



Near-infrared spectroscopy for predicting chemical composition and classifying local chicken meat

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ABSTRACT

Near-infrared (NIR) spectroscopy was tested to predict meat quality and classify chicken parts, sex, and diet in a local chicken breed. Sixty chicks (both sexes) were reared under identical conditions until 120 days, then assigned to a low-lipidic (LL, 3.85% EE, ether extract) and a high-lipidic diet (HL, 9.49% EE). At 150 days, breast and thigh samples were collected, and physical traits –pH and color– and proximate composition –moisture, crude protein (CP), ether extract (EE), and ash– were determined using wet chemistry methods. Samples were scanned intact, ground, and freeze-dried with a benchtop NIR spectrometer (1100–2500 nm), and prediction models were developed on both fresh and dry matter bases. External validation (70/30% split) was applied for pH, yellowness, EE, and ash, whereas cross-validation was performed separately for breast and thigh for redness, lightness, moisture, and CP. Model performance was evaluated using the coefficient of determination in validation (R^2_{VAL}) and cross-validation (R^2_{CV}), and the ratio of performance to deviation (RPD). Physical traits and ash showed poor predictability (R^2_{VAL} and $R^2_{CV} < 0.68$; $RPD < 1.74$), while chemical traits, particularly EE in freeze-dried samples, achieved excellent prediction ($R^2_{VAL} > 0.95$; $RPD > 3$). Partial Least Squares-Discriminant Analysis enabled perfect discrimination of breast and thigh (100%), high accuracy for sex ($> 89\%$), but limited discrimination for diet ($< 67\%$). Overall, NIR spectroscopy demonstrated its potential as a rapid, non-destructive tool for predicting relevant chemical quality traits such as protein content and EE enhancing traceability in local poultry production systems.

1. Introduction

In recent years, consumer awareness of poultry meat quality has increased substantially, leading to a higher demand for poultry products produced through alternative farming methods (Franzoni et al., 2021). Moreover, consumers' interest in local products helps preserve local breeds, which have substantial environmental and socio-cultural importance due to their benefits to local communities while addressing the growing perception among more welfare-conscious consumers of the negative impacts associated with intensive farming system. In this context, there is a growing scientific and commercial interest in local chicken breeds, which are generally characterized by lower growth rates, longer rearing periods, and meat qualities that align with consumer expectations for higher welfare and traditional production systems. These breeds represent valuable models for studying the relationship between genotype, production system, and product quality.

Within this framework, the Bionda Piemontese (BP) is an autochthonous chicken breed of the Piedmont region in north-western Italy. The breed is characterized by a light blonde plumage, a single red comb which is straight in the rooster and bent at the back in hens, wattles and earlobes are also red, and the legs are yellow. Although it is considered a dual-purpose breed, chickens are primarily raised for meat production, achieving a carcass rate of 1.8 kg for roosters and 1.3 kg for hens at five months of age (Bongiorno et al., 2022). The low slaughter yield classifies this breed as a slow-growing chicken breed (Soglia et al., 2020). However, native slow-growing breeds exhibit distinct compositional and textural characteristics that contribute to the uniqueness of their meat (Cappone et al., 2026). Therefore, this distinctive quality combined with slow growth performance characterizing local breeds, results in higher production costs, making the meat less accessible to all consumers.

Meat quality is a broad concept that involves the evaluation of different traits such as meat color, pH level, drip loss, intramuscular fat,

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and its nutritional properties, which include protein, ether extract, and moisture contents (Baéza et al., 2022; Xiao et al., 2024). The traditional methods used for this determination have the disadvantage of being destructive, labor-intensive, costly, and time-consuming, thus making them not suitable for local poultry commerce (Barbin et al., 2015). This has led to increased interest in near-infrared (NIR) spectroscopy, which offers a more efficient alternative. Near-infrared spectroscopy is widely applied for quantitative analysis as a fast routine method of chemical constituents such as protein, moisture, and fat content in products of animal and vegetable origin (Stuart, 2004; Yang et al., 2021). Regarding the poultry industry, NIR analysis prediction models based on the chemical composition and physical parameters have been applied mainly in broiler strains (Fodor et al., 2024). Since meat from commercial lines and local breeds differs significantly, it is important to evaluate the applicability of these models to local breeds as well (Cappone et al., 2026). Furthermore, discriminant analysis enhances the application of NIR for verifying and controlling the adulteration and purity of samples (Murray et al., 2001). Near-infrared-based discrimination has been effectively used for identifying different meat species (Cozzolino & Murray, 2004; McElhinney et al., 1999; Thyholt et al., 1998) and for classification purposes in commercial food products (Fumière et al., 2000; Hervás et al., 1994). In the poultry industry, this approach has been applied to distinguish among chicken parts (such as breast, thigh, and drumstick) (Fumière et al., 2000; Nolasco-Perez et al., 2018), between slow-growing and fast-growing lines (Ding et al., 1999; McDevitt et al., 2005), and among different feeding (McDevitt et al., 2005; Prieto et al., 2009). In this perspective, such analysis could become a valuable tool for local breeds, enabling rapid meat authentication and contributing to the prevention of food fraud.

Therefore, this study aimed to evaluate the potential of NIR spectroscopy to predict chicken meat quality and discriminate chicken parts, sex, and diet in a local chicken breed, the Bionda Piemontese, which is the most widespread in Piedmont region (Castillo et al., 2021). Moreover, NIR measurements were performed on different meat preparations (intact, ground, and freeze-dried), to assess the method's effectiveness and provide a recommendation the most suitable sample presentation.

2. Materials and methods

2.1. Poultry samples and sample collection

This study utilized the same experimental chicken population previously investigated by Stoppani et al. (2025). Briefly, a total of 60 chickens, from Bionda Piemontese (BP) breed, equally divided by sex (30 males and 30 females), were assigned to two different diets: a Low Lipidic content diet (LL, ether extract, EE=3.6%) and a High Lipidic content diet (HL, EE=9.3%). The ingredient and chemical composition of both diets were identical to those reported by Stoppani et al. (2025) (Table 1). Each dietary group was replicated three times per sex, with five birds per replicate.

Chickens were slaughtered at 150 days of age in a commercial abattoir. Twenty-four hours after slaughtering, chickens were cut into quarters, and one whole leg and half-breast fillet were kept for the following analyses. The experimental protocol was developed in compliance with the current European Directive (2010/63/EU) concerning the care and use of animals for scientific purposes and received approval from the Bioethical Committee of the University of Turin (Prot. ID:251833).

2.2. Physicochemical analysis

Twenty-four hours after slaughtering, pH and color parameters were measured on all thigh and breast fillet samples, as described in Stoppani et al. (2025). Briefly, pH and color parameters were measured with a pH-meter SevenGO SG2TM (Mettler-Toledo, Schwerzenbach, Switzerland) and a Minolta CR-200 Chroma Meter (Konica Minolta,

Table 1

Ingredients (g/kg as fed) and formulated chemical composition of the diets from Stoppani et al. (2025).

Ingredients	LL	HL
Corn meal	700	625
Soybean meal	224	239
Palm kernel oil	–	60
Sunflower seed oil	40	40
Calcium carbonate	12	12
Dicalcium phosphate	10	10
Vitamin-mineral premix ¹	10	10
Soybean oil	5	5
Cane molasses	5	5
Sodium chloride	2	2
Sodium bicarbonate	1	1
Metabolizable energy (kcal/kg)	2983	3264
Chemical composition (formulated)		
Dry matter (%)	88.66	86.51
Crude protein (%)	17.00	17.00
Ash (%)	4.81	4.80
Ether extract (%)	3.85	9.49
Crude fiber (%)	4.06	3.99

Abbreviations: LL: Low Lipidic; HL: High Lipidic

¹ Premix contained the following nutrients (units are expressed per kg of diet): vitamin A, 15,000 IU; vitamin D3, 3,000 IU; vitamin E, 25 IU; vitamin K3, 5 mg; vitamin B₁, 2 mg; vitamin B₂, 7 mg; vitamin B₆, 4 mg; vitamin B₁₂, 25 mg; pantothenic acid, 11.04 mg; nicotinic acid, 35 mg; folic acid, 1 mg; biotin, 15 µg; choline chloride, 250 mg; Cu, 1.6 mg; Mn, 60 mg; Zn, 45 mg; Fe, 80 mg; I, 0.4 mg; Se, 0.15 mg.

Chiyoda, Japan), respectively. Subsequently, the fresh samples were frozen at -20 °C and sent to the Agricultural and Animal Production Laboratory of the Universitat Autònoma de Barcelona (Spain), where they were thawed and analyzed by NIR spectroscopy within 24 hours after thawing. All samples were processed in the same way and spectra collected in three presentation forms: intact, ground, and freeze-dried. After the NIR measurements, the freeze-dried samples were examined through chemical methods.

Freeze-dried samples of whole leg and breast cuts were analyzed for moisture (#950.46), crude protein (CP, #2001.11), and ash (#920.153) contents according to AOAC International (2000) procedures; and for ether extract (EE, #960.39), determined following the guidelines of AOAC International (2003). All chemical analyses were performed in duplicate. Proximate composition results were expressed as g/100 g of fresh matter (FM) and as g/100 g of dry matter (DM). The distribution of the data was evaluated by visual inspection of histograms generated in R.

2.3. Near-infrared spectroscopy analysis

Both breast fillet and thigh samples were analyzed with a NIRSystems 5000 (FOSS®, Hillerød, Denmark) scanning monochromator, operating in reflectance mode. The spectral range was between 1,100 and 2,500 nm, with a spectral interval of 2 nm. Both breast and thigh samples were presented in three different physical forms for NIR measurement: intact, ground, and freeze-dried.

Twenty-four hours after thawing, fresh intact samples were prepared for spectral acquisition. Prior to scanning, the skin and excess water on the surface were removed. The spectral scans were collected at room temperature by cutting a piece (8 × 5 × 1 cm) from a specific point of the breast and thigh to minimize variations. The thigh pieces were selected to be free of bones and tendons, and all samples were presented using a plastic film. Fig. 1 shows both the selected portion of the sample and the scanning setup with the plastic film.

Then, leg samples were manually deboned, and the major tendons removed. Fresh breast and leg muscles were ground separately using a kitchen chopper for 10 sec. Approximately 5 g of each ground sample were scanned and after being wrapped in a plastic film (Fig. 2).



Fig. 1. Preparation and spectral acquisition of fresh meat samples. Standardized piece ($8 \times 5 \times 1$ cm) cut from the breast (left) and thigh (middle). Right: scanning setup showing the sample covered with a plastic film during near-infrared (NIR) analysis.



Fig. 2. Spectral acquisition of ground samples. Scanning setup showing the ground sample covered with a plastic film during near-infrared (NIR) analysis.

Finally, the ground samples were freeze-dried with a Telstar® (LyoAlfa 15, Barcelona, Spain). The freeze-dried breast samples were first milled with a laboratory mill (CT293 Cyclotec™ 6500 FOSS®, Hillerød, Denmark), and then ground at a high-speed action (4 g/sec, impeller 10.000 rpm) to pass a fine mesh screen (1 mm in diameter). Although the high-volume air flow provided self-cleaning action, a cleaning step was performed between all samples to minimize cross-contamination. This technique is useful to prepare samples with low fat (up to 10%) and low moisture content (up to 15%). Leg dried samples contained up to 10% of fat; therefore, they were milled using a grinder. For better homogeneity of the milled thigh samples, a 1.6 mm sieve was used to filter the larger parts. Then, about 2-3 g of freeze-dried samples were scanned using a ring cup (35 mm diameter) with a quartz glass (Fig. 3).

All the scans were acquired in duplicate, resulting in a total of 240 spectra (60 chickens $\times$ 2 anatomical sites [thigh and breast] $\times$ 2 replicate scans). For intact and ground samples, the process involved turning them upside-down. In the case of freeze-dried samples, the sample cup was manually rotated approximately 180° relative to the previous scan.



Fig. 3. Spectral acquisition of freeze-dried sample. Scanning setup showing the freeze-dried sample using a ring cup during near-infrared (NIR) analysis.

2.4. Chemometric analysis

Chemometric analysis to predict the physicochemical traits was performed using WinISI 4.10 software (Infrasoft International, Port Matilda, PA, USA), as reported by Albanell et al. (2021). This software is specifically developed to treat spectral data and to be able to implement the calibration models developed on the device used to acquire the spectra. If the prediction models developed are accurate enough, we will be able to obtain the predicted trait values for each sample instantly.

A first exploratory spectral data analysis confirmed not clustering of samples, confirming that all 120 spectra could be used to develop the prediction models for pH, b^* , EE, and ashes. However, color parameters a^* and L^* , moisture, and CP clustered separately for breast and thigh. Therefore, prediction models for pH, b^* , EE, and ashes were based on an external-validation approach, with 70% of the samples included in the calibration (CAL) set and 30% of the samples in the validation (VAL) set. The calibration and validation sets had similar means and standard deviations for all the evaluated traits, ensuring that the variables were uniformly distributed across both datasets. On the other hand, prediction models for color parameters a^* and L^* , moisture, and CP were developed separately for breast ($n = 60$) and thigh ($n = 60$), based on cross-validation (CV) approach.

The calibration equations were developed using the full spectra on the global equation program of WinISI software version 4.10. The spectral data and the laboratory reference values were regressed by using modified partial least-square regression (MPLS) and the raw spectrum of all samples was preprocessed applying several combinations of scattering correction (D, Detrend; SNV, standard normal variate; SNV+D; and MSC, multiplicative scatter correction) and derivative (1,4,4,1; 1,5,5,1; 1,10,10,1; 1,10,4,1; 1,12,2,2; 2,4,4,1; 2,5,5,1; 2,10,10,1; 2,10,4,1; 2,12,2,2; where the first digit is the number of the derivative, the second is the gap over which the derivative is calculated, the third is the number of data points in the first smoothing, and the fourth is the number of data points in the second smoothing) resulting in 40 regression equations per parameter. Processing of the data before modeling is very important because it can reduce spectral fluctuations caused by scattering and variance (Pizarro et al., 2004). The optimum calibration model was also chosen on the lowest standard error of calibration (SEC) and standard error of prediction (SEP), and the highest coefficients of determination (R^2_{CAL} , R^2_{CV} , and R^2_{VAL}) (He et al., 2013). Moreover, the bias (mean difference between the predicted and the reference data) and the slope in the external validation dataset were also calculated, and should be closer to 0 and 1, respectively. In general, an R^2 value between 0.66 and 0.81 indicates a proximate quantitative estimation of the reference value, values between 0.82 and 0.90 indicate a good estimation, and values > 0.91 indicate an excellent estimation. Additionally, calibration performance was assessed using the ratio of performance to deviation (RPD), calculated as the ratio of SD of the validation samples to the SEP. According to Williams (2014), a

calibration with $RPD \leq 1.9$ is considered unsuitable; values between 2.0 and 2.4 indicate performance suitable for rough screening; values between 2.5 and 2.9 provide a fair prediction that can be used for screening, and values ≥ 3.0 indicate good prediction and can be used for quantitative analysis.

Additionally, a partial least squares regression-discriminant analysis (PLS-DA) of NIR spectra was also performed to classify chicken parts, sex, and dietary treatment through R software v. 4.3.1 using “caret” and “waves” packages (Hershberger et al., 2021; Kuhn, 2008). To build the PLS-DA models, the dataset was split into a train (70%) and a test (30%) set, balancing the number of samples according to the considered variables (chicken part, sex, and dietary treatment). Chemometric models evaluated included raw spectral data (Raw data), and spectral data after applying 12 different correction methods: standard normal variate (SNV) with and without first (SNVD1) or second derivative (SNVD2); first (D1) or second (D2) derivative; Savitzky-Golay (SG) smoothing, and its combination with SNV (SNVSG), first derivative (SGD1), and first or second derivative, window 5 and 11 (SG.D1W5; SG.D2W5; SG.D1W11; SG.D2W11). The best prediction models were selected based on the highest classificatory accuracy, which in NIR-based discriminant analyses is typically reported between 90% and 95% for food authentication tasks (Cozzolino & Murray, 2004; Fumière et al., 2000).

3. Results

3.1. Spectra characterization

Fig. 4 displays the average spectra profiles of the different sample presentations divided for meat cuts (Fig. 4A), sex (Fig. 4B), and dietary treatments (Fig. 4C). The raw spectra of intact and ground samples were similar, with a greater absorbance in intact than in ground samples. On the other hand, the spectrum of freeze-dried samples differed markedly from those of intact and ground samples, showing lower absorbance due to water removal, with a spectral pattern primarily driven by dry-matter components. Moreover, the spectral pattern between sex, chicken part or diets was similar. Almost the same absorbance was observed between sex (Fig. 4B) and diet (Fig. 4C) categories, whereas breast had a greater absorbance than thigh for intact and ground samples, and a lower absorbance for freeze-dried samples (Fig. 4A).

3.2. Distribution of physicochemical traits

A preliminary analysis of the data distribution is essential to ensure accurate NIR calibration. In this study, strong effect was given by the meat cut, resulting in the creation of two distinct clusters (breast and thigh) for a^* and L^* , moisture, and CP, values (Fig. 5). Except for these traits, all other variables were analyzed as a single dataset.

3.3. Quantitative NIR prediction equations

Descriptive statistics for pH, b^* , ether extract (EE), and ash content of the dataset were analyzed using an external validation approach to develop the prediction models (Table 2). On the other hand, Table 3 showed the descriptive statistics for a^* , L^* , moisture, and protein (CP) of the dataset used to build the prediction models for breast and thigh separately. Best prediction models were obtained with different mathematical treatments and scatter corrections (Tables 4 and Table 5).

3.3.1. pH, yellowness, EE, and ash prediction models with the complete dataset

The prediction models developed on intact samples show promising results for EE in FM ($R^2_{CAL} = 0.84$) and DM ($R^2_{CAL} = 0.86$). However, only the model for EE (DM) met the threshold established for RPD ($R^2_{VAL} = 0.76$; RPD = 2.08), making it suitable for rough screening. Nevertheless, in ground samples the prediction models improved with respect to the intact samples, reaching an RPD > 2.5 for EE in both FM ($R^2_{VAL} = 0.88$;

RPD = 2.89) and DM ($R^2_{VAL} = 0.86$; RPD = 2.65). More accurate prediction models were achieved with freeze-dried samples for ash ($R^2_{VAL} = 0.82$; RPD = 2.34 on FM basis, and $R^2_{VAL} = 0.75$; RPD = 2.15 on DM basis) and EE content ($R^2_{VAL} = 0.95$; RPD = 7.27 on FM basis, and $R^2_{VAL} = 0.96$; RPD = 4.71 on DM basis). Conversely, prediction models for pH and yellowness (b^*) did not achieve acceptable accuracy in any of the sample presentation tested (Table 4).

3.3.2. Redness, lightness, moisture, and CP prediction models for breast and thigh

Prediction models revealed an unsatisfactory performance for intact samples for all four parameters (a^* , L^* , moisture, and CP; Table 5). For ground samples, only CP content of the breast expressed in DM reached the threshold established for RPD ($R^2_{CV} = 0.84$; RPD = 2.53) to consider it suitable for screening. The best prediction models were obtained with freeze-dried samples, to predict CP expressed as intact matter for the thigh ($R^2_{CV} = 0.89$; RPD = 3.11), and expressed in DM for both, breast ($R^2_{CV} = 0.97$; RPD = 5.86) and thigh ($R^2_{CV} = 0.90$; RPD = 3.18) (Table 5).

3.4. Discriminant analysis

Discriminant analysis was performed to test the ability of NIR in predicting chicken part (breast or thigh), sex (male or female), and dietary treatment (LL or HL). The best discriminant model for each category (chicken part, sex, and dietary treatment) is displayed in Table 6 according to the different sample preparations and the test set. Several mathematical treatments and scatter corrections led to similar performance of the classificatory model (Table 6). Results showed that PLS-DA analysis performed on NIR spectra to detect chicken parts had 100% accuracy applying several scatter corrections in all sample preparations. Regarding sex discrimination, the analysis revealed a slightly lower accuracy in all sample preparations ($> 89\%$) compared to chicken part models, with the greatest accuracy obtained in freeze-dried samples (97%). However, it was not possible to build accurate prediction models to differentiate between the dietary treatments. For dietary discrimination, the best prediction model was obtained in freeze-dried, which reached a 67% accuracy.

4. Discussion

4.1. NIR spectra differs based on sample presentation

Between 1100 and 2500 nm, the NIR spectra of chicken meat samples are mainly shaped by the overtone and combination vibrations of O–H, C–H, and N–H bonds (Xiaobo et al., 2010). This wavelength range is characterized by absorption bands of water (around 1450 nm, corresponding to the first overtone of O–H stretching, and at 1940 nm, related to the combination of O–H stretching and bending), lipids (1200–1250 nm, 1720–1760 nm, and 2300–2340 nm, reflecting C–H stretching and deformation modes), and proteins (2050 and 2180 nm) (Cozzolino & Murray, 2004; Fodor et al., 2024). Therefore, this spectral window provides valuable information on relevant meat constituents—moisture, fat, and protein—and highlights the structural differences between intact, ground, and freeze-dried samples. These differences are mainly due to the strong absorption of water in the NIR region, but also to different sample presentation. Sample presentation affects the NIR spectrum, due to differences in tissue organization that influence the information obtained from the sample, such as myofibrillar refringence, myoglobin content, protein precipitation in the sarcoplasm, macroscopic surface reflectance properties of cut meat, and sarcomere length (Cozzolino & Murray, 2002). In particular, intact samples tend to exhibit higher apparent absorbance, likely due to the orientation of muscle fibers, which can act as optical waveguides and facilitate light propagation through the tissue. This effect arises from the combined influence of light absorption and scattering: the structural heterogeneity of intact tissue and the compartmentalized distribution of water create refractive

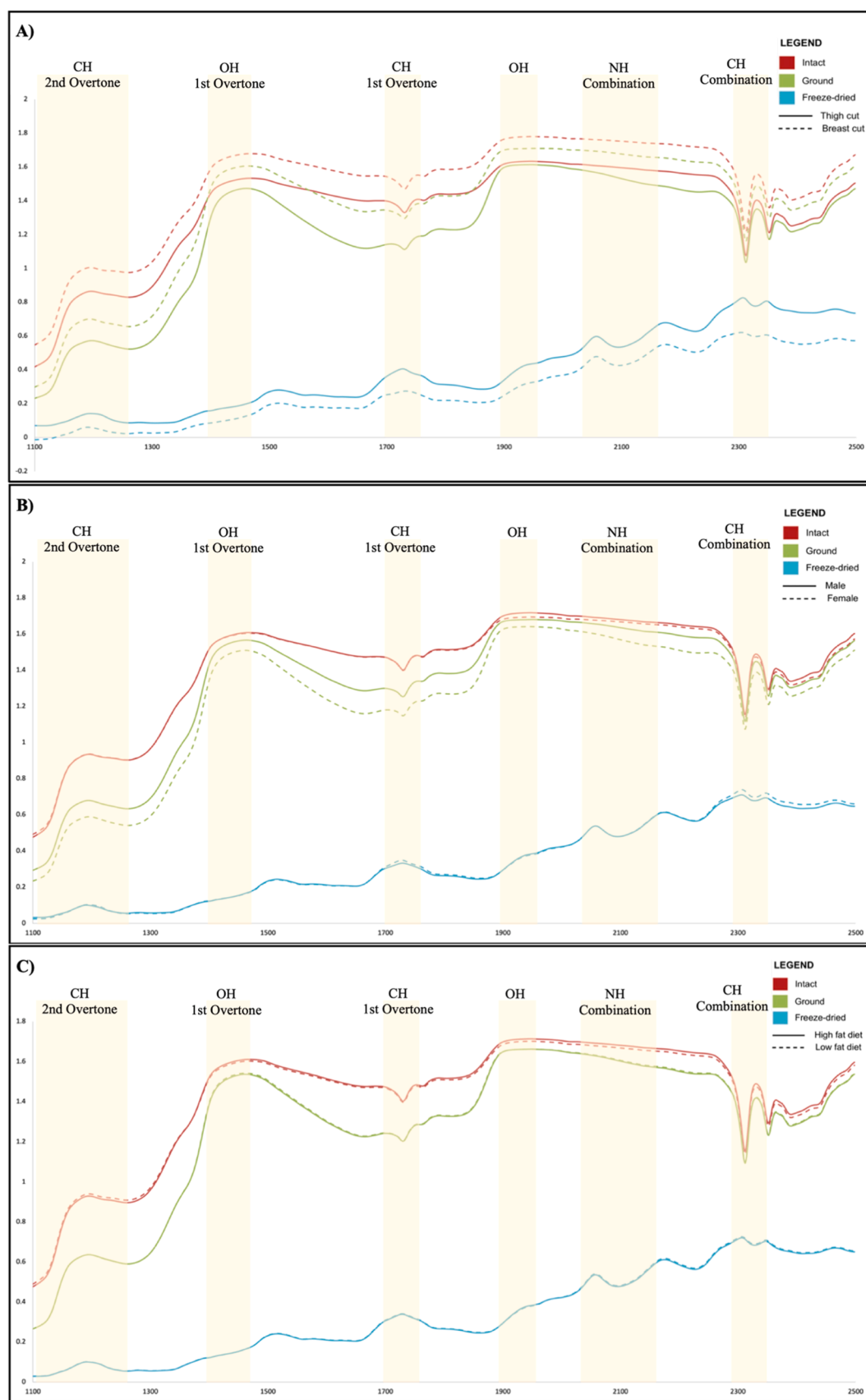


Fig. 4. Average spectra profile of intact, ground, and freeze-dried samples with the attribution of the main infrared bands. (A) Average spectra according to meat cut (thigh and breast); (B) to sex (male and female); (C) to dietary treatment (high-fat and low-fat diets). The x-axes represent the wavelength range (nm), while the y-axes represent the absorbance (Log1/reflectance).

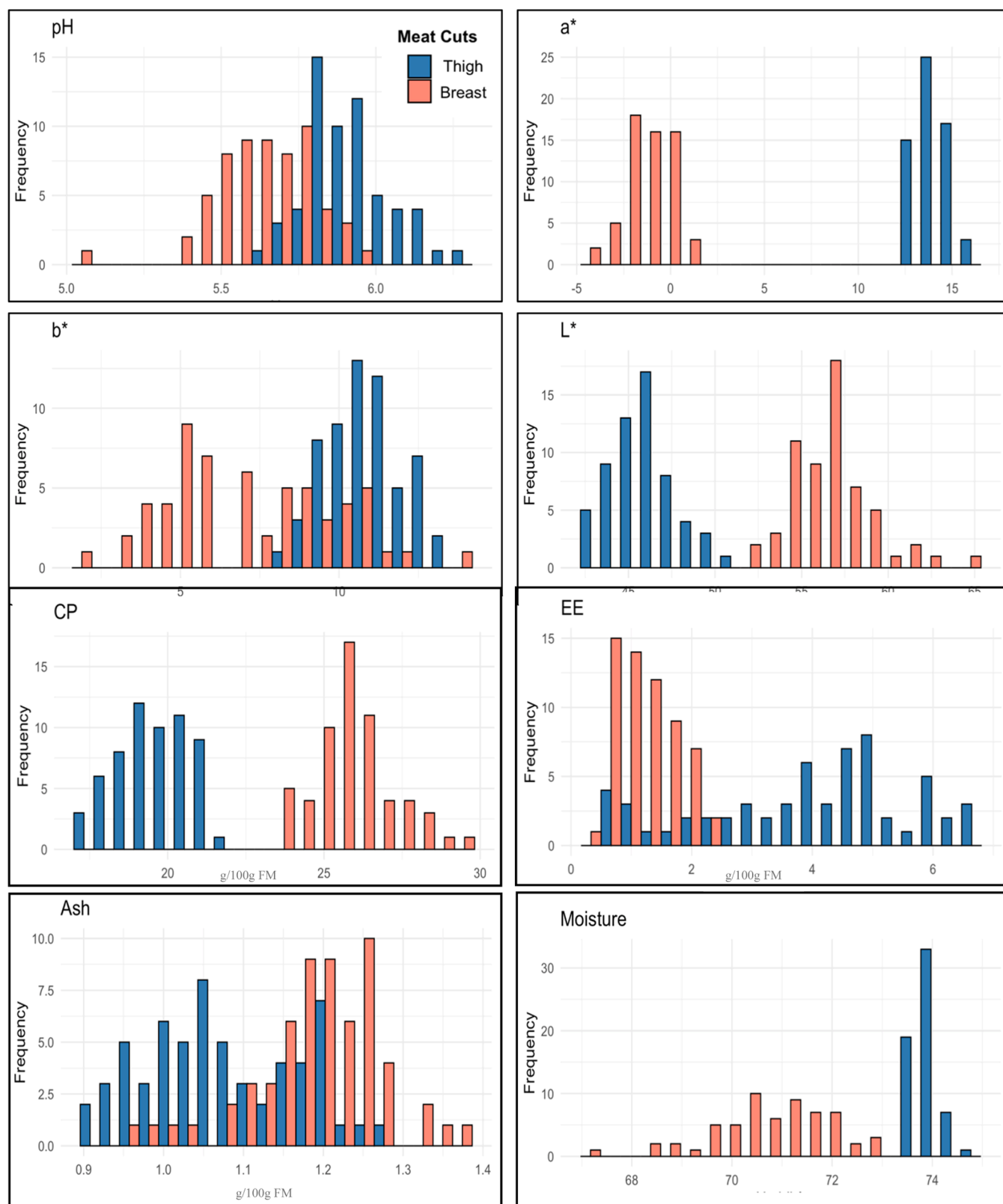


Fig. 5. Distribution of data collected in breast and thigh samples for pH, color (a^* : redness; b^* : yellowness; L^* : lightness), crude protein (CP), ether extract (EE), ashes, and moisture. CP, EE, and ash are presented on a fresh matter (FM) basis.

index mismatches, increasing photon path length and, consequently, apparent absorbance. As a result, intact and ground samples, which retain their natural moisture, exhibit higher absorbance in the water-related bands, whereas freeze-dried samples, having lost most of

their water, show reduced absorbance and a spectral profile dominated by protein, lipids, and other dry matter components.

Table 2

Descriptive statistics of calibration and validation datasets for pH, yellowness (b^*) color, ether extract (EE), and ash content in fresh meat (FM) in Bionda Piemontese chicken.

Constituent	Calibration set (n = 96)			Validation set (n = 24)		
	Mean $\pm$ SD ¹	Min ²	Max ³	Mean $\pm$ SD ¹	Min ²	Max ³
pH	5.78 $\pm$ 0.21	5.06	6.29	5.77 $\pm$ 0.18	5.41	6.20
b^*	9.12 $\pm$ 2.68	3.22	13.72	8.67 $\pm$ 3.03	1.73	13.01
EE (g/100g FM)	2.57 $\pm$ 1.80	0.45	6.76	2.68 $\pm$ 2.15	0.51	6.59
Ash (g/100g FM)	1.14 $\pm$ 0.13	0.92	1.87	1.12 $\pm$ 0.11	0.90	1.28

¹ SD= Standard Deviation

² Min= Minimum value

³ Max= Maximum value

Table 3

Descriptive statistics of calibration datasets for redness (a^*) color, color lightness (L^*), moisture, and crude protein (CP) content in fresh meat (FM) of Bionda Piemontese chicken.

Constituent	Breast set (n = 60)			Thigh set (n = 60)		
	Mean $\pm$ SD ¹	Min ²	Max ³	Mean $\pm$ SD ¹	Min ²	Max ³
a^*	-1.29 $\pm$ 1.18	-4.00	0.94	14.03 $\pm$ 0.83	12.59	16.28
L^*	56.51 $\pm$ 2.38	51.69	64.30	45.91 $\pm$ 1.95	42.32	51.06
Moisture	70.77 $\pm$ 1.20	67.19	72.91	73.91 $\pm$ 0.24	73.52	74.79
CP (g/100g FM)	25.87 $\pm$ 1.25	23.43	29.29	19.62 $\pm$ 1.14	17.11	21.57

¹ SD= Standard Deviation

² Min= Minimum value

³ Max= Maximum value

Table 4

Fitting statistics¹ of near-infrared spectroscopy prediction models for pH, yellowness (b^*) color, ether extract (EE) and ash content in fresh (FM) and dry matter (DM) of Bionda Piemontese chicken meat were developed using external validation on intact, ground, and freeze-dried samples.

Trait	Calibration					Validation							
	Math Treatment	Scatter correction ²	N _{CAL}	R ² _{CAL}	SEC	R ² _{CV}	SE _{CV}	N _{VAL}	R ² _{VAL}	SEP	Bias	Slope	RPD
<i>Intact sample</i>													
pH	1,4,4,1	D	93	0.69	0.11	0.59	0.13	23	0.48	0.13	-0.03	0.84	1.49
b^*	2,5,5,1	SNV	93	0.58	1.67	0.45	1.67	24	0.51	2.21	-0.61	1.21	1.38
EE (FM)	1,4,4,1	SNV+D	93	0.84	0.74	0.75	1.00	23	0.71	1.12	0.09	1.02	1.88
EE (DM)	1,10,10,1	D	91	0.86	2.67	0.78	3.34	23	0.76	3.98	0.03	0.97	2.08
Ash (FM)	1,4,4,1	D	92	0.74	0.05	0.60	0.07	23	0.66	0.06	-0.00	1.07	1.72
Ash (DM)	1,4,4,1	D	95	0.39	0.22	0.20	0.25	23	0.40	0.25	-0.03	1.21	1.40
<i>Ground sample</i>													
pH	2,5,5,1	SNV	93	0.64	0.12	0.64	0.13	24	0.57	0.13	-0.03	0.74	1.39
b^*	1,4,4,1	MSC	95	0.57	1.76	0.57	1.90	23	0.54	1.79	-0.10	0.98	1.69
EE (FM)	1,10,10,1	SNV+D	86	0.97	0.32	0.96	0.42	23	0.88	0.74	-0.12	1.02	2.89
EE(DM)	1,5,5,1	SNV+D	86	0.97	1.17	0.97	1.39	23	0.86	3.17	-0.27	1.03	2.65
Ash (FM)	1,12,2,2	SNV+D	91	0.73	0.06	0.70	0.06	23	0.71	0.06	-0.01	0.86	1.77
Ash (DM)	1,12,2,2	SNV+D	92	0.68	0.16	0.46	0.20	23	0.73	0.18	-0.03	1.01	1.91
<i>Freeze-dried sample</i>													
pH	2,5,5,1	D	92	0.81	0.09	0.77	0.10	23	0.41	0.14	-0.01	0.62	1.32
b^*	2,12,2,2	MSC	90	0.88	0.91	0.81	1.24	23	0.68	1.74	-0.29	0.94	1.74
EE (FM)	2,4,4,1	SNV+D	86	1.00	0.10	1.00	0.11	23	0.95	0.47	-0.10	0.47	7.27
EE (DM)	2,4,4,1	SNV+D	87	1.00	0.44	1.00	0.48	23	0.96	1.78	-0.32	1.02	4.71
Ash (FM)	2,4,4,1	SNV+D	92	0.72	0.05	0.73	0.06	23	0.82	0.05	0.00	0.93	2.34
Ash (DM)	1,10,10,1	SNV+D	92	0.67	0.16	0.65	0.16	23	0.75	0.16	0.01	1.00	2.15

¹ N_{CAL}= number of samples used for calibration; R²_{CAL}= coefficients of determination in calibration; SEC= standard error of calibration; R²_{CV}= coefficients of determination in cross-validation; SE_{CV}= standard error of cross-validation; N_{VAL}= number of samples used for the validation; R²_{VAL}= coefficients of determination in validation; SEP= standard error of prediction; RPD= ratio of performance to deviation.

² SNV, standard normal variate; D, detrend; MSC, multiplicative scatter correction; and SNV+D combination of SNV and D.

4.2. Effect of sample distribution on prediction models

Robust NIR calibration models require datasets with a wide and representative range of variation for all measured traits, displaying a uniform distribution (Chariskou et al., 2022; Fernández-Cabanás et al., 2023). Adequate variability allows the chemometric models to generalize to new samples and reduces prediction errors (Chu et al., 2005). In BP chickens, breast and thigh muscles are known to differ in color (Dabbou et al., 2019), as well as in protein and fat content (Bongiorno et al., 2022). These differences can lead to distinct data distributions, which influence model performance. When a given parameters is strongly affected by the anatomical origin of the sample, as observed in the present study, combining datasets may lead to two clearly differentiated data-point clouds, forcing the slope of the prediction model. In such cases, developing separate calibration models for each muscle cut is more appropriate. This was evident for traits such as a^* , L^* , moisture, and CP, which showed different distribution between breast and thigh samples. The separation of the dataset based on the anatomical region for these parameters allows the models to minimize noise associated with compositional and structural heterogeneity. On the other hand, parameters such as pH, b^* , EE, and ashes displayed comparable distributions across the two cuts, covering both cuts the complete range. For these traits, merging the dataset did not lead to force the slope of the prediction model, allowing to increase the sample size to enhance model robustness and stability (Ezenarro & Schorn-García, 2025). Therefore, these findings highlight the importance of evaluating the distribution of each trait prior to model development.

4.3. Unit of measurement (Fresh or Dry Matter) impact model performance

The chemical parameters in terms of ash, CP, and EE are typically reported on a dry matter basis, analyzing freeze-dried samples (Abeni & Bergoglio, 2001; Berzaghi et al., 2005; McDevitt et al., 2005). However, expressing these parameters directly on a fresh matter (FM) basis (g/100 g FM) offers a more practical advantage, especially for regulatory compliance and consumer information, as it aligns with how nutritional

Table 5

Fitting statistics¹ of near-infrared spectroscopy prediction models of redness (a^*) color, color lightness (L^*), moisture, and crude protein (CP) content in fresh (FM) and dry matter (DM) in Bionda Piemontese chicken meat on intact, ground, and freeze-dried samples developed in cross-validation.

Trait	Meat cut	Math treatment	Scatter correction ²	R^2_{CAL}	SEC	R^2_{CV}	SE _{CV}	RPD
Intact sample								
a^*	breast	1,5,5,1	MSC	0.90	0.37	0.39	0.84	1.40
	thigh	2,4,4,1	D	0.27	0.64	0.16	0.68	1.22
L^*	breast	1,10,10,1	D	0.30	1.55	0.00	1.95	1.22
	thigh	1,10,10,1	D	0.68	1.01	0.25	1.61	1.21
Moisture	breast	2,4,4,1	D	0.23	0.98	0.00	1.28	0.94
	thigh	2,4,4,1	D	0.44	0.15	0.16	0.19	1.30
CP (FM)	breast	1,4,4,1	SNV+D	0.37	1.02	0.00	1.30	0.96
	thigh	1,12,2,2	D	0.75	0.61	0.41	0.87	1.31
CP (DM)	breast	1,12,2,2	SNV	0.40	1.61	0.20	1.80	1.12
	thigh	1,12,2,2	D	0.73	2.24	0.42	3.08	1.35
Ground sample								
a^*	breast	2,4,4,1	D	0.39	0.91	0.29	0.97	1.22
	thigh	2,4,4,1	D	0.23	0.72	0.14	0.76	1.09
L^*	breast	2,4,4,1	D	0.19	1.58	0.03	1.70	1.40
	thigh	2,4,4,1	D	0.08	1.88	0.00	2.01	0.97
Moisture	breast	2,4,4,1	D	0.12	1.05	0.00	1.16	1.03
	thigh	1,4,4,1	MSC	0.20	0.18	0.11	0.19	1.26
CP (FM)	breast	2,4,4,1	D	0.18	1.07	0.11	1.11	1.12
	thigh	2,10,10,1	D	0.70	0.63	0.62	0.69	1.64
CP (DM)	breast	1,4,4,1	MSC	0.89	0.68	0.84	0.80	2.53
	thigh	2,5,5,1	D	0.72	2.22	0.63	2.50	1.66
Freeze-dried sample								
a^*	breast	2,4,4,1	D	0.92	0.36	0.87	0.92	1.28
	thigh	2,4,4,1	SNV+D	0.28	0.68	0.07	0.76	1.09
L^*	breast	1,4,4,1	D	0.52	1.46	0.40	1.60	1.49
	thigh	1,5,5,1	D	0.70	1.08	0.53	1.31	1.49
CP (FM)	breast	1,10,10,1	D	0.27	1.03	0.16	1.10	1.14
	thigh	1,4,4,1	D	0.91	0.35	0.89	0.37	3.11
CP (DM)	breast	1,10,10,1	D	0.99	0.25	0.97	0.35	5.86
	thigh	2,4,4,1	MSC	0.92	1.22	0.90	1.31	3.18

¹ N_{CAL} = number of samples used for calibration; R^2_{CAL} = coefficients of determination in calibration; SEC = standard error of calibration; R^2_{CV} = coefficients of determination in cross-validation; SE_{CV} = standard error of cross-validation; RPD = ratio of performance to deviation.

² SNV, standard normal variate; D, detrend; MSC, multiplicative scatter correction; and SNV+D combination of SNV and D.

Table 6

Models' accuracy obtained in the test set ($n = 36$) to discriminate chicken part, sex and dietary treatment using different pre-processing methods on different sample presentations.

	Sample presentation	Scatter correction ¹	Correctly classify
Chicken part (Breast vs Thigh)	Intact	Raw, SNV, SNVD1, SNVD2, D1, SG, SNVSG, SGD1, SG.D1W5, SG.D1W11, SG.D2W5, SG.D2W11	100%
	Ground	SNV, SNVD1, SNVD2, D1, D2, SG, SNVSG, SG.D1W5, SG.D2W5	100%
	Freeze-dried	Raw, SNVD1, D1, SG	100%
Sex (Male vs Female)	Intact	SGD1, SG.D1W11	89%
	Ground	SNVD1	91%
	Freeze-dried	Raw, SNV, D1, SG, SNVSG, SGD1, SG.D1W11	97%
Dietary treatment (Low lipid diet vs High lipid diet)	Intact	SNV, SNVSG	57%
	Ground	Raw, SNV	60%
	Freeze-dried	SG.D2W11	67%

¹ Raw, Raw data; SNV, standard normal variate; D1, first derivative; D2, second derivative; SG, Savitzky-Golay smoothing; SNVD1, combination of SNV and D1; SNVD2, combination of SNV and D2; SNVSG, combination of SNV and SG; combination of SG with first (SGD1) and second derivatives (SGD2), window 5 and 11 (SG.D1W5; SG.D2W5; SG.D1W11; SG.D2W11).

values are displayed on commercial labels in the meat industry (Fumière et al., 2000; Prevotnik et al., 2004). In this study, both DM and FM units were employed to accommodate the range of meat preparations analyzed, including intact, ground, and freeze-dried preparations. This

dual approach facilitated greater flexibility and robustness in the development of calibration models, as it accounted for the variability introduced by differing physical states of the meat. In general, very similar results were obtained for all the chemical parameters expressed as DM or FM. However, for ground and freeze-dried breast samples, CP could be reliably predicted only when expressed on DM basis. These results indicate that water probably only impairs the accuracy of protein content. Moreover, in the case of freeze-dried samples, expressing results on a DM basis is preferable, as it avoids the introduction of additional variability associated with moisture correction factors. Including such corrections may reintroduce uncertainty into the model, thereby reducing its predictive performance despite the inherent stability of the freeze-dried matrix.

4.4. Poor performance of NIR model to predict color and pH

Prediction NIR models for both color and pH traits did not meet the standard threshold established by Williams (2014) of $RPD \leq 1.9$, indicating an unstable and unreliable prediction for screening. The unsatisfactory results in predicting color have also been reported by other studies, possibly due to the greater subjectivity of such factors (Abeni & Bergoglio, 2001; Prieto et al., 2009). In fact, variability may have been introduced by differences in the measurement location selected by the operator on the sample surface, further affecting the consistency of the results. However, more accurate predictions for color parameters were achieved when a broader spectral range was used. For instance, De Marchi et al. (2011) found that visible-NIR predictions were suitable for screening purposes for a^* ($R_{CV} = 0.88$; $RPD = 2.14$) and b^* ($R_{CV} = 0.93$; $RPD = 2.82$) values in intact chicken breast, although unreliable prediction was observed for L^* value ($R_{CV} = 0.69$; $RPD = 1.40$). Hence, these results indicate that visible spectroscopy (400–750 nm) can be a

valuable tool for meat color characterization, particularly for chromatic coordinates.

A similar trend was observed for pH value across all sample preparations. The low estimation ability found in the present study is consistent with previous research, which has attributed this limitation to the minimal variation in pH measurements in chicken meat. For example, [Berzaghi et al. \(2005\)](#) confirmed that NIR models were not able to predict meat pH at 24h and 7 days in laying hens. Similarly, although excellent R^2 were reported in broiler breast samples using a visible-NIR instrument, the RPD remained ≤ 1.9 , indicating insufficient reliability for practical use ([Liu et al., 2004](#)).

It should also be noted that pH and color were measured at 24 hours post-slaughter, whereas NIR spectra were acquired after thawing. This time difference may explain the unsatisfactory results for these parameters, as both color and pH are known to change over time and after freezing–thawing ([Zhang et al., 2017](#); [Zhang et al., 2023](#)). Nevertheless, these findings suggest that visible spectroscopy (400–750 nm) can still be useful for characterizing meat color, particularly for chromatic coordinates. Overall, the results confirm that NIR spectroscopy cannot reliably predict color or pH traits in chicken meat under the conditions evaluated.

4.5. Ash prediction accurate only in freeze-dried samples

Ash prediction was unreliable in intact and ground samples; however, a greater accuracy was observed in freeze-dried samples, with R^2_{VAL} between 0.70–0.82 and RPD between 2.2 and 2.3 for both for fresh (FM) and dry matter (DM). This indicates that NIR model was able to provide reliable predictions for freeze-dried samples suitable for rough screening, according to the evaluation criteria of [Williams \(2014\)](#), who classified models with RPD values between 2.0 and 2.4 as adequate for screening purposes. In contrast, performance in intact and ground samples was limited, likely due to water interference. This is a promising result because it is the first time it is achieved a prediction model for ash content in poultry meat accurate enough for rough screening ([Abeni & Bergoglio, 2001](#); [Berzaghi et al., 2005](#)). The improved performance observed could be attributed to the use of a combined dataset including both breast and thigh meat.

4.6. Crude protein and ether extract predicted successfully

In freeze-dried samples, the models for CP and EE achieved excellent accuracy, supporting their suitability for precise quantitative applications. Specifically, CP showed excellent performance expressed on a DM basis in terms of RPD and based on [Williams \(2014\)](#) thresholds (RPD ≥ 3). At the same time, EE achieved even greater accuracy on both FM and DM bases, with RPD ranging from 4 to 7. The strong predictive capability of NIR to estimate CP and EE parameters reflected the fact that the main differences in the NIR region are primarily attributed to lipid and protein absorption bands ([Ding et al., 1999](#)). Moreover, fair predictive ability (RPD = 2.5 – 2.9, according to [Williams, 2014](#)) was obtained for EE in ground samples, whereas CP reached this level only in ground breast samples. This offers a practical advantage for routine analysis, as it allows accurate predictions for those traits without requiring freeze-drying, a process that is time-consuming and not always feasible in applied settings. However, the observed differences in prediction accuracy between breast and thigh samples are likely driven by variations in data distribution, as demonstrated for CP, where distinct value ranges and heterogeneity affected model performance.

Overall, these findings highlight that separating breast and thigh samples for traits with distinct distributions can improve calibration accuracy, whereas combining datasets may be appropriate when traits are similarly distributed in both matrices. In this context, NIR spectroscopy emerges as a viable alternative to wet chemistry methods for both ground and freeze-dried samples, provided that dataset structure and sample heterogeneity are carefully considered during model

development.

4.7. Successful discrimination of chicken parts and sex, not dietary treatment

The successful discrimination of chicken parts could be explained by the better discrimination capacity of the model for chicken parts than for sex and dietary treatment. Successful discrimination between chicken parts aligns with previous studies, which reported that meat cuts are more easily distinguishable in intact samples ([Nolasco-Perez et al., 2018](#); [Soumeh et al., 2025](#)) and that ground meat preparations also allow for discrimination between breast and thigh or drumstick portions ([Nolasco-Perez et al., 2018](#)). Moreover, the present study demonstrated that meat cuts discrimination can also be successfully performed on freeze-dried samples, which are useful for authenticating samples of processed or preserved meat products. This capability is particularly valuable for distinguishing between breast and thigh meat, which often differ in composition and quality traits. These promising results suggest that this technique could be integrated into an analytical system of surveillance for certified meat products.

In contrast with [McDevitt et al. \(2005\)](#), who reported that meat from fast- and slow-growing broiler and layer-type chickens could be classified according to dietary treatment but not by sex, the present study achieved accurate models to discriminate sex but not diet. Their unsatisfactory result for sex may be attributed to the age of the animals, possibly due to their sexual immaturity. In our study, chickens were slaughtered at five months of age and exhibited clear sex-related differences in chemical composition, particularly in CP and EE content. The higher accuracy obtained in predicting sex for freeze-dried samples is likely due to the enhanced concentration and homogeneity of chemical components after freeze-drying. Removal of water may amplify spectral differences in proteins, lipids, and other metabolites that reflect the observed sex-related variations. Although both freeze-dried and ground samples are destructive, intact samples showed the worst performance because tissue heterogeneity, due to water content and limited light penetration, reduces the signal-to-noise ratio and mask compositional differences. By contrast, no significant differences were observed between dietary treatments ([Stoppani et al., 2025](#)), which may explain the lack of discrimination observed in our dataset. The differences in meat chemical composition between sexes may have caused the variations in NIR absorption, as the spectra are mainly characterized by O–H, N–H, and C–H absorption bands ([Xiaobo et al., 2010](#)). Consequently, diet-related discrimination would only be expected when feeding regimes markedly affect these spectral features, as reported by [Berzaghi et al. \(2005\)](#) in chicken meats differing in fatty acid composition.

Accurate prediction of anatomical parts, sex, and dietary treatments is particularly important for preventing food fraud, as male and female meat, breast and thigh cuts, and products from different diets can differ in market value or labeling requirements. NIR spectroscopy provides a rapid, non-destructive tool to ensure the authenticity and correct labeling of meat products.

5. Conclusions

This study is the first to apply NIR spectroscopy to the local chicken breed Bionda Piemontese, demonstrating its potential to predict certain meat quality traits in local markets. Freeze-dried samples remained the most accurate predictions of chemical traits, particularly crude protein and fat. Nevertheless, NIR spectroscopy proved effective for fat prediction in ground samples, offering a practical advantage by eliminating the need for sample preparation and enabling direct analysis of the meat. In contrast, pH and color were not reliably predicted, likely due to low variability and subjective measurement. In addition, the results suggest that meat cuts should be analyzed separately, as breast and thigh showed different prediction accuracies. The choice of unit of measurement (fresh basis vs dry matter) had a limited overall impact; however,

for freeze-dried samples, expressing results on a dry matter basis improved prediction accuracy by better aligning the reference values with the spectral information. Different mathematical treatments and scatter correction techniques should be tested for each parameter in order to select the most appropriate to develop optimal prediction or classification models. NIR also effectively distinguished muscle type and sex, with the highest accuracy in freeze-dried samples, but could not discriminate the experimental diet tested. These results highlight the potential of NIR as a rapid and non-destructive tool for meat authentication, particularly for verifying product identity and detecting potential food fraud related to mislabeling of cut or sex, which in local breeds, exhibit different meat quality characteristics. Overall, these results suggest that, with an increased sample size and improved calibration, NIR spectroscopy could replace traditional analytical methods for Bionda Piemontese, offering a rapid, cost-effective approach that is particularly valuable for local markets where minimizing analysis costs is essential, while supporting nutritional labeling, product authenticity, and quality control. Future studies could extend this research to other local breeds to evaluate whether the observed NIR predictions and discriminations are consistent across different genetic backgrounds.

Ethical approval

All animal procedures were conducted in accordance with the ethical standards and guidelines for the care and use of animals for scientific purposes. The experimental protocol was developed in compliance with the current European Directive (2010/63/EU) concerning the care and use of animals for scientific purposes and received approval from the Bioethical Committee of the University of Turin (Prot. ID:251833).

Data availability statement

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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CRediT authorship contribution statement

Nadia Stoppani: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Elena Albanell:** Writing – review & editing, Supervision, Methodology. **Valeria Zambotto:** Methodology. **Chiara Bianchi:** Methodology. **Dominga Soglia:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Achille Schiavone:** Writing – review & editing, Supervision. **Carmen L. Manuelian:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Methodology, Conceptualization.

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.

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