



Feasibility of geographic authentication of Sicilian pistachios by Fourier-Transform Infrared spectroscopy

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ABSTRACT

Quality and traceability are essential to food industry, spurring adoption of non-invasive methods for rapid assessment of key product attributes. This study investigates the feasibility of portable Fourier-Transform Infrared spectroscopy with attenuated total reflectance (FTIR-ATR) for fast, in-situ and non-destructive authentication of the geographical origin of Sicilian pistachios (*Pistacia vera* L.). A pilot set of pistachio kernels harvested in 2023 was analysed, including samples from the “Pistacchio Verde di Bronte” and “Pistacchio di Raffadali” production districts – both recognized with the Protected Designation of Origin (PDO) label – and samples from an uncertified Sicilian growing area. These samples were subsequently compared with additional Sicilian and foreign pistachio kernels, mostly harvested in 2025, to validate the proposed authentication model. The method successfully disentangled Sicilian pistachios from foreign samples and distinguished Sicilian pistachios of the same origin harvested in different years, demonstrating robustness in detecting variations due to geographic origin and harvesting year. Complementary physico-chemical analysis, including moisture content, cotyledon colour, and total polyphenol content, revealed differences between Sicilian and foreign samples consistent with the spectral discrimination, thus corroborating the identification of the chemical features responsible for sample discrimination. These findings support the potential of FTIR-ATR spectroscopy for assessing the quality and geographical origin of pistachio nuts.

1. Introduction

Pistachio (*Pistacia vera*) is one of the most known and consumed nuts in the world. Its kernels are consumed raw or roasted and used in the production of desserts, ice creams, cakes, spreadable creams and some meat products. It has a high nutritional value, being particularly rich in proteins (18.1–20.5%) and fats (45–56.1%) (CREA, 2019; FoodData Central, 2023) and represents a good source of dietary fiber, minerals, vitamins and polyphenolic compounds (Arena et al., 2019).

Thanks to its ability to survive in adverse pedo-climatic and hydro-logic conditions, the *Pistacia vera* tree is traditionally cultivated in soils unsuitable for other crops (Bellomo and Fallico, 2007). Pistachio world top-producers are United States (400,070 tons), Iran (241,668 tons),

Turkey (239,289 tons) and China (81,699 tons) (FAOSTAT, 2022). In Italy, pistachio is grown almost exclusively in Sicily, within the Bronte, Adrano and Biancavilla municipalities (province of Catania), around the Mt. Etna where the volcanic soil and climate conditions allow to get nuts with bright green colour and aromatic taste, particularly appreciated in international markets (Vitale et al., 2013). Specifically, pistachio production in Catania accounts for approximately 90% (36403 tons), whereas lower outputs are recorded in Agrigento (5.7% - 2375 tons), Caltanissetta (4.9% - 2024 tons), Palermo (0.9% - 358 tons) and Messina (0.1% - 55 tons) (ISTAT, 2023). The unique pedo-climatic conditions of this region, combined with traditional cultivation methods, result in Sicilian pistachios having a peculiar organoleptic and nutritional quality different to foreign products. This excellence has been recognized by the

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European Union by the Protected Designation of Origin (PDO) certification, granted both to pistachio cultivated in Catania province (Pistacchio Verde di Bronte PDO) and to that in Agrigento and Caltanissetta provinces (Pistacchio di Raffadali PDO). This certification guarantees the geographical origin and ensures compliance with strict quality standards and traditional agricultural practices (Regulation EU No 1151/2012).

The high market value of the Sicilian PDO-certified pistachios has made them vulnerable to fraudulent practices, such as the mislabelling and substitution with lower-quality and non-certified foreign nuts. Counterfeiting poses a serious threat to both consumer trust and the economic sustainability of the Sicilian producers, underscoring the need for analytical approaches to authenticate geographical origin. Up to now, identification was based on chemical markers tracing the production area of vegetable crops, given that the chemical composition is strongly influenced by several factors, including fruit ripeness, weather conditions, genotype and agronomical practices (Panebianco et al., 2023, 2024; Rabadán et al., 2019). In this context, various analytical techniques associated with chemometrics, including the liquid- or gas-chromatography-based techniques and the Nuclear Magnetic Resonance (NMR) spectroscopy, have been used to discriminate pistachios from different countries worldwide (e.g. Italy, Greece, Turkey, Iran and USA) by detecting differences in the composition of fatty acid, phytosterol, pigment and phenolic compounds (Arena et al., 2007; Boukid et al., 2019; Mannino et al., 2019; Rotondo et al., 2020; Saitta et al., 2014). Other authors have highlighted the possibility of authenticating the pistachio's geographical origin also by comparing the elemental profiles detected through atomic spectrometry, such as Inductively coupled plasma atomic emission spectrometry (ICP-AES) and Inductively coupled plasma mass spectrometry (ICP-MS) (Anderson and Smith, 2005; Kalogiouri et al., 2021). These techniques are accurate and sensitive, but they require expensive instrumentation and complex procedures for sample preparation, processing and analysis, involving the destruction of the analysed matrix. These limitations make it difficult to apply them in the food production, fast processing and distribution chain, highlighting the need for faster and in-situ alternatives. Among them, vibrational spectroscopic techniques - including Fourier Transform Infrared spectroscopy in the mid-infrared range ($4000\text{--}400\text{ cm}^{-1}$) - have attracted attention since they require minimum sample preparation and are fast, accurate, relatively inexpensive and potentially non-destructive analytical tools. Comparative studies have reported that mid-infrared spectroscopy can outperform other vibrational techniques (e.g., near-infrared, Raman spectroscopy and near-infrared hyperspectral imaging) in predicting chemical, structural and rheological properties of complex food matrices since it captures variability due to cultivar, post-harvest conditions and processing treatments, providing suitability for comprehensive food characterization (Lan et al., 2020, 2022). These characteristics make FTIR spectroscopy particularly suitable for use along the food production and distribution chain, where consistent and continuous workflow is required (Cozzolino, 2022).

FTIR spectroscopy and related advanced imaging techniques have been successfully applied to verify the geographical origin and the quality of several food products, like oils, cereals, dairy products, nuts, fruit and fresh vegetables, or to provide information on their chemical composition and structural heterogeneity (Cebi et al., 2023; Lan et al., 2025; Li et al., 2025; Wang et al., 2021). As concerning the pistachio, Aykas and Menevseoglu (2021) have reported the ability of portable FTIR systems to reveal adulteration by detecting the presence of powdered green pea and peanut, added as adulterants, mixed with pistachio powder, whereas Valasi and Pappas (2021) have applied the FTIR technique to differentiate pistachio samples from two different harvest years. However, to our knowledge, the FTIR spectroscopy has never been applied for tracing the geographical origin of Sicilian pistachio samples. Previous studies have examined the composition of pistachio samples coming from countries with notable different climatic

and pedological conditions, by employing the near infrared spectroscopy (Cevoli and Fabbri, 2024; Vitale et al., 2013).

Therefore, considering the strong impact of this product on the island's economy and the need to protect high-quality certified products from fraud, we started developing a reliable, non-invasive and fast analytical procedure based on a portable FTIR-ATR device for the authentication of pistachio geographical origin. The primary aim of this study was to evaluate the ability of this technique, combined with multivariate data analysis, to distinguish PDO-certified pistachios from Bronte and Raffadali among themselves and from other Sicilian and foreign samples, a topic still scarcely explored for PDO products. Thus, it would represent a first feasibility study, by using a portable FTIR system, for authenticating these two PDO-certified products with respect to other Sicilian and non-Sicilian pistachio samples.

2. Materials and methods

The study was conducted along three main stages. In the first step, a preliminary feasibility study was performed to validate the methodological approach, to assess the sensitivity of a portable FTIR-ATR spectrometer in comparison with a bench-top system and to determine the most suitable sample preparation method. In the second step, PDO-certified and uncertified pistachio samples from three Sicilian growing areas, harvested in 2023, were analysed. These samples were used as a training set to build the classification model and to assess the possibility of specifically tagging PDO samples. In the final step, additional pistachio samples from Sicily and foreign countries, harvested in two different years (2023 and 2025), were introduced as an external test set to validate the model's robustness, assessing its ability to discriminate samples based on geographical origin and harvest year. Statistical analyses were therefore conducted following a structured step-by-step protocol, including data dimensionality reduction, spectroscopic feature extraction, model definition and model validation. Selected physico-chemical parameters, including moisture content, cotyledon colour, and total polyphenol content, were measured on the same sample-set. These parameters were evaluated in relation to FTIR-ATR absorption bands to better understand their contribution to sample discrimination.

2.1. Pistachio sample and collection

A total of 53 pistachio samples were analysed in this study. The sample-set included: (a) one PDO-certified "Pistacchio Verde di Bronte" sample, used to validate the experimental procedure (feasibility study); (b) 19 Sicilian pistachio samples harvested in 2023 from the PDO production zones of Raffadali and Bronte (Agrigento and Catania provinces) and a non-certified area in Milazzo (Messina province), used as a training set to build the classification model; (c) 20 pistachio samples from the Sicilian PDO zones and 13 foreign pistachio samples originating from Greece, Spain, United States and Iran, used as an external test set to validate the model. Most of the external samples were harvested in 2025, while three were collected in 2023. Fig. 1 shows the geographical production areas of Sicilian pistachios used in this study. Table 1 provides detailed information on all the pistachio samples, including the number of samples, origin, harvest year, cultivar and sample identifier code.

Pistachio samples were obtained both from local markets and producers, as well as from the Cooperativa Produttori Pistacchio Smeraldo Bronte s.r.l. (Catania, Italy). Samples (800–900 g per sample) were vacuum-packed and stored in the dark at $15 \pm 2^\circ\text{C}$ prior to analysis.

2.2. Sample preparation

Before spectroscopic analysis, pistachio samples were manually peeled. For sample-sets a and b, analyses were performed on both peeled whole and finely ground kernels, thus generating two sub-samples for



Fig. 1. Area of origin of Sicilian pistachio samples collected for this study (A). Pistachio orchards located in Raffadali (B), Bronte (C) and Milazzo (D). In A the markers indicate the sampling sites. Samples from PDO districts were collected within the geographical boundaries defined by the respective production regulations (Ministero dell'Agricoltura, della Sovranità alimentare e delle Foreste, 2016). The image was freely downloaded from USGS EROS (Earth Resources Observatory and Science-EROS-Center, <http://eros.usgs.gov/#>).

Table 1
Pistachio samples used in this study.

Sample-set	N. of samples	Origin	PDO district	ID*	Cultivar	Harvest year
a	1	Italy (Bronte)	Bronte	T1	Bianca	2022
b	5	Italy (Raffadali)	Raffadali	C1-C5	Bianca	2023
	11	Italy (Bronte)	Bronte	C6-C8; C10-C17	Bianca	2023
	1	Italy (Bronte)	Bronte	C9	Larnaka	2023
	1	Italy (Milazzo)	-	C18	Bianca	2023
	1	Italy (Milazzo)	-	C19	Aegina	2023
c	3	Italy (Raffadali)	Raffadali	S1-S3	Bianca	2025
	3	Italy (Bronte)	Bronte	S4-S6	Bianca	2023
	14	Italy (Bronte)	Bronte	S7-S20	Bianca	2025
	2	Greece	-	S21, S22	Aegina	2025
	1	Spain	-	S23	-	2025
	6	United States	-	S24-S29	Kerman	2025
	4	Iran	-	S30-S33	Kerman	2025

* Sample identifier (ID)

each sample. Conversely, the sample-set c was analysed only as peeled whole kernels, as this approach proved to be the most suitable and rapid based on the previous results.

For powder preparation, approximately 30 g of peeled pistachio kernels were finely ground using a mortar and pestle for a few minutes to obtain a homogeneous material. The resulting powder was then distributed into eight plastic containers, forming samples with a surface area of about 1 cm² and a thickness of approximately 2 mm. To ensure consistent sample density, the powder was gently pressed to achieve a final density of $\rho = 0.58 \text{ g/cm}^3$. The same preparation procedure was applied to all powdered samples to ensure consistency for all the FTIR-ATR measurements.

2.3. FTIR-ATR instrumentation

FTIR-ATR spectra were obtained using two infrared spectrometers: a table-top system (Thermo Fisher Scientific Nicolet 380), used only in the preliminary feasibility study, and a portable device (Agilent Cary 630®).

The table-top instrument works in attenuated total reflectance (ATR) by a Smart Orbit diamond cell and is controlled by the OMNIC software (Thermo Electron Corp., Madison, WI, USA). The portable system is equipped with different modules working in transmission, diffuse reflectance and single-reflection attenuated total reflectance. The latter module, implementing a diamond sampling window, has been employed in the present study. Thanks to the instrument small dimensions (16 × 31 × 13 cm) and weight (4.8 kg), it can be easily transported for performing in-situ measurements. The system is managed by the Agilent MicroLab PC software (Agilent Technologies Inc., Danbury, CT, USA).

2.4. FTIR-ATR measurement protocol

Measurements were first conducted on the sample-set a (sample T1, Table 1) for the validation of experimental procedure; then, they were extended to all samples under study (sample-sets b and c).

For the whole kernel analysis, each pistachio kernel was split into two halves by a longitudinal cut to obtain flat surfaces. Measurements

were taken on both halves, placing the pistachio surface directly on the ATR diamond window, aligning the seeds with the axis of the optical slit, as reported in (Vitale et al., 2013). The powdered sub-samples were positioned in a similar way, keeping them inside their containers. Careful attention was paid to ensure constant contact pressure between the sample surface and the ATR crystal throughout the measurement campaign.

For each measurement, spectra were collected by averaging 64 scans in the 4000–400 cm^{-1} (table-top spectrometer) or 4000–650 cm^{-1} range (portable spectrometer), with a resolution of 4 cm^{-1} . For each sample, measurements were replicated 8–16 times to account for sample heterogeneity. Specifically, for the samples analysed in whole kernel form, 3–4 seeds per sample were selected and two measurements were acquired at different positions on each half of the seed, yielding 12–16 spectra per sample (3–4 seeds \times 2 halves \times 2 measurements = 12–16 replicates). As regards the pistachio powdered sub-samples, eight spectra per sample were acquired (2 spectra per container, for a total of 4 containers = 8 replicates). All the spectra were normalized and averaged within each sub-sample, prior to statistical analysis.

2.5. Experimental validation (feasibility study)

The methodology was validated through a preliminary feasibility study on a single pistachio sample (sample T1, sample-set a, Table 1), by reiterating measurements on both whole and powdered kernels, by using the portable and bench-top spectrometers.

In detail, for each instrument, 12 measurements were carried out on whole pistachio kernels (4 measurements per seed, for a total of 3 seeds) and further 12 on powders (3 measurements per container, for a total of 4 containers). The resulting datasets were compared to assess reproducibility and consistency within the same measurement conditions, as well as across different measurement configurations, including instrument type and sample texture.

To quantify internal consistency under the same measurement conditions, the root mean square (RMS) was calculated with respect to the spectrum obtained averaging the 12 measurements. In detail, the RMS values were calculated for each sub-sample (pistachio kernels/pistachio powder) and for each of the 12 measurements (replicates) performed on each sub-sample, by using the formula:

$$\text{RMS} = \sqrt{\frac{\sum (x_i - \mu_i)^2}{N}} \quad (1)$$

where x_i represents the absorbance value of each replicate at the channel i , μ_i the average absorbance value calculated for the 12 replicates at the channel i and N the total number of channels associated with each replicate (1868 channels for measurements performed with the table-top spectrometer in the wavelength range of 4000–400 cm^{-1} and 1798 channels for those performed by the portable apparatus in the range of 4000–650 cm^{-1}).

To assess consistency across different measurement configurations (portable apparatus versus table-top spectrometer) and to highlight differences in the absorption bands in relation to the sample form (whole kernel versus powder), normalized FTIR-ATR spectra acquired using the two different instruments, representative of the two sub-samples, were compared. In this case, for both kernels and pistachio powder, the RMS dispersion of the spectra (replicates) obtained by the table-top spectrometer was estimated with respect to the mean spectrum obtained by the portable spectrometer, and vice versa. To perform the RMS comparison between spectra obtained by the two instruments, a cubic line interpolation was performed using the XLSTAT package (XLSTAT, 2023.3.1). Finally, Pearson's correlation coefficients were calculated to confirm the coherence between table-top and portable measurements.

2.6. Physico-chemical analysis of pistachio samples

On pistachio samples from sample-sets b and c (Table 1), some physico-chemical quality parameters, including moisture content, CIE $L^* a^* b^*$ colour parameters and total polyphenol content (TPC), were determined.

Since FTIR-ATR measurements were performed without the pistachio seed coat, the total polyphenol content (TPC) was determined on peeled pistachio kernels to facilitate comparison between the two different analytical procedures, considering the different concentration of phenolic compounds between the skin and the seed (Grace et al., 2016). At the same time, the possibility of identifying the geographical origin of the samples based on these parameters was also investigated.

The moisture content was determined by gravimetric method. Kernels were finely crushed for 30 s in a grinder to obtain a homogeneous product and 5 g of the resulting powder was placed in an oven (Heathermoven, Thermo Fisher Scientific Inc., USA) at 105°C until the dry weight was constant (Ballistreri et al., 2009). For each sample, measurements were performed in triplicate and the average moisture content (%) \pm standard deviation was calculated.

Colour parameters were determined using a portable colorimeter (CR-400, Konica Minolta Sensing Inc., Japan) with a repeatability of $\Delta E^*ab \leq 0.07$ and a measuring spot size of 8 mm. The chromaticity of the samples was recorded in the $L^* a^*$ and b^* colour space as established by the "Commission Internationale de l'Éclairage" (CIE). The L^* coordinate measures the amount of light reflected ranging from 100 (white) to 0 (black), whereas a^* and b^* indicate the colour's position along the red-green and yellow-blue axes, respectively (Backhaus et al., 2011). Other chromatic parameters recognized by the CIELab system are the coordinates C (chroma) and h (hue angle), which are related to the a^* and b^* values [$h = \arctan(b^*/a^*)$; $C = (a^{*2} + b^{*2})^{1/2}$] (Weatherall and Coombs, 1992). For each sample, L^* , a^* , b^* , C and h coordinates were measured on eight different pistachio peeled kernels, on the inner portion of the cotyledons, performing two measurements at two different points (for a total of 16 measures per sample). For each chromatic coordinate, the average value and the standard deviation were calculated.

As concerning the total polyphenol content (TPC), for each sample, an amount of 2 g of peeled and fine triturated pistachio seeds was mixed with 25 mL of methanol 0.1% HCl (Methanol, HPLC PLUS gradient grade, Carlo Erba reagents, Italy; HCl, 37% super pure-for trace analysis, Carlo Erba reagents, Italy) stirred by a vortex-mixer (Minishaker MS2, IKA, USA) for 5 min and ultrasonicated (Ultrasonic cleaning instrument, FALC, Italy) for 10 min at room temperature. After the extraction process, the resulting solution was centrifuged (Thermo Scientific SL, USA) at 7000 \times g for 10 min, and the supernatant was separated. Then, an aliquot of supernatant (5 mL) was brought to a final volume of 25 mL with distilled water. TPC was quantified using the Folin-Ciocalteu colorimetric method as described by Fernández-Agulló et al. (2013), with some modifications. One milliliter of each diluted extract was mixed with 5 mL of 10% Folin-Ciocalteu reagent (Carlo Erba, Italy) and 4 mL of 7.5% sodium carbonate (PanReac ApplyChem, Italy) and let to react in the dark for 2.5 h at room temperature. After the incubation, the absorbance of the solution was measured against a blank sample at 765 nm using the Lambda 25 UV-VIS spectrometer (PerkinElmer model, Waltham, WA, USA). Quantification was carried out according to the standard curve of gallic acid. The results were expressed as gallic acid equivalent (GAE) per 100 grams of dry matter (mg/100 g of DM). All measurements were performed in triplicates.

2.7. Statistical analysis

Clustering of the pistachio samples characterized by mid-infrared spectroscopy, as well as the spectral features involved in the cluster discrimination, were first investigated through Principal Component Analysis (PCA) and then validated by Linear Discriminant Analysis

(LDA). PCA was performed using the XLSTAT package (XLSTAT, 2023.3.1), while LDA analysis was conducted by using the scikit-learn library in Python (<https://www.python.org>) and the *lda* function from the MASS package in R (<https://www.r-project.org>). The combination of unsupervised PCA and supervised LDA facilitated dimensionality reduction and the evaluation of classification performance based on dataset features.

Before performing statistical analysis, all measurements were normalized by dividing the single absorbance values by their sum. The method of normalization to sum, as reported by Khalighi et al. (2024), helps address issues arising from small drifts in source intensity (instrumental changes over time), variations in contact area and pressure, and slight changes in effective path length due to sample morphology, enhancing data consistency. The normalization was applied to all measurements, replicated 8–16 times.

As a first step, PCA was applied to pistachio samples harvested in 2023 year (sample-set b, Table 1) to reveal their clustering patterns. Separate analyses were performed for whole kernels and powdered samples. Accordingly, two different data matrices were built by reporting in the columns the normalized mean absorbance values corresponding to each wavenumber (for a total of 1798 variables/channels) and labelling the rows (observations) by the nineteen pistachio samples from Raffadali, Bronte and Milazzo (matrix size = 19×1798). To refine the PCA and reveal the contribution of different functional groups and molecular structures to sample clustering, additional analyses were performed on four selected subsets of the FTIR spectra. These subsets were defined based on the presence of characteristic vibrational features and were extracted either as single continuous spectral regions or as combinations of non-contiguous spectral intervals. For analyses, the combination of 3050–2800 and 1800–1700 cm^{-1} intervals (matrix size = 19×189 , A); the 1700–1130 cm^{-1} region (matrix size = 19×307 , B); the 1130–650 cm^{-1} region (matrix size = 19×259 , C) and the combination of 3050–2750 and 1800–1130 cm^{-1} intervals (matrix size = 19×469 ; D) were considered. In all analyses, PCA was applied to standardized data matrices. To validate the clustering assessed by PCA, the normalized spectra acquired for the pistachio kernels were analysed by LDA over the full spectral range from 4000 to 650 cm^{-1} .

In the final and crucial step of the study, the overall performances and, specifically, the predictive ability of the model was evaluated by projecting new pistachio samples from Sicily and other countries (sample-set c, Table 1) onto the PCA space defined by the sample-set b employed for model calibration. The projection of the sample-set c, not included in the model calibration, allowed us to assess whether they were positioned consistently within the clusters defined by the sample-set b, thereby providing a further validation of the classification approach. Since most of the sample-set c were collected in a different harvest year from the sample-set b, the model was tested with respect to both temporal and geographical variability. Notably, the predictive analysis was performed considering the whole field kernels and it was applied both to spectra covering the entire wavenumber range from 4000 to 650 cm^{-1} and to the combination of 3050–2750 cm^{-1} and 1800–1130 cm^{-1} spectral intervals.

As concerning the physico-chemical parameters (moisture, colour parameters and total polyphenol content), experimental data were statistically analysed through one-way analysis of variance (ANOVA; Minitab Statistical Software, V.22) with Tukey's honest significance difference test ($\alpha = 0.05$) and compared. ANOVA was carried out on individual experimental measurements and on mean values calculated from samples grouped according to origin region and harvest year. Principal component analysis (PCA) was then carried out on the dataset consisting of individual experimental values (matrix size = 52 samples \times 7 parameters).

3. Results and discussion

3.1. Experimental validation (feasibility study)

Mid-infrared spectroscopy is sensitive to the vibrational modes of molecular functional groups in the 4000–400 cm^{-1} region, providing a detailed chemical fingerprint of the sample. Fig. 2 shows representative FTIR-ATR spectra of the pistachio sample used in the preliminary feasibility study (sample-set a, T1, Table 1), analysed in whole form and as ground powder. The main absorption bands, with assignments to the major functional groups, are summarized in Table 2.

Prior to analysing the pistachios from different geographic origins, measurement reproducibility was assessed. Intra-set reproducibility was observed among the replicated spectra (12 replicates) within each group of measurements (whole and powdered kernels analysed with either the table-top or portable spectrometer). The replicates exhibited nearly identical spectral profiles across the entire range when superimposed, indicating minimal variability within each group (Fig. S1). This result was quantitatively confirmed by root mean square (RMS) analysis, which yielded values consistently below 10^{-3} for both pistachio kernels and powders (ranging from 1.9×10^{-5} to 3.9×10^{-4}) (Figs. S2 and S3, graphs B and C, intra-instrumental comparisons, blue bars).

The good agreement between spectra acquired using the table-top and portable infrared spectrometers supported the robustness of the FTIR-ATR measurements. Despite the portable instrument is operating in a slightly different spectral range (specifically between 4000 and 650 cm^{-1}), the differences between spectra from the two instruments appeared negligible. The averaged normalized spectra from the two instruments exhibited highly similar profiles, overlapping closely across the spectral range (Figs. S2 and S3, graphs A). Consistently, also in this case, RMS values were found always lower than 10^{-3} (ranging from 1.3×10^{-4} to 4.6×10^{-4}) (Figs. S2 and S3, graphs B and C, inter-instrumental comparisons, red bars). The strong agreement between spectra from the two different devices was further validated in the whole range through correlation scatter plots, reported in Fig. S4. Pearson's correlation coefficients, calculated on the averaged spectra, were $r = 0.9958$ for the pistachio kernel and $r = 0.9949$ for the pistachio powder. This comprehensive analysis demonstrates the high reproducibility and consistency of the spectral measurements across both instruments, validating the feasibility of the analytical approach for both laboratory and portable applications. The use of portable infrared spectroscopy represents a significant advantage for on-site quality assessment of food products in different segments of the food supply chain, as it enables fast sample identification without the need for complex and high-cost laboratory-based analyses (Cebi et al., 2023).

A further comparison highlighted the effect of sample texture on the spectral shape. Clear differences between whole and powdered pistachio kernels were observed, especially in the fingerprint region (1200–900 cm^{-1}) and in the spectral regions associated with triglycerides (3000–2800 cm^{-1} and around 1700 cm^{-1}) (Fig. 2). These results indicate that sample preparation significantly affects the FTIR-ATR spectral features and must be carefully considered in authentication studies, as previously reported by Lamas et al. (2025), who demonstrated that grinding markedly changes the spectral response of almond kernels, influencing the performance of chemometric discrimination models.

Overall, the low intra-set variability, the agreement between instruments and the clear spectral differences induced by sample texture validated the robustness of the proposed approach. Based on these results, measurements were extended to the full sample-set, consisting of the samples harvested in 2023 and 2025, using the "Agilent Cary 630 (R)" portable spectrometer, obtaining the crucial advantage of determining the chemical profile directly *in-situ*.

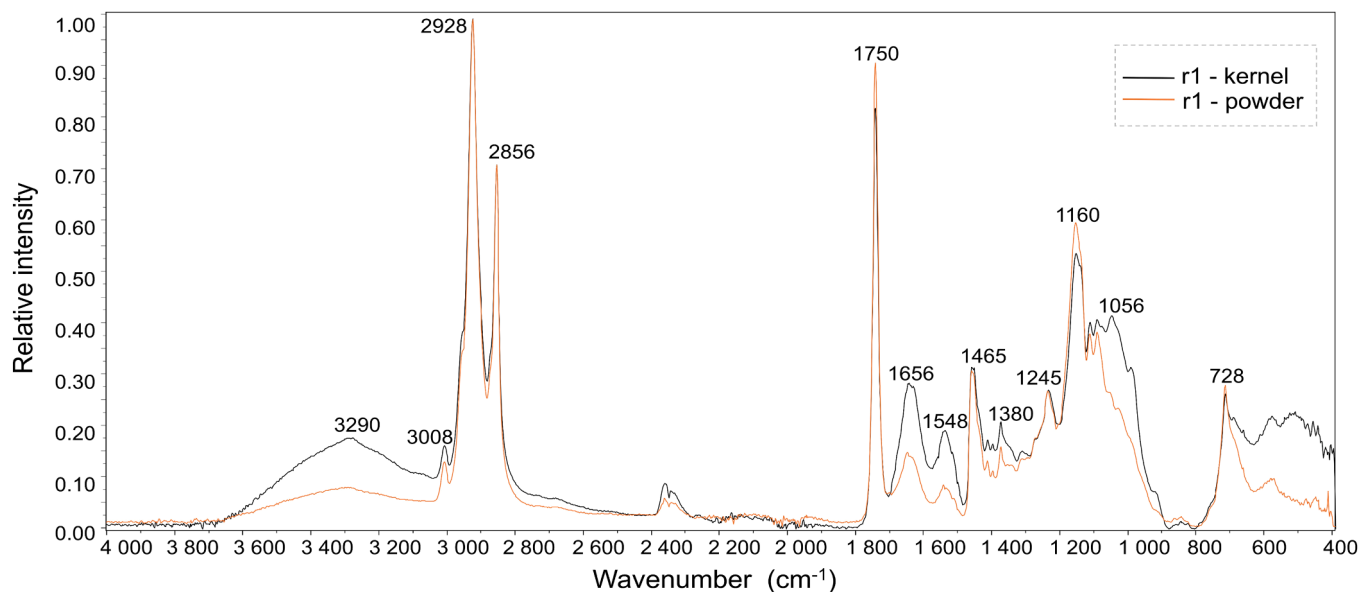


Fig. 2. Typical FTIR-ATR spectra of pistachio kernels, analysed in whole and powdered form, obtained using the Thermo Fisher Scientific Nicolet 380 table-top spectrometer (spectra normalized to the maximum intensity). Spectra referred to the sample T1, used for the experimental validation (sample-set a, Table 1).

Table 2

Summary of the FTIR-ATR bands detected on non-powdered and powdered pistachio kernels with respective attribution.

Position (cm ⁻¹)	Attribution	Molecule	Ref.
Functional group region (4000–1500 cm⁻¹)			
3645–3050	OH, NH stretching	Water, polysaccharides, proteins	Wadood et al. (2020) Manfredi et al. (2018); Valasi and Pappas (2021)
3008	<i>cis</i> -CH group stretching at C=C	Unsaturated fatty acids	Fediuc and Oroian (2025)
2928, 2856	-CH ₂ asymmetric and symmetric stretching	Lipids	Manfredi et al. (2018); Valasi and Pappas (2021)
1750	-C=O stretching	Lipids	Aykas and Menevseoglu (2021)
1656, 1548	C=O stretching; N-H bending/C-N stretching (“Amide I”, “Amide II”)	Proteins	Manfredi et al. (2018); Valasi and Pappas (2021)
1600	C=O– and aromatic C=C stretching; OH bending	Pectins, phenolic compounds	Lucarini et al. (2019)
Fingerprint region (1500–400 cm⁻¹)			
1465	CH ₂ bending	Lipids and proteins	Wadood et al. (2020)
1417	CH rocking (<i>cis</i> -olefins)	Lipids	Dogan et al. (2007); Valasi and Pappas, (2021)
1380	CH ₃ bending	Lipids and proteins	Fediuc and Oroian (2025) Dogan et al. (2007); Valasi et al. (2020)
1245	-C-O stretching	Triglycerides	Fediuc and Oroian (2025)
1160	-C-O (ester) stretching and -CH ₂ bending	Triglycerides	Fediuc and Oroian (2025); Valasi and Pappas (2021)
1143	aromatic C-H stretching	Phenolic compounds	Lucarini et al. (2019)
1300–900	C-O and C-C stretching; C-O-H bending	Carbohydrates	Valasi and Pappas (2021); Wang et al. (2021)

3.2. FTIR-ATR analysis for assessing Sicilian pistachio origin

3.2.1. Authentication of Sicilian pistachio samples harvested in 2023

In the second step of the study, FTIR-ATR measurements were performed on pistachio samples harvested in 2023 from Bronte, Raffadali and Milazzo (sample-set b, Table 1). A complementary X-ray Fluorescence (XRF) analysis has been carried out on the same pistachio samples, as already done for tomato fruits in (Panebianco et al., 2022), reinforcing and synergizing the present FTIR-ATR results at the level of elemental composition (Panebianco et al., 2025).

Fig. S5 reports the FTIR-ATR spectra of three pistachio samples, each collected from one of the three different Sicilian harvesting areas, analysed in whole form and as ground powder. The differences in the spectral profile associated with pistachio texture (kernel vs powder) were confirmed, clearly appearing in the fingerprint region and in the “Amide I” and “Amide II” bands (for details of band assignments, see Table 2). On the other hand, the spectra appeared to be very similar upon visual inspection when compared according to their geographical

origin, apart from slight differences in the intensity ratios of the bands falling in the 1700–1500 cm⁻¹ spectral region (Fig. S5). Consequently, the pistachio composition (connected to the territory) has been investigated through multiparametric statistical analysis (PCA) to authenticate the origin by disentangling different clusters.

The PCA carried out on dataset originating from pistachio kernels highlighted differences among the samples collected in the different Sicilian farmlands (Fig. 3A). The first three principal components explained the 90.57% of the total variance (F1 = 52.79%; F2 = 25.32%; F3 = 12.46%). In general, pistachio samples tended to cluster according to their geographical origin, by generating three groups corresponding to Raffadali, Bronte and Milazzo. Most of pistachio samples from Raffadali district were located in the upper-left quadrant of the score plot, whereas the samples from Bronte district and Milazzo clustered in the upper-right and in the lower quadrants, respectively (Fig. 3A). We do not expect clear clustering, since unsupervised PCA analysis is primarily aimed at reducing the dimensionality (i.e., simplifying the data space) rather than distinctly categorizing or separating the data.

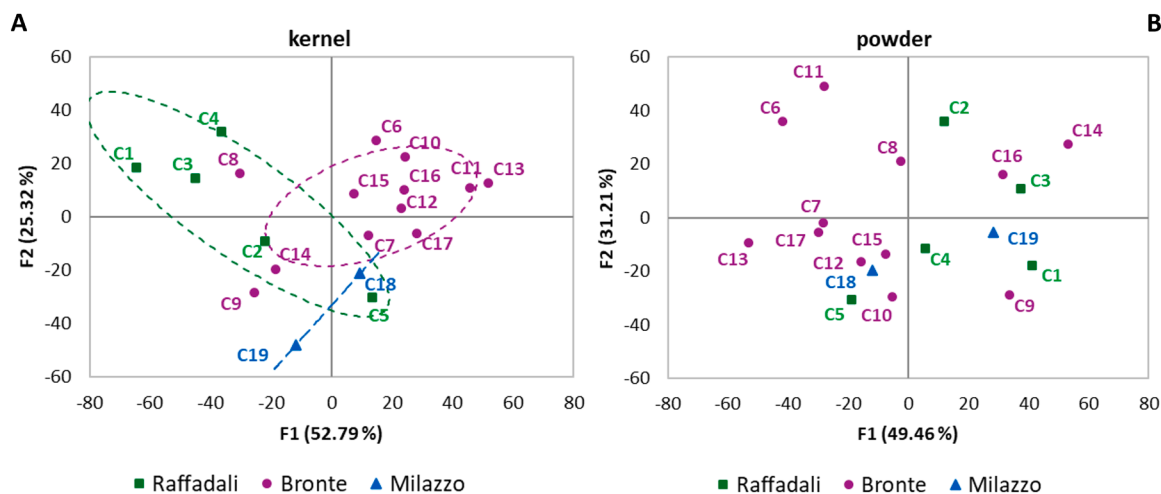


Fig. 3. Score plots (F2 versus F1) obtained by analysing the spectra of pistachio whole kernels (A) and powders (B) from samples harvested in Sicily in 2023 (sample-set b). The markers, distinguished by different colour and shape, represent the samples collected in Raffadali (C1–C5, green squares), Bronte (C6–C17, purple dots) and Milazzo (C18–C19, blue triangles). Dotted lines represent the 50% confidence ellipses for each group.

Clustering evidence was lost when PCA was performed on the dataset deduced for the pistachio powder spectra. In this case, as shown by F1–F2 score plot in the Fig. 3B, the samples from different Sicilian growing areas overlapped with each other, hindering discrimination. This outcome is related to the different textures, which result in distinct spectral shapes (kernel vs powder), as previously described and illustrated in Fig. S5. Similarly, other studies have reported significant variations in authentication outcomes of almond seeds when analysed in different forms (shell, kernel, endocarp or powder), highlighting the strong relationship between sample preparation, spectral features and discrimination performance (Lamas et al., 2025; Scappaticci et al., 2023). From an experimental point of view, during FTIR measurements, the infrared light irradiates the surface of pistachio powder, where fats are mainly present as a result of oil extraction following grinding. Consequently, the yields of the spectral regions related to fats (due to the separation of the oily components) are more prominent than those related to non-lipid compounds. In this context, performing measurements directly on unprocessed seeds leads to a better characterization, thanks to the inclusion of a greater number of discriminant compounds. This leads to a plethora of practical applications aimed at preventing pistachio counterfeiting, in support of the traceability system, simplifying the sample preparation procedure. As already reported in literature (Cozzolino, 2022), applying the vibrational spectroscopy techniques on unprocessed samples offers the advantage of determining the chemical composition directly *in-situ*, in field or during food manufacturing (e.g., process control), allowing for fast, easy and non-destructive sample authentication.

Principal Component Analysis was also performed using non-averaged spectra from pistachio samples, so that each seed (for whole kernels) or each container (for powders) was treated as an independent sample in the statistical analysis (Fig. S6). Results were fully comparable with those derived from the analysis of the averaged spectra (Fig. 3A and B), confirming the robustness of the proposed approach and indicating that intra-sample variability does not affect the overall discrimination performance.

To reveal the spectral features involved in the cluster discrimination (for pistachio kernels), the loadings of the first three principal components were analysed and plotted (Fig. 4). As already known, the loading plot can be used to detect the characterizing vibrational bands causing group differences, as it highlights the weights of each chemical functional group on the principal components (Gautam et al., 2015). High absolute loading values suggest that the spectral region significantly influences the respective components, whereas loading values near to

zero indicate that the spectral region provides a minor contribution (Sampaio et al., 2017). The loading plots in Fig. 4 show that the discriminant spectral features responsible for the clustering of whole pistachio kernels were mainly represented by the spectral bands assigned to vibrations of CH_3 , CH_2 , CO and CH groups in proteins, lipids and phenolic compounds falling in the “fingerprint region” between 1500 and 1130 cm^{-1} (peaks at 1465 , 1380 , 1245 , 1160 and 1143 cm^{-1}) and by the bands associated with lipid bond vibrations at 2928 , 2856 and 1750 cm^{-1} (Table 2). This is evidenced by the negative loading values of F1 and F2 for these spectral regions (Fig. 4A and B). Recent FTIR-ATR studies on nuts, edible-oils and plant-derived foods have reported similar band assignments at these wavenumbers, highlighting the importance of lipids, proteins and phenolic components in sample discrimination (Christodoulou et al., 2025; Fediuc and Oroian, 2025; Manfredi et al., 2018; Valasi et al., 2020). Other spectral characteristics affected by pistachio growing area were the absorption bands associated with functional groups of proteins, pectins and phenolic compounds falling in the spectral region between 1700 and 1500 cm^{-1} . As a matter of fact, the loading representation for F2 points out significant negative peaks also at 1656 and 1548 cm^{-1} , which correspond to “Amide I” and “Amide II” bands originating from protein vibrations, and in the spectral interval around 1600 cm^{-1} where the bands originating from stretching of C=O – and C=C groups in pectins and phenolic compounds fall (Fig. 4B, Table 2). Also in this case, the attribution of these bands is consistent with recent FTIR investigations on protein-rich food matrices, where Amide I and II vibrations have been identified as major contributors to spectral variability and sample discrimination in multivariate models (Keithellakpam et al., 2025). Conversely, spectra were similar to each other for the content of water and carbohydrates and in the spectral zones dominated by the background signal, as suggested by spectral positions dominated by F1 and F2 loading positive peaks in the plot. As a matter of fact, the highest positive loading values for F1 were found in the spectral regions without structures and associated with the background (spectral regions ranging approximately from 4000 to 3600 cm^{-1} , 3200 – 3020 cm^{-1} and 2800 – 1770 cm^{-1}), whereas those for F2 occurred in the spectral regions between 3600 and 3100 cm^{-1} and 1130 – 950 cm^{-1} , characterizing the absorption bands associated with water and carbohydrates (Fig. 4A and B, Table 2). In food matrices with high lipid and protein content, such as nuts, the spectral response of major components (lipids and proteins) generally dominates FTIR spectra, often reducing the spectral contribution of water and carbohydrates in multivariate discrimination models, as reported in FTIR investigations of complex food matrices dominated by fats and proteins

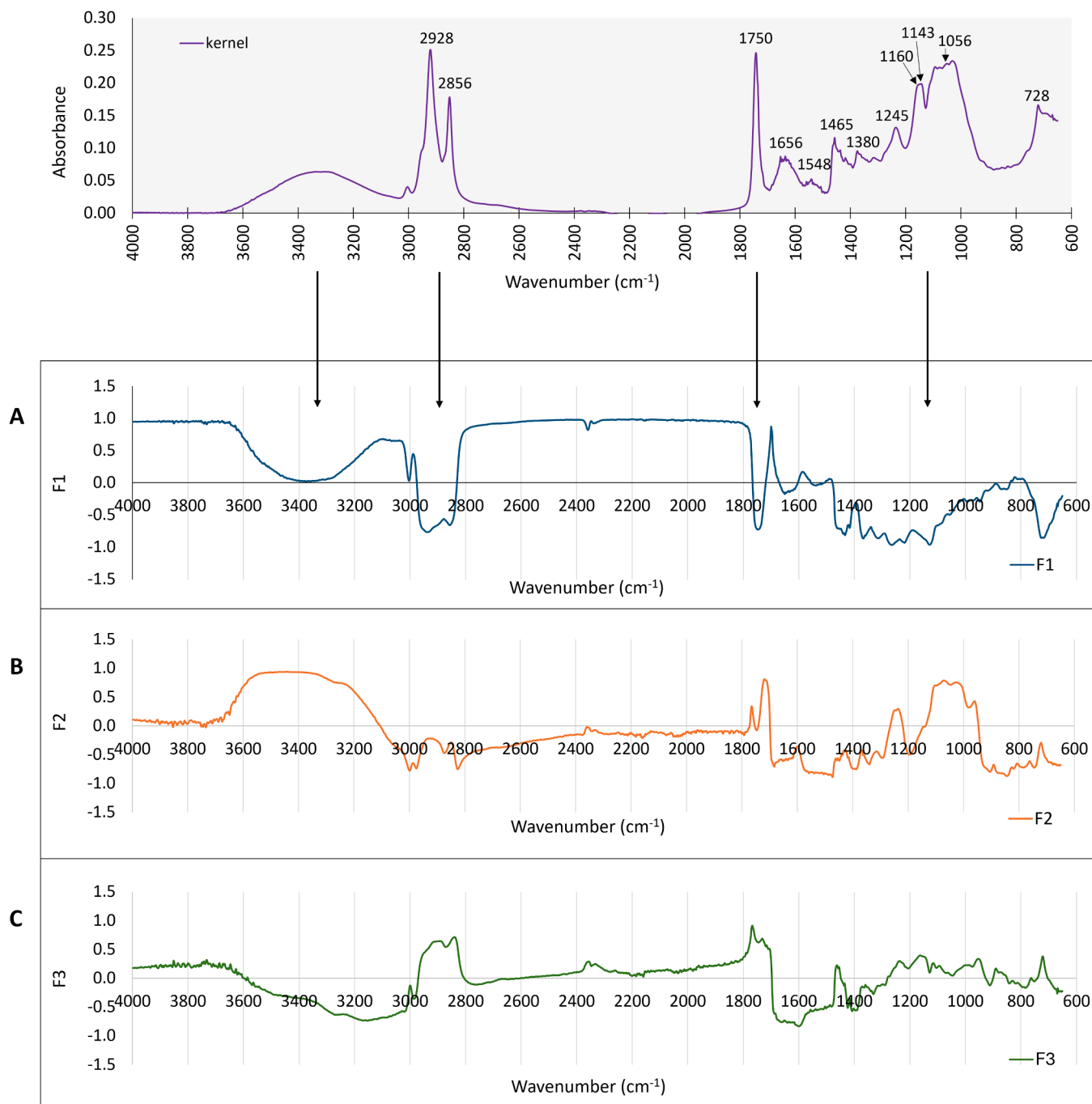


Fig. 4. Loading plots of F1 (A), F2 (B) and F3 (C) principal components. PCA was carried out on dataset consisting of full spectra associated with Sicilian pistachio kernels harvested in 2023 (sample-set b).

(Christodoulou et al., 2025). Finally, regarding the loadings of F3, positive and negative peaks were associated with fat and protein-related bands at wavenumbers 2928, 2856, 1750, 1656 and 1548 cm⁻¹ (Fig. 4C). Taken together, these results suggest that lipids, proteins, pectins and phenolic compounds make an important contribution to dataset clustering, in agreement with the literature reported above. The spectral regions with high or low contributions to pistachio discrimination, identified through the loading analysis, were reanalysed individually or in combination by new PCA to improve cluster characterization. These spectral intervals were used to generate the matrices A, B, C and D, as described in the 2.7 section. The best discrimination between Raffadali, Bronte and Milazzo groups was achieved by simultaneously analysing the fingerprint region and the bands

associated with protein and lipid vibrations (matrices B and D), whereas neither fat-related bands alone (matrix A) nor the spectral region dominated by carbohydrate absorptions (matrix C) provided discriminatory ability (Fig. 5). Excluding spectral intervals with low discriminatory power improved clustering, reaching near-linear separability among the three geographical sub-classes (see for comparison Fig. 3A).

PCA results confirmed the key role of the absorption bands associated with fats, pectins, proteins and phenolic compounds in pistachio kernel discrimination, highlighting that discrimination relied on the overall molecular profile rather than on a single compound. Therefore, it is reasonable to hypothesize that differences among the samples from Raffadali, Bronte and Milazzo are due to variations in the concentration of these compounds, affected in turn by the growth environment and

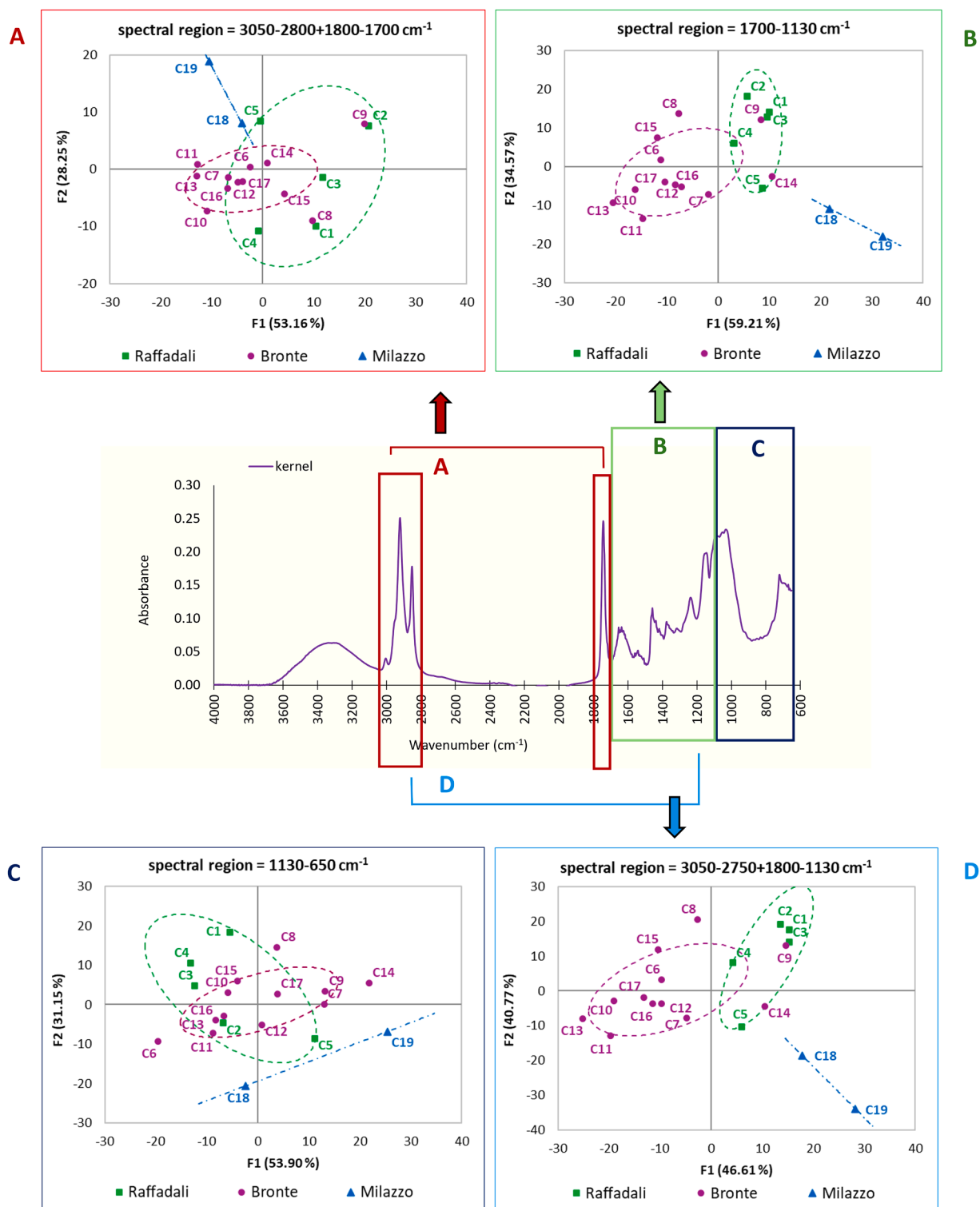


Fig. 5. Comparison of F2 versus F1 score plots obtained by analysing specific regions of the spectra associated with the Sicilian pistachio samples harvested in 2023 (sample-set b, whole kernels). The markers, distinguished by colour and shape, represent the samples collected in Raffadali (C1-C5, green squares), Bronte (C6-C17, purple dots) and Milazzo (C18-C19, blue triangles). Dotted lines represent the 50% confidence ellipses for each group.

agronomic practices during cultivation. Regarding this aspect, it was reported that climatic conditions (temperature, humidity, rainfall rate), geographical location (latitude, longitude and altitude), irrigation and soil fertility affect the fatty acids profile and polyphenol content in different tree nuts, including pistachio (Bolling et al., 2011; Noguera-Artiaga et al., 2020; Rabadán et al., 2019). Our results agree with previous studies performed on peanut samples by Wang et al.

(2021), who demonstrated that the geographical production area mainly influences total oil and protein contents, leading to differences in the FTIR spectra. The strong relationship between food composition and territory of origin was also reported by other researchers with the help of chromatography-based techniques. On pistachio seeds, for example, Arena et al. (2007) and Boukid et al. (2019) found that fatty acid and phytosterol composition was influenced by growing location, whereas

Bellomo and Fallico (2007), Mannino et al. (2019) and Saitta et al. (2014) reported appreciable differences linked to the territory of origin due to the content of coloured pigments (anthocyanins, chlorophylls, lutein) and phenolic compounds. The clustering assessed by PCA was cross-checked by Linear Discriminant Analysis (LDA), which enabled post-hoc validation of the proposed model. LDA correctly discriminated the pistachio samples associated with Raffadali, Bronte and Milazzo clusters according to geographical origin, providing consistent results with respect to the PCA plots reported in Fig. 5B and D (Fig. 6). Among the analysed samples, pistachio seeds from Milazzo showed the greatest differences in terms of chemical profile, while samples from Raffadali and Bronte displayed more similar characteristics, as evidenced by their positions in the plot (larger distances between groups indicate larger differences in composition). Model invariance was assessed by repeating LDA in R statistical software, yielding consistent results (Fig. S7).

3.2.2. Validation of the methodology using an external dataset

In the final step of the work, the robustness and applicability of the developed model were further evaluated by projecting an external dataset of Sicilian and non-Sicilian pistachio samples harvested in 2023 and 2025 (sample-set c, Table 1) onto the PCA space defined by the training set consisting of the samples harvested in 2023 (sample-set b, Table 1). By freezing the model on the dataset associated to the sample-set b, this approach allowed us to assess clustering consistency according to geographical origin and to evaluate the effect of harvest year on sample positioning.

Focusing on the projection of the Sicilian samples used as external test set (sample-set c), a coherent clustering according to geographical origin was observed, as these samples mainly fall near the clusters defined by the Sicilian samples harvested in 2023 (sample-set b), remaining clearly separated from the samples of foreign origin (Fig. 7A). However, a systematic shift was observed for the Bronte samples harvested in 2025 (represented in the figure by red squares) with respect to the Bronte samples harvested in 2023 (represented by red dots). This shift can be attributed to the effect of harvest year. Specifically, the samples from Bronte harvested in 2025 were mainly located at negative F1 and F2 scores, whereas their counterparts harvested in 2023 were mainly characterized by positive values along both principal components. Conversely, the Bronte samples harvested in 2023 used as external test set (samples S4, S5 and S6) projected entirely within the cluster defined by the Bronte samples harvested in 2023 (sample-set b used for training), confirming the internal consistency of the model

when temporal variability is minimized.

This behaviour was particularly evident when we proceeded to combine all the Sicilian samples in a single dataset. In this case, Fig. 7B reports the clustering obtained by projecting foreign samples onto the combined data matrix of Sicilian samples harvested in 2023 and 2025 years (sample-set b + c, Table 1). Again, the agreement among samples collected in the same harvest year supported the validity of the experimental procedure, while the observed shift highlighted the sensitivity of the model to time-harvesting variability. The model was further validated by randomly splitting the Sicilian pistachio samples harvested in 2023 and 2025 into a training set and an external test set, so that a portion of the samples was used for training and the remaining samples, together with samples of foreign origin, were used for testing. Also in this case, samples were grouped by geographical origin, with external test samples from Sicily generally matching the corresponding training set according to harvest year (Fig. S8). In this context, the results demonstrated that geographical authentication of “Pistacchio Verde di Bronte” within the Sicilian dataset (with respect to PDO “Pistacchio di Raffadali” or non-certified Sicilian pistachios) is no longer possible when harvest time vary, as Sicilian samples form a coherent group (Fig. 7A and B). Nevertheless, Sicilian samples remained clearly separated from non-Sicilian ones, indicating that geographical origin continues to play a major role in overall spectral differentiation despite the additional variability associated with harvest conditions.

Our findings agree with previous studies demonstrating the effectiveness of FTIR-ATR spectroscopy combined with chemometric analysis in disentangling the origin of similar food matrices, such as almonds and peanuts (Scappaticci et al., 2023; Wang et al., 2021). Beyond geographical authentication, FTIR spectroscopy has also proven effective for cultivar traceability purposes, allowing discrimination of samples from the same or nearby production zones based on varietal differences (Lamas et al., 2025; Manfredi et al., 2018). Similarly, if the “harvest time” variable is considered, other studies have also shown the ability of technique to reveal compositional differences associated with harvest season. For example, Valasi et al. (2020) and Valasi and Pappas (2021) demonstrated that pistachio oils and kernels from Greece could be successfully discriminated according to harvest year.

Concluding, the experimental results highlighted two different levels of sample discrimination, depending on the specific dataset composition: the first one was reached by using a fully coherent dataset of samples collected in the same harvesting period, the second one by including all the Sicilian samples regardless the harvesting season. In the

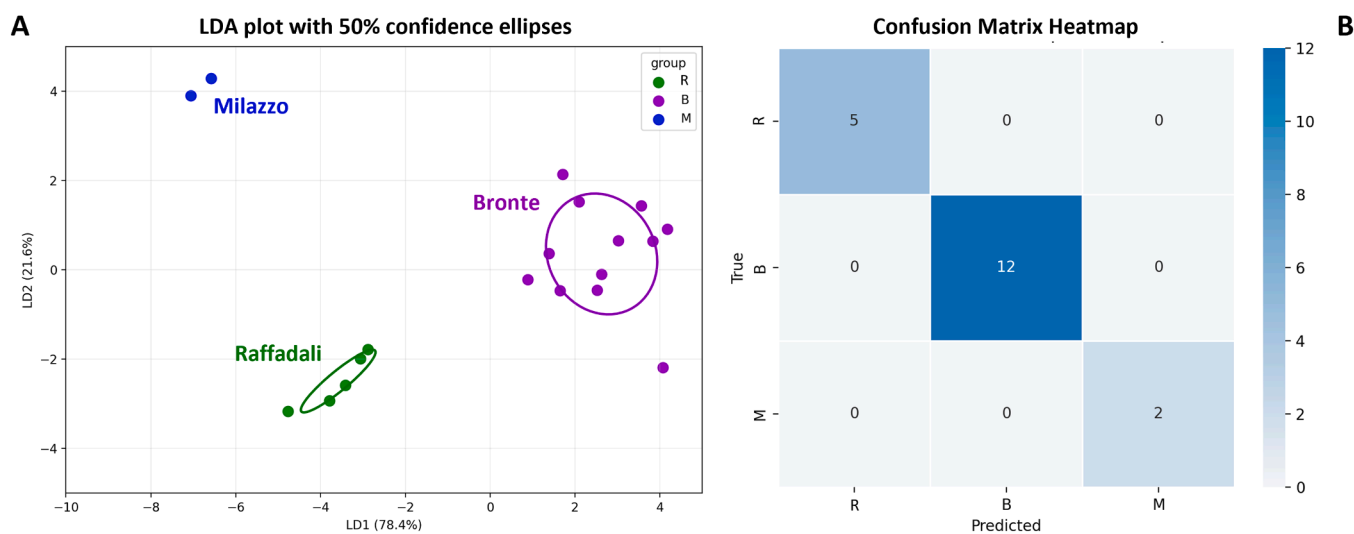


Fig. 6. LDA results obtained from the full spectra of pistachio samples harvested in 2023 (sample-set b) from Raffadali (in green), Bronte (in purple) and Milazzo (in blue). The LDA score plot is shown on the left (A), while the corresponding confusion matrix is reported on the right (B). Confidence ellipses (50%) are superimposed on the clusters. LDA was performed by using the scikit-learn library in Python (<https://www.python.org>).

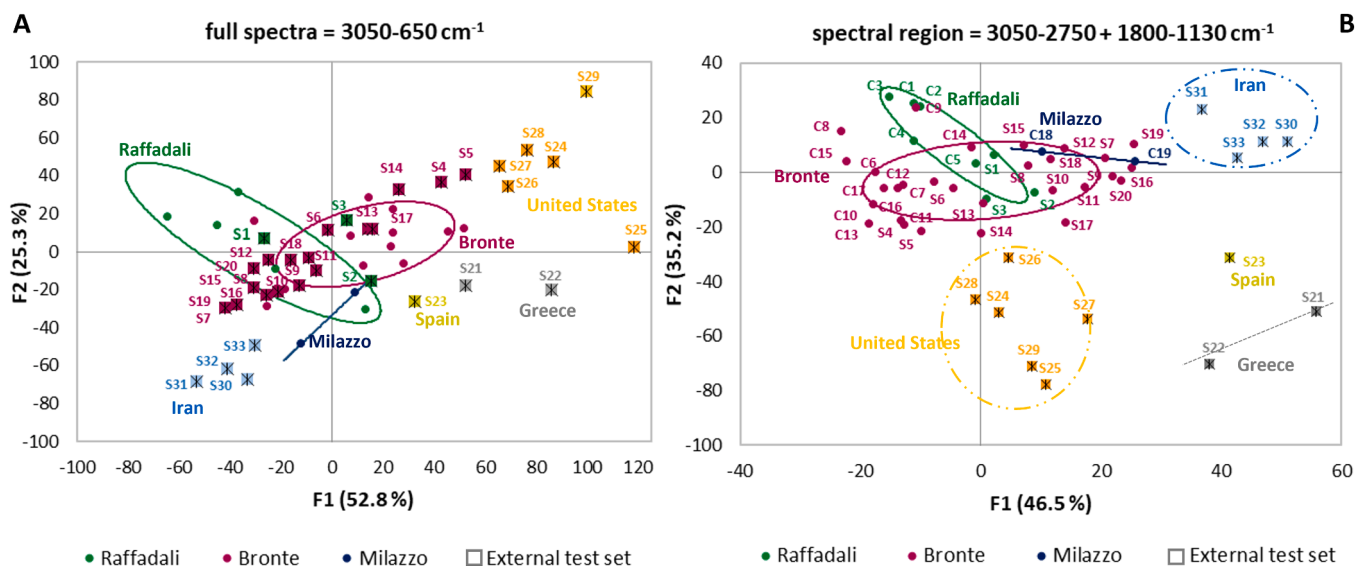


Fig. 7. Assessment of the model's predictive ability using external datasets. In A) projection of 33 external samples (Sicilian and non-Sicilian samples, sample-set c) onto the matrix of samples collected in 2023 used for training (sample-set b), considering the full spectrum. In B) projection of 13 external samples of foreign origin onto the matrix of Sicilian samples collected in 2023 and 2025 (sample-set b + c) used for training, considering combinations of non-contiguous spectral intervals (3050–2750 and 1800–1130 cm⁻¹). The lines around each group indicate the 50% confidence regions.

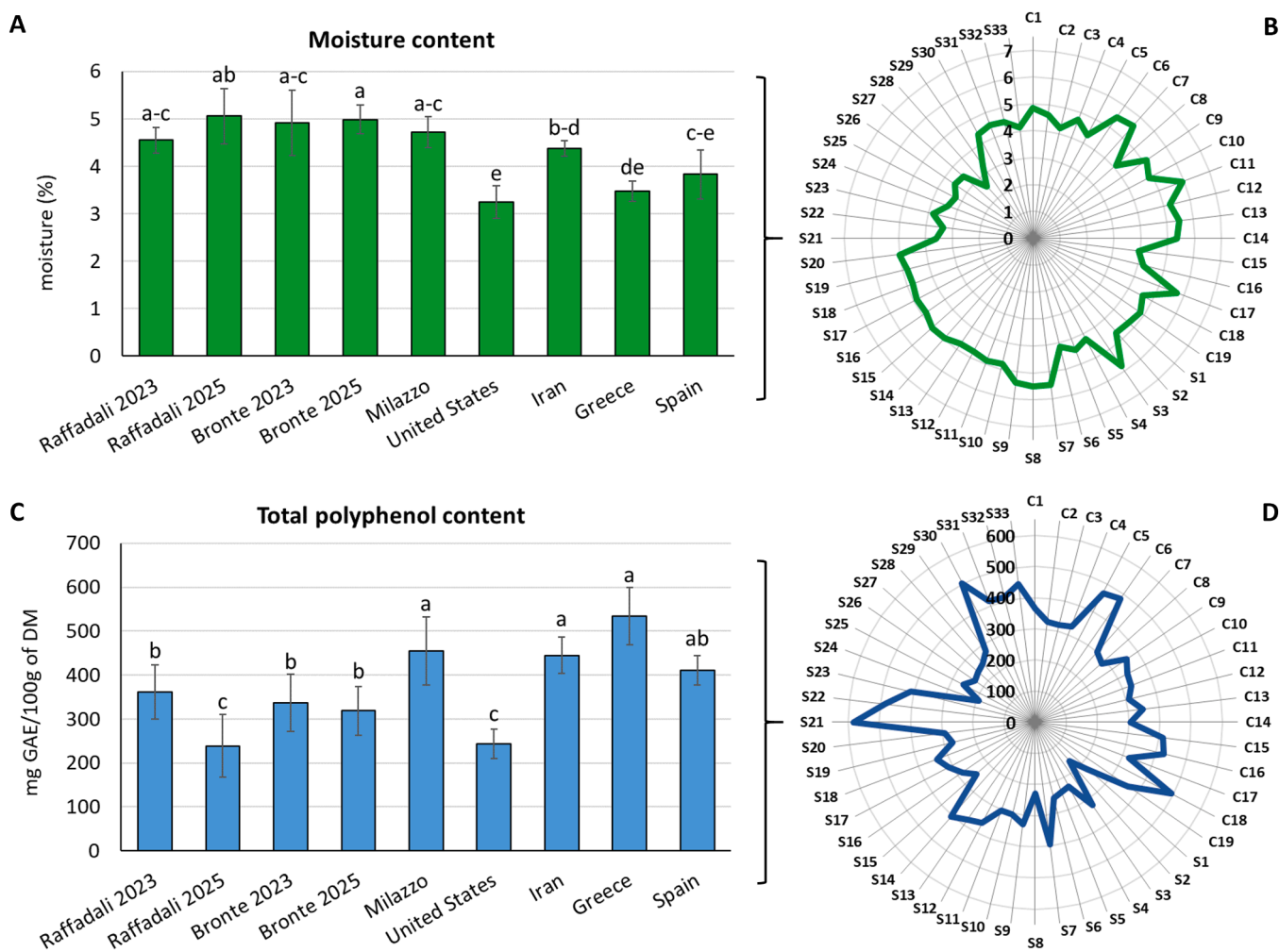


Fig. 8. Results of physico-chemical analyses. In A) and C) mean values of moisture (%) and total polyphenol content of samples (mg GAE/100 g of DM) grouped by origin regions and harvest year. Bars show the standard deviation. Columns with different letters are significantly different according to Tukey's honest significant difference test ($\alpha = 0.05$). In B) and D) variability of moisture (%) and total polyphenol content across all samples.

first case it was possible to reveal a local clustering, disentangling pistachios from different growing zones of Sicily, whereas in the second case it was possible to reveal just a global clustering (Sicilian vs foreign samples), since the local features correlation was destroyed by the variability linked to the harvest year.

3.2.3. Physico-chemical analysis of pistachio samples

As previously reported, the concentration of pigments, polyphenols and the colour parameters are important discriminating feature of pistachios and are often used to authenticate its geographical origin (Bellomo and Fallico, 2007; Saitta et al., 2014). These parameters, as well as moisture content, change during ripening and drying process and affect pistachio quality, providing valuable information on their stability and antioxidative properties (Ballistreri et al., 2009). For this reason, to evaluate the possibility of discriminating the geographical origin of the pistachio samples and to cross-check and complement characterization results by FTIR-ATR analysis, moisture content, colour parameters and total polyphenol content were determined.

The average moisture content (%) of the samples was reported in Fig. 8A, whereas the moisture values determined for the individual samples are reported in Fig. 8B and in Table S1. Most Italian pistachio samples showed similar moisture levels, ranging between 4% and 6%, according to literature and PDO production regulation. These results are similar to those reported in literature (Arena et al., 2007). In this regard, it was reported that moisture content of fresh pistachio kernels decreases from 40–50% to about 4–6% during the drying process, leading to an increased shelf-life and stability (Saber et al., 2024). The pistachio

samples from Iran (S30-S33) had a similar moisture content respect to the Sicilian ones (4.1–4.5%), whereas pistachios from Greece, Spain and United States (S21-S29) had a significantly lower moisture content (ranging between 2.6% and 3.8%) (Fig. 8B, Table S1).

Regarding the determination of the total polyphenol content (TPC), the mean values are shown in Fig. 8C, whereas the TPC values determined for the individual samples are reported in Fig. 8D and in Table S1. The phenolic profile of pistachio seeds has been characterized by several authors using HPLC/DAD/MS identifying benzoic acid derivatives (protocatechuic acid, gallic acid, 4-hydroxybenzoic acid), anthocyanins, flavan-3-ols, proanthocyanidins, flavonols, isoflavons, flavanones, stilbenes and phenolic acid as the main components (Arena et al., 2019; Ballistreri et al., 2009; Kelebek et al., 2020; Moreno-Rojas et al., 2022). It is well known that the total phenolic content in pistachio skin is significantly higher than that in peeled pistachio in the Italian products (Mannino et al., 2019; Tomaino et al., 2010). Discrimination among unpeeled pistachio varieties based on their phenolic content and their geographical origin has also been reported (Mannino et al., 2019). As shown in Fig. 8D and in Table S1, most Sicilian samples showed similar TPC values (ranging from 166 to 494 mg GAE/100 g dry matter). These values were lower than those of Iran and Greek pistachio samples and similar to those of samples from United States and Spain.

The CIE $L^* a^* b^*$ coordinates, in particular a^* and b^* , are directly correlated with chlorophyll content (Fernández-León et al., 2012), suggesting that colour parameters are a reliable method to estimate these pigments (Sanmartín et al., 2011). The measured color parameters reflected differences between Sicilian pistachios and foreign pistachios

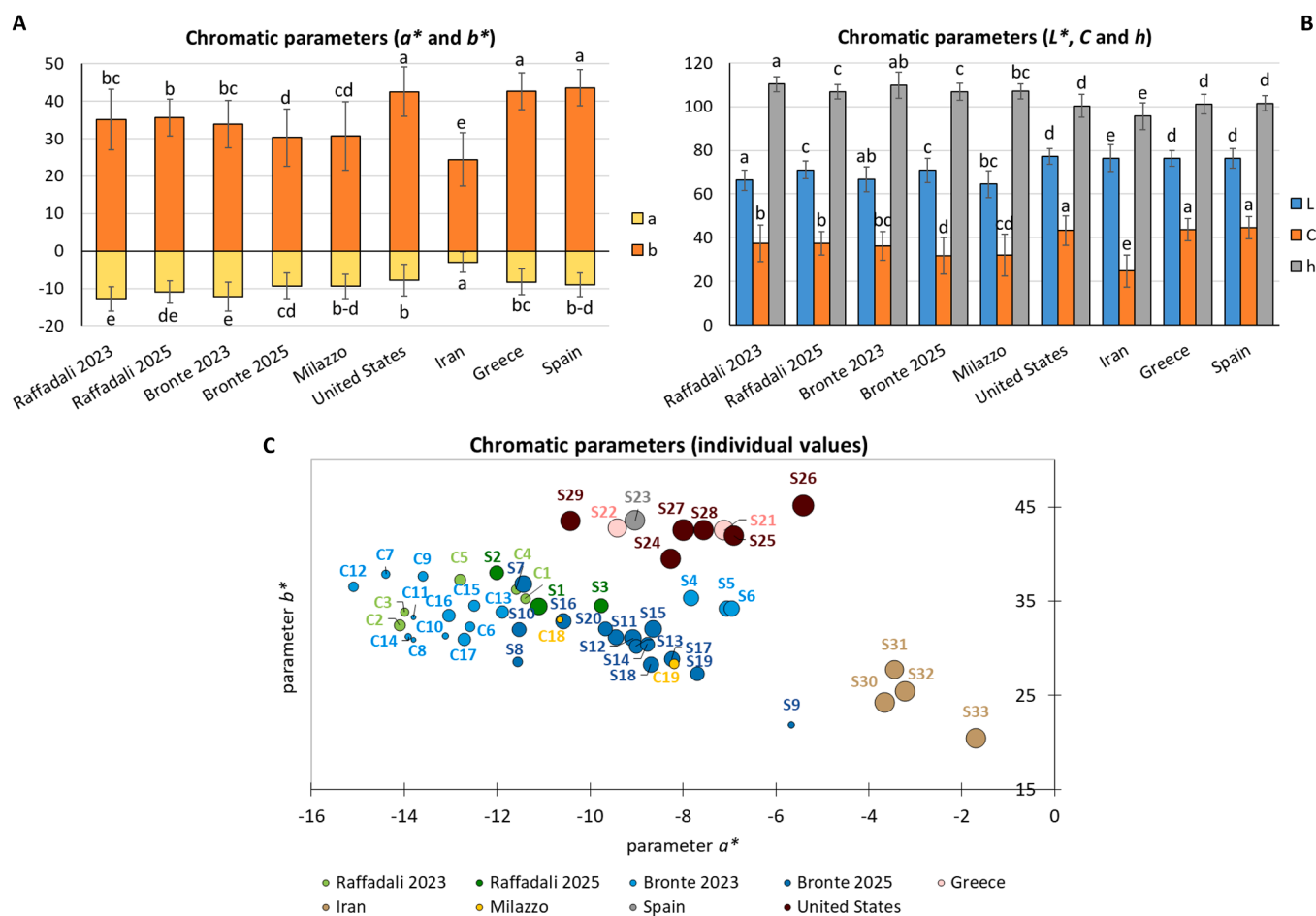


Fig. 9. Results of physico-chemical analyses. In A) and B) trend of the mean values of a^* , b^* and L^* , C and h color parameters of samples grouped by origin regions and harvest year. Bars show the standard deviation. Columns with different letters are significantly different according to Tukey's honest significant difference test ($\alpha = 0.05$). In C) bubble chart showing the relationships between L^* , a^* and b^* chromatic coordinates associated with samples.

due to the different chlorophylls levels (Bellomo and Fallico, 2007; Mannino et al., 2019; Saitta et al., 2014);). It is well known that Bronte and Raffadali pistachios remain green even when fully ripe, whereas other varieties generally appear yellow or pale green (Bellomo and Fallico, 2007; Boukid et al., 2019). As observed for moisture content, the colour of Sicilian pistachio was similar, whereas it differed significantly from that of foreign samples (Fig. 9A and B, Table S2).

The relationships between the CIE L^* , a^* and b^* chromatic coordinates corresponding to each sample (expressed as mean values) and the grouping of samples as a function of their geographical origin were more clearly highlighted by means of the bubble chart reported in Fig. 9C. The bubble's size was used to represent the amount of light reflected by kernels (the larger the bubble, the greater the L^* parameter), whereas the a^* and b^* variables were plotted along the x- and y-axes. As shown in this figure, colour parameters divided samples into three clusters: Sicilian pistachios (located in the center-left region of the plot), Iranian (right region) and Spanish/Greek/American (upper region). A shift in the a^* value was also observed for most Sicilian samples harvested in 2023 (marked with light blue and green dots) compared to those collected in 2025 (marked with dark blue and green dots). Thus, these results highlighted color differences related both to the origin of the samples and to the harvest year (2023 vs. 2025).

Principal Component Analysis (PCA) showed the correlation between physico-chemical parameters and geographical origin of pistachio samples (Fig. 10). The coordinates a^* , b^* and hue angle (h) were the most closely related to chlorophyll content (Conesa et al., 2019). The most contribute to the variance of Sicilian pistachio was given from h colour coordinate (Fig. 10A), while L^* , a^* and b^* contributed to the separation of non-Sicilian pistachios. In Fig. 10B, it is possible to see three different clusters: one grouping together Sicilian samples, one consisting of Iranian samples and one grouping American, Greece and Spanish pistachios. In the PCA space, Sicilian and Iranian samples occupied distinct regions, while USA samples overlapped with the Mediterranean samples (Greek and Spanish) in the region characterized by negative F1 and positive F2 scores, indicating similar properties. Overall, the PCA demonstrates that the analysed parameters are significantly correlated with the geographical origin, allowing a clear discrimination. The observed clustering pattern based on physico-chemical parameters strongly support the FTIR multivariate analysis (Fig. 7). In particular, the same clustering trend separating

Sicilian and non-Sicilian samples was observed, especially in selected spectral regions, indicating that the differences highlighted in the fingerprint region are supported by the physico-chemical parameters. Overall, these findings confirm that the physico-chemical attributes of the pistachio kernels are consistent with the spectral differences captured by FTIR-ATR analysis. The strong agreement between chemometric clustering based on FTIR data and that derived from traditional parameters demonstrates the reliability of FTIR spectroscopy as a rapid, non-destructive tool for tracing the geographical origin of pistachio seeds.

4. Conclusion

This study explored the potential of FTIR-ATR spectroscopy for characterizing pistachio seeds, with a specific focus on Sicilian PDO-certified pistachio kernels from the Bronte and Raffadali districts. The proposed approach enabled reliable discrimination of Sicilian pistachios from other geographical origins and showed sensitivity to variations across different harvest years, highlighting its applicability for both local and international traceability. The robustness of the methodology was verified through a careful optimization of sample preparation and measurement procedures, resulting in good statistical reproducibility, low variability among replicates and consistency across different instruments. Classification performance, evaluated through cross-validation and projection of held-out samples (external sample-set), confirmed the predictive ability of the model for authenticating samples of unknown or suspicious origin.

To date, the application of FTIR spectroscopy with portable instrumentation for characterizing PDO-certified Sicilian pistachios has not yet been extensively investigated, either at a small geographical scale (within the same region) or at a broader international scale. Consequently, we believe that the present study provides new insights into the authentication and safeguarding of this high-quality product. Moreover, the simplicity, portability, and non-destructive nature of this methodology, along with its ability to obtain identification-relevant information directly from whole pistachio kernels without any sample pre-treatment, make it highly promising for real-time applications in the field and along industrial processing lines.

According to the spectral bands generated by the different classes of compounds, it was possible to tag the most important spectral

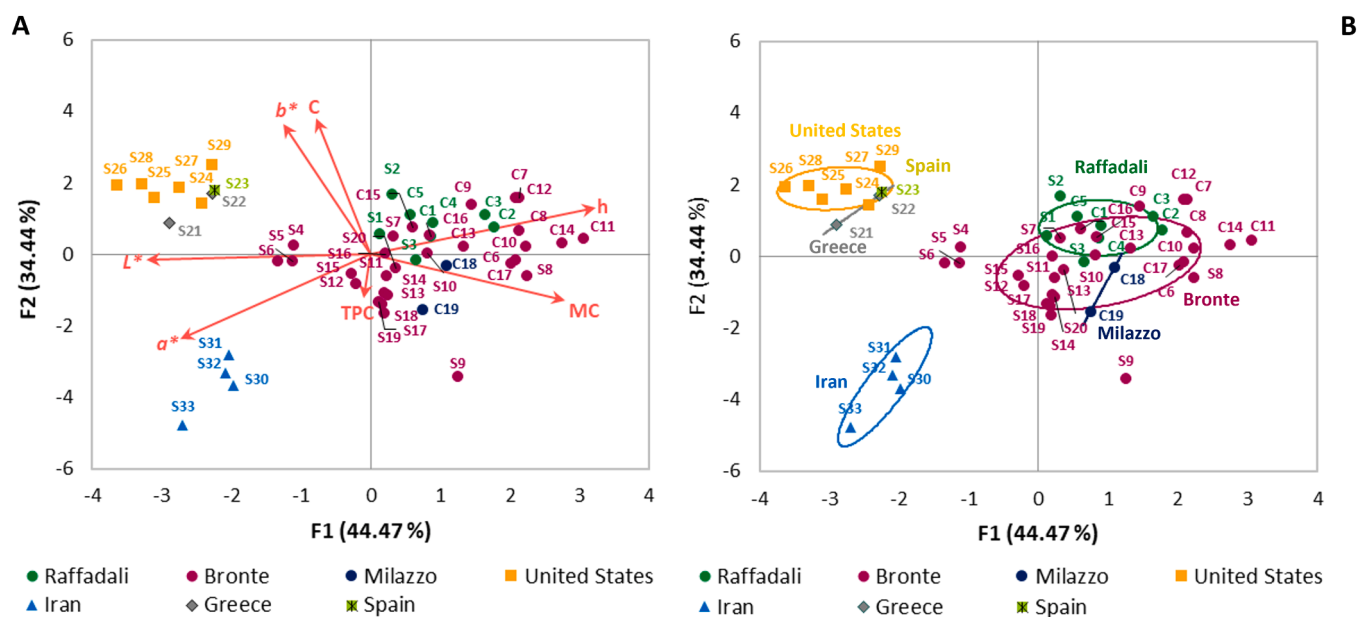


Fig. 10. Biplot obtained from PCA analysis, with contribute of moisture content (MC), total phenolic compound (TPC) and color coordinates (L^* , a^* , b^* , C and h) on variance (A) and score plot obtained from PCA analysis (B). The lines around each group indicate the 50% confidence regions.

components responsible for clustering, making a bridge to standard chemical analysis results. Beyond the contribution of lipids and proteins, sample discrimination was driven by differences in pigments responsible for color and in polyphenolic content, which give rise to characteristic spectral features in the fingerprint region, as evidenced by the results of conventional physico-chemical analyses. The strong consistency between chemometric analysis of FTIR-ATR spectra and conventional physico-chemical parameters, even when considering only the fingerprint region, confirmed the suitability of FTIR spectroscopy as a rapid and non-destructive tool for pistachio origin authentication.

In the future, we plan to extend measurements to a greater number of samples from Sicilian and international sites to make this approach applicable on a larger scale, developing calibration models to quantify key chemical constituents, including moisture, polyphenols, pigments and lipids.

CRedit authorship contribution statement

Salvina Panebianco: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Maria Cristina Caggiani:** Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis. **Elena Arena:** Writing – review & editing, Validation, Resources, Methodology. **Germana Barone:** Writing – review & editing, Validation, Resources. **Gabriella Cirvilleri:** Writing – review & editing, Resources, Methodology, Conceptualization. **Biagio Fallico:** Writing – review & editing, Validation, Resources, Methodology. **Claudio Finocchiaro:** Writing – original draft, Methodology, Investigation. **Gabriele Lanzafame:** Writing – original draft, Methodology, Investigation. **Paolo Mazzoleni:** Writing – review & editing, Resources, Methodology, Conceptualization. **Agatino Musumarra:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Maria Grazia Pellegriti:** Writing – review & editing, Validation, Methodology, Formal analysis, Conceptualization.

Ethical statement - plant collection

This experimental research does not involve plant species at risk of extinction. Pistachio samples for this study were kindly provided by farms' owners, without any payment and formal request for the collection of plant material. Plant collection was carried out in accordance with EU and Italian guidelines and regulations.

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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.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jfca.2026.109117](https://doi.org/10.1016/j.jfca.2026.109117).

Data availability

Data will be made available on request.

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