# Comparison of hyperspectral imaging and spectrometers for prediction of cheeses composition

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**Abstract**

Artisanal cheeses are part of the heritage and identity of different countries or regions. In this work, we investigated the spectral variability of a wide range of traditional Brazilian cheeses and compared the performance of different spectrometers to discriminate cheese types and predict compositional parameters. Spectra in the visible (vis) and near infrared (NIR) region were collected, using imaging (vis/NIR-HSI and NIR-HSI) and conventional (NIRS) spectrometers, and it was determined the chemical composition of seven types of cheeses produced in Brazil. Principal component analysis (PCA) showed that spectral variability in the vis/NIR spectrum is related to differences in color (yellowness index) and fat content, while in NIR there is a greater influence of productive steps and fat content. Partial least squares discriminant analysis (PLSDA) models based on spectral information showed greater accuracy than the model based on chemical composition to discriminate types of traditional

Brazilian cheeses. Partial least squares (PLS) regression models based on vis/NIR-HSI, NIRS, NIR-HSI data and HSI spectroscopic data fusion (vis/NIR + NIR) demonstrated excellent performance to predict moisture content (RPD > 2.5), good ability to predict fat content (2.0 < RPD < 2.5) and can be used to discriminate between high and low protein values (~1.5 < RPD < 2.0). The results obtained for imaging and conventional equipment are comparable and sufficiently accurate, so that both can be adapted to predict the chemical composition of the Brazilian traditional cheeses used in this study according to the needs of the industry.

**Keywords:** artisanal cheeses; denomination of origin; non-destructive technologies; visible-near infrared (vis/NIR) spectroscopy; chemometrics; data fusion.

## 1. Introduction

Historical aspects, regional characteristics, manufacturing technologies, microbial diversity and dairy species contributed to the emergence of different cheeses with unique characteristics that reflect the cultural identity of certain populations. In Brazil, there is a wide type of traditional cheeses that are part of the heritage and identity of different regions, including the North (Marajó cheese), Northeast (Butter and Coalho), South (Colonial and Serrano), Southeast (Araxá, Campo das Vertentes, Cerrado, Canastra and Serro) and Center (Caipira) of the country (Margalho et al., 2021).

Traditional Brazilian cheeses have gained greater visibility in recent years, especially through the granting of Geographical Indications (GI), awards in national and international competitions (such as the World Cheese Awards) and, above all, through the approval of new regulations. To date, five geographical indications have been granted in the Indication of Origin (IP) modality, which recognizes the traditional area of cheese production, historical connotation and economic relevance (Serro, Canastra, Colônia Witmarsum, Marajó and

Cerrado); and a GI in the Denomination of Origin (DO) modality, which recognizes the specificities of the geographic environment, including natural factors (suitable climate and native pastures) and human factors, which provide the product with unique characteristics (the terroir, such as Serrano cheese “Campos de Cima da Serra”) (Brasil, 1996a). In 2018, through new regulations, the “Arte” seal was created, which now allows the retail sale of artisanal cheeses made with raw milk throughout the national territory (Brasil, 2018).

The GIs and the “Arte” seal connect characteristic cheeses to their place of origin and, through know-how passed down through generations, recognizes their own identity and adds value, which increases the interest of other producers in regulating their products. Consequently, it is necessary to know the characteristics of artisanal cheeses and identify markers that can assist in the certification of products with geographical indication and in the identification of fraudulent products (Santos et al., 2017).

Different methodologies based on chemical composition (Fitztum et al., 2023; Santos et al., 2017) and gas chromatograph (Margalho et al., 2021), Mid-Infrared Spectroscopy (MIR), Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) (Silva et al, 2023), and inductively coupled plasma optical emission spectrometer (ICP-OES) (Andrade et al., 2022) were applied to characterize and discriminate cheeses according to the type or region of production. Despite presenting promising results, it is necessary to investigate new techniques that allow obtaining reliable, fast, and low-cost results.

Techniques based on vibrational spectroscopy in the visible/near infrared range (vis/NIR) have consolidated applications in the food industry (Ayvaz et al., 2021; Stocco et al., 2019; Wiedemair et al., 2019; Marinoni et al., 2017; Madalozzo et al., 2015; Karoui et al., 2006;) and has been investigated as a tool for authentication. Vis/NIR spectroscopy provides a spectrum of the sample, often called “fingerprint”, which can be used to extract information related to its composition. It is characterized as being non-destructive, avoiding the use of chemicals and allowing rapid characterization and measurement of several attributes simultaneously at any stage of the production chain and directly on the surface of the cheese.

However, the potential of vis/NIR can be compromised in heterogeneous matrices, such as ripened cheeses, as conventional spectrometers provide information from a small portion of the sample. To overcome this difficulty, it is possible to use vis/NIR hyperspectral imaging (HSI), which combines in a single device the advantages of conventional spectroscopy and artificial vision, allowing the simultaneous acquisition of information related to composition (spectral information) and its distribution within the sample (spatial information) (Amigo et al., 2019). Considering the shortages of studies in this field, there is a need for further investigation comparing the application of hyperspectral imaging and conventional spectrometers to predict the composition of a wider type of cheeses and without the need for sample preparation.

Due to the large amount of information generated by hyperspectral imaging and conventional spectroscopy, a data mining step (also known as chemometrics) becomes necessary to extract only the relevant information from spectra. Spectral pre-processing steps are performed to remove/minimize the influence of undesirable phenomena that affect the spectral measurement, such as light scattering, particle size and morphology effects and detector artifacts. However, there is no standard method, and a trial-and-error approach is required for a specific application. Additionally, a wavelength selection step can be performed to develop simpler models, reducing data processing time and allowing industrial applications (Pasquini, 2018; Amigo, 2019). Finally, algorithms are applied to investigate spectral variability and develop models to predict chemical compounds of interest.

Thus, this study compared the performance of NIRS and hyperspectral imaging (vis/NIR-HSI and NIR-HSI) devices, as well as the fusion of HSI spectroscopic data (vis/NIR + NIR) to discriminate and predict the chemical composition of a wide type of Brazilian traditional cheeses. In this work, we investigate the relationship of cheese processing steps with spectral variability, and the influence of instrument technology (image and point measurement), spectral region (vis and NIR) and spectral preprocessing methods on the performance of classification and predictive models.

## 2. Material and methods

### 2.1. Brazilian traditional cheese samples

Seventy-two samples of seven types of Brazilian traditional cheeses were obtained from producers, supermarkets and markets located in three regions of Brazil were analyzed: Northeast, Southeast and South. The sample set (Table 1) included the main types of traditional Brazilian cheeses and considered different production technology, curd heat treatment (uncooked and cooked), curd melting, maturation (ripened or not ripened), chemical composition and structural characteristics of the cheese matrix (soft and hard cheeses) to address a wider sample variation and allow effective evaluation of the ability of vis/NIR devices to predict chemical composition.

**Table 1.** Description of the Brazilian traditional cheeses included in the sampling.

| Region | Cheese type (Identification) | Production aspects | | Regulatory patterns |
|---|---|---|---|---|
| | | **Production technology** | **Distinctive steps** | |
| Northeast | Coalho integral (CO) and light (CL) | Enzymatic coagulation, cutting, stirring, whey drainage, cooking (45–55 °C), and pressing | Not ripened | Moisture: 36.0 –54.9%; FTS: 35.0 a 60.0%[1] |
| | Butter cheese integral (BC) and light (BL) | Spontaneous coagulation, desorption, washing with water and/or milk and melting (at 85°C for at least 15 min) with butteroil | Melted | Moisture: ≤54.9 %; FTS: 25.0 – 55.0%[1] |
| | Minas Frescal (MF) | Enzymatic coagulation, supplemented or not with the action of lactic acid bacteria, whey drainage and packaging | Not ripened | Moisture: >55.0%; FTS: 25.0 – 44.9%[2] |
| South/ Southeast | Minas Araxá (AR) | Coagulation with endogenous culture and industrial rennet, cutting, agitation, molding, and superficial salting. The cheese is turned daily during shelf maturation | Ripened (14 days) | Moisture: ≤ 45.9%[3] |
| | Minas Canastra (CT) | Coagulation with natural dairy culture and industrial rennet, cutting, stirring, molding, pressing, and salting | Ripened (14 days) | Moisture: ≤ 45.9%[3] |

| | | | | |
|---|---|---|---|---|
| | Minas Padrão (light) (MP) | Enzymatic coagulation, complemented by the action of lactic acid bacteria, heating (32–42°C), draining, pressing, and salting | Ripened (20 days) | Moisture: 36 a 45.9%. FTS: 42 a 57%[4] |
| | Colonial (CN) | Coagulation with industrial rennet, heating (30–45°C), whey drainage, molding, pressing, and salting | Ripened (10 days) | Moisture: 36.0 a 45.9%; FTS 45.0 a 59.9%[5] |

[1]Brasil (2001), [2]Brasil (1997), [3]Minas Gerais (2008), [4]Brasil (2020), [5]Rio Grande do Sul (2023).

## 2.2. Image and spectral data collection

### 2.2.1. Sample treatment and morphological features

To investigate the spectral changes associated with the cheese process, especially ripening, the cheeses were sectioned horizontally (20 mm in height) and two cylinder of 25 mm in diameter were removed from the center (internal surface) towards the rind (external surface), with the aid of a stainless-steel sampler.

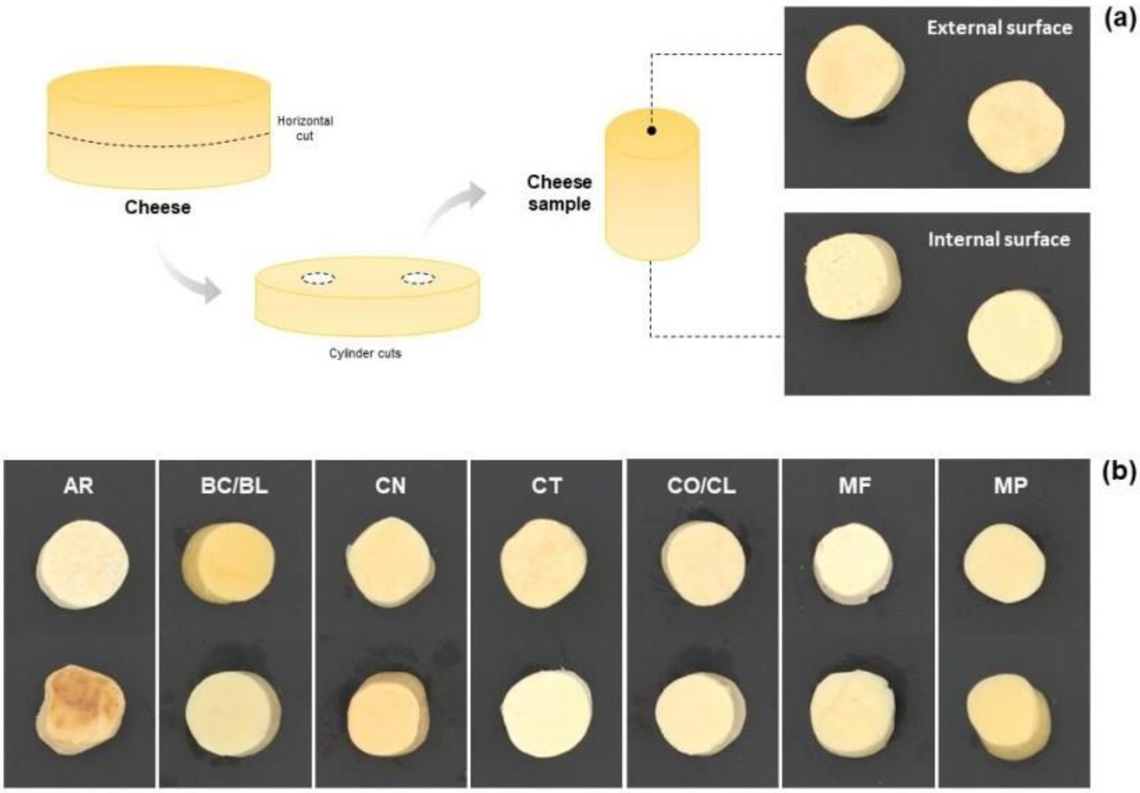


**Fig. 1.** Representation of obtaining cheese samples for spectral acquisition (a) and the types of traditional Brazilian cheeses (b) included in the sampling.

### 2.2.2. Portable NIR spectrometer (NIRS)

Spectra were recorded using a portable NIR spectrophotometer (MicroNIR Pro Lite 1700, VIAVI, Santa Rosa, California, USA), previously calibrated, scanning the wavelength range of 908 and 1676 nm (spectral resolution of 6.2 nm). Reflectance spectra were corrected by means of a two-point calibration. For that, a white reference spectrum (Spectralon, Labsphere Inc., North Sutton, USA) and a dark current spectrum were acquired in each collection session. Finally, two spectra were acquired at random locations on the outer surface (totaling 144 spectra) and two spectra on the inner surface (totaling 144 spectra) of each cheese cylinder.

### 2.2.3. Hyperspectral imaging (HSI)

Hyperspectral images of Brazilian traditional cheeses were acquired in the visible and near infrared spectral region (vis/NIR – HSI) using a Specim IQ camera (Spectral Imaging Ltd., Oulu, Finland) with a spectral range of 397 to 1004 nm (512 × 512 pixels and FWHM of 7 nm). This camera is based on the pushbroom principle and has a mobile, portable, and autonomous design (integrated operating system and controls), which allows the acquisition of images in different environments. This camera has a certified reflectance device made of teflon to perform the calibrations. Also, near-infrared (NIR – HSI) hyperspectral images were acquired using a laboratory system composed of a Xenics® XEVA-USB InGaAs camera (320 × 256 pixels; Xenics Infrared Solutions, Inc., Leuven, Belgium), a spectrograph (Specim ImSpector N17E Enhanced; Spectral Imaging Ltd., Oulu, Finland) covering the spectral range between 884 – 1717 nm (3.25 nm spectral resolution), a mirror scanner (Spectral Imaging Ltd., Oulu, Finland) and a computer system with instrumental acquisition software SpectralDAQ v. 3.62 (Spectral Imaging Ltd., Oulu, Finland). A “white reference” image (W, 100% reflectance) was acquired from a white ceramic tile (Labsphere Inc., North Sutton, USA), and a “dark reference” image (B, 0% reflectance) was obtained with the light source off and the camera covered with its opaque cap.

The cheese cylinders were placed on a dark surface and illuminated by two 70 W tungsten iodine halogen lamps (Prilux ®, Barcelona, Spain) separated 50 cm and oriented at 45° from the area of image. The distance between the camera and the sample was 45 cm. The camera covers an angle of 24º with a speed of 5.6 degrees/sec. For each cheese cylinder, an image of the outer surface and one of the inner surfaces was recorded. After the calibration and segmentation processes, the average spectra of the region of interest (ROI) were extracted using Matlab (R2019; Mathworks, Natick, USA).

#### 2.2.4. HSI spectroscopic dataset fusion

Given the complementary nature of the information provided by the visible (vis) and near infrared (NIR) spectra, the average vis/NIR-HSI (397 to 1004 nm) and NIR-HSI (884 to 1717 nm) spectra were sectioned at the 950 nm wavelength and unified into a new matrix (397 to 1717 nm) to increase reach and improve classification and prediction models.

### 2.3. Chemical composition, colorimetric parameters, and texture

#### 2.3.1. Chemical composition

The samples were crushed until obtaining a homogeneous state and then were subjected to the following analyses (g/100 g of cheese): moisture, fat, proteins, and ash, according to the Association of Official Analytical Chemists protocols (AOAC, 2012). After, fat was calculated in dry matter (g/100g) for comparison with the legislation and carbohydrates were calculated by subtracting the moisture, fat, protein, and ash contents from the total composition (100%).

The results of moisture (g/100g of cheese) and fat in dry matter (FDM) (g/100g of dry matter) were used to classify the cheeses according to the Brazilian legislation (Brasil, 1996b), in cheeses of low (moisture < 35.9%), medium (36.0 – 45.9%), high (46.0 – 54.9%) and very high moisture (> 55.0%); and in skimmed (fat < 10.0%), low fat (10.0 – 24.9%), semi-fat (25.0 – 44.9%), full fat (45.0 – 59.9%) or extra fat (> 60%).

**2.3.2. Fatty acids**

For the quantification of fatty acid methyl esters, the sample preparation used were the same as those reported by Sant'Ana et al. (2019). The identification and quantification of fatty acid methyl esters was performed with a gas chromatograph equipped with flame ionization detection (GC-FID) (QP2010-plus, Shimadzu, Kyoto, Japan) and a fused silica capillary column (SP-2560, 100 m × 0.25 mm × 0.20 µm, Supelco, Bellefonte, PA, USA). The injector and detector were kept at 250 and 280 °C, respectively. The temperature program was as follows: 50 °C for 1 min; ramped to 150 °C at 50 °C/min and held for 20 min; then ramped to 190 °C at 1 °C/min, held for 1 min; and ramped to 220 °C at 2 °C/min, held for 30 min. Helium was used as the carrier gas at 1 mL/min constant flow rate, and 1 µL of the sample was injected.

The identification of peaks of fatty acid methyl esters was performed by comparing the retention times with the standards (FAME Mix, 37 components) under the same analysis conditions.

**2.3.3. Instrumental color**

Instrumental color was determined by measuring the coordinates L* (lightness), a* (greenish for negative and reddish for positive values) and b* (bluish for negative and yellowish for positive values), using a digital colorimeter (CM-2600D, illuminant D65 Konica Minolta

Sensing Inc., Osaka, Japan), calibrated with a white ceramic standard. Then, the chroma ($C_{ab}^{*}$) was calculated, which corresponds to the saturation or intensity of the color, and hue angle ($h_{ab}$), according to CIE (1978). The Yellowness index (YI) of the samples was calculated using Equation (1) (Francis & Clydesdale, 1975).

$$YI = 142.86 \frac{b^*}{L^*} \qquad \text{Equation (1)}$$

### 2.3.4. Statistical analysis

One-way Analysis of Variance (ANOVA) was applied to chemical composition and instrumental color parameters to identify whether they influenced by cheese types. Tukey's multiple mean comparison test ($p < 0.05$) was applied to identify differences between cheese types, using Sisvar software version 5.7 (Lavras, Minas Gerais, Brazil).

## 2.4. Multivariate analysis

### 2.4.1. Spectral preprocessing

Different pre-processing methods were applied to the vis/NIR spectra to correct effects of the random noise, light scattering, and changes in the baseline. Savitzky-Golay smoothing (SG), Standard Normal Variate (SNV) and Savitzky-Golay derivatives ($1^{st}$ SG and $2^{nd}$ SG) (both derivatives were used with a 13-point window and second order polynomial filtering) were tested alone or in combinations (SG + SNV, SNV + $1^{st}$ SG and SNV + $2^{nd}$ SG). The best results obtained for each equipment and chemical component are presented in the results. All pre-processing and post analysis were performed using PLS Toolbox 8.9.1 from Eigenvector Research, Inc. (Manson, WA, USA) to Matlab R2019a (Mathworks, Natick, USA).

### 2.4.2. Principal Component Analysis (PCA)

Principal Component Analysis (PCA) was initially performed to investigate the influence of the spectral acquisition surface (external and internal surface of the cheese) on the grouping of cheeses. It was observed that both surfaces allow visualization of groupings between samples and explain close variance percentages. Thus, the external surface spectra (144 spectra, two for each sample) were chosen for the PCA and other multivariate analyzes for maintaining the integrity of the samples and the non-destructive nature of the spectroscopic method. Finally, Principal Component Analysis (PCA) was applied to the complete spectra and informative region (spectrum region with peaks and absorption differences between samples) to investigate spectral variability of the cheeses and correlate the grouping of samples with the cheese type, differences between the chemical composition and distinctive steps related to production (ripening, curd heating and curd melting). PCA models were developed using singular value decomposition (SVD) algorithm (95% confidence level) and outliers were detected and eliminated using Hotelling's residual Q and $T^2$ values.

### 2.4.3. Classification and prediction models

Classification models based on the Partial Least Squares Discriminant Analysis (PLSDA) method were constructed to discriminate the seven types of traditional Brazilian cheeses. Furthermore, regression models (PLSR) were built to predict the chemical composition of the cheeses: moisture, fat, proteins, and ash.

Initially, the classification and prediction models were developed using the full spectrum and then a variable selection step was applied to build reduced models. Selection of optimal wavelengths was performed by two approaches: (1) Informative region and (2) Interval Partial Least Squares algorithm (iPLS). The selection of the informative region was performed by visual inspection of the spectrum, considering regions with higher peaks and differences between cheese samples. For the vis/NIR-HSI data, the spectrum was divided into two informative regions: 387 – 780 nm (visible region) and 780 – 1004 nm (infrared region); and in the NIRS and NIR-HSI data, were studied the regions of 1100 – 1600 nm and 1050 – 1350, 1600 – 1680 nm (Medeiros et al, 2023). The iPLS was performed considering one wavelength per interval and limited to 10 intervals. In addition to the classification models based on spectral information, a model based on chemical composition was built to compare the efficiency of spectral techniques with traditional chemical methods.

In both models, 70% of the samples (50 samples) were used and the remaining 30% (22 samples), containing at least two samples of each cheese type, were used as an independent set to test the predictive capacity of the models. The optimal number of latent variables (LV) in the classification and prediction models was chosen using the lowest average classification error in cross-validation (leave-one-out) and lowest root mean squared error of cross validation (leave-one-out) (RMSECV), respectively.

The performance of PLS-DA models was evaluated by sensitivity (fraction of samples that belong to a class and are properly accepted) (Eq. (1)), specificity (fraction of samples that do not belong to a class and are correctly rejected) (Eq. (2)), accuracy (ratio between the number of samples correctly classified, regardless of the class, and the total number of samples) (Eq. (3)) and error (Eq. (4)).

$$Sensitivity = \frac{TP}{TP + FN} \qquad (Eq.\ 1)$$

$$Specificity = \frac{TN}{FP + TN} \qquad (Eq.\ 2)$$

$$Accuracy = \frac{TP + TN}{TP + TN + FP + FN} \times 100 \qquad (Eq.\ 3)$$

$$Error = \frac{FP + FN}{TP + FP + TN + FN} \times 100 \qquad (Eq.\ 4)$$

where TP is true positive, TN is true negative, FN is false negative, and FP is false positive.

The performance of the calibration models was evaluated by the coefficient of determination ($R^2$) and Root Mean Square Error (RMSE) for the calibration ($R_C^2$, RMSEC) and cross-validation ($R_{CV}^2$, RMSECV) and the predictive capacity of the models was evaluated using the coefficient of determination ($R_P^2$), Root Mean Square Error (RMSEP) and Performance Ratio to Deviation (RPD = SD/RMSEP, where SD is the standard deviation of the chemical component content) (Nicolaï et al., 2007).

## 3. Results and discussion

### 3.1. Chemical composition and instrumental color parameters

Brazilian traditional cheeses showed significant differences ($p < 0.05$) for the evaluated compositional parameters (Table 1): Moisture (34.35 – 54.43%), fat (9.07 – 36.47%), fat in total solids (17.50 – 55.88%), protein (18.78 – 28.35%), carbohydrates (0.39 – 10.19%) and ash (1.64 – 5.45%).

The main differences for moisture content were observed for Minas Canastra (CT), which had the lowest moisture content. This cheese undergoes a minimum maturation period of 14 days, where a series of biochemical events take place, responsible for its flavor and texture, as well as for the reduction of moisture. The highest moisture contents were observed for light butter cheese (BL), Minas Frescal (MF) and Minas Padrão light (MP) cheeses. MF cheese is characterized by being marketed fresh (not matured), and BL and MP cheeses are characterized by a reduction in fat content, implying an increase in moisture content. According to Brazilian legislation (Brasil, 1996b), the analyzed cheeses are classified as low cheeses (hard cheeses) (< 35.9%) to high cheeses (soft cheeses) (46.0 – 54.9%) moisture content and comply with their respective technical regulations.

The lowest levels of fat were observed for BL and MP cheese (11.48 and 13.51%), while the highest fat content was observed for BC cheese (29.25%). These differences are mainly attributed to the ingredients used during cheese production. Although these cheeses are produced from skimmed milk, butter cheese has as its main characteristic the addition of butteroil during the melting process of the coagulated mass. This butter, typical of the northeast region of Brazil, is obtained by heating cream (110 to 120°C) until complete melting and contains a minimum fat content of 98.5%, contributing to a higher fat content in the butter cheese. According to the fat content in the dry extract, the analyzed cheeses are classified between lean (10–24.9 %) and full fat (45–59.9 %) (Brasil, 1996b) and are in accordance with the provisions of their respective technical regulations.

The main difference in protein content was observed for MP cheese (27.31%) (highest content) and is related to the incorporation of whey protein concentrate, which is used to improve the yield or sensory properties of low-fat cheeses. All other cheeses had protein contents between 22.74 and 24.96% and are in line with reports by other authors (Costa et al., 2022; Margalho et al., 2021).

The lowest carbohydrate contents were observed for matured cheeses (Minas - Canasta and Colonial) (2.15 – 2.84¨%) and for Coalho cheese (2.58%). The amount of carbohydrates in the cheese is influenced by the concentration of lactose in the milk, type and amount of coagulant, as well as aspects related to the curd washing step, such as washing time, amount of water and particle size (Hayaloglu & McSweeney, 2014; Ibáñez et al., 2020). In addition, in mature cheeses, lactose is metabolized by lactic acid bacteria, releasing glucose and galactose, and synthesizing organic acids, resulting in low carbohydrate content (Bezerra et al., 2017). The highest carbohydrate contents were observed for light cheeses (BL, MP and CL) (4.82 to 8.66%). This result can be attributed to the lower fat content present in these cheeses, which consequently alters the amount of other constituents.

The main differences in ash content were observed for butter cheese (BC and BL) (2.11 and 2.60%) and CL (4.18%) and may be related to some particular steps during manufacture of these cheeses. In butter cheese, the milk is coagulated by the action of acids and then subjected to draining, washing, and melting with butteroil. Acid coagulation causes colloidal calcium phosphate solubilization (Masotti et al., 2020) and washing can promote the leaching of minerals into the whey, contributing to the reduction of mineral content. On the other hand, in the production of CO cheese, as well as in CL, MP and MF cheeses, calcium chloride is added as an ingredient to provide ideal conditions for milk coagulation (improves gel firmness and reduces coagulation time), resulting in an increase in mineral content (Koutina et al., 2016). Minas traditional AR and CT cheeses had statistically equal mineral contents ($p < 0.05$) to cheeses with added calcium chloride. Although these cheeses do not have addition of calcium chloride, this increase may be related to differences in the percentage of salt added in the technological process and the concentration of solids during maturation.

The composition intervals presented in this study are in accordance with those reported by Margalho et al. (2021) for 402 samples of 11 types of Brazilian artisan cheeses and are close to those reported by authors who obtained robust models to predict compositional attributes in a wide type of cheeses (Ayvaz et al., 2021; Stocco et al., 2019). Thus, it can be inferred that the sampling used in this study and the models developed from these data are representative of the compositional variability of Brazilian traditional cheeses.

**Table 2.** Chemical composition, instrumental color parameters and yellowness index (YI) of Brazilian traditional cheeses.

| Region | Cheese samples | Chemical composition (%) | | | | | | Color parameters (CIELAB units) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Moisture** | **Fat** | **FDM** | **Protein** | **Carb** | **Ash** | **L*** | $C^*_{ab}$ | $h_{ab}$ | **YI** |
| Northeast | Coalho - Integral (CO) | 45.46$^{bc}$ ±2.62 | 23.34$^{bc}$±2.17 | 42.73$^{ab}$ ±2.42 | 24.73$^{ab}$ ±1.12 | 2.58$^{de}$± 1.46 | 3.89$^{ab}$ ±0.66 | 89.1$^{ab}$ ± 1.5 | 25.3$^{ab}$ ± 2.8 | 100.2$^{ab}$ ± 1.0 | 40.6$^{abc}$ ± 4.7 |
| | Coalho - Light (CL) | 44.78$^{bcd}$ ±2.83 | 21.98$^{c}$±6.29 | 39.38$^{b}$ ±9.41 | 24.23$^{b}$±1.77 | 4.82$^{b}$ ± 1.39 | 4.18$^{a}$ ±0.62 | 90.9$^{a}$ ± 0.6 | 22.5$^{ab}$ ± 2.2 | 90.8$^{d}$ ± 0.5 | 35.4$^{bc}$ ± 3.4 |
| | Minas Frescal (MF) | 46.65$^{b}$ ±2.33 | 22.95$^{bc}$±0.61 | 43.12$^{ab}$ ±2.87 | 22.74$^{b}$ ±2.43 | 4.42$^{bc}$ ± 0.90 | 3.25$^{bc}$ ±0.21 | 90.8$^{a}$ ± 4.9 | 20.4$^{b}$ ± 2.2 | 98.4$^{bc}$ ± 5.3 | 31.8$^{c}$ ± 4.5 |
| | Butter cheese - Integral (BC) | 40.71$^{cd}$±3.84 | 29.25$^{a}$ ±4.57 | 49.06$^{a}$±4.66 | 24.56$^{b}$±1.24 | 3.36$^{bcde}$±1.01 | 2.11$^{d}$±0.31 | 69.3$^{d}$ ± 4.9 | 25.9$^{ab}$ ± 6.4 | 105.0$^{a}$ ± 2.5 | 51.6$^{a}$ ± 12.6 |
| | Butter cheese - Light (BL) | 54.45$^{a}$ ±0.15 | 11.48$^{d}$±0.12 | 25.19$^{c}$±0.28 | 23.72$^{b}$±0.24 | 7.76$^{a}$±0.32 | 2.60$^{cd}$±0.02 | 67.4$^{d}$ ± 2.6 | 21.1$^{ab}$ ± 0.7 | 96.7$^{bc}$ ± 0.7 | 44.5$^{abc}$ ± 3.3 |
| South/ Southeast | Minas Araxá (AR) | 42.54$^{bcd}$ ±2.27 | 26.78$^{abc}$±1.73 | 46.65$^{a}$ ±3.07 | 23.11$^{b}$±2.21 | 3.93$^{bcd}$±0.69 | 3.64$^{ab}$±0.36 | 81.2$^{bc}$ ± 10.3 | 27.3$^{a}$ ± 6.9 | 90.6$^{d}$ ± 8.3 | 49.7$^{ab}$ ± 19.9 |
| | Minas Canastra (CT) | 40.12$^{d}$ ±4.90 | 28.12$^{ab}$ ±3.47 | 46.86$^{a}$ ±2.83 | 24.96$^{ab}$ ± 2.20 | 2.84$^{cde}$ ±0.94 | 3.95$^{ab}$ ±0.86 | 83.2$^{abc}$ ± 7.9 | 19.7$^{b}$ ± 2.3 | 95.9$^{bcd}$ ± 2.9 | 33.9$^{c}$ ± 5.7 |
| | Minas Padrão light (MP) | 46.55$^{b}$ ±1.10 | 13.51$^{d}$±3.37 | 25.17$^{c}$ ±5.86 | 27.31$^{a}$ ±0.96 | 8.66$^{a}$ ± 1.45 | 3.98$^{ab}$±0.27 | 79.6$^{c}$ ± 1.7 | 22.8$^{ab}$ ± 3.2 | 94.2$^{cd}$ ± 0.9 | 40.2$^{abc}$ ± 5.6 |
| | Colonial (CN) | 43.87$^{bcd}$±2.14 | 26.29$^{abc}$±3.27 | 45.82$^{a}$ ±3.89 | 24.30$^{b}$ ±1.26 | 2.15$^{e}$ ±1.51 | 3.89$^{ab}$ ±0.44 | 83.8$^{abc}$ ± 1.9 | 25.6$^{ab}$ ± 4.7 | 97.8$^{bc}$ ± 3.8 | 43.3$^{abc}$ ± 9.2 |
| | Range | 34.35 – 54.43 | 9.07 – 36.47 | 17.50 – 55.88 | 18.78 – 28.35 | 0.39 – 10.19 | 1.64 – 5.45 | 60.9 – 94.7 | 17.0 – 38.5 | 77.2 – 109.5 | 26.1 – 82.4 |

FDM = Fat in dry matter and Carb = Carbohydrate. Values in the same column followed by different letters are significantly different by ANOVA test ($p < 0.05$).

**Table 3.** Fatty acids profile of Brazilian traditional cheeses.

| Region | Cheese samples | Fatty acids profile (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **C14:0** | **C16:0** | **C16:1** | **C18:0** | **C18:1** | **C18:2** | **C18:3** | **SFAs** | **MUFAs** | **PUFAs** |
| Northeast | Coalho - Integral (CO) | Colonial | 10.66$^{ab}$±1.10 | 10.35$^{ab}$±0.4 9 | 31.19$^{bc}$±2.35 | 32.91$^{abc}$±0.85 | 1.33$^{b}$±0.70 | 2.94$^{ab}$±1.09 | 13.55$^{ab}$±1.37 | 12.02$^{bcd}$±0.59 | 29.24$^{ab}$±2.8 |
| | Coalho - Light (CL) | (CN) | 10.65$^{ab}$±0.76 | 8.36 – | 31.41$^{bc}$±0.16 | 27.05 – 38.30 | 1.50$^{b}$±0.98 | 0.81 – 5.04 | 13.33$^{ab}$±0.84 | 8.32 – 16.66 | 3 |
| | Minas Frescal (MF) | Range | 11.58$^{ab}$±1.79 | 13.15 | 32.73$^{abc}$±2.54 | | 2.41$^{ab}$±1.32 | | 12.51$^{abc}$±1.55 | | 28.61$^{ab}$±1.9 |
| | Butter cheese - Integral (BC) | | 9.77$^{b}$±1.10 | | 29.22$^{c}$±1.62 | | 1.71$^{ab}$±1.12 | | 14.65$^{a}$±1.65 | | 6 |
| | Butter cheese - Light (BL) | | 11.02$^{ab}$±0.09 | | 31.85$^{abc}$±0.01 | | 1.17$^{b}$±0.04 | | 13.77$^{ab}$±0.07 | | 25.76$^{bc}$±3.8 |
| South/ Southeast | Minas Araxá (AR) | | 12.07$^{a}$±0.85 | | 36.08$^{a}$±2.39 | | 1.93$^{ab}$±1.02 | | 11.69$^{bcd}$±0.80 | | 3 |
| | Minas Canastra (CT) | | 11.95$^{a}$±0.57 | | 33.56$^{ab}$±3.38 | | 2.82$^{ab}$±1.31 | | 9.82$^{d}$±1.53 | | 30.67$^{a}$±2.97 |
| | Minas Padrão light (MP) | | 11.03$^{ab}$±0.78 | | 32.85$^{abc}$±1.80 | | 3.60$^{a}$±1.27 | | 10.55$^{cd}$±0.56 | | 29.33$^{ab}$±0.0 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 4 | 22.60$^{c}$±2.56 | 2.32$^{ab}$±0.23 | 2.10$^{ab}$±0.14 | 1.21$^{ab}$±0.31 | 65.91$^{b}$±2.99 | 30.57$^{b}$±2.67 | 3.53$^{a}$±0.44 |
| | 24.78$^{bc}$±1.76 | 2.36$^{ab}$±0.21 | 2.15$^{ab}$±0.42 | 1.08$^{abc}$±0.33 | 66.59$^{b}$±1.68 | 30.12$^{b}$±1.49 | 3.17$^{abc}$±0.26 |
| | 27.14$^{abc}$±1.99 | 2.00$^{ab}$±0.07 | 2.17$^{ab}$±0.18 | 0.91$^{abc}$±0.40 | 69.08$^{ab}$±5.37 | 28.17$^{ab}$±4.82 | 3.27$^{abc}$±0.31 |
| | 26.34$^{abc}$±0.34 | 1.94$^{ab}$±0.28 | 1.87$^{b}$±0.47 | 1.35$^{a}$±0.32 | 64.06$^{b}$±2.92 | 32.38$^{b}$±2.45 | 3.50$^{ab}$±0.62 |
| | 20.29 – 34.58 | 2.49$^{a}$±0.42 | | 1.15$^{abc}$±0.20 | 66.35$^{b}$±0.13 | 30.50$^{b}$±0.00 | 3.16$^{abc}$±0.13 |
| | | 1.18 – 2.83 | | 0.54$^{bc}$±0.38 | 72.77$^{a}$±2.94 | 24.53$^{b}$±2.74 | 2.71$^{bc}$±0.45 |
| | | | | 0.70$^{abc}$±0.30 | 68.86$^{ab}$±2.37 | 27.60$^{ab}$±1.47 | 2.64$^{c}$±0.34 |
| | | | | 0.79$^{abc}$±0.56 | 66.61$^{b}$±3.06 | 30.74$^{b}$±2.94 | 2.65$^{c}$±0.28 |
| | | | | 0.52$^{c}$±0.19 | 67.71$^{ab}$±0.82 | 29.28$^{ab}$±1.30 | 3.01$^{abc}$±0.52 |
| | | | | 0.00 – 1.77 | 59.24 – 76.15 | 21.29 – 36.59 | 2.20 – 4.17 |

In Table 2, Polar coordinates chroma ($C^*_{ab}$) and hue ($h_{ab}$) are shown instead of the Cartesian coordinates a* and b*, as they explain color better from a psychophysical point of view. Chroma in cheeses is almost entirely due to the influence of the b* component. In addition, indicating the hue value ($h_{ab}$) alone shows whether the cheese has a more yellowish or more orange appearance without the need to evaluate a* and b* simultaneously. Finally, the yellowness index (YI), which depends on b* and L*, has been shown as a common indicator of cheese color in scientific literature. Brazilian artisanal cheeses showed significant differences ($p < 0.05$) for the instrumental color parameters: lightness (60.9 – 94.7), chroma (17.0 – 38.5), hue (77.2 – 109.5) and yellowness index (26.1 – 82.4). Standard deviation values showed that there is also a large variation in color parameters for the same type of cheese, which may be related to differences in the production process and storage conditions. The yellow color of the cheese ($h_{ab} \sim 90$) is mainly due to the presence of carotenoids, such as β-carotene ($C_{40}H_{56}$), lutein ($C_{40}H_{56}O_2$) and β-cryptoxanthin ($C_{40}H_{56}O$) (Gentili et al., 2013). In this study, a relationship was observed between the brightness and the productive aspects of the cheeses, where the unripened cheeses (CO and MF) were characterized by lighter colors (higher values of lightness), the ripened cheeses by intermediate colors and the melted by darker colorations (lower lightness values). Regarding the yellowness index, which takes into account the color parameter b* and brightness, it was observed that BC is more yellow, while MF and AR cheeses are more whitish.

Table 3 presents the results of the fatty acid profile of traditional Brazilian cheeses, where statistically significant differences are observed ($p < 0.05$). In general, the samples consist mainly of saturated fatty acids (SFAs) (59.24 – 76.15%), with emphasis on myristic acid (C14:0, 8.36 – 13.15%), palmitic acid (C16:0, 27.05 – 38.30%) and stearic (C18:0, 8.32 – 16.66%), followed by monounsaturated fatty acids (MUFAs) (21.29 – 36.59%), especially oleic (C18:1, 20.29 – 34.58%). It is possible to observe that cheeses produced in the Northeast region (CO, CL, BC, BL and MF) have a different fatty acid profile than cheeses from the South and Southeast regions (AR, CT, MP and CN), as they have lower percentages of SFAs and higher levels of PUFAs. The fatty acid profile of cheeses is directly related to the composition of milk fat (rearing system, animal feeding and time of year), as well as cheese processing, especially microbiota and maturation period (Jesus et al., 2023; 2021). Thus, these compositional differences associated with the production process and producing region can be investigated to certify the origin of the cheeses.

**3.2. Spectral profile**

The mean absorbance spectra of Brazilian traditional cheeses (Fig. 2) are comparable to those previously reported for other cheese types (Malegori et al., 2021; Reis et al., 2022; Medeiros et al., 2023). It is possible to observe different absorption bands in the visible (450 nm) and near infrared (970, 1150, 1210, 1230, 1390, 1450, 1500, 1526 and 1660-1690 nm) regions of the pre-processed spectra related to the main organic compounds present in cheese.

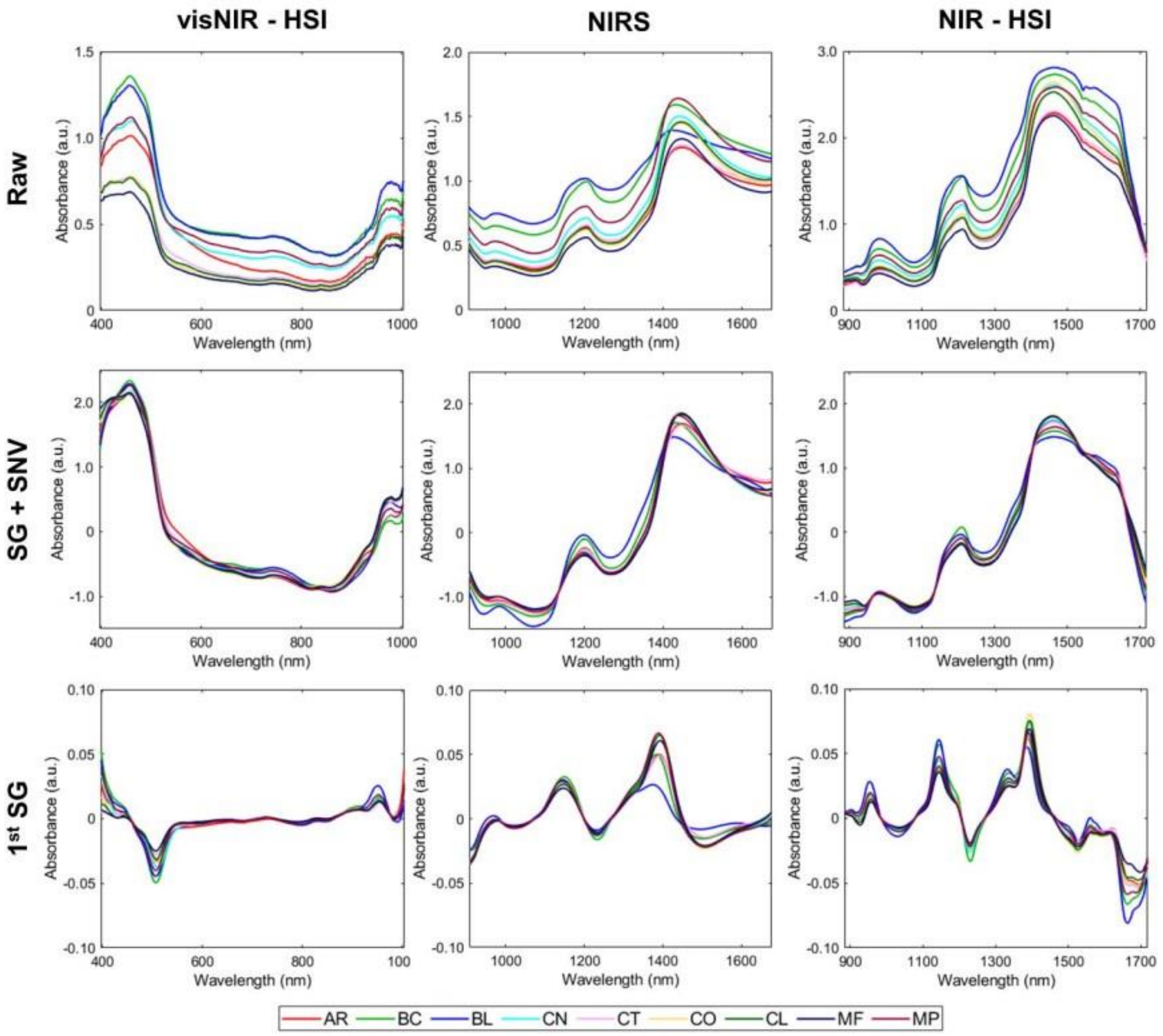


**Fig. 2.** vis/NIR and NIR spectra of Brazilian traditional cheeses raw, preprocessed with Savitzky-Golay smoothing combined with SNV (SG + SNV) and with the first derivative of Savitzky-Golay (1$^{st}$ SG).

The absorption peak at 450 nm can be attributed to the presence of carotenoids responsible for the yellow color of cheeses (Britton et al., 2004), such as β-carotene, lutein and β-cryptoxanthin, and/or the presence of riboflavin (vitamin B2) (Becker et al., 2003). The absorption bands at 970 and 1450 nm correspond to second and O–H stretching in the first overtone, characteristic of water. The absorption bands at 740, 1150, 1230, 1340-1390 and 1690 nm correspond to C–H stretching at the fourth, second and first overtone (–CH, –CH2, –CH3), present in CH2 groups in the acid chain fatty acids and their terminal CH3 groups, as well as CH and CH2 present in the glycerol fraction. The absorption bands at 1170 and 1660 nm are attributed to C–H vibrations in the second and first overtone and are associated with the presence of unsaturated fatty acids (–HC=CH–) in the aliphatic chains of fat, especially oleic acid (C18 :1) (Osborne et al., 1993). The peaks at 910 and 1210 nm, attributed to C–H vibrations at the third and first overtone, and at 1500 and 1530 nm, attributed to N–H vibrations at the first overtone, are associated with protein content, such as casein (Frank & Birth, 1982; Osborne et al., 1993).

**3.3. Principal Components Analysis (PCA)**

Principal Component Analysis (PCA) (Fig. 3) was applied to NIRS and HSI spectra (vis/NIR, NIR and data fusion) to explore spectral variations among Brazilian traditional cheeses. The results are shown according to three groupings: cheese type, production steps (ripening and curd melting) and composition. The influence of curd heating was also investigated, but it was not observed sample separation regarding this feature.

The two-dimensional representation (PC1 × PC2) of the PCA scores showed that the NIR-HSI spectra explained a higher percentage of cheese variability (93.64%), followed by NIRS data (92.41%), vis/NIR-HSI (86.78 %) and the fusion of HSI spectroscopic data (77.26%). Although there is a difference in the variability explained by the first two components (PC1 and PC2), the PCAs performed with the different data allow the visualization of the separation of some cheese types, especially butter cheese (BC) and Minas Padrão light cheese (MP). The overlapping of the other cheeses implies that there are other factors, in addition to the type of cheese, that influence the spectral variability of the cheeses and, consequently, the separation of the samples.

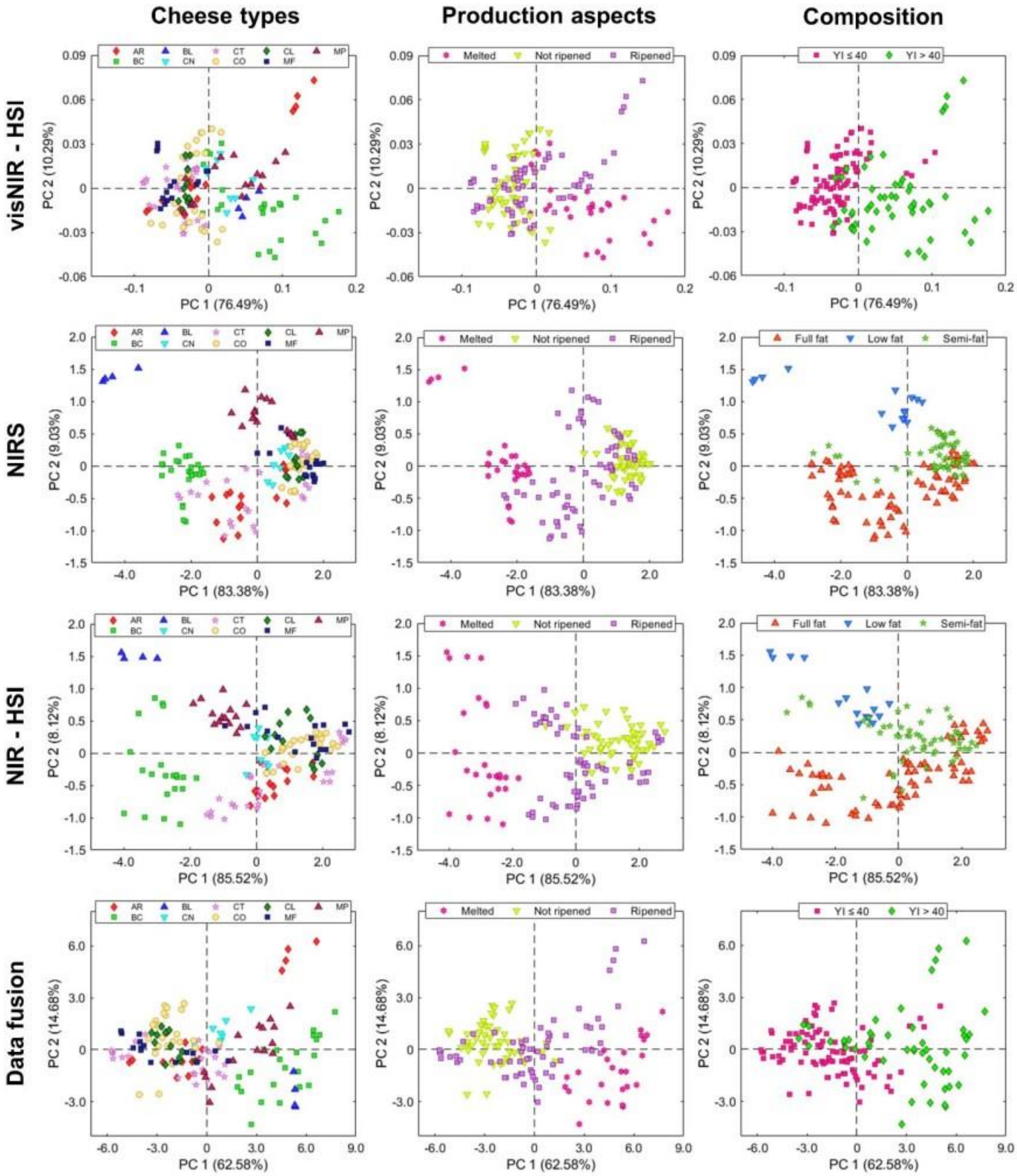


**Fig. 3.** PCA score plot of Brazilian traditional cheeses according to type, production (ripening and curd heating), and compositional aspects (fat content and yellowness index).

In the PCA performed with the vis/NIR-HSI spectral data (397- 1004 nm, 1st SG) (Fig. 3) it is possible to observe that the spectral variability represented by the scores of first principal component was influenced to a greater extent by the color of the samples, where cheeses with lower yellowness index (YI ≤ 40) were characterized by negative scores on PC1 and the highest yellowness index (YI > 40) by positive scores. This result is in line with the loadings plot (Fig. S2a), where it is possible to observe that variations in PC1, as well as in PC2, are associated with the content of carotenoids and/or riboflavin (440 nm) (Britton et al., 2004). The compositional aspects of the cheeses, especially the fat content, influenced the separation of samples in PC3 scores (Fig. S1 in supplementary material), where cheeses with lower fat content were characterized by negative scores and higher fat cheeses by positive scores. The biggest contributors to the separation in this main component (Fig. S2a in supplementary material) are associated with the content of carotenoids and riboflavin (430, 500 and 530 nm) (Britton et al., 2004; Becker et al., 2003) and the fat content (940 nm) (Osborne et al, 1993).

In PCA models performed with NIRS (1100 – 1600 nm, SNV + $2^{nd}$ SG) and NIR-HSI data (1100 – 1600 nm, SNV + $1^{st}$ SG) (Fig. 3) the first principal component (PC1) is associated with changes in protein structure (especially casein) due to cheese processing. In non-ripened cheeses (CO, CL and MF), located in the positive region of PC1 scores, the enzymatic action of industrial rennet promotes limited proteolysis of k-casein (cleavage of the $Phe_{105}$-$Met_{106}$ peptide bond) separating it into two macropeptides (Herbert et al., 1999). In ripened cheeses (AR, CT, MP and CN), distributed mainly between the negative and intermediate scores regions, in addition to enzymatic coagulation, changes occur during ripening that break cheese proteins into oligopeptides, which can additionally be degraded into shorter peptides and amino acids (Boran et al. 2023). In melted cheeses (BC and BL), spread in the negative part of PC1 scores, the main aspects of production are acid coagulation promoted by the natural microbiota of milk and the melting stage. The latter has a greater impact on the structure of the proteins, since melting salts (citrates, polyphosphates, or sodium bicarbonate) are used that promote the peptization of casein, separating its large hydrophobic aggregates into smaller units (Garcia et al., 2023). Thus, it is possible to infer that the extent of changes in protein structure increases in the negative sense of PC1 scores (not ripened < ripened < melted). This behavior was also observed by Herbert et al. (1999) when studying the influence of milk coagulation types (acid, enzymatic and mixed) on tryptophan emission fluorescence spectral data, where the first principal component separated the samples according to modifications in the micellar structure. The second principal component describes the variability associated with the fat content, where it is possible to separate the samples with the highest (full fat) and lowest (low fat) content in the negative and positive part of PC2 scores, respectively. The wavelengths (Fig. S2b and S2c in supplementary material) that contributed to the separation of the samples towards PC1 and PC2 scores are associated with moisture content (1450 nm), fat (1140, 1170, 1210, 1320, 1390 and 1410 nm) and proteins (1190 and 1510 nm) (Osborne et al., 1993)

From an exploratory point of view, the fusion of HSI spectroscopic data (vis/NIR + NIR) (397 – 1600 nm, SNV + $1^{st}$ SG) did not promote sample separation beyond what was observed for the separate techniques. In the first principal component (PC1) scores it is possible to observe a trend of sample separation according to the structural modifications of the proteins, as observed in the PCA performed with the NIR data, while the influence of the yellowness index is also observed, like the observed in the PCA for the vis/NIR data. In the loadings of PCA applied to the data fused (Fig. S3d in supplementary material) it is possible to observe that the wavelengths that influenced the separation in PC1 and PC2 scores are the same ones reported in vis/NIR-HSI and NIR-HSI separately, which are associated with the carotenoid content (490 nm), water (970 nm), fat (1140, 1230, 1330 and 1410 nm) and proteins (1500nm) (Osborne et al., 1993).

### 3.4. Classification models

PLS-DA classification models (Table 4) based on spectral information (NIR, vis/NIR-HSI, NIR-HSI and HSI data fusion) were constructed to discriminate traditional Brazilian cheeses. The performances of these models were compared with the model based on chemical composition data to investigate the potential of spectroscopy as a tool to assist in the certification of these products. The chemical composition parameters used in the construction of the model were the fatty acids C6:0, C8:0, C10:0, C12:0, C14:0, C18:0, C18:1, C18:3, SFAs and MUFAs), as they provided better prediction results.

It is possible to observe that the models performed with spectral information presented better classification performances, with higher sensitivity (≥ 0.75) and specificity (≥ 0.84) values and lower error rates (11 - 18%), compared to the model built only with chemical composition information (33% error rate). This result is justified by the large amount of information contained in the spectra, which is influenced by the composition and productive aspects, as observed in the PCA. Comparing the results of the three devices, the best classification index was obtained using information in the near infrared region (NIRS), where the sensitivity and specificity values were 0.95 and 0.84, respectively, and the classification rate 89% correct. Fusion of HSI spectroscopic data did not improve cheese discrimination (84% accuracy).

**Table 4.** Figures of merit of PLSDA models to classify Brazilian traditional cheese types.

| Equipment | Pre-processing | Variables | LV | Calibration | | | Prediction | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | **Sens** | **Spec** | **Acc** | **Sens** | **Spec** | **Acc** |
| Chemical composition | Autoscale | Fatty acids* | 3 | 0.85 | 0.76 | 0.81 | 0.57 | 0.76 | 0.67 |
| vis/NIR-HSI | $2^{nd}$ + SNV | iPLS | 5 | 0.94 | 0.93 | 0.82 | 0.75 | 0.89 | 0.82 |
| NIRS | $1^{st}$ SG | 1050 - 1350, 1600 - 1680nm | 5 | 0.96 | 0.90 | 0.93 | 0.95 | 0.84 | 0.89 |
| NIR-HSI | $1^{st}$ SG | iPLS | 4 | 0.94 | 0.88 | 0.91 | 0.85 | 0.85 | 0.85 |
| Data fusion | $1^{st}$ SG | 780 - 1600 nm | 6 | 0.88 | 0.89 | 0.89 | 0.81 | 0.87 | 0.84 |

*C6:0, C8:0, C10:0, C12:0, C14:0, C18:0, C18:1, C18:3, SFAs e MUFAs.

The distribution of validation samples into classes (Fig. 4) showed that the largest classification errors in the model based on chemical composition, the largest errors were observed for CO/CL and MF cheeses, which were incorrectly classified as AR cheese. In models based on spectral information, the largest errors were observed for samples of CT and for coalho cheeses (CO/CL), where samples of CT cheese were identified as AR or coalho cheese (CO/CL), and coalho samples (CO/CL) were incorrectly classified as MF or MP. The wavelengths that contributed to the classification of cheeses in the visible region are related to the lipid fraction (930 nm), carotenoids (450 nm) and water (970 nm). In the infrared region, the wavelengths with the greatest contribution are centered in spectral regions associated with the content of water (970 3 1440 nm), fat (930, 1150, 1220 and 1275 nm) and proteins (1540 nm).

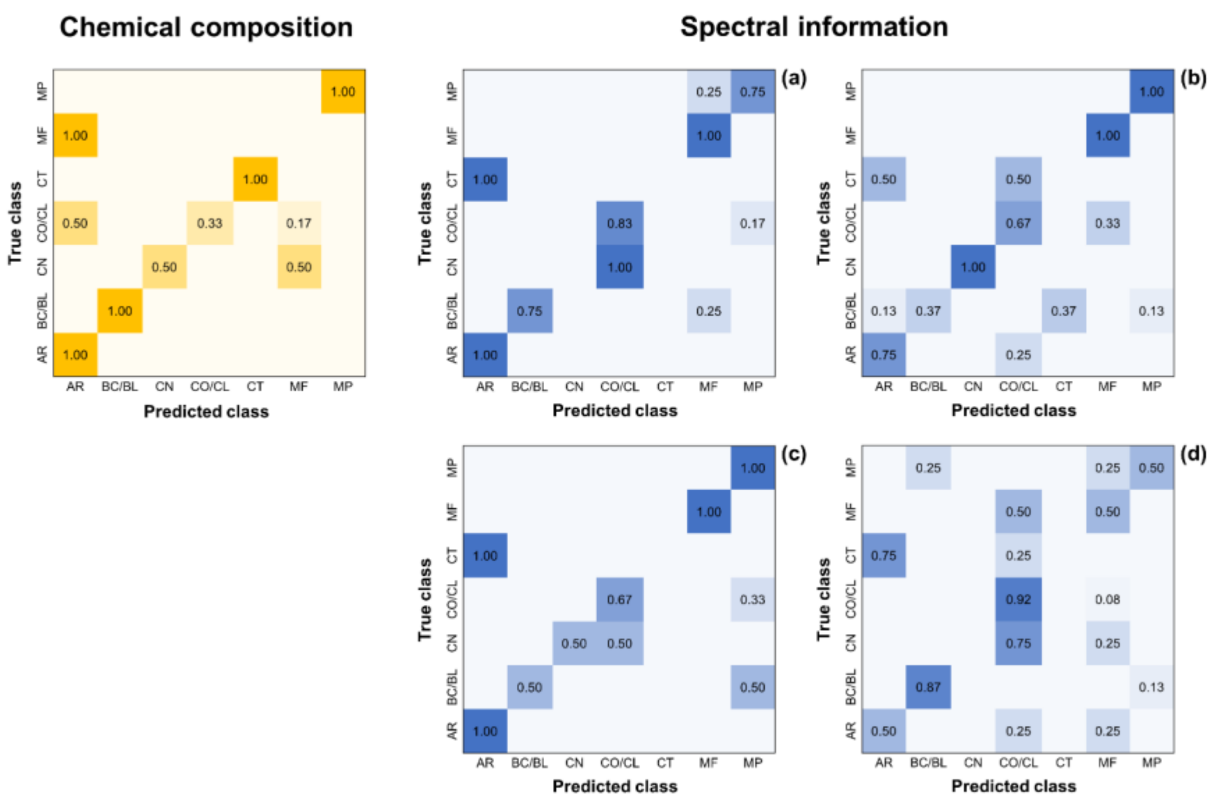

**Fig. 4.** Confusion matrix (external validation) of PLS-DA models based on chemical composition data and spectral information: vis/NIR-HIS (a), NIRS (b), NIR-HIS (c) and HSI spectroscopic dataset fusion (d).

### 3.5. Prediction models

PLS regression models were developed to compare the performance of three spectrometers (NIR, vis/NIR-HSI and NIR-HSI) and fusion spectroscopic data (vis/NIR-HSI and NIR-HSI) in predicting moisture, fat, protein and ash in Brazilian traditional cheeses. The influence of pre-processing and spectral region on the statistical parameters of the models was investigated, so that the best results are presented in Table 5. Figures of merit for the ash models are not reported here because they did not performed values sufficiently relevant regarding model performance.

In our study it was observed that only the model developed to predict protein content using the NIRS spectrum presented better results with the raw spectrum (without pre-processing). Even in this case, the best result was obtained by reducing the spectrum from 908 - 1676 nm to 1100 -1660 nm. Thus, in general, the models performed with pre-processed spectra and/or with the selection of wavelengths related to the target chemical attribute showed superior predictive performance than those using the raw data (full spectrum without pre-processing). When the calibration and cross-validation results of the models obtained for the different instruments were compared, the fusion of the spectral data showed slightly better results. However, the performance of the devices to predict in the external set varied according to the chemical components.

**Table 5.** Figures of merit of PLSR models to predict moisture, fat, and protein content in Brazilian traditional cheeses.

| Equipment | Pre-processing | Spectral range | LV | Calibration | | Cross-validation | | Prediction | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $R_C^2$ | RMSEC | $R_{CV}^2$ | RMSECV | $R_P^2$ | RMSEP | RPD |
| ***Moisture*** | | | | | | | | | | |
| vis/NIR-HSI | SNV + 1st SG | 780 - 1000 nm | 4 | 0.88 | 1.65 | 0.86 | 1.79 | 0.84 | 1.34 | 2.54 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| NIRS | 1st SG | iPLS | 5 | 0.87 | 1.70 | 0.86 | 1.73 | 0.85 | 1.36 | 2.51 |
| NIR-HSI | SNV + 1st SG | iPLS | 5 | 0.89 | 1.54 | 0.88 | 1.66 | 0.90 | 1.32 | 2.59 |
| Dafa fusion | SNV + 1st SG | 397 - 1717 nm | 5 | 0.91 | 1.38 | 0.88 | 1.62 | 0.90 | 1.27 | 2.69 |
| ***Fat*** | | | | | | | | | | |
| vis/NIR-HSI | 1st SG | 397 - 1007 nm | 4 | 0.87 | 2.28 | 0.83 | 2.58 | 0.84 | 2.17 | 2.49 |
| NIRS | SNV + 2nd SG | 1100 - 1600 nm | 5 | 0.88 | 2.14 | 0.85 | 2.39 | 0.85 | 2.17 | 2.49 |
| NIR-HSI | SG + SNV | 1050 - 1350, 1600 - 1680 nm | 7 | 0.87 | 2.29 | 0.83 | 2.56 | 0.82 | 2.52 | 2.08 |
| Dafa fusion | 1st SG | 397 - 1600 nm | 6 | 0.90 | 1.98 | 0.87 | 2.24 | 0.81 | 2.33 | 2.25 |
| ***Protein*** | | | | | | | | | | |
| vis/NIR-HSI | SNV + 1st SG | iPLS | 5 | 0.73 | 1.10 | 0.69 | 1.16 | 0.57 | 0.99 | 1.50 |
| NIRS | Raw | 1100 - 1600 nm | 6 | 0.80 | 0.94 | 0.72 | 1.12 | 0.73 | 1.01 | 1.47 |
| NIR-HSI | SNV + 1st SG | iPLS | 5 | 0.83 | 0.88 | 0.79 | 0.97 | 0.78 | 0.93 | 1.58 |
| Dafa fusion | SNV + 1st SG | 397 - 1717 nm | 5 | 0.84 | 0.81 | 0.78 | 0.95 | 0.67 | 0.91 | 1.62 |

The best model to predict the moisture content was obtained from the fusion of the HSI spectroscopic data set (vis/NIR + NIR), where an $R_P^2$ of 0.90 and RMSEP of 1.27% were obtained. The variables that most contributed to this performance are centered around 508, 1120, 1140, 1170, 1225, 1320, 1395, 1510, 1560 and 1660 nm and are associated with the content of other chemical components present in the cheese, such as fat, proteins, and carotenoids (Britton et al., 2004; Osborne et al., 1993). Although the visible region has contributed to this result, it is possible to obtain similar results for the prediction of moisture content from the other equipment ($R_P^2$ between 0.84 and 0.90 and RMSEP close to 1.3) using only the near infrared region. According to the RPD values, all models can be applied to predict the moisture content with good performance (RPD between 2.5 and 3.0).

The best models to predict fat content were obtained with NIRS and vis/NIR-HSI spectra, where $R_P^2$ ~0.85 and RMSEP of 2.17% were observed. The variables that contributed to the performance of the NIRS model are mainly associated with the CH, $CH_2$ and $CH_3$ groups present in the aliphatic chains of fat (1170, 1215, 1360, 1395, 1415 nm) and, to a lesser extent, with structures present in proteins (1430 nm) and water (1450 nm). In the vis/NIR model, a strong contribution of wavelengths associated with fat content (950 nm) was also observed. However, it is interesting to emphasize that the greatest contribution was observed in the visible spectrum (430 and 505 nm), which is associated with carotenoid content. This

result is expected, because in addition to carotenoids contributing to the yellowish color of cheeses, they are solubilized in the fat fraction. Similarly, Stocco et al. (2019) observed that models built with the vis/NIR region (350 – 1830 nm) presented similar performances to those based on the NIR region (1100 – 1830 nm) ($R_P^2$ of 0.85 and RPD of 2.03), when comparing the performance of two spectrometers and spectrum intervals to predict the fat content in cheeses. All models predicting fat content showed RPD between 2.0 and 2.5, indicating that they can be used for screening purposes.

The fusion of the HSI spectroscopic dataset (vis/NIR + NIR) showed the best result for predicting protein content, with calibration and prediction errors close to 0.9%. The wavelengths that contributed to the performance of the model are associated with protein content (1510 nm) and structures associated with carotenoids (508 nm) and fat (1145, 1225, 1395 and 1660 nm). It is possible to predict the protein content without loss of performance ($R_P^2$ of 0.78 and error of 0.93%) using the NIR-HSI spectra and to obtain approximate results using the other equipment (between 0.57 and 0.73 and errors of ~1.0%). However, according to the RPD values, only models based on the fusion of spectroscopic data and NIR-HSI are indicated for estimating the protein content, as they are able to distinguish between high and low levels of this compound (1.5 < RPD < 2.0).

Few studies available in the scientific literature have investigated the use of different spectrometers and spectral ranges in the vis and NIR ranges to predict chemical characteristics of a wide type of cheeses. This number is even lower when looking for studies that developed models from spectra acquired with whole cheese (without any sampling, grinding or other preparation). The discussion, therefore, focuses mainly on studies that used this last criterion and was carried out in terms of RMSEP and RPD, since the coefficient of determination ($R^2$) is influenced by the range of reference values.

Wiedemair et al (2019) compared the performance of models based on vis/NIR (740 – 1070 nm) and NIR (800 – 2500 nm) spectra to predict the composition of hard and semi-hard cheeses (n = 46). These authors reported results in accordance to this current work and observed better performance for moisture prediction using the NIR spectrum (RMSEP of 1.10 and RPD of 5.60). On the other hand, fat content was predicted with lower error (RMSEP of 1.19 and RPD of 7.75) from vis/NIR spectra. Stocco et al. (2019) developed PLS models to predict compositional characteristics of a wide type of cheeses (197 samples from 37 categories) using a portable vis/NIR spectrometer (350 - 1830 nm) and obtained performance (RMSEP) very close to those reported in this study to predict fat ( 2.03%, 350 - 1830 nm) and proteins (1.57%, 850 - 1050 nm), and slightly lower for moisture prediction (2.00%, 1100 - 1830 nm). Ayvaz et al (2021) when predicting the composition of Ezine cheese from different species (n = 81) using an FTIR spectrophotometer (10000 – 4000 $cm^{-1}$) obtained superior results for moisture (RPD of 3.38) and similar results for fat (RPD of 2.14) and proteins (RPD of 1.47).

The results obtained in this study were lower than those reported for predicting fat content in Emmental cheese (n = 91, RMSEP of 0.39% and RPD of 3.82) (Karoui et al., 2006), Grana Padano (n = 190, RMSEP of 1.19%) (Marinoni et al., 2017) and ricotta (n = 19, RMSECV of 1.9%) (Madalozzo et al., 2015). The smaller errors obtained in these studies are justified by the high homogeneity of the sample set, since only one category of cheese was included. The results are also lower than those obtained for models based on spectra acquired with crushed/grated cheese. This result is expected, as predicting cheese composition from the outer surface spectrum is more challenging. Although grinding produces better performances (Ayvaz et al., 2021; Wiedemair et al., 2019; Marinoni et al., 2017), it confronts the non-destructive character of vis/NIR spectroscopy and limits its application in the rapid authentication of cheeses with of origin or protected geographical indication.

### 3.6. Chemical maps of moisture, fat, and protein

The best models obtained for the vis/NIR-HSI and NIR-HSI data were applied to each pixel from ROI of the images to visualize the distribution of moisture, fat, and protein in the cheese samples, obtaining a pseudo-color image, or chemical map. Fig. 5 shows some maps of the spatial distribution of the predicted chemical properties in different types of cheese. The values presented in the lower corner of each cheese cylinder correspond to the absolute error (%), calculated by subtracting the reference value and that predicted by the model.

One of the greatest advantages of using hyperspectral imaging, compared to RGB imaging and conventional spectroscopy, is that it is possible to observe the concentration distribution of the modeled analyte. In the RGB image (Fig. 5) one can observe differences in the color and texture of the external surface of the samples due to the presence of different types of cheese, while the compositional differences are practically imperceptible. On the other hand, hyperspectral imaging allows you to clearly visualize differences in composition by the color intensity of each pixel in the chemical map.

Comparing the spatial distribution maps vis/NIR-HSI and NIR-HSI it is possible to observe a difference in the homogeneity and color intensity of the pixels for the same sample. This observation may be related to the difference in resolution between the images and the heterogeneous distribution of analytes in the sample. Despite this, there are no major differences between the results obtained by the reference method and those predicted by the two devices.

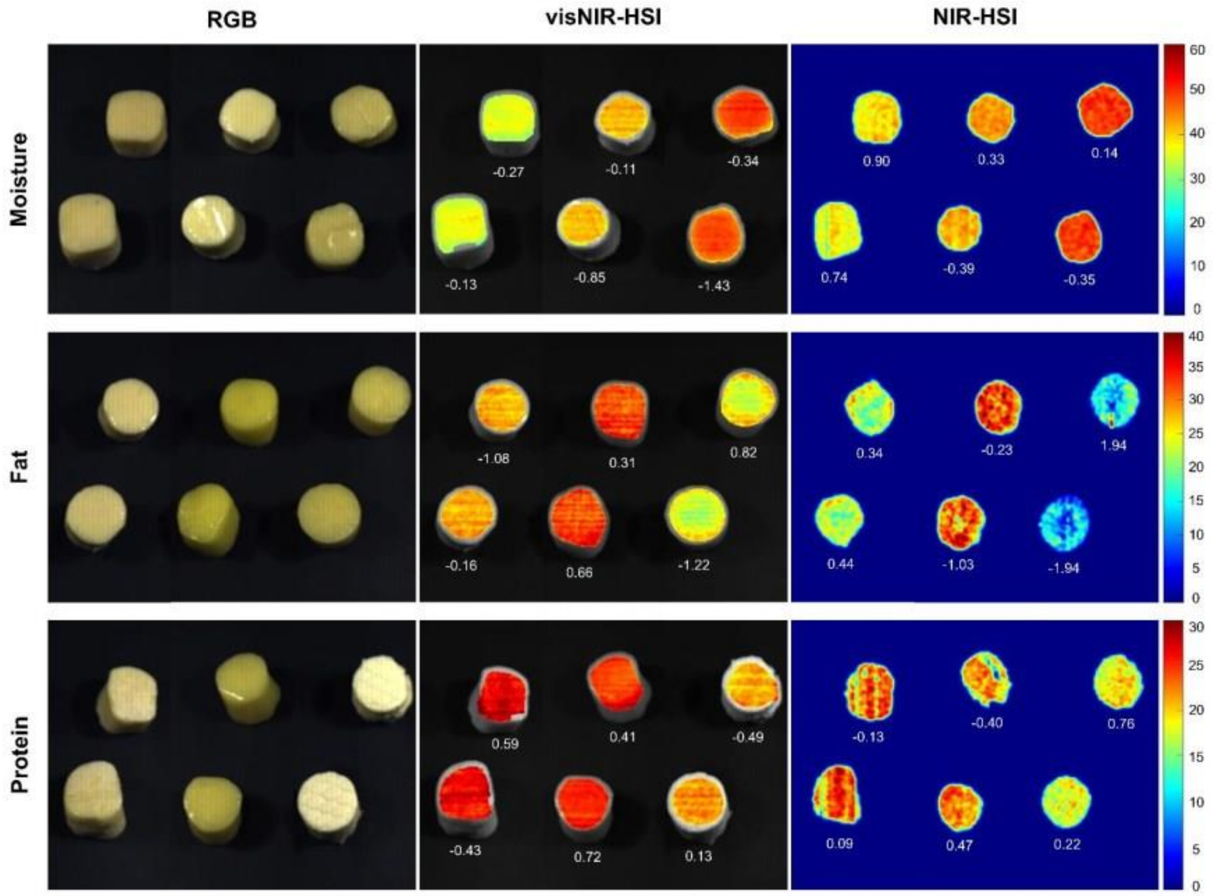


**Fig. 5.** RGB images and spatial distribution maps of moisture, fat and protein content based on vis/NIR-HSI and NIR-HSI data.

It is important to mention that the vis/NIR-HSI and NIR-HSI models (based on the spectra acquired on the external surface) presented a low average error to predict the composition of the samples from the images of the internal part of the cheese, with average absolute errors of 0.32 and 0.00% for moisture, 1.01 and 0.31% for fat, and 0.33 and 0.06% for protein, respectively. These results indicate that, even though some cheeses have composition gradients due to proteolytic, lipolytic and dehydration phenomena that occur during maturation, the information obtained from the external surface is sufficient to predict its composition satisfactory accuracy. Hence, these models can be used to predict the composition of Brazilian cheeses used in this study and also enhanced including additional types of cheese and can be useful to study compositional changes that occur during maturation.

## 4. Conclusion

This study compared the feasibility of hyperspectral imaging (vis/NIR-HSI, NIR-HSI and data fusion) and conventional (NIRS) spectrometers to characterize Brazilian traditional cheeses. Principal component analysis (PCA) showed that the spectral variability of Brazilian traditional cheeses in the vis/NIR is related to differences in color and fat content, while in the NIR there is a greater influence of productive aspects and fat content.

The PLS-DA models showed that the spectrum in the near infrared region (NIRS) has relevant information to discriminate the types of Brazilian artisanal cheeses, indicating that this technique can be used as a useful tool in investigating the authenticity of cheeses according to the region of origin.

The PLS models demonstrated that it is possible to efficiently predict the chemical composition (moisture, fat, and protein) of a wide type of cheeses. Hyperspectral imaging equipment (vis/NIR-HSI and NIR-HSI) can be recommended, especially when there is great heterogeneity within the sample and/or when there is interest in studying and visualizing compositional changes, as the general quantitative results obtained are comparable to the technique punctual (NIRS). The latter, in turn, is more attractive from an economic and operational point of view, due to lower cost, portability and shorter processing time. Finally, and in this case, the fusion of HSI spectroscopic data (vis/NIR + NIR) did not provide significant improvement of the predictive capability to justify its recommendation, because despite providing better performances to predict moisture and proteins, these are not substantially greater to justify its use.

## 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.

## Acknowledgments

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. Maria Lucimar da Silva Medeiros acknowledges scholarship funding from CNPq (300145/2022-5) and Santander international mobility program for graduate students. Prof. Douglas Fernandes Barbin is CNPq research fellow (308260/2021-0).

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