.

The measurements were taken using four portable NIR spectrometers: MicroNIR 1700 ES (VIAVI, Milpitas, CA, USA), Enterprise Sensor (TellSpec, Toronto, ON, Canada), SCiO Sensor (Consumer Physics, Tel Aviv, Israel), and microPHAZIR (Thermo Fisher Scientific, Waltham, MA, USA). A benchtop spectrometer, the NIRFlex N-500 (Büchi, Flawil, Switzerland), chosen for its robustness and good performance in various applications, was also used as a reference for the evaluation of the portable devices [34–36]. Each instrument features distinct technical specifications, levels of portability, and key functional elements. From the optical point of view, predominantly they have different wavelength selectors, which largely define the operational spectral ranges and overall sizes of the

sensors. It should be noted that all the instruments used are equipped with a tungsten halogen lamp and InGaAs detector except the SCiO Sensor, which features an LED lamp and Si photodiode detector. The recording configurations used were those recommended for each device as reported by Hernández-Jiménez et al. [27], who also provide detailed characteristics of the key attributes of these instruments.

For all the devices, the detector window was applied directly to the surface of the same slice for static recording. To avoid the possible evaporation of water in the samples, the measurements were taken consecutively with all the equipment. Spectra were measured at six points on the lean muscle and four points on the fat for each slice. For each recording area, the mean of all replicates was calculated to obtain an average spectrum of the fat and an average spectrum of the lean meat for each of the 60 sliced ham samples.

### *2.4. Statistical Analyses*

#### 2.4.1. Spectra Pre-Treatment

To compare the predictive performance of the different instruments, quantitative models were developed by applying the same spectral treatments to the sets of spectra obtained by all of the evaluated spectrometers. The spectra obtained in lean muscle and those obtained in fat were processed and compared independently. The spectra were expressed in absorbance values by calculating log(1/R) with R being the reflectance values. The software used to develop all the models was WinISI 4.10.

The wavelength range of each of the five evaluated instruments is different; the full measurement range that each instrument can record was used in this study. The replicate spectra recorded for each sample were averaged to obtain a single spectrum per sample and instrument. Different combinations of spectra pre-treatments aimed at the suppression of scattering effects (Standard Normal Variate (SNV), DeTrend (DT), and SNV + DT, and effective resolution enhancement combined with smoothing (first- and second-order derivatives by the Savitzky–Golay algorithm) were applied to the spectra included in the calibration set. These pre-treatments reduce anomalies and noise and thus improve the predictive capability of the model, neutralize wavelength-dependent trends, and better expose the analytical information present in the spectra [37,38].

The mathematical treatments applied with WinISI software are described by four digits (a,b,c,d), where a is the derivative order, b is the derivative gap, c is the smoothing segment, and d is a second smoothing segment. In this study, the mathematical treatments applied were the following: without derivative (0,0,1,1), with a first derivative (1,5,5,1) and two second derivatives with different segments (2,5,5,1) and (2,10,10,1), based on the results obtained in previous research [39,40].

#### 2.4.2. Development of Calibration Models

The predictive model used was partial least squares (MPLS) regression, a supervised dimensionality reduction technique for solving regression problems. It extracts latent variables that capture joint variation between the data (X) and a target variable (y). These latent variables are maximally predictive of y, allowing for efficient analysis with reduced dimensions. The involved dimensionality reduction step itself is similar to the principal component analysis (PCA) as the samples are projected on the score space; the difference is that the relationship between the data (independent variables) and a target variable during dimension reduction is considered [41]. This also enables the detection of outliers according to the Mahalanobis distance (H) of each sample from the population center [42]. In the present study, the spectra with an H-distance > 3.5 were considered outliers. Outlying samples were also eliminated for chemical reasons under the T-criterion, i.e., those samples that showed significant differences between the reference value and the predicted value for a T-value $\geq$ 2.5 [43].

The number of latent variables was chosen to minimize calibration and cross-validation errors and was different depending on the constituent to be calibrated. For this work, a maximum of 9 latent variables were prefixed for the generation of the models, and

a maximum of two passes were carried out to eliminate spectral and chemical outliers using the H- and T-criteria, respectively. The predictive ability of the model was tested by cross-validation. In analytical problems with the number samples similar to those available in this study ($n \leq 60$), leave-one-out cross-validation (LOO CV) is often recommended [44] without the need for external validation. However, LOO CV is computationally heavy in the processing of large data sets (i.e., large number of data points per spectrum), such as those delivered by the NIRFlex N-500 [45]. Alternatively, the k-fold CV approach is less computationally expensive than LOO CV and even more robust against overfitting when each sample is represented by one observable (averaged spectra). As demonstrated by Turgut et al. [45], k-fold cross-validation delivers consistent results; however, the selected number of folds might be meaningful when different spectrometers are evaluated. Therefore, a more sophisticated approach was employed in this study with the model internally validated using a 6-fold CV, as the processed spectral data did not present any major problems given the sample size and relative homogeneity of the data set. The choice of the cross-validation method—within a reasonable range of folds considering the sample count (e.g., 4 to 7 folds)—should not affect the final estimate of the optimal model complexity and, consequently, the choice of a 6-fold CV grants proper validity to the results. Additionally, calibration was carried out six times, according to the number of groups, and internal validation was performed each time by a group of samples that was not taken into account in the model. The resulting performance of the predictions was compared by the highest multiple correlation coefficient (R-squared, RSQ) and the lowest standard error of calibration and cross-validation (SEC and SECV).

## 3. Results and Discussion

### 3.1. Spectral Characteristics

Figure 1 shows the average of the raw spectra measured both on the lean muscle and on the fat for each piece of equipment. The spectral interval ranged from 740 nm (shortest wavelength acquired by SCiO Sensor) to 2500 nm (longest wavelength acquired by NIRFlex N-500). Within the overlapping wavelength range (1000 to 1700 nm) where most devices collected spectra, a general similarity in the absorption line shapes can be observed (Figure 1). Higher absorbance values are seen in the regions associated with C-H groups from fatty acids (around 1200 nm, 1700–1750 nm, and 2300–2350 nm, as highlighted in yellow in Figure 1) and O-H groups linked to moisture (around 1000 nm, 1400–1450 nm, and 1950 nm, as highlighted in blue in Figure 1) [46,47]. These features are particularly strong in the case of the NIRFlex N-500 benchtop spectrometer, followed by the Enterprise Sensor.

Regarding the spectra of fat, higher spectral intensities are observed around 1200 nm, a wavelength related to absorption in the second overtone of the C-H stretching vibration, associated with intramuscular fat [48,49], and at 1700–1750 nm, corresponding to the region of $CH_2$ stretching first overtones and binary combination bands, i.e., the signal highly indicative of fat and saturated fatty acids. Further characteristic features can be identified between the 2300 and 2350 nm region measured by the NIRFlex N-500. The bands corresponding to absorption of the C-H combinations associated with unsaturated fatty acid content are observed there [50]. Differences associated with moisture are clearly observable in the 1400–1450 nm and 1950–2000 nm bands with markedly higher intensity in the lean muscle spectra than in the fat spectra due to the third, second, and first overtones of the O-H stretching mode [51]. The absorption at around 1000 nm is also indicative of O-H bonds, and it can be concluded that the spectra of lean meat showed stronger absorption than the spectra of fat; this behavior manifests a relatively stronger magnitude for the SCiO Sensor. Previous studies [52,53] on Iberian ham have shown that different fatty acids exhibit similar absorption bands due to the high number of shared absorbing molecular groups (-CH2-), a similarity that is particularly reflected in regression models. Nevertheless, the absorption bands at 1696 and 1720 nm have been related to the particular case of linoleic acid [54].

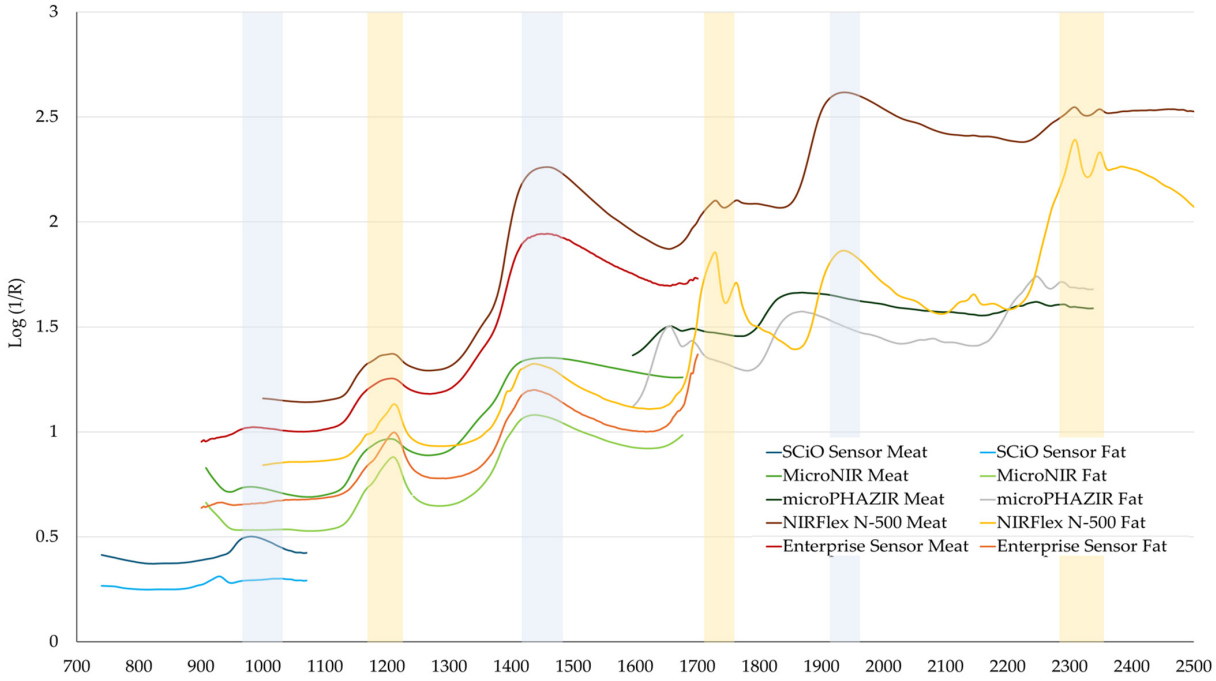


**Figure 1.** Average of the 60 raw spectra registered in the lean meat and in the fat part of the slice with the different equipment. Yellow bands correspond to the absorbance of C-H groups related to fatty acids. Blue bands correspond to the absorbance of O-H groups related to moisture.

### *3.2. Fatty Acid Composition*

The results of the lipid profile for the 28 fatty acids that could be quantified in at least 80% of the ham samples and the sums by functional groups (SFAs: total saturated fatty acids, MUFAs: total monounsaturated fatty acids, PUFAs: total polyunsaturated fatty acids, n3: total n3 PUFAs, n6: total n6 PUFAs) are shown in Table 1. It can be seen that both the values of the individual fatty acids and the sums show a wide range of variability. This is due to the fact that the hams come from animals reared in three different farms with different times in the fattening stage (*montanera*) and of different genetic purity (100% Iberian and 50% Iberian). As reported in other studies, the time in *montanera* influences the lipid profile of the animals [33]. In addition, genetic purity also affects the fatty acid profile, with significantly higher percentages of monounsaturated fatty acids and significantly lower percentages of polyunsaturated fatty acids being observed in 100% Iberian pigs [55]. The values of the different fatty acids and summates are within the values previously described for the *Biceps femoris* muscle of 100% Iberian and 50% Iberian animals raised in fattening systems similar to that of the present study [56,57].

**Table 1.** Fatty acids analyzed in Iberian hams, expressed as % by weight of total FAs.

| Fatty Acid | Mean | Min | Max | SD | CV |
|---|---|---|---|---|---|
| C12:0 | 0.09 | 0.07 | 0.20 | 0.02 | 20.88 |
| C13:0 | 0.00 | 0.00 | 0.04 | 0.01 | 247.33 |
| C14:0 | 1.34 | 1.03 | 2.06 | 0.15 | 11.36 |
| C14:1 n5 | 0.03 | 0.01 | 0.10 | 0.01 | 36.45 |
| C15:0 | 0.03 | 0.02 | 0.08 | 0.01 | 26.90 |
| C15:1 | 0.00 | 0.00 | 0.07 | 0.01 | 613.80 |
| C16:0 | 21.91 | 19.05 | 24.91 | 1.26 | 5.74 |
| C16:1 | 4.53 | 3.15 | 5.68 | 0.58 | 12.86 |
| C17:0 | 0.18 | 0.14 | 0.24 | 0.02 | 12.68 |
| C17:1 | 0.19 | 0.12 | 0.27 | 0.03 | 14.75 |
| C18:0 | 8.23 | 6.27 | 10.77 | 0.97 | 11.74 |
| C18:1 n9t | 0.23 | 0.13 | 0.34 | 0.04 | 18.69 |
| C18:1 | 50.68 | 46.38 | 53.61 | 1.61 | 3.17 |
| C18:1 n7 | 4.66 | 3.43 | 5.48 | 0.48 | 10.27 |

**Table 1.** *Cont.*

| Fatty Acid | Mean | Min | Max | SD | CV |
|---|---|---|---|---|---|
| C18:2 n6t | 0.02 | 0.00 | 0.17 | 0.02 | 98.91 |
| C18:2 n6 | 5.38 | 3.81 | 7.20 | 0.90 | 16.73 |
| C20:0 | 0.17 | 0.12 | 0.48 | 0.04 | 26.96 |
| C18:3 n6 | 0.02 | 0.01 | 0.12 | 0.01 | 66.45 |
| C20:1 n9 | 0.05 | 0.00 | 0.11 | 0.03 | 72.91 |
| C18:3 n3 | 1.33 | 1.10 | 1.54 | 0.12 | 8.92 |
| C21:0 | 0.07 | 0.04 | 0.12 | 0.01 | 18.78 |
| C20:2 n6 | 0.25 | 0.19 | 0.32 | 0.04 | 14.57 |
| C22:0 | 0.03 | 0.02 | 0.05 | 0.01 | 19.42 |
| C20:3 n6 | 0.06 | 0.04 | 0.08 | 0.01 | 15.77 |
| C22:1 n9 | 0.10 | 0.07 | 0.28 | 0.03 | 28.39 |
| C20:3 n3 | 0.25 | 0.00 | 0.34 | 0.05 | 22.10 |
| C20:4 n6 | 0.08 | 0.02 | 0.18 | 0.03 | 45.21 |
| C23:0 | 0.00 | 0.00 | 0.04 | 0.01 | 187.08 |
| C22:2 n6 | 0.04 | 0.00 | 1.32 | 0.17 | 463.49 |
| C24:0 | 0.02 | 0.00 | 0.05 | 0.01 | 44.39 |
| C20:5 n3 | 0.00 | 0.00 | 0.03 | 0.00 | 464.18 |
| C24:1 n9 | 0.01 | 0.00 | 0.23 | 0.03 | 387.71 |
| C22:6 n3 | 0.06 | 0.00 | 0.13 | 0.02 | 24.89 |
| SFAs | 32.06 | 28.38 | 37.25 | 2.10 | 6.54 |
| MUFAs | 60.47 | 56.39 | 62.91 | 1.53 | 2.53 |
| PUFAs | 7.45 | 5.72 | 10.16 | 1.10 | 14.74 |
| n3 | 1.64 | 1.35 | 1.93 | 0.15 | 9.17 |
| n6 | 5.81 | 4.22 | 8.33 | 0.97 | 16.67 |

Min: minimum value, Max: maximum value, SD: standard deviation, CV: coefficient of variation, SFAs: total saturated fatty acids, MUFAs: total monounsaturated fatty acids, PUFAs: total polyunsaturated fatty acids, n3: total n3 PUFAs, n6: total n6 PUFAs.

### *3.3. Regression Models*

The calibration equations for the different fatty acids were calculated using the fatty acid profile of the intramuscular fat determined by gas chromatography (GC) as a reference analysis and the spectra recorded either in the lean meat or in the fat of the slice as a predictor variable, in order to evaluate which sampling area is more appropriate for prediction. The development of the equations was carried out both using the raw spectra and using the spectra treated with different methods as described in Section 2.4.1. Systematic evaluation of different combinations of pre-treatments is desirable in order to optimize the method used in a specific application and efficiently evaluate different validation sets [39]. In total, 16 combinations of spectral pre-treatments were performed for each fatty acid or summation, sampling area, and piece of equipment.

After the optimization of the pre-treatment, it was possible to calibrate 24 individual fatty acids and the summates for any of the five devices used with an RSQ > 0.5. When analyzing which treatment provided the best statistical parameters for obtaining the prediction equation for each fatty acid, piece of equipment, and place of recording of the spectra (Supplementary Materials, Table S1), it is observed that the application of the second derivative, specifically the variant denoted as (2,5,5,1), provided the best calibration results in 61 out of the 145 equations obtained in this work, followed by the variant denoted as (1,5,5,1), with a first derivative, which allowed obtaining acceptable equations for 48 out of the 145 equations. As for the scatter treatments, the DT treatment was the most suitable as it allowed the obtainment of 46 equations fulfilling the criteria, followed by the SNV treatment, which was successful for 39 equations. Finally, the combination of smoothing and scatter treatments that yielded the highest number of acceptable equations was DeTrend (2,5,5,1) with a second derivative, with 25 equations out of the total 145, and the first derivative without scatter treatment (i.e., 'None') (1,5,5,1) with 20 equations out of 145.

Other studies have also reported the best approaches to obtaining fatty acid equations from subcutaneous fat with second derivative and smoothing treatments [29], in agreement with the results observed for other products derived from Iberian pork (*chorizo* and *salchichón*) [58]. In Iberian pork loins, González-Martín et al. [59] also obtained almost all the calibration equations for the prediction of fatty acids with the spectra treated with

a second derivative combined with DeTrend. The application of the second derivative to NIR spectra has been shown to be particularly suitable for the prediction of many meat constituents [60]. On the one hand, it minimizes the differences between the maximum and minimum absorbances of different samples by reducing scattering effects and increasing the resolution of the peaks of the spectra [61]. On the other hand, its application on the spectra reveals different peaks that would be related to fat (962–968 nm) and that would improve the prediction of fatty acids [52].

### 3.4. Fatty Acid Profile Prediction Using Fat Spectra

Table 2 shows the main statistical descriptors of the best regression equations obtained for each of the fatty acids predicted by any of the five NIR spectrometers evaluated here, using the spectra recorded in the fatty part of the Iberian ham slices. The number of outliers removed during calibration ranged from 0 to 5, representing between 0% and 8% of the initial calibration set, which is an acceptable range of values [58].

The number of fatty acids that could be successfully calibrated strongly depended on the particular NIR instrument used. Thus, with the benchtop spectrometer NIRFlex N-500, which is the most robust and offers the widest wavelength range, 24 fatty acids could be calibrated with RSQ values higher than 0.5 (0.54–0.99). However, the RSQs in cross-validation only exceed 0.5 in five of the equations. Another parameter that can be used to evaluate and compare the calibration performance between devices is RPD = SD/SEC. For the benchtop instrument, 10 of the 24 equations could be classified as good for calibration by obtaining an RPD > 2. These include the following fatty acids: lauric acid (C12:0), myristoleic acid (C14:1), palmitoleic acid (C16:1), oleic acid (C18:1), *trans*-vaccenic acid (C18:1n7), linoleic acid (C18:2n6), arachidic acid (C20:0), *cis*-8,11,14-eicosadienoic acid (C20:2n6), the summation of saturated fatty acids (SFAs), and the summation of monounsaturated fatty acids (MUFAs) with RPD values between 2.03 and 2.98. Another 10 equations were considered suitable for analytical purposes as they had an RPD > 3; in fact, they were between 3.13 and 8.75. These included palmitic acid (C16:0), heptadecenoic acid (C17:1), stearic acid (C18:0), α-linolenic acid (C18:3n3), heneicosanoic acid (C21:0), erucic acid (C22:1n9), lignoceric acid (C24:0), the summation of polyunsaturated fatty acids (PUFAs), n3 PUFAs, and n6 PUFAs [16,62].

The Enterprise Sensor (TellSpec) allowed the second highest number of successful prediction equations to be obtained, with 16 predicted fatty acids or summations. All of them could also be calibrated with the NIRFlex N-500, except behenic acid (C22:0), which could only be predicted with this portable device. Comparing the predictive performance of this miniaturized spectrometer with that of the benchtop instrument, a lower value of the RSQ and RPD statistics and higher calibration errors can be observed. The RSQ values were between 0.51 and 0.86 with RPD values > 2 for 5 out of the 16 equations, i.e., those corresponding to C16:1, C18:2 n6, C22:0, MUFAs, and n6 fatty acids.

Regarding the SCiO Sensor and MicroNIR devices, calibration equations with an RSQ > 0.5 could be obtained for 12 and 10 fatty acids (or summations), respectively, 10 of which were common to both devices: C16:0, sC18:0, C18:2 n6, C18:3 n3, C20:n6, SFAs, MUFAs, PUFAs, n3, and n6. C20:0 and C24:0 acids could only be predicted by the SCiO Sensor and not by MicroNIR. As far as the parameters related to predictive performance are concerned, the SCiO Sensor obtained RSQs between 0.55 and 0.81, with six of the equations presenting an RSQ > 0.7, and RPDs between 1.50 and 2.32. In the case of MicroNIR, the RSQ values were between 0.51 and 0.72, but only one equation presented a value >0.7, and the RPDs were slightly lower than those obtained with the SCiO, between 1.43 and 1.90. Therefore, both devices were able to calibrate a lower number of fatty acids and achieved lower statistical parameter values compared to the benchtop instrument. A comparison between the SCiO Sensor and Enterprise Sensor shows that, although fewer fatty acids can be calibrated with the SCiO Sensor, there were six with an RSQ > 0.7 in both cases, obtaining RPD > 2 values for three components (SFAs, PUFAs, and n6) with the SCiO Sensor instead of the five (C16:1, C18 n:6, C22:0, PUFAs, and n6) shown by the Enterprise

Sensor. However, it is worth noting that the MicroNIR device was the only portable device to show RSQ values in cross-validation equal or higher than 0.5 for individual fatty acids. But for the fatty acid summates, RSQcv values > 0.5 were obtained for the total n6 fatty acids with the Enterprise Sensor device and for the SFAs and MUFAs summates for the MicroNIR device.

Finally, only five fatty acids could be calibrated with the microPHAZIR instrument, including pentadecanoic acid C15:0—which was only calibrated with this device—C16:0, C17:1, C20:0, and C24:0. The RSQ and RPD values were between 0.50 and 0.69 and between 1.14 and 1.80, respectively, with the lowest performance of all the devices compared. Moreover, while all the devices used allowed the obtainment of calibration equations for the fatty acid summations according to their degree of unsaturation, with the microPHAZIR device, it was not possible to calibrate any sum. It appears that the particularly narrow wavelength region in which this handheld spectrometer operates poses a considerable limiting factor for fatty acid prediction. The bands corresponding to fatty acids have been described to be around 1210 nm, related to the second CH overtone; 1726 and 1760 nm, associated with the first CH overtone; and 2308 nm, associated with the second CH overtone [58]. All these bands are outside the recording range of this equipment.

Figure 2 shows the prediction plots for the sum of the MUFAs. This parameter was chosen because it could be successfully predicted by all instruments except the microPHAZIR device. In addition, a high MUFA content is characteristic of Iberian products and is directly related to the type of feed received by the animals during the fattening period. Monounsaturated fatty acids are also of great interest to the processing industry since their presence plays a key role in the sensory profile. The NIRFlex N-500 instrument provided better RSQ values than the SCiO, Enterprise, and MicroNIR devices.

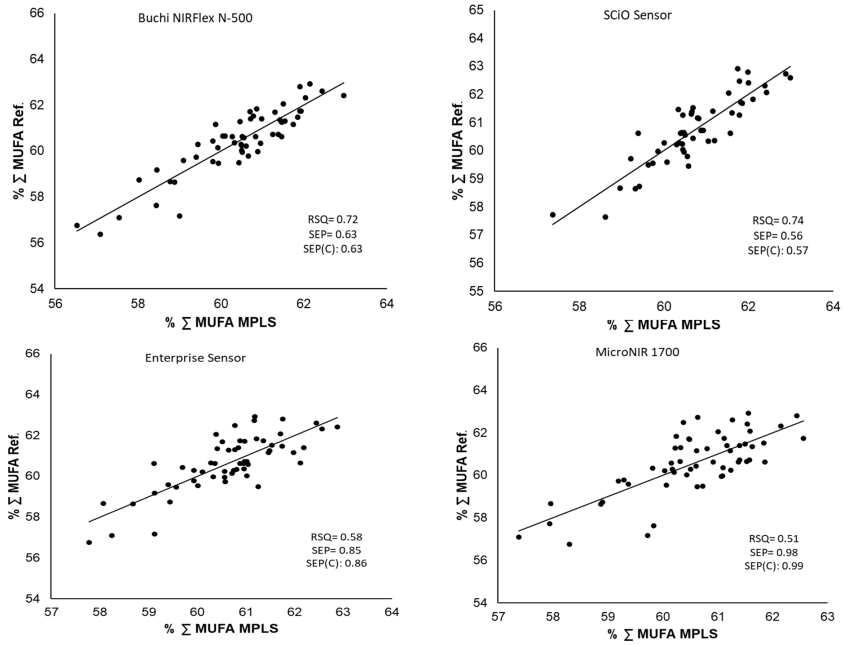


**Figure 2.** Linear regression plot of the measured versus predicted values within the calibration set for monounsaturated fatty acids with different devices using NIR spectra measured in the fat zones of the samples.

### *3.5. Fatty Acid Profile Prediction Using Lean Meat Spectra*

Similar to the case of fat, the calibration equations were calculated using the spectra measured in the lean meat part of the ham slice as well; their corresponding statistical descriptors are shown in Table 3. In this case, the number of outliers removed during calibration ranged from 0 to 8 (in two equations) representing between 0% and 13% of the initial calibration set.

The benchtop spectrometer (NIRFlex N-500) again allowed the obtainment of the highest number (a total of 24) of equations with an RSQ > 0.5, with values ranging from 0.59 to 0.99 and RPDs ranging from 1.56 to 10.40. Of the total of 24 equations, those obtained for C12:0, C14:0, C14:1 n-5, C16:0, C18:0, C18:1, C18:2 n6, C20:0, C18:3 n3, C20:3n6, C20:3 n3, and all sums (SFAs, MUFAs, PUFAs, n3, and n6) can be considered as suitable for analytical purposes (RPD > 3); this means that almost all the main fatty acids can be successfully predicted. As previously observed for the fat, the next highest number of predictive equations was obtained for the Enterprise Sensor with a total of 19 calibrated components, with RSQ values between 0.50 and 0.93 and RPDs between 1.42 and 3.67. In this case, only the equations obtained for the C18:2 n6, C20:0, and the total n6 fatty acids are classified as suitable for analytical purposes for presenting RPD > 3 [16]. Of these calibrated constituents, all were common with the benchtop except heptadecanoic acid (C17:0), C17:1, and elaidic acid (C18:1 n9t), which had not been possible to calibrate with the NIRFlex N-500. As far as the other instruments are concerned, 14 equations were successfully obtained for the MicroNIR instrument, 12 equations for the microPHAZIR instrument, and 9 equations for the SCiO Sensor. Regarding the statistical metrics, low calibration errors are observed in all cases, but these were slightly higher than those of the benchtop unit in most cases.

The RSQ values obtained for the microPHAZIR equipment were between 0.52 and 0.92, and the equations obtained for the fatty acids C17:0, C17:1, C18:1 n9t, C18:1 n7, and the sum of MUFAs presented an RPD value > 2, which means that they can be considered as good. It should be noted that the C18:1n7 fatty acid could only be calibrated with the PHAZIR instrument. In the case of the MicroNIR equipment, the RSQ values ranged between 0.50 and 0.70, while for the SCiO Sensor, these values ranged between 0.52 and 0.61, and the RPD values were below 2 for both equipment in all cases.

Figure 3 shows the prediction graphs for the sum of the MUFAs. In this case, the SCiO was the only equipment that does not allow this summation to be calibrated. The linear regression of the calculated values against the values obtained in the calibration showed, as previously observed for the fat recording, the good performance of the NIRFlex N-500 equipment compared to the portable equipment, followed by the microPHAZIR.

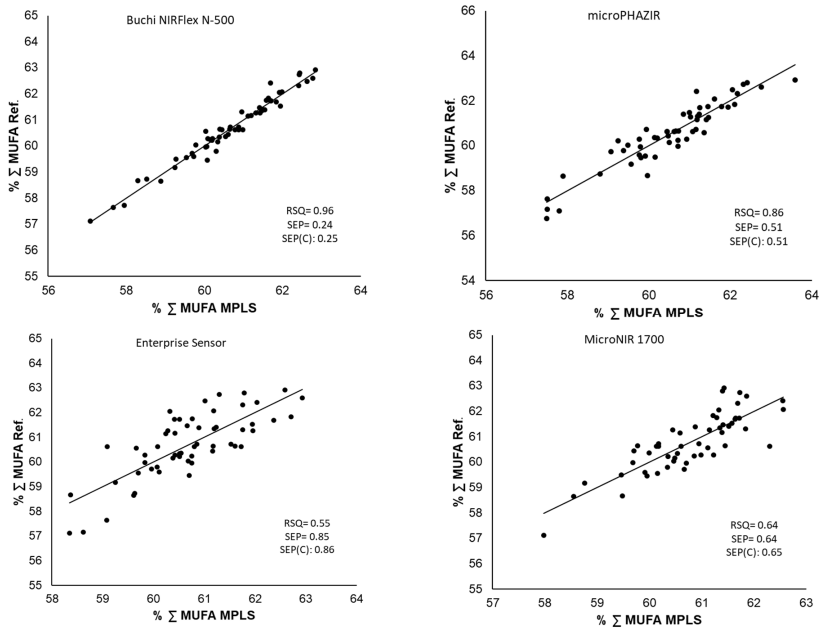


**Figure 3.** Linear regression plot of the measured versus predicted values within the calibration set for monounsaturated fatty acids with different devices using the NIR spectra measured in the lean meat zones in the samples.

**Table 2.** Statistical descriptors for the best calibration equations obtained from the NIR spectra of fat for the five devices.

| Fatty Acid | NIRFlex N-500 | | | | | | MicroPHAZIR | | | | | | SCiO Sensor | | | | | | Enterprise Sensor | | | | | | MicroNIR | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | SEC | RPD | RSQ | SECV | RSQcv | N | SEC | RPD | RSQ | SECV | RSQcv | N | SEC | RPD | RSQ | SECV | RSQcv | N | SEC | RPD | RSQ | SECV | RSQcv | N | SEC | RPD | RSQ | SECV | RSQcv |
| C12:0 | 55 | 0.00 | 2.03 | 0.75 | 0.01 | 0.02 | | | | | | | | | | | | | | | | | | | | | | | | |
| C14:0 | 57 | 0.06 | 1.81 | 0.70 | 0.09 | 0.30 | | | | | | | | | | | | | | | | | | | | | | | | |
| C14:1 n5 | 58 | 0.00 | 2.32 | 0.82 | 0.01 | 0.15 | | | | | | | | | | | | | 57 | 0.00 | 1.57 | 0.60 | 0.01 | 0.05 | | | | | | |
| C15:0 | | | | | | | 58 | 0.003 | 1.40 | 0.50 | 0.00 | 0.13 | | | | | | | | | | | | | | | | | | |
| C16:0 | 57 | 0.34 | 3.55 | 0.92 | 0.87 | 0.47 | 57 | 0.730 | 1.56 | 0.59 | 0.89 | 0.37 | 59 | 0.64 | 1.91 | 0.73 | 1.19 | 0.03 | 58 | 0.73 | 1.69 | 0.65 | 0.99 | 0.34 | 57 | 0.73 | 1.67 | 0.64 | 0.85 | 0.50 |
| ∑ C16:1 | 59 | 0.23 | 2.51 | 0.84 | 0.46 | 0.37 | | | | | | | | | | | | | 57 | 0.22 | 2.66 | 0.86 | 0.47 | 0.31 | | | | | | |
| C17:0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| C17:1 | 59 | 0.01 | 3.85 | 0.93 | 0.03 | 0.00 | 57 | 0.015 | 1.59 | 0.60 | 0.02 | 0.09 | | | | | | | | | | | | | | | | | | |
| C18:0 | 60 | 0.26 | 3.66 | 0.93 | 0.90 | 0.12 | | | | | | | | | | | | | 59 | 0.58 | 1.65 | 0.63 | 0.90 | 0.11 | | | | | | |
| C18:1 | 57 | 0.51 | 2.98 | 0.89 | 0.98 | 0.58 | | | | | | | 53 | 1.00 | 1.55 | 0.58 | 1.31 | 0.27 | 57 | 0.93 | 1.61 | 0.61 | 1.43 | 0.06 | 58 | 0.64 | 1.52 | 0.56 | 0.79 | 0.33 |
| C18:1 n7 | 60 | 0.20 | 2.39 | 0.83 | 0.38 | 0.37 | | | | | | | | | | | | | 57 | 0.22 | 1.98 | 0.75 | 0.41 | 0.06 | | | | | | |
| C18:2 n6 | 57 | 0.34 | 2.53 | 0.84 | 0.57 | 0.56 | | | | | | | 58 | 0.45 | 1.98 | 0.75 | 0.81 | 0.18 | 58 | 0.38 | 2.34 | 0.82 | 0.67 | 0.41 | 58 | 0.53 | 1.63 | 0.62 | 0.65 | 0.43 |
| C20:0 | 58 | 0.01 | 2.69 | 0.86 | 0.01 | 0.12 | | | | | | | 58 | 0.02 | 1.53 | 0.58 | 0.02 | 0.38 | | | | | | | | | | | | |
| C18:3 n6 | 58 | 0.00 | 1.52 | 0.55 | 0.00 | 0.15 | 57 | 0.021 | 1.14 | 0.54 | 0.03 | 0.22 | | | | | | | | | | | | | | | | | | |
| C18:3 n3 | 56 | 0.02 | 6.26 | 0.97 | 0.06 | 0.69 | | | | | | | 58 | 0.08 | 1.49 | 0.55 | 0.11 | 0.16 | 59 | 0.08 | 1.48 | 0.54 | 0.11 | 0.16 | 56 | 0.07 | 1.79 | 0.69 | 0.08 | 0.53 |
| C21:0 | 58 | 0.00 | 3.68 | 0.93 | 0.01 | 0.31 | | | | | | | | | | | | | 56 | 0.01 | 1.62 | 0.62 | 0.01 | 0.07 | | | | | | |
| C20:2 n6 | 58 | 0.01 | 2.49 | 0.84 | 0.03 | 0.38 | | | | | | | 59 | 0.02 | 1.75 | 0.67 | 0.04 | 0.01 | | | | | | | 56 | 0.02 | 1.69 | 0.65 | 0.02 | 0.48 |
| C22:0 | | | | | | | | | | | | | | | | | | | 57 | 0.00 | 2.09 | 0.77 | 0.00 | 0.06 | | | | | | |
| C20:3 n6 | 58 | 0.01 | 1.48 | 0.54 | 0.01 | 0.12 | | | | | | | | | | | | | | | | | | | | | | | | |
| C22:1 n9 | 59 | 0.00 | 4.67 | 0.95 | 0.01 | 0.18 | | | | | | | | | | | | | | | | | | | | | | | | |
| C20:3 n3 | 57 | 0.03 | 1.46 | 0.53 | 0.04 | 0.13 | | | | | | | | | | | | | | | | | | | | | | | | |
| C22:2 n6 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| C24:0 | 54 | 0.00 | 8.75 | 0.99 | 0.01 | 0.20 | 56 | 0.004 | 1.80 | 0.69 | 0.01 | 0.04 | 53 | 0.00 | 1.50 | 0.55 | 0.01 | 0.23 | 53 | 0.00 | 1.45 | 0.51 | 0.01 | 0.37 | | | | | | |
| SFAs | 60 | 0.85 | 2.47 | 0.84 | 1.65 | 0.37 | | | | | | | 59 | 0.95 | 2.14 | 0.78 | 1.67 | 0.31 | 56 | 1.28 | 1.55 | 0.59 | 1.75 | 0.22 | 54 | 0.96 | 1.90 | 0.72 | 1.19 | 0.56 |
| MUFAs | 56 | 0.66 | 2.22 | 0.80 | 1.15 | 0.38 | | | | | | | 52 | 0.63 | 1.96 | 0.74 | 1.07 | 0.23 | 57 | 0.88 | 1.55 | 0.58 | 1.23 | 0.17 | 57 | 1.02 | 1.43 | 0.51 | 1.19 | 0.33 |
| PUFAs | 56 | 0.32 | 3.13 | 0.90 | 0.64 | 0.60 | | | | | | | 55 | 0.44 | 2.32 | 0.81 | 0.80 | 0.38 | 58 | 0.47 | 2.30 | 0.81 | 0.79 | 0.46 | 56 | 0.61 | 1.69 | 0.65 | 0.71 | 0.51 |
| n3 | 58 | 0.03 | 5.30 | 0.96 | 0.11 | 0.36 | | | | | | | 59 | 0.09 | 1.63 | 0.63 | 0.15 | 0.00 | 57 | 0.10 | 1.47 | 0.54 | 0.12 | 0.28 | 57 | 0.09 | 1.53 | 0.57 | 0.12 | 0.32 |
| n6 | 57 | 0.23 | 3.93 | 0.94 | 0.62 | 0.53 | | | | | | | 55 | 0.42 | 2.12 | 0.78 | 0.65 | 0.46 | 57 | 0.38 | 2.52 | 0.84 | 0.67 | 0.50 | 57 | 0.53 | 1.69 | 0.65 | 0.67 | 0.42 |

N: number of samples after removing the outliers; SEC: standard error of calibration; SECV: standard error of cross-validation; RSQ: multiple correlation coefficient of calibration; RPD: ratio performance deviation; RSQcv: multiple correlation coefficient of cross-validation.

**Table 3.** Statistical descriptors for the best calibration equations obtained from the NIR spectra of lean meat for the five devices.

| Fatty Acid | NIRFlex N-500 | | | | | | MicroPHAZIR | | | | | | SCiO Sensor | | | | | | Enterprise Sensor | | | | | | MicroNIR | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | SEC | RPD | RSQ | SECV | RSQcv | N | SEC | RPD | RSQ | SECV | RSQcv | N | SEC | RPD | RSQ | SECV | RSQcv | N | SEC | RPD | RSQ | SECV | RSQcv | N | SEC | RPD | RSQ | SECV | RSQcv |
| C12:0 | 56 | 0.00 | 7.90 | 0.98 | 0.01 | −0.07 | | | | | | | | | | | | | 53 | 0.00 | 1.82 | 0.69 | 0.01 | −0.1542 | | | | | | |
| C14:0 | 56 | 0.03 | 3.06 | 0.89 | 0.10 | 0.08 | 59 | 0.07 | 1.67 | 0.64 | 0.11 | 0.07 | | | | | | | 57 | 0.05 | 2.09 | 0.77 | 0.09 | 0.26 | | | | | | |
| C14:1 n5 | 56 | 0.00 | 3.53 | 0.92 | 0.00 | 0.18 | 56 | 0.00 | 1.61 | 0.62 | 0.01 | −0.04 | | | | | | | | | | | | | | | | | | |
| C15:0 | 57 | 0.00 | 2.86 | 0.88 | 0.00 | 0.12 | | | | | | | | | | | | | | | | | | | | | | | | |
| C16:0 | 59 | 0.37 | 3.28 | 0.91 | 1.05 | 0.24 | | | | | | | 54 | 0.79 | 1.47 | 0.53 | 0.88 | 0.42 | 57 | 0.43 | 2.64 | 0.86 | 0.80 | 0.51 | 59 | 0.86 | 1.47 | 0.54 | 1.01 | 0.35 |
| ∑ C16:1 | 58 | 0.27 | 1.97 | 0.74 | 0.49 | 0.13 | 59 | 0.35 | 1.61 | 0.61 | 0.53 | 0.13 | | | | | | | | | | | | | | | | | | |
| C17:0 | | | | | | | 57 | 0.01 | 2.26 | 0.80 | 0.02 | 0.32 | | | | | | | 57 | 0.02 | 1.42 | 0.50 | 0.02 | 0.23 | 59 | 0.01 | 1.58 | 0.60 | 0.02 | 0.34 |
| C17:1 | | | | | | | 57 | 0.01 | 2.51 | 0.84 | 0.03 | 0.12 | | | | | | | 56 | 0.02 | 1.51 | 0.56 | 0.03 | −0.1011 | | | | | | |
| C18:0 | 59 | 0.30 | 3.13 | 0.90 | 0.84 | 0.21 | 60 | 0.67 | 1.45 | 0.52 | 1.05 | −0.19 | | | | | | | | | | | | | 56 | 0.63 | 1.49 | 0.55 | 0.75 | 0.35 |
| C18:1 n9t | | | | | | | 59 | 0.01 | 3.49 | 0.92 | 0.03 | 0.35 | | | | | | | 53 | 0.03 | 1.51 | 0.56 | 0.04 | 0.12 | 57 | 0.03 | 1.45 | 0.53 | 0.03 | 0.22 |

**Table 3.** *Cont.*

| Fatty Acid | NIRFlex N-500 | | | | | | MicroPHAZIR | | | | | | SCiO Sensor | | | | | | Enterprise Sensor | | | | | | MicroNIR | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **N** | **SEC** | **RPD** | **RSQ** | **SECV** | **RSQcv** | **N** | **SEC** | **RPD** | **RSQ** | **SECV** | **RSQcv** | **N** | **SEC** | **RPD** | **RSQ** | **SECV** | **RSQcv** | **N** | **SEC** | **RPD** | **RSQ** | **SECV** | **RSQcv** | **N** | **SEC** | **RPD** | **RSQ** | **SECV** | **RSQcv** |
| C18:1 | 58 | 0.37 | 4.13 | 0.94 | 1.29 | 0.27 | 58 | 0.73 | 2.08 | 0.77 | 1.26 | 0.30 | | | | | | | 57 | 0.79 | 1.92 | 0.73 | 1.43 | 0.10 | 59 | 1.14 | 1.42 | 0.50 | 1.37 | 0.27 |
| C18:1 n7 | | | | | | | 58 | 0.22 | 2.09 | 0.77 | 0.39 | 0.23 | | | | | | | | | | | | | | | | | | |
| C18:2 n6 | 56 | 0.13 | 6.27 | 0.97 | 0.58 | 0.52 | | | | | | | 53 | 0.51 | 1.59 | 0.61 | 0.56 | 0.51 | 58 | 0.27 | 3.38 | 0.91 | 0.65 | 0.49 | 57 | 0.59 | 1.49 | 0.55 | 0.85 | 0.06 |
| C20:0 | 58 | 0.00 | 10.40 | 0.99 | 0.02 | −0.01 | 60 | 0.02 | 1.81 | 0.69 | 0.03 | 0.22 | 55 | 0.02 | 1.42 | 0.50 | 0.02 | 0.36 | 54 | 0.01 | 3.41 | 0.91 | 0.03 | 0.18 | | | | | | |
| C18:3 n6 | 59 | 0.00 | 2.25 | 0.81 | 0.00 | 0.06 | | | | | | | | | | | | | 56 | 0.00 | 1.75 | 0.68 | 0.00 | 0.37 | 57 | 0.00 | 1.44 | 0.53 | 0.00 | 0.26 |
| C18:3 n3 | 59 | 0.03 | 3.94 | 0.94 | 0.10 | 0.26 | | | | | | | 56 | 0.08 | 1.44 | 0.52 | 0.09 | 0.34 | 60 | 0.08 | 1.47 | 0.54 | 0.11 | 0.13 | 57 | 0.07 | 1.75 | 0.67 | 0.08 | 0.53 |
| C21:0 | 58 | 0.00 | 2.32 | 0.81 | 0.01 | −0.31 | | | | | | | | | | | | | | | | | | | | | | | | |
| C20:2 n6 | 60 | 0.02 | 1.56 | 0.59 | 0.03 | 0.14 | | | | | | | 54 | 0.02 | 1.48 | 0.54 | 0.03 | 0.26 | 60 | 0.02 | 1.49 | 0.55 | 0.03 | 0.13 | | | | | | |
| C22:0 | 59 | 0.00 | 1.71 | 0.65 | 0.00 | 0.31 | | | | | | | | | | | | | | | | | | | | | | | | |
| C20:3 n6 | 58 | 0.00 | 8.00 | 0.98 | 0.01 | 0.45 | | | | | | | | | | | | | 59 | 0.01 | 1.45 | 0.52 | 0.01 | 0.31 | | | | | | |
| C22:1 n9 | 58 | 0.01 | 1.76 | 0.68 | 0.01 | −0.32 | | | | | | | 55 | 0.01 | 1.45 | 0.53 | 0.01 | 0.28 | | | | | | | 56 | 0.01 | 1.49 | 0.55 | 0.01 | 0.39 |
| C20:3 n3 | 56 | 0.00 | 9.60 | 0.99 | 0.03 | 0.53 | | | | | | | | | | | | | 57 | 0.02 | 1.96 | 0.74 | 0.04 | 0.28 | 55 | 0.02 | 1.80 | 0.69 | 0.03 | 0.52 |
| C24:0 | 58 | 0.00 | 1.76 | 0.68 | 0.01 | −0.15 | 55 | 0.00 | 1.78 | 0.68 | 0.01 | 0.26 | | | | | | | 56 | 0.01 | 1.42 | 0.50 | 0.01 | 0.01 | | | | | | |
| SFAs | 58 | 0.27 | 7.26 | 0.98 | 1.45 | 0.43 | | | | | | | 57 | 1.39 | 1.53 | 0.57 | 2.00 | 0.10 | 58 | 0.87 | 2.18 | 0.79 | 1.61 | 0.27 | 58 | 1.31 | 1.62 | 0.62 | 1.66 | 0.37 |
| MUFAs | 57 | 0.25 | 5.30 | 0.96 | 1.16 | 0.19 | 55 | 0.54 | 2.64 | 0.86 | 1.35 | 0.08 | | | | | | | 56 | 0.89 | 1.50 | 0.55 | 1.20 | 0.17 | 53 | 0.69 | 1.68 | 0.64 | 0.78 | 0.53 |
| PUFAs | 57 | 0.09 | 11.11 | 0.99 | 0.75 | 0.48 | | | | | | | 52 | 0.63 | 1.57 | 0.59 | 0.69 | 0.49 | 58 | 0.49 | 2.25 | 0.80 | 0.82 | 0.43 | 57 | 0.73 | 1.48 | 0.54 | 0.88 | 0.33 |
| n3 | 57 | 0.03 | 5.17 | 0.96 | 0.11 | 0.42 | | | | | | | | | | | | | | | | | | | 56 | 0.08 | 1.84 | 0.70 | 0.11 | 0.45 |
| n6 | 56 | 0.14 | 6.71 | 0.98 | 0.62 | 0.53 | | | | | | | 52 | 0.55 | 1.58 | 0.60 | 0.60 | 0.50 | 57 | 0.27 | 3.67 | 0.93 | 0.64 | 0.55 | | | | | | |

N: number of samples after removing the outliers; SEC: standard error of calibration; SECV: standard error of cross-validation; RSQ: multiple correlation coefficient of calibration; RPD: ratio performance deviation; RSQcv: multiple correlation coefficient of cross-validation.

### 3.6. Comparison of Spectra Sampling Zone: Fat vs. Lean Meat

When comparing the efficiency of the spectrometers based on the sampling zone (fat vs. lean tissue), the NIRFlex N-500 performed well in both zones, successfully calibrating 24 fatty acids. Notably, the spectrometer achieved calibration for C15:0 and C22:0 in the lean tissue zone, which could not be calibrated successfully using spectra from the fat zone. However, it was unable to predict certain fatty acids such as C17:1 and C18:1n7 (the latter being one of the major fatty acids in the sample, as shown in Table 1). In terms of model performance, the RSQ values (a measure of the goodness of fit of the calibration model) were >0.7 in more than 80% of the equations obtained from both the lean and fat tissue zones, which indicate strong calibration quality. However, when assessing the RSQ in cross-validation (RSQcv), it was found that the RSQcv values were lower for spectra recorded in the lean tissue zone compared to the fat zone. The RSQcv is particularly important as it measures the predictive accuracy of the model on unseen data or how well the model generalizes to new samples. A value close to 1 would indicate that the model can predict the fatty acid concentrations in new samples very accurately, which is ideal for practical applications. In this case, the lower RSQcv in the lean tissue zone suggests that although the model fits well to the training data, it may not generalize as effectively when the lean zone is analyzed. This trend seems to reflect the differences in the homogeneity of the sample within the respective zones considered here. The lean muscle tissue generally has a more complex composition, with varying amounts of water, protein, and fat distributed unevenly across different regions of the tissue. This variation makes it more challenging to create a predictive model that generalizes well to new samples. In contrast, the fat zone tends to have a more consistent and homogenous composition (mostly fat), which allows for better calibration and more stable, generalized predictions. The fat is more uniform across the tissue, making it easier for the model to capture consistent absorption features and reduce prediction errors.

Thus, this discrepancy in the zone-specific modeling highlights the importance of careful selection of the sampling zone (fat vs. lean) in model development, as it can influence the predictive performance of the calibration equations. Furthermore, a clear impact of instrument-related characteristics can be seen in this context. Spectrometers with a wider sample spot should perform better in the case of more inhomogeneous samples. Therefore, while the NIRFlex N-500 was successful in calibrating a broad range of fatty acids across both zones, further optimization is needed for better predictive performance, especially in the lean tissue zone.

In the case of the Enterprise Sensor, the number of constituents for which successful prediction equation could be established was higher when the recording was made in the lean meat with 19 constituents compared to the 16 calibrated in the fat. No equation was obtained for C14:0 and C18:2 n6 in the fat recording and for C16:1, C18:1n7, and the sum of n3 in the recording in the meat zone. RSQ values > 0.7 were observed in 50% of the equations in both locations, but only RSQcv values > 0.5 were obtained for the sum of n6 in both the fat and lean zone and of C16:0 in the lean-zone recordings.

In the case of the microPHAZIR equipment with the recording of the lean zone, 12 equations were obtained, including all the major fatty acids—except C18:3n3—and MUFAs. In the case of the spectra taken with this equipment in the fat zone, only five fatty acids are predictable, among which only C16:0 is found among the major fatty acids, but none of the summations. In addition, no predictive equation was obtained for RSQ values > 0.7.

MicroNIR also shows this behavior, i.e., a lower number of predicted constituents from the fat spectrum (10) with respect to the lean zone (14) and only one equation with an RSQ > 0.7 in each recording zone. It should be noted that this equipment is the one that presented the highest number of equations with an RSQcv > 0.5 with respect to the rest of the portable equipment.

Finally, the SCiO presented the opposite tendency, so that the number of equations obtained from the fat spectra was greater than in the meat zone with the prediction of

12 fatty acids, among which are all the summative and the majority C16:0, C18:2 n6, and C18:3 n3 versus 9 fatty acids predicted in the lean meat zone and 50% of the equations with RSQ values > 0.7 but no RSQcv > 0.5.

In order to assess the suitability of the equations developed, the SEC and SECV values must also be taken into consideration. Specifically, the RSQcv and SEC are relevant because a higher value of the RSQcv and a lower value of the SEC are indicative of a better predictive fit and less error in the calibrated value, thus obtaining a more accurate and reliable model. On the other hand, SEC and SECv values provide information on how the model fits the calibration data and how it behaves with data that are not part of the calibration training. Comparison between the two data may result in detecting poor predictive reliability due to overfitting when the SEC is significantly lower than the SECv. To determine a correct fit, the SEC and SECv values must be very close to confirm a tight and reliable model for the prediction of samples external to the calibration set.

The SEC in all equations took very low values in general, with slightly higher values in the fat recording zone for the NIRFlex N-500, microPHAZIR, and Enterprise Sensor devices. On the contrary, for the SCiO Sensor equipment, the SEC values were slightly higher in most of the equations for the spectra taken in the lean meat zone, while for the MicroNIR equipment, the behavior of the errors was similar in both zones. Finally, the cross-validation errors (SECv) presented different behaviors with respect to the SEC; in some of the cases, the behavior of both was maintained, and in other cases, it was much higher. These errors are comparable with those reported in the literature for the fatty acids C18:0, C18:2, and C18:3 [28]. The analysis of the results obtained seems to show that the number of equations obtained for the calibration of fatty acids is always higher when the recording is carried out on lean meat. This fact is especially significant in the microPHAZIR equipment that records in the longer wavelength zone (1596–2395 nm). On the other hand, only the spectral zone of 740–1070 nm (SCiO equipment) offers a greater number of calibration equations in samples when the recording is performed on fat. This appears to indicate that higher wavelengths are more suitable for lean and lower wavelengths for fat.

It should be noted that fatty acids are muscle components that are strongly influenced by animal nutrition and to a lesser extent by genotype. In turn, they are associated with many sensory attributes of meat and meat products [63]. Specifically, in Iberian ham, a high content of infiltrated fat directly related to a higher oleic acid content and lower PUFA content is pursued, which is reflected in the product through the properties of smoothness and shine. In addition, a high oleic and palmitoleic acid content is associated with less hardness, dryness, and fibrousness in the cured product [57]. Therefore, the determination of the lipid profile can be interesting to look at for correlations with sensory parameters of interest in this type of cured product.

## 4. Conclusions

The results obtained in this work evidence the potential of NIR spectroscopy, including miniaturized sensors, which can be considered as an alternative to conventional gas chromatographic analysis for the determination of the lipid profile in Iberian ham. The results showed that it was possible to predict, using spectra recorded in both meat and fat, the most abundant fatty acids and their summations. All of them showed RSQ values > 0.7 when using the NIRFlex N-500 benchtop instrument for both the meat and fat spectra recording sites. The Sensor Enterprise gave an RSQ > 0.7 for C18:2 and PUFA prediction using the spectra of fat and for C16:0, C18:1, C18:2, SFAs, and PUFAs using the spectra of meat; the ScIO for C16:0, C18:2, SFAs, MUFAs, and PUFAs for records taken in the fat; the microPHAZIR for C18:1 and MUFAs; and MicroNIR only for PUFAs when using the spectra of meat in both cases.

Of the five spectrometers used, the best results were obtained with the use of the benchtop instrument (NIRFlex N-500) because 20 equations with an RSQ > 0.7 were obtained for both the muscle and fat, and it can be considered as a reference for evaluating the analytical performance of portable spectrometers in the examined application. It was shown that

portable instruments offer good performance levels as well, indicating full suitability for on-site operation in industry; the Enterprise Sensor (TellSpec), followed by the MicroNIR device, appeared particularly promising, both of which record in the range between ca. 900 and 1700 nm. Thus, for the first device, it was possible to obtain 19 equations (9 with an RSQ < 0.7) in muscle and 14 equations (5 with an RSQ < 0.7) in fat, and for the second, it was possible to obtain 16 equations in muscle and 10 equations in fat, but only one at both recording sites had an RSQ < 0.7. Of the two spectra measurement zones that were considered at the sample surface, it was observed that the highest number of successful regressions and the highest RSQ values were obtained when the spectra were taken on the lean meat. An exception for this trend was observed only for the SCiO sensor, which yielded better results when the spectra were recorded on the fatty part where 12 equations (6 with an RSQ < 0.7) were obtained. These results could be related to the fact that the absorption range of this equipment is located in the water absorption zone, which could make prediction difficult in the case of samples with a higher water content. The results also revealed that measurements performed in both zones delivered complementary information on lipid composition. The preliminary study of feasibility confirms the suitability of employing NIR spectroscopy, and miniaturized sensors in particular, for comprehensive analysis of fatty acids in Iberian ham including on-site application. However, further research is needed; in particular, it would be necessary to improve the statistical basis by increasing the number of samples, including controlled samples that differ in their fatty acid composition to broaden the range of application and improve the precision of the calibrations. This would allow the obtainment of a more robust method for its generalized application in the Iberian pork sector.

**Supplementary Materials:** The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/app142210680/s1, Table S1: Mathematical pre-treatment for obtaining the best prediction equation for each fatty acid, piece of equipment, and place of recording of the spectra.

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