



Application of near infrared spectroscopy combined with chemometrics to authenticate local cultivar flour content in the production of protected geographical indication “Galician bread”

M. Pilar España-Fariñas^a, Patricia Cazón^{a,b,*}, Luis Urquijo-Zamora^c,
Santiago Pereira-Lorenzo^{d,e}, María Ángeles Romero-Rodríguez^{a,d}

^a Areas of Nutrition and Food Science and Food Technology, Department of Analytical Chemistry, Nutrition and Food Science, Faculty of Sciences, Campus Terra, University of Santiago de Compostela, 27002, Lugo, Spain

^b Dairy Products and Food Technology Centre (APLTA), Campus Terra, University of Santiago de Compostela, Spain

^c Department of Crop Production, Agricultural Research Center of Mabegondo, 15318, A Coruña, Spain

^d Instituto de Biodiversidade Agraria e Desenvolvemento Rural (IBADER), Campus Terra, University of Santiago de Compostela, 27002, Lugo, Spain

^e Department of Crop Production and Engineering Projects, Escuela Politécnica Superior, Campus Terra, University of Santiago de Compostela, 27002, Lugo, Spain

ARTICLE INFO

Keywords:

Food authentication
Near infrared spectroscopy
Chemometrics
Galician bread
Local wheat cultivars
Protected geographical indication

ABSTRACT

The Protected Geographical Indication (PGI) “Galician Bread” safeguards the traditional production of Galician wheat bread, recognized for its distinctive quality, which requires the use of at least 25 % flour from the local wheat cultivars ‘Caaveiro’ and ‘Callobre’. The aim of this study was to evaluate the potential of near-infrared spectroscopy (NIR) to ensure the authenticity, through quantitative analysis, of this high-value food product. A total of 160 mixtures samples of local wheat cultivars and commercial flours were prepared, ranging from 0 to 100 % of ‘Caaveiro’ or ‘Callobre’ content. Spectral data, acquired in the range 900–1700 nm, was analyzed with chemometric tools, using principal components analysis (PCA) and partial least squares regression (PLSR). The mathematical models were developed using different preprocessing techniques, with the most effective model employing a combination of de-trending (DT) and multiplicative scatter correction (MSC). The optimal model achieved high predictive accuracy, with a prediction determination coefficient (R_p^2) of 0.965, a root mean square error of prediction (RMSEP) of 5.561 % and a residual predictive deviation (RPD) of 5.292. The results demonstrated the effectiveness of NIR combined with chemometrics as a rapid, non-destructive and reliable method for predicting the content of local cultivars in wheat flour. This approach not only supports the authenticity of PGI “Galician Bread” by quantifying the presence of ‘Caaveiro’ or ‘Callobre’ flours in wheat mixtures, but also enables routine quality control and offers a valuable solution for both producers and regulatory bodies to ensure the traceability and integrity of the product.

1. Introduction

Bread has been a popular staple food worldwide and available in many types for thousands of years (Paciulli et al., 2021). Despite technological advancements, bread remains a highly traditional product, deeply rooted in its places of origin and the customs of certain regions (Mondal & Datta, 2008). In Spain, there is a close relationship between bread and its importance in the sustenance of population, as well as a strong connection with the traditions of the country (Luengo et al., 2023). The particular geographical features, climate and available crops

in each Spanish region have resulted in a high diversity of bread-making processes and specialties.

In Galicia (NW Spain), the Protected Geographical Indication (PGI) “Galician Bread” has been registered in 2019 (European Commission, 2019). This certification ensures that traditional Galician bread is made with soft wheat flour (*Triticum aestivum* L.) and at least 25 % of the flour must come from local wheat varieties. Based on studies conducted with local varieties of wheat at the Mabegondo Agrarian Research Center (CIAM), two varieties of soft wheat were selected and registered based on their agronomic behavior and grain quality for the production of

* Corresponding author. Areas of Nutrition and Food Science and Food Technology, Department of Analytical Chemistry, Nutrition and Food Science, Faculty of Sciences, Campus Terra, Universidade de Santiago de Compostela, 27002, Lugo, Spain.

E-mail address: patricia.cazon.diaz@usc.es (P. Cazón).

<https://doi.org/10.1016/j.foodcont.2025.111409>

Received 27 February 2025; Received in revised form 29 April 2025; Accepted 5 May 2025

Available online 5 May 2025

0956-7135/© 2025 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Galician bread, ‘Caaveiro’ and ‘Callobre’ (Urquijo, 2018). Thus, the “Galician Bread” is a traditional bread characterized by its crispy crust, soft crumb and slightly acidic flavor. These distinctive properties are attributed to the use of wheat flour from local cultivars and traditional production methods that include prolonged fermentation with sourdough.

Due to the high quality associated with products bearing Protected Geographical Indication (PGI) labels, they are often linked to higher selling prices and provide greater financial benefits to producers compared to the corresponding standard products (Dias & Mendes, 2018). Consequently, there is a high risk of fraudulent labeling, unfair competition and the partial substitution of raw materials with lower-quality alternatives (Fanelli et al., 2021). Therefore, it is crucial to protect these high-quality products against potential commercial fraud.

The analytical methods used to control food authenticity require robust techniques that can be employed by regulatory authorities. Regarding the bread produced under the PGI “Galician Bread”, different methodologies have been developed in recent years to ensure its authenticity, such as methods based on molecular biology (Ramos-Cabrer et al., 2022), polarized light microscopy (Fernández-Canto et al., 2023), or the combination of microscopy with neural networks (Fdez-Vidal et al., 2024). However, all these studies have focused on the local cultivar ‘Caaveiro’ and have not considered the other registered local Galician wheat variety, ‘Callobre’. Moreover, all these techniques involve laborious, expensive and time-consuming methods that must be carried out by specialized professionals to identify flour varieties, making routine control to prevent commercial fraud more difficult. As a result, infrared spectroscopic methods coupled with chemometric analysis have emerged as a robust alternative in the evaluation of food authenticity. Until now, the most versatile method for evaluating authenticity, discrimination or traceability in different food matrices has been near-infrared (NIR) spectroscopy due to its advantages in terms of cost, speed, simplicity, high performance, portability, and versatility (Cozzolino, 2016; Sadat et al., 2019).

Near-infrared spectroscopy is a technique that operates in the NIR portion of the electromagnetic spectrum, ranging from 780 to 2500 nm, which lies between the visible light spectrum and the mid-infrared spectrum (Pasquini, 2018). NIR spectra contain information about X–H chemical bonds, such as C–H, O–H, and N–H (Manley, 2014). Since all molecules containing hydrogen can be detected in an NIR spectrum, a wide range of organic materials can be analyzed using this technique (Zhang et al., 2022). Therefore, near-infrared spectroscopy is widely used for the analysis of food products, being especially valuable for studying bulk materials with minimal sample preparation (Lohumi et al., 2015).

The application of NIR spectroscopy in the evaluation of food authenticity has recently been studied in various food matrices, including honey (Guelpa et al., 2017), rice (Teye et al., 2019), apple (Eisenstecken et al., 2019), cocoa (Pinto et al., 2024), black tea (Firmani et al., 2019), artichokes (Mir-Marqués et al., 2016), meat (Alamprese et al., 2013), gum Arabic (Dong et al., 2017) and common spices (Shawky et al., 2024), among others. Wheat-derived matrices have also generated significant interest in latest years. Several studies evaluated adulteration in wheat flour, such as the incorporation of additives (Dong et al., 2024; Liu et al., 2019; Shi et al., 2023) or other food matrices (Duarte et al., 2022; Unuvar et al., 2023). On the other hand, the discrimination of wheat varieties (Miralbés, 2008) and its geographical origin (Wadood et al., 2019) has also garnered interest in literature. Furthermore, in a recent work, Candeias et al. (2025) developed a method based on NIR spectroscopy to detect and quantify cassava starch in commercial wheat flour, aiming to support the Brazilian bill proposal advocating for the creation of the “Brazilian bread” by mandating the incorporation of cassava by-products into wheat flour.

However, to date, there is no evidence that NIR technology has been applied to quantify the presence of registered local Galician cultivars in wheat flour mixtures. Therefore, the aim of this study was to develop

predictive mathematical models using near-infrared spectroscopy (NIR) technology, in combination with chemometric tools, to ensure the authenticity of “Galician Bread” under the PGI protection, by quantifying flour derived from the two registered local cultivars ‘Caaveiro’ and ‘Callobre’.

2. Materials and methods

2.1. Flour samples

The samples analyzed were supplied by the company Da Cunha. All the flour used belonged to soft wheat (*Triticum aestivum* L.). A total of 10 samples were used: 4 obtained from grains of the local cultivar ‘Callobre’, 4 from the local cultivar ‘Caaveiro’ and 2 from commercial wheat flours.

The local cultivars (‘Callobre’ and ‘Caaveiro’) were grown under two production systems, organic and conventional, using a completely randomized block design with 4 replicates per system. The experimental plots (10 × 20 m) were located at CIAM (Xunta de Galicia) (43° 14′ N, 8° 15′ W) in the northwestern region of Spain. In the organic system, chicken manure (8.5 t/ha) was used, and weeds were controlled with a cultivator. In the conventional system, mineral fertilizers (350 kg/ha of 8-15-15 fertilizer and 120 kg/ha of calcium ammonium nitrate) and specific phytosanitary products were applied for weed and foliar disease control.

The cereal grains from the cultivars ‘Callobre’ and ‘Caaveiro’ were milled using a stone mill, while the commercial flour was processed with an electric mill, following the typical procedures used in the production of PGI “Galician Bread”. All samples were hermetically sealed and stored at room temperature until further use.

2.2. Sample preparation

A total of 10 different wheat flour types were used in the preparation of the samples: 4 from the local cultivar ‘Caaveiro’ (organic and conventional), 4 from ‘Callobre’ (organic and conventional), and 2 commercial wheat flours (one organic and one conventional).

A detailed breakdown of the flour types is provided in Table 1.

The experimental design involved preparing flour mixtures, systematically varying the ratios between each of the eight local cultivar flours and the two commercial flours across ten distinct proportions: 10:90, 20:80, 25:75, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, and 90:10. Each mixture was prepared to a final weight of 10 g. Additionally, a 25:75 ratio was included, as 25 % represents the minimum requirement specified in the PGI “Galician Bread” regulations. This design resulted in a total of 160 flour samples, as summarized in Fig. 1. All mixtures were prepared independently and analyzed separately.

Table 1
Description of wheat flour samples used in the study.

Sample Type	Cultivar	Production System	Number of Flour Types
Local cultivar flour	‘Caaveiro’	Organic	2
Local cultivar flour	‘Caaveiro’	Conventional	2
Local cultivar flour	‘Callobre’	Organic	2
Local cultivar flour	‘Callobre’	Conventional	2
Commercial flour	Commercial wheat flour	Organic	1
Commercial flour	Commercial wheat flour	Conventional	1

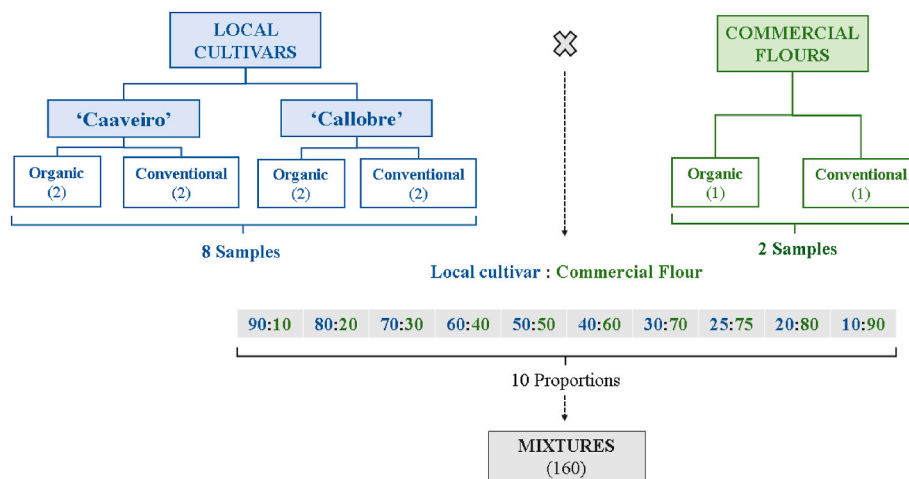


Fig. 1. Sample preparation process for obtaining near-infrared (NIR) spectra.

2.3. Near infrared spectral data acquisition

The Proximate™ spectrophotometer (Büchi Labortechnik AG, Flawil, Switzerland, SN 1100157273) was used. Daily calibration of the Proximate™ spectrophotometer was performed using the certified white reference supplied by Büchi, in accordance with the manufacturer's protocol, to ensure consistent spectral response and measurement accuracy. The spectra were obtained in diffuse reflection mode within the range of 900–1700 nm, using the NIRWise™ software.

Each sample was measured with the spectrophotometer at room temperature and scanned two times after rotating the glass container. For each reading, the instrument performed 200 scans over 15 s at a frequency of 50 Hz while the sample container rotated continuously, allowing the light beam to interact with different areas of the sample surface. The flour samples were placed in the glass sample holder and 10 g of sample was added, an amount sufficient to form a uniform, opaque layer, ensuring complete surface coverage without empty spaces.

2.4. Chemometric and statistical analysis

2.4.1. Spectral data preprocessing

For chemometric analysis Unscrambler® Version 10.5 software (Camo, Oslo, Norway) was used. The preprocessing of the original spectral data is essential to remove background information and noise, improving the modeling effect (Blanco & Villarroya, 2002; Candolfi et al., 1999). Therefore, to achieve reliable, accurate, and stable calibration models, it is crucial to preprocess the spectral data before modeling.

In this study, five spectral preprocessing techniques were applied comparatively, namely: De-trending (DT), baseline correction (BC), multiplicative scatter correction (MSC), standard normal variate (SNV) (Rinnan et al., 2009) and Savitzky-Golay (SG) smoothing (Savitzky & Golay, 1964). Combinations of these preprocessing techniques were also evaluated.

The spectral dataset (170 samples, where 160 belong to mixtures and 10 to pure flours) was downloaded and preprocessed. After that, the data was divided into two subsets: a calibration set (113 samples), used for the development of the calibration models, and a prediction set (57 samples), used to evaluate the actual predictive capability of the constructed models. To select the members of each set and achieve a 2:1 calibration/prediction set division (Teye et al., 2019), for every three samples, two spectra were randomly selected for the calibration set, while the remaining sample was assigned to the prediction set to avoid bias. The partition was performed using The Unscrambler® software, ensuring a random but stratified distribution across all mixture

proportions.

2.4.2. Calibration model development

For the qualitative analysis, an initial exploration data analysis was performed using Principal Component Analysis (PCA). Subsequently, the spectra were divided into 5 categorical variables, each containing 12 spectral datasets. These sample groups were: 100 % commercial flour, 100 % local cultivar flour, 0–25 % local cultivar flour, 30–60 % local cultivar flour, and 70–90 % local cultivar flour. PCA was performed to reduce the dimensionality of the dataset and to identify spectral outliers using the F-residual and Hotelling- T^2 values with a 5 % cut off set.

In addition, calibration mathematical models were developed using Partial Least Squares Regression (PLSR), establishing a linear correlation between the spectroscopic absorbance intensities and the corresponding concentrations of flour from a local cultivar. The performance of the resulting PLSR models was compared. The validation of all the calibration models was carried out through full cross-validation. Moreover, these models were also applied to the prediction set to evaluate their actual predictive ability of the percentage of flour from the 'Caaveiro' or 'Callobre' varieties in the samples.

Both the number of principal components (7 PCs) used in the PCA classification and the number of factors (7) for constructing PLS models for the quantification of the two registered local cultivars were derived from the default configuration of the Unscrambler® software.

2.4.3. Model evaluation

The predictive capabilities of the models were evaluated through statistical indicators, including the root mean square error of calibration (RMSEC), the root mean square error of cross-validation (RMSECV), the root mean square error of prediction (RMSEP), the calibration determination coefficient (R^2_c), the cross-validation determination coefficient (R^2_{cv}), the prediction determination coefficient (R^2_p), and the residual predictive deviation of cross-validation (RPD). The only parameter not calculated by Unscrambler® was RPD, which was obtained using Microsoft Excel 2016 (Microsoft Corporation, Washington), according to Equation (1).

$$RPD = \frac{SD}{RMSEP} \quad (\text{Equation 1})$$

The RPD and coefficients of determination (R^2) serve as essential indicators for evaluating model performance; higher values reflect stronger predictive capabilities (Nicolai et al., 2007; Urbano-Cuadrado et al., 2004). In contrast, a lower RMSE value indicates improved regression accuracy, as it indicates reduced prediction error (Burns & Ciurczak, 2007).

3. Results and discussion

3.1. Near infrared spectra characterization

The raw average spectra of the different categories of flour samples based on the local cultivar content ('Callobre' or 'Caaveiro') are highlighted in Fig. 2.

The spectra of all analyzed samples of flour, both pure and mixed, revealed a similar spectral pattern. The main absorption bands are located between 900 and 1144 nm, 1180–1216 nm, and 1428–1700 nm. Absorptions in the NIR region are associated with overtone and combination bands of fundamental molecular vibrations of organic groups such as C–H, O–H, N–H, and C=O (Green, 2003; Nawrocka & Lamorsk, 2013). Therefore, the observed variations may be caused by differences in the content of the main constituents of wheat flour.

Most of the relevant spectral bands and wavelength ranges have already been assigned in the literature to correspond to different nutritional parameters (Benes et al., 2022; Manley, 2014; Wadood et al., 2019; Zhao et al., 2014). The band at 1425 nm corresponds to the first overtone of O–H bond stretching, which is associated with moisture content. Meanwhile, the bands at 1195 nm, attributed to the second overtone of C–H bond stretching, and at 1360 nm, which combines C–H double stretching and C–H bending, are related to the presence of lipids in the sample. The absorption peaks at 1466 nm and 1585 nm are linked to the first overtone of O–H stretching, associated with starch. Additionally, the absorption band between 965 and 990 nm may be caused by the second overtone of N–H bond stretching, associated with proteins.

3.2. Chemometric analysis

3.2.1. PCA

To perform an initial exploration data analysis, Fig. 3 shows the score plot of the Principal Component Analysis (PCA) using NIR spectra without applying pretreatments.

The two principal components (PC1 and PC2) explain the variability in the spectral data. PC1 accounts for 97 % of the total variance, while PC2 explains only 2 %. Thus, most of the discrimination between the samples is represented along the PC1 axis. The 100 % commercial flour samples (blue squares) are grouped towards the left, while the 100 % local cultivar flour samples (red circles) are clearly differentiated

towards the right, indicating that they have distinctive spectral characteristics separating them from other categories. Moreover, the mixtures grouped into the 0–25 %, 30–60 %, and 70–90 % local cultivar categories show a gradual distribution between the two extreme categories (100 % commercial and local cultivar). This reflects a linear trend in the data corresponding to the increasing proportions of local varieties 'Caaveiro' or 'Callobre' in the mixtures. Furthermore, the spectral regions that contributed most significantly to PC1 were located around 980 nm, 1200 nm and 1450 nm, which correspond to absorption regions typically associated with water, starch and protein. These findings suggest that the separation observed along PC1 is driven by real compositional differences between flour samples, particularly related to cultivar, and not by noise.

3.2.2. PLSR prediction models

Table 2 presents the most important statistical indicators of the PLSR models obtained using raw spectra and different spectral preprocessing techniques to determine the performance of the models in predicting the proportion of local varieties 'Caaveiro' or 'Callobre' in the wheat flour samples. The accuracy of the regression models and the optimal preprocessing method was selected based on R^2 values close to one, lower RMSE values and RPD values greater than three (Bellon-Maurel et al., 2010).

All the models developed to predict the content of the local varieties 'Caaveiro' and 'Callobre' in the wheat flour samples exhibited a good coefficient of determination for the calibration and prediction sets, with R_C^2 and R_P^2 values above 0.94, even for the regression using raw spectral data. However, preprocessing methods significantly improved the results.

Among the regression models obtained with individual preprocessing methods, DT stood out with an R_P^2 of 0.962 and an RPD of 5.123, demonstrating high accuracy. Combinations such as DT + SNV and DT + MSC were the most effective, achieving the best results ($R_P^2 = 0.965$ and RPD >5). In contrast, methods like BC and Smoothing, even in combination, were less effective, with RPD values below 3 in some cases. These results confirm the importance of spectral pretreatment before modeling to improve the quality and robustness of predictive models.

The results indicate that the best PLSR model, selected based on higher R_P^2 , lower RMSEP and higher RPD values, was the combination of DT + MSC. This model demonstrated the best predictive capabilities to ensure the authenticity of "Galician Bread" under PGI protection by

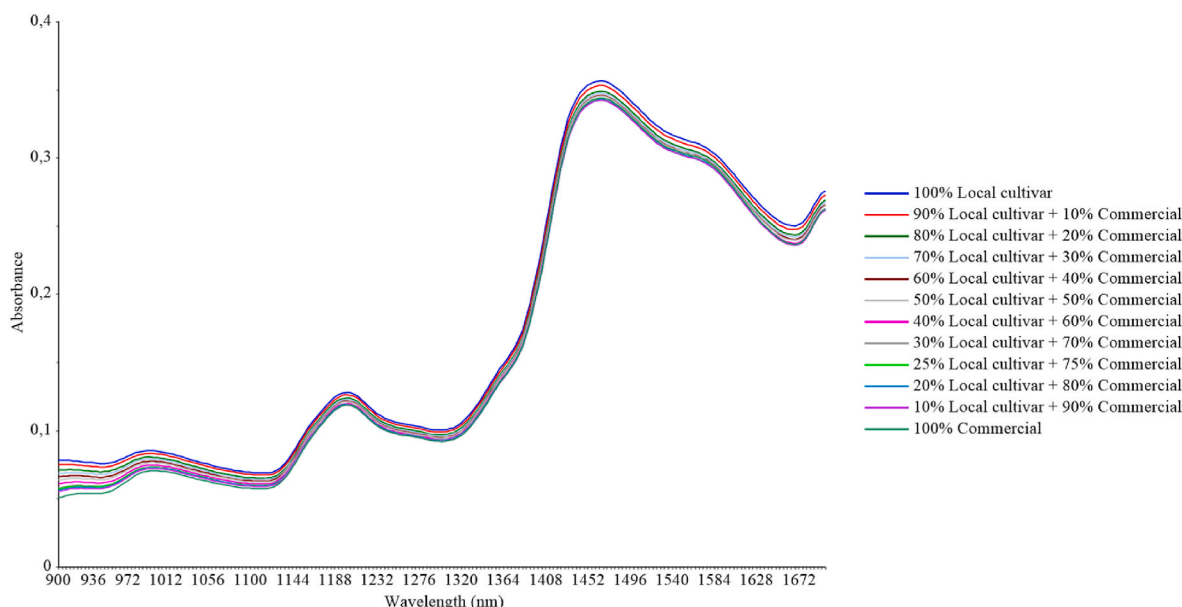


Fig. 2. Average near Infrared (NIR) spectra of different sample types based on local cultivar flour content in the 900–1700 nm region.

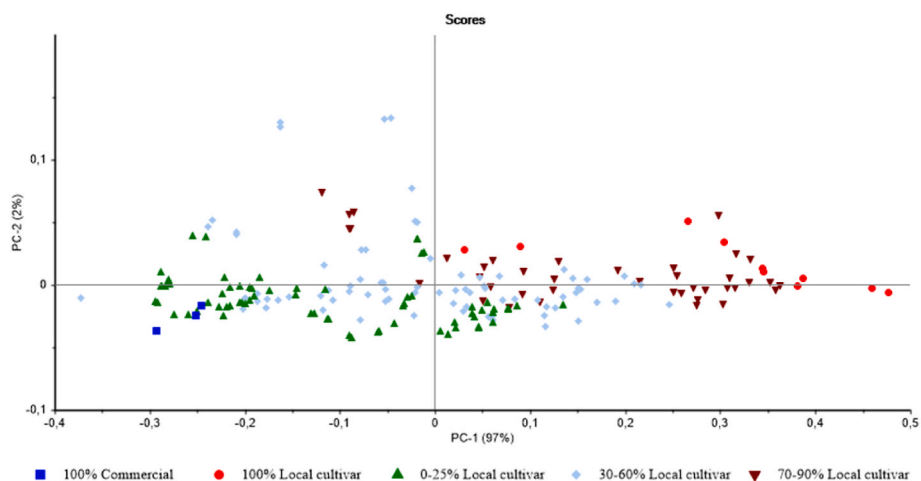


Fig. 3. Principal component analysis (PCA) scores plot of the raw spectral data of the 5 categorical variables: 100 % commercial flour, 100 % local cultivar flour, 0–25 % local cultivar flour, 30–60 % local cultivar flour, and 70–90 % local cultivar flour.

Table 2

Results of partial-least square regression models for predicting local cultivar flour in wheat flour samples using near infrared (NIR) absorbance spectra with different pretreatment methods.

Pretreatment	Calibration		Cross-Validation		Prediction		
	R ² _c	RMSEC	R ² _{cv}	RMSECV	R ² _p	RMSEP	RPD
Raw spectra	0.940	7.182	0.932	7.675	0.950	6.588	4.467
DT	0.962	5.690	0.955	6.275	0.962	5.745	5.123
BC	0.952	6.420	0.946	6.858	0.840	11.836	2.487
SNV	0.947	6.725	0.940	7.254	0.947	6.780	4.341
MSC	0.948	6.702	0.940	7.244	0.947	6.793	4.333
Smoothing	0.939	7.262	0.930	7.813	0.911	8.830	3.333
DT + SNV	0.967	5.352	0.962	5.750	0.965	5.571	5.283
DT + BC	0.953	6.345	0.948	6.699	0.954	6.346	4.638
DT + MSC	0.966	5.371	0.961	5.796	0.965	5.561	5.292
DT + Smoothing	0.959	5.908	0.954	6.291	0.878	10.323	2.851

R²_c: coefficient of determination for calibration; RMSEC: root mean square error of calibration (%); R²_{cv}: coefficient of determination for cross-validation; RMSEC: root mean square error of cross-validation (%); R²_p: coefficient of determination for prediction; RMSEP: root mean square error of prediction (%); RPD: ratio of prediction to deviation; DT: De-trending; BC: baseline correction; SNV: standard normal variate; MSC: multiplicative scatter correction (MSC); Smoothing: Savitzky-Golay (SG) smoothing.

quantifying the content of local varieties ‘Caaveiro’ and ‘Callobre’ in the wheat flour.

Figs. 4 and 5 present the PLSR prediction plots, which confirm the linear mathematical relationships between the actual percentage values of a registered local cultivar in wheat flour (X) and the corresponding

predicted values (Y) using the best PLSR model developed with DT + MSC as preprocessing techniques for the calibration set (Fig. 4) and the prediction set (Fig. 5).

In general, the results reported in the present work align with other studies evaluating authenticity in flour samples. Ndlovu et al. (2019)

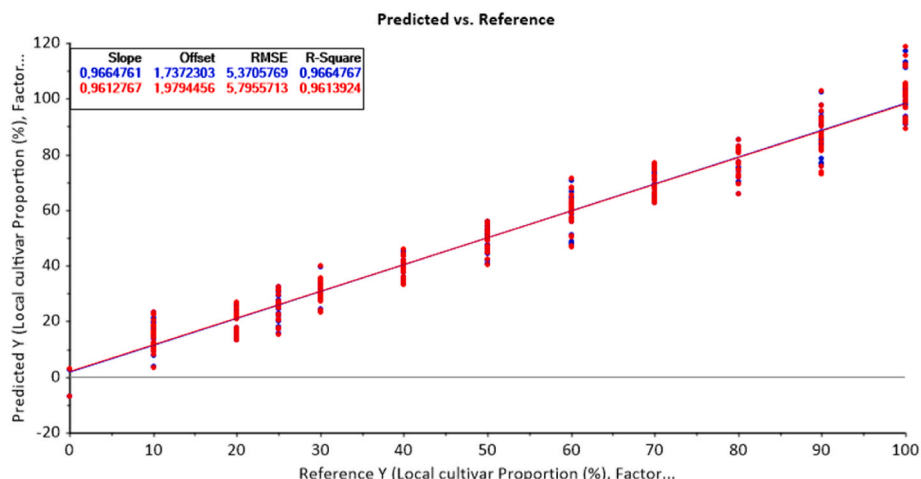


Fig. 4. Predicted against reference values for the calibration data set obtained using the best PLSR model developed with DT + MSC as preprocessing techniques.

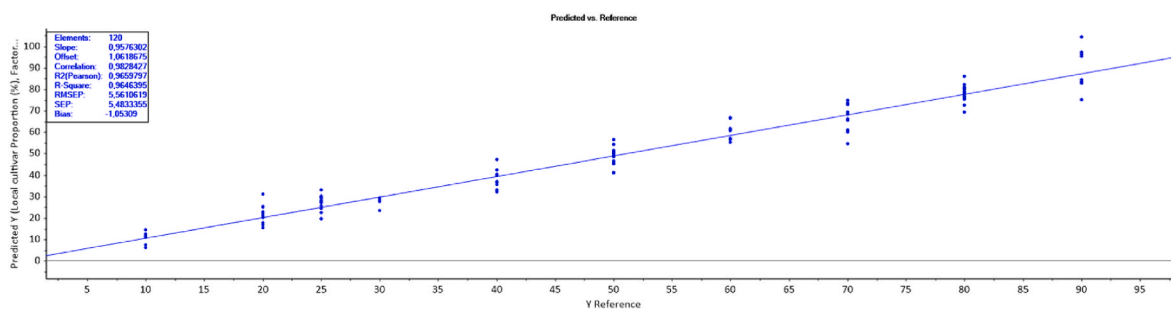


Fig. 5. Predicted against reference values for the prediction data set obtained using the best PLSR model developed with DT + MSC as preprocessing techniques.

developed PLSR models using Vis-NIR spectra combined with chemometric tools to detect the adulteration of unripe banana flour with wheat flour. The best PLSR model, obtained by applying the Savitzky-Golay second derivative pretreatment, achieved R^2 values exceeding 0.99 and an RPD of 12.021. Similarly, Huang et al. (2018) developed a PLSR predictive model based on NIR spectroscopy to determine the potato flour content in potato-wheat flour blends, achieving a R^2 of 0.9995. More recently, Ayvaz et al. (2021) investigated methods to detect the adulteration of einkorn flour with common wheat flour. For this purpose, they employed NIR spectroscopy combined with chemometrics to analyze flour and bread mixtures. The results indicated that NIR was highly effective in detecting adulteration in both matrices. Specifically, for bread, PLSR models developed achieved correlation coefficients of 0.94, standard errors below 2.64 %, and an RPD value of 10.1. For flour, it achieved R^2 of 0.99, a RMSEP of 0.73 % and a RPD of 14.1.

Moreover, the detection levels achieved in this investigation are similar to those obtained with previous studies on ‘Caaveiro’ wheat with microscopy, which correctly classified ($R^2 = 0.9574$), with a maximum difference of 7 % with respect to the percentage of real mixture (Fernández-Canto et al., 2023), and at the same level with ddPCR based on SSRs (Ramos-Cabrer et al., 2022). The main deviations were due to the difficulty of homogenizing the samples for the analysis.

4. Conclusions

This study highlights the potential of near-infrared spectroscopy (NIR) combined with chemometric tools as a robust and reliable method for authenticating the local wheat cultivars ‘Caaveiro’ and ‘Calobre’ content in PGI “Galician Bread”. The predictive PLSR models developed, particularly those using a combination of de-trending (DT) and multiplicative scatter correction (MSC), exhibited high accuracy and strong predictive capabilities, with a R^2 of 0.965 and an RPD of 5.292. These results confirm the usefulness of NIR spectroscopy as a rapid, non-destructive and cost-effective analytical tool to quantitatively assess the presence of local cultivars in wheat flour mixtures, thus supporting the verification of authenticity in this popular and traditional food product. This methodology offers a practical solution for routine quality control in preventing commercial fraud in Galician bread.

Although the milling procedures within the flour groups were standardized, no quantitative granulometric analysis was performed. Future research should investigate the influence of particle size distribution on spectral response and model performance. Furthermore, although the samples in this study were analyzed shortly after preparation and without prolonged storage, the potential effects of prolonged storage times on flour quality and spectral stability remain unexplored. Future studies should focus on developing methods to evaluate the impact of long-term storage conditions, specifically variations in relative humidity and temperature, on the physicochemical stability of flour and, consequently, on the quality of the final product.

This study was primarily focused on ensuring compliance with PGI “Galician Bread” regulations. However, future research could explore the development of models capable of distinguishing between the

specific cultivars “Caaveiro” and “Calobre”. Although the current model satisfies regulatory requirements, differentiating between these cultivars would improve the method’s specificity and broaden its potential applications for varietal authentication.

CRedit authorship contribution statement

M. Pilar España-Fariñas: Writing – original draft, Validation, Methodology, Investigation, Data curation, Conceptualization. **Patricia Cazón:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Luis Urquijo-Zamora:** Writing – review & editing, Supervision, Methodology. **Santiago Pereira-Lorenzo:** Writing – review & editing, Supervision, Project administration. **María Angeles Romero-Rodríguez:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

Funding

This research was funded by the “Cátedra do Pan e do Cereal”, Institutional Cátedra of the University of Santiago de Compostela (Ref. 2018-Ad003) and the Spanish Ministry of Science and Innovation, Proyectos de Generación de Conocimiento 2021–2023 (PID2021-123905OB-I00). The author Patricia Cazón is grateful for the financial support of the Xunta de Galicia postdoctoral fellowship (Nº. ED481D-2024-015).

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.

Acknowledgements

The authors would like to thank the Da Cunha Group by the samples and the support of the “Cátedra do Pan e do Cereal”.

Data availability

Data will be made available on request.

References

- Alamprese, C., Casale, M., Sinelli, N., Lanteri, S., & Casiraghi, E. (2013). Detection of minced beef adulteration with Turkey meat by UV–vis, NIR and MIR spectroscopy. *LWT - Food Science and Technology*, 53(1), 225–232. <https://doi.org/10.1016/j.lwt.2013.01.027>
- Ayvaz, H., Korkmaz, F., Polat, H., Ayvaz, Z., & Barış Tuncel, N. (2021). Detection of einkorn flour adulteration in flour and bread samples using computer-based image analysis and near-infrared spectroscopy. *Food Control*, 127, Article 108162. <https://doi.org/10.1016/j.foodcont.2021.108162>
- Bellon-Maurel, V., Fernandez-Ahumada, E., Palagos, B., Roger, J.-M., & McBratney, A. (2010). Critical review of chemometric indicators commonly used for assessing the quality of the prediction of soil attributes by NIR spectroscopy. *TrAC, Trends in Analytical Chemistry*, 29(9), 1073–1081. <https://doi.org/10.1016/j.trac.2010.05.006>

- Benes, E., Biró, B., Fodor, M., & Gere, A. (2022). Analysis of wheat flour-insect powder mixtures based on their near-infrared spectra. *Food Chemistry X*, 13, Article 100266. <https://doi.org/10.1016/j.fochx.2022.100266>
- Blanco, M., & Villarroya, I. (2002). NIR spectroscopy: A rapid-response analytical tool. *TRAC, Trends in Analytical Chemistry*, 21(4), 240–250. [https://doi.org/10.1016/S0165-9936\(02\)00404-1](https://doi.org/10.1016/S0165-9936(02)00404-1)
- Burns, D. A., & Ciurczak, E. W. (Eds.). (2007). *Handbook of near-infrared analysis*. CRC Press. <https://doi.org/10.1201/9781420007374>
- Candeias, D., de Barros, S. R., Lyra, W., Fernandes, D. D., & Diniz, P. H. (2025). Assessing the quality of wheat flour blended with cassava starch using a handheld NIR Spectrophotometer and chemometrics. *Journal of the Brazilian Chemical Society*. <https://doi.org/10.21577/0103-5053.20240090>
- Candolfi, A., De Maesschalck, R., Jouan-Rimbaud, D., Hailey, P. A., & Massart, D. L. (1999). The influence of data pre-processing in the pattern recognition of excipients near-infrared spectra. *Journal of Pharmaceutical and Biomedical Analysis*, 21(1), 115–132. [https://doi.org/10.1016/S0731-7085\(99\)00125-9](https://doi.org/10.1016/S0731-7085(99)00125-9)
- Cozzolino, D. (2016). Near-infrared spectroscopy and food authenticity. In *Advances in food traceability techniques and technologies* (pp. 119–136). Elsevier. <https://doi.org/10.1016/B978-0-08-100310-7.00007-7>
- Dias, C., & Mendes, L. (2018). Protected designation of origin (pdo), protected geographical indication (PGI) and traditional speciality guaranteed (tsg): A bibliometric analysis. *Food Research International*, 103, 492–508. <https://doi.org/10.1016/j.foodres.2017.09.059>
- Dong, X., Dong, Y., Liu, J., Wang, C., Bao, C., Wang, N., Zhao, X., & Chen, Z. (2024). Identification and quantitative detection of illegal additives in wheat flour based on near-infrared spectroscopy combined with chemometrics. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 323, Article 124938. <https://doi.org/10.1016/j.saa.2024.124938>
- Dong, Y., Sørensen, K. M., He, S., & Engelsen, S. B. (2017). Gum Arabic authentication and mixture quantification by near infrared spectroscopy. *Food Control*, 78, 144–149. <https://doi.org/10.1016/j.foodcont.2017.02.002>
- Duarte, E. S. de A., de Almeida, V. E., da Costa, G. B., de Araújo, M. C. U., Vêras, G., Diniz, P. H. G. D., & Fernandes, D. D. de S. (2022). Feasibility study on quantification and authentication of the cassava starch content in wheat flour for bread-making using NIR spectroscopy and digital images. *Food Chemistry*, 368, Article 130843. <https://doi.org/10.1016/j.foodchem.2021.130843>
- Eisenstecken, D., Stürz, B., Robatscher, P., Lozano, L., Zanella, A., & Oberhuber, M. (2019). The potential of near infrared spectroscopy (NIRS) to trace apple origin: Study on different cultivars and orchard elevations. *Postharvest Biology and Technology*, 147, 123–131. <https://doi.org/10.1016/j.postharvbio.2018.08.019>
- European Commission. (2019). Regulation 2019/2182 of 16 December 2019 entering a name in the register of protected designations of origin and protected geographical indications. *Official Journal of the European Union*, L330 [Pan Galego (PGI)] <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32019R2182>
- Fanelli, V., Mascio, I., Miazzi, M. M., Savaio, M. A., De Giovanni, C., & Montemurro, C. (2021). Molecular approaches to agri-food traceability and authentication: An updated review. *Foods*, 10(7), 1644. <https://doi.org/10.3390/foods10071644>
- Fdez-Vidal, X. R., Fernández-Canto, N., Romero-Rodríguez, M.Á., Ramos-Cabrer, A. M., Pereira-Lorenzo, S., & Lombardero-Fernández, M. (2024). Neural networks allow the automatic verification of the type of flour, analysing the starch granule morphology, to ensure the protected geographical indication 'Galician Bread'. *Food Control*, 158, Article 110198. <https://doi.org/10.1016/j.foodcont.2023.110198>
- Fernández-Canto, N., Romero-Rodríguez, M.Á., Ramos-Cabrer, A. M., Pereira-Lorenzo, S., & Lombardero-Fernández, M. (2023). Polarized light microscopy guarantees the use of autochthonous wheat in the production of flour for the protected geographical indication 'Galician Bread'. *Food Control*, 147, Article 109597. <https://doi.org/10.1016/j.foodcont.2022.109597>
- Firmani, P., De Luca, S., Bucci, R., Marini, F., & Biancolillo, A. (2019). Near infrared (NIR) spectroscopy-based classification for the authentication of Darjeeling black tea. *Food Control*, 100, 292–299. <https://doi.org/10.1016/j.foodcont.2019.02.006>
- Green, J. (2003). Optical spectroscopy. In *Handbook of spectroscopy* (pp. 279–296). Wiley. <https://doi.org/10.1002/3527602305.ch17>
- Guelpa, A., Marini, F., du Plessis, A., Slabbert, R., & Manley, M. (2017). Verification of authenticity and fraud detection in South African honey using NIR spectroscopy. *Food Control*, 73, 1388–1396. <https://doi.org/10.1016/j.foodcont.2016.11.002>
- Huang, Y., Xu, F., Hu, H., Dai, X., & Zhang, H. (2018). Development of a predictive model to determine potato flour content in potato-wheat blended powders using near-infrared spectroscopy. *International Journal of Food Properties*, 21(1), 2030–2036. <https://doi.org/10.1080/10942912.2018.1502199>
- Liu, Y., Sun, L., Ran, Z., Pan, X., Zhou, S., & Liu, S. (2019). Prediction of talc content in wheat flour based on a near-infrared spectroscopy technique. *Journal of Food Protection*, 82(10), 1655–1662. <https://doi.org/10.4315/0362-028X.JFP-18-582>
- Lohumi, S., Lee, S., Lee, H., & Cho, B.-K. (2015). A review of vibrational spectroscopic techniques for the detection of food authenticity and adulteration. *Trends in Food Science & Technology*, 46(1), 85–98. <https://doi.org/10.1016/j.tifs.2015.08.003>
- Luengo, E., Pastor, J., & Saldaña, G. (2023). Traditional breads from Spain. In *Traditional European breads* (pp. 343–366). Springer International Publishing. https://doi.org/10.1007/978-3-031-23352-4_16
- Manley, M. (2014). Near-infrared spectroscopy and hyperspectral imaging: Non-destructive analysis of biological materials. *Chemical Society Reviews*, 43(24), 8200–8214. <https://doi.org/10.1039/C4CS00062E>
- Mir-Marqués, A., Elvira-Sáez, C., Cervera, M. L., Garrigues, S., & de la Guardia, M. (2016). Authentication of protected designation of origin artichokes by spectroscopy methods. *Food Control*, 59, 74–81. <https://doi.org/10.1016/j.foodcont.2015.05.004>
- Miralbés, C. (2008). Discrimination of European wheat varieties using near infrared reflectance spectroscopy. *Food Chemistry*, 106(1), 386–389. <https://doi.org/10.1016/j.foodchem.2007.05.090>
- Mondal, A., & Datta, A. K. (2008). Bread baking – a review. *Journal of Food Engineering*, 86(4), 465–474. <https://doi.org/10.1016/j.jfoodeng.2007.11.014>
- Nawrocka, A., & Lamorsk, J. (2013). Determination of food quality by using spectroscopic methods. In *Advances in agrophysical research*. InTech. <https://doi.org/10.5772/52722>
- Ndlovu, P. F., Magwaza, L. S., Tesfay, S. Z., & Mphahlele, R. R. (2019). Rapid visible–near infrared (Vis–NIR) spectroscopic detection and quantification of unripe banana flour adulteration with wheat flour. *Journal of Food Science and Technology*, 56(12), 5484–5491. <https://doi.org/10.1007/s13197-019-04020-0>
- Nicolai, B. M., Beullens, K., Bobelyn, E., Peirs, A., Saeys, W., Theron, K. I., & Lammertyn, J. (2007). Nondestructive measurement of fruit and vegetable quality by means of NIR spectroscopy: A review. *Postharvest Biology and Technology*, 46(2), 99–118. <https://doi.org/10.1016/j.postharvbio.2007.06.024>
- Paciulli, M., Littardi, P., Rinaldi, M., & Chiavaro, E. (2021). Wheat bread in the mediterranean area: From past to the future. In *Cereal-based foodstuffs: The backbone of mediterranean cuisine* (pp. 47–88). Springer International Publishing. https://doi.org/10.1007/978-3-030-69228-5_3
- Pasquini, C. (2018). Near infrared spectroscopy: A mature analytical technique with new perspectives – a review. *Analytica Chimica Acta*, 1026, 8–36. <https://doi.org/10.1016/j.aca.2018.04.004>
- Pinto, A., Deryck, A., Lima, G. V., de Oliveira, A. C., Moura, F. G., Barbin, D. F., Fernández Pierna, J. A., Baeten, V., & Rogez, H. (2024). Advances in the individual authentication of cocoa beans: Vis/NIR spectroscopy as a tool to distinguish fermented from unfermented beans and classify genotypes in the eastern Amazonia. *Food Control*, 164, Article 110559. <https://doi.org/10.1016/j.foodcont.2024.110559>
- Ramos-Cabrer, A. M., Fernández-Canto, N., Almeida-García, F., Gorostidi, A., Lombardero-Fernández, M., Romero-Rodríguez, M.Á., & Pereira-Lorenzo, S. (2022). Traceability of the local cultivar 'Caaveiro' in flour mixtures used to produce Galician bread by simple sequence repeats and droplet digital polymerase chain reaction technology. *International Journal of Food Science and Technology*, 57(11), 7085–7098. <https://doi.org/10.1111/ijfs.16048>
- Rinnan, Å., van den Berg, F., & Engelsen, S. B. (2009). Review of the most common pre-processing techniques for near-infrared spectra. *TRAC, Trends in Analytical Chemistry*, 28(10), 1201–1222. <https://doi.org/10.1016/j.trac.2009.07.007>
- Sadat, A., Corradini, M. G., & Joye, I. J. (2019). Molecular spectroscopy to assess protein structures within cereal systems. *Current Opinion in Food Science*, 25, 42–51. <https://doi.org/10.1016/j.cofs.2019.02.001>
- Savitzky, A., & Golay, M. J. E. (1964). Smoothing and differentiation of data by simplified least squares procedures. *Analytical Chemistry*, 36(8), 1627–1639. <https://doi.org/10.1021/ac60214a047>
- Shawky, E., Nahar, L., Nassief, S. M., Sarker, S. D., & Ibrahim, R. S. (2024). Spice authentication by near-infrared spectroscopy: Current advances, limitations, and future perspectives. *Trends in Food Science & Technology*, 148, Article 104522. <https://doi.org/10.1016/j.tifs.2024.104522>
- Shi, S., Feng, J., Ma, Y., Cao, C., Li, L., & Jiang, Y. (2023). Rapid determination of two illegal additives in wheat flour by near-infrared spectroscopy and different key wavelength selection algorithms. *Lebensmittel-Wissenschaft & Technologie*, 189, Article 115437. <https://doi.org/10.1016/j.lwt.2023.115437>
- Teye, E., Amuah, C. L. Y., McGrath, T., & Elliott, C. (2019). Innovative and rapid analysis for rice authenticity using hand-held NIR spectrometry and chemometrics. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 217, 147–154. <https://doi.org/10.1016/j.saa.2019.03.085>
- Unuvar, A., Boyacı, I. H., Yazar, S., & Koksel, H. (2023). Rapid detection of common wheat flour addition to durum wheat flour and pasta using spectroscopic methods and chemometrics. *Journal of Cereal Science*, 109, Article 103604. <https://doi.org/10.1016/j.jcs.2022.103604>
- Urbano-Cuadrado, M., Luque de Castro, M. D., Pérez-Juan, P. M., García-Olmo, J., & Gómez-Nieto, M. A. (2004). Near infrared reflectance spectroscopy and multivariate analysis in enology. *Analytica Chimica Acta*, 527(1), 81–88. <https://doi.org/10.1016/j.aca.2004.07.057>
- Urquijo, L. (2018). Cómo recuperar los ecotipos autóctonos? In M. A. Romero Rodríguez, & S. Pereira Lorenzo (Eds.), *Respuestas ás preguntas sobre o pan e o cereal do país* (pp. 33–38). Monografías país. Monografías do Ibader – Serie Recursos Alimentarios 1. Ibader. Universidad de Santiago de Compostela. Lugo (coord).
- Wadood, S. A., Guo, B., Zhang, X., & Wei, Y. (2019). Geographical origin discrimination of wheat kernel and white flour using near-infrared reflectance spectroscopy fingerprinting coupled with chemometrics. *International Journal of Food Science and Technology*, 54(6), 2045–2054. <https://doi.org/10.1111/ijfs.14105>
- Zhang, S., Liu, S., Shen, L., Chen, S., He, L., & Liu, A. (2022). Application of near-infrared spectroscopy for the nondestructive analysis of wheat flour: A review. *Current Research in Food Science*, 5, 1305–1312. <https://doi.org/10.1016/j.crf.2022.08.006>
- Zhao, H., Guo, B., Wei, Y., & Zhang, B. (2014). Effects of grown origin, genotype, harvest year, and their interactions of wheat kernels on near infrared spectral fingerprints for geographical traceability. *Food Chemistry*, 152, 316–322. <https://doi.org/10.1016/j.foodchem.2013.11.122>