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Emphasizing the Potential of Attenuated Total Reflectance–Fourier Transform Infrared (ATR-FTIR) Spectroscopy Combined with Chemometrics, for Classification of Greek Grape Marc Spirits

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Abstract: Grape marc spirits, such as the Greek tsipouro/tsikoudia, reflect the cultural heritage of winemaking traditions worldwide. This study explored the application of Attenuated Total Reflectance–Fourier Transform Infrared (ATR-FTIR) spectroscopy combined with chemometrics for its potential as a fast classification methodology for spirit characterization. ATR-FTIR spectra from thirty-nine products revealed distinctive bands corresponding to various chemical constituents, such as alcohols, organic acids, water, carbohydrates, and phenols. Principal Component Analysis (PCA) was performed on all acquired ATR-FTIR data and 78.50% of the total variance in the data was explained. Also, partial least squares–discriminant analysis (PLS-DA), used for the classification of products based on their major geographic origin, gave a correct classification of 89.5% for the north and 83.3% for the south of Greece. Classification of the type of distillations used was with 74.36% accuracy. Significant markers were identified through analysis, such as those associated with the O-H bending vibrations of phenols or alcohols, contributing to the discrimination of grape marc spirits from Crete when compared with the other four main geographical origin designations. By combining ATR-FTIR spectroscopy with chemometrics, this research gave insights into the origins and compositional variations of the spirits, providing an opportunity for a quality control assessment tool.

Keywords: attenuated total reflectance–fourier transform infrared (ATR-FTIR); classification; grape marc spirits; tsipouro/tsikoudia; chemometrics

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1. Introduction

The historical significance of grape marc spirits is deeply rooted in the cultural tapestry of winemaking traditions, offering a journey of technology and tradition through the ages. Each variant, whether the Iberian Peninsula's Orujo, Italy's Grappa, Cyprus's Zivania, or the diverse expressions of grape marc worldwide, encapsulates the essence of its region and the craftsmanship of its makers [1]. These spirits emerge as liquid ambassadors, reflecting the historical evolution of viticulture and the utilization of grape pomace—a byproduct of winemaking that, rather than being discarded, undergoes meticulous distillation to yield beverages of distinct character [2]. The global diversity of grape marc spirits is a testament to the terroir-driven approach, where geographical and climatic conditions shape the unique qualities of the pomace. EU Regulation 1576/89 established overarching production protocols and set standard analytical composition limits for traditional alcoholic beverages. Additionally, it delineated specific production methods and stricter analytical parameters tailored to particular geographical denominations [3]. Further guidance on procedural matters such as labeling and geographical indications for European grape marc distillates was outlined in EU Regulation 2019/78.

Greece, in accordance with Annex III of Regulation (EC) No. 110/2008, designated four viticultural regions for the production of protected designation of origin (PDO) grape marc spirits. Those regions include tsipouro of Thessaly, tsipouro of Macedonia, tsikoudia of Crete, and tsipouro of Tyrnavos [1]. The first three derive their names from their respective geographical areas, while Tyrnavos, situated in the Thessaly region, serves as a significant viticultural hub. Before 1988 in Greece, production licenses for tsipouro were restricted to certain days of the year and typically granted only to farmers, often grape growers residing in economically disadvantaged areas. However, the landscape has evolved significantly since then, with tsipouro gaining recognition and popularity among consumers worldwide, including wine enthusiasts. Homemade tsipouro, now available commercially, is typically consumed in its undiluted form, boasting an ethanol content usually exceeding 50% v/v. Conversely, commercially produced tsipouro undergoes dilution with water before bottling, resulting in an alcohol content ranging between 37.5% and 50% vol. [3,4].

The surge in the popularity of grape marc spirits has, unfortunately, given rise to authenticity challenges [5–7]. Quality control of alcoholic beverages can be conducted through two distinct approaches: sensory and instrumental quality control [5,8,9]. Although sensory information will always be important and relevant, instrumental quality control methods might offer several advantages in terms of reproducibility speed and not being susceptible to adaptation or fatigue [8–10]. Nevertheless, for practical application in the alcoholic beverage industry, those methods must be swift, cost-effective, and capable of delivering reproducible results through continuous operation [11].

Techniques such as UV, Vis, near-infrared (NIR), mid-infrared (MIR), Raman, Fluorescence, nuclear magnetic resonance spectroscopy, and voltammetric techniques have emerged as promising solutions to address the challenges previously outlined [12–17]. Adulteration and counterfeiting pose threats to the distinctive characteristics of these spirits, underscoring the need for advanced analytical methods. Traditional methods like gas chromatography are time-consuming and have a lengthy, complex, and labor-intensive sample preparation process with increased analysis times and capital equipment requirements. In contrast, Fourier Transform Infrared (FT-IR) and MIR spectroscopy appear simpler, faster, and more available with reduced analysis times and instrument costs for comprehensive analysis. Specifically, MIR spectroscopy offers rapid and high-resolution analysis of grape marc spirits, with the fingerprint region (1800–1200 cm^{-1}) revealing unique signatures. Operating between 4000 and 600 cm^{-1}, mid-infrared spectroscopy explores overtone and combination bands, offering nuanced insights into molecular composition. As the demand for authentic and unadulterated beverages grows, those advanced analytical techniques serve as invaluable tools in uncovering the true essence of grape marc spirits. Attenuated Total Reflectance–Fourier Transform Infrared (ATR-FTIR) was chosen due to its widespread use as an analytical instrument, offering the advantage of rapidly generating data with minimal or no sample preparation. ATR-FTIR has been a method of choice in food characterization previously, due to its widespread use as an analytical instrument, offering the advantage of rapidly generating data with minimal or no sample preparation. Additionally, it has the capability to provide key information about the chemicals present in the sample. Previous applications of FTIR data, in conjunction with Principal Component Analysis (PCA), have successfully classified various substances such as vegetable oils, fruit juices, and coffee [18–21], while it was recently suggested as a valid tool for wine authenticity and traceability evaluation [22].

Previously, we utilized novel sensory methodologies [2] and Gas Chromatography–Mass Spectrometry (GC-MS) analyses combined with chemometrics [1] to understand product differentiation within that complex category of spirits. GC-MS analyses were also applied in other studies of grape marc spirits from Greece [3] and also recently together with chemometrics [23]. Schiavone and co-workers (2020) studied the authentication of Grappa spirits by mid- and near-infrared spectroscopies coupled with chemometrics. Nevertheless, to our knowledge there has not been any other published study on FTIR analyses of grape marc spirits from Greece, previously.

The main objective of this work was to study thirty-nine (39) samples of Greek grape marc spirits from diverse distillation processes [24] and specifically and/or broadly defined origin areas [1,23], employing ATR-FTIR spectroscopy band transformation. Additionally, the study aimed to assess the capability of FTIR spectroscopy combined with chemometric tools such as PCA and partial least squares–discriminant analysis (PLS-DA) for classifying Greek grape marc spirits through meaningful insights into the quality information of a representative set of samples.

2. Material and Methods

2.1. Materials

Thirty-nine (39) grape marc spirits were collected from all over Greece, representing all major Greek PDOs (Table 1). The samples were either provided by the producers themselves or purchased in retail markets. Information regarding the grape variety used and production techniques was mostly directly obtained in parallel, from the producers themselves. In the case of samples bought from retailers, information was taken from product labels. Among those products, 29 were produced by local distilleries of the regions, and the remaining 10 were homemade (traditional grape marc spirits), while 20 tsipouro/tsikoudia spirits were made from a single grape variety and 19 were obtained by the distillation of the grape marc from multiple grape varieties. Last but not least, 10 products came out of single distillation, 20 out of double, and 10 out of smaller-scale home distillation (Table 1).

Table 1. Grape marc spirits used in the study. Coding, specific and major regions of origin, and distillation processes used in their making.

Spirit Code	Specific Origin	Major Region	Type (Tsipouro/Tsikoudia)	Distillation Process	Code	Varieties
T1	Tyrnavos	North	Tsipouro	double distillation	CAD2	Moschato Mavro
T2	Tyrnavos	North	Tsipouro	double distillation	CAD2	Moschato Mavro
T3	Tyrnavos	North	Tsipouro	double distillation	CAD2	Moschato Mavro
T4	Tyrnavos	North	Tsipouro	double distillation	CAD2	Moschato Mavro
T5	Tyrnavos	North	Tsipouro	double distillation	CAD2	Malagouzia
T6	Tyrnavos	North	Tsipouro	double distillation	CAD2	Roditis
T7	Thessaly	North	Tsipouro	double distillation	CAD2	Muscat, Roditis, Savatiano
T8	Thessaly	North	Tsipouro	double distillation	CAD2	Xinomavro, Cabernet sauvignon, Chardonnay, Muscat
T9	Thessaly	North	Tsipouro	double distillation	CAD2	Savatiano, Roditis, Muscat
T10	Peloponnese	South	Tsipouro	single distillation	CAD1	Roditis
T11	Peloponnese	South	Tsipouro	double distillation	CAD2	Malagouzia, Chardonnay, Merlot
T12	Peloponnese	South	Tsipouro	single distillation	CAD1	Agiorgitiko, Moschofilero, Muscat
T13	Peloponnese	South	Tsipouro	double distillation	CAD2	Moschofilero
T14	Peloponnese	South	Tsipouro	single distillation	CAD1	Moschofilero, Agiorgitiko
T15	Peloponnese	South	Tsipouro	double distillation	CAD2	Agiorgitiko
T16	Thrace	North	Tsipouro	double distillation	CAD2	Roditis, Moschato Mavro
T17	Thrace	North	Tsipouro	single distillation	CAD1	Muscat, Cabernet Sauvihnon, Syrah, Merlot
T18	Thrace	North	Tsipouro	single distillation	CAD1	Muscat, Merlot, Syrah

Table 1. *Cont.*

Spirit Code	Specific Origin	Major Region	Type (Tsipouro/Tsikoudia)	Distillation Process	Code	Varieties
T19	Epirus	North	Tsipouro	double distillation	CAD2	Debina
T20	Macedonia	North	Tsipouro	double distillation	CAD2	Malagousia
T21	Macedonia	North	Tsipouro	single distillation	CAD1	Muscat, Asyrtiko, Cabernet Sauvignon
T22	Central Greece	North	Tsipouro	single distillation	CAD1	Sabbatiano
T23	Central Greece	North	Tsipouro	single distillation	CAD1	Savatiano, Muscat, Chardonnay
T24	Crete	South	Tsikoudia	double distillation	CAD2	Moschato Mavro
T25	Crete	South	Tsikoudia	double distillation	CAD2	Moschato Mavro
T26	Crete	South	Tsikoudia	double distillation	CAD2	Moschato Mavro
T27	Crete	South	Tsikoudia	single distillation	CAD1	Moschato Mavro
T28	Cyclades	Island	Tsipouro	double distillation	CAD2	Asyrtiko
T29	Cyclades	Island	Tsipouro	double distillation	CAD2	Asyrtiko, Mavrotragano
T30	Tyrnavos	North	Tsipouro	home distillation	HD	Moschato Mavro
T31	Tyrnavos	North	Tsipouro	home distillation	HD	Moschato Mavro
T32	Tyrnavos	North	Tsipouro	home distillation	HD	Moschato Mavro
T33	Tyrnavos	North	Tsipouro	home distillation	HD	Moschato Mavro
T34	Tyrnavos	North	Tsipouro	home distillation	HD	Moschato Mavro
T35	Tyrnavos	North	Tsipouro	home distillation	HD	Moschato Mavro
T36	Crete	South	Tsikoudia	home distillation	HD	Romeiko
T37	Crete	South	Tsikoudia	home distillation	HD	Romeiko
T38	Central Greece	North	Tsipouro	home distillation	HD	Malagouzia, Muscat, Roditis
T39	Central Greece	North	Tsipouro	home distillation	HD	Savatiano, Roditis

2.2. Fourier Transform Infrared Spectroscopy with Attenuated Total Reflectance (ATR-FTIR)

Fourier Transform Infrared Spectroscopy with Attenuated Total Reflectance (ATR-FTIR) was obtained using an IRAffinity-1S FTIR spectrometer (Shimadzu, Kyoto, Japan). The IRE used was diamond. The samples and the background spectra were screened from 4000 to 499 cm^{-1} and the average of 20 scans at 4 cm^{-1} resolution was recorded, while the ATR reference was adjusted to 3284.77 cm^{-1}. The FTIR spectra of tsipouro/tsikoudia spirit samples were subjected to ATR correction, normalization, smoothing, and peak picking treatment. Data processing and analysis was performed by LabSolutions IR software (version 2.21) [25].

2.3. Statistical Analysis

The statistical analysis of Principal Component Analysis (PCA) and partial least squares (PLS) was performed using XLSTAT Addinsoft statistical and data analysis solutions (New York, NY, USA). PCA was initially employed to map all thirty-nine products, based on their total ATR FTIR data. Outlier detection and removal were conducted according to PCA analysis and specifically according to the values on the PC1 axis. Subsequently, the ATR-FTIR spectral data from all spirits were subjected to Variable Importance in Projection (VIP) in conjunction with partial least squares analyses to determine which of the FTIR variables were the most relevant to utilize in further classification analyses, according to different production parameters. Namely, the parameters of raw materials (grapes), specific and major regions of origin, as well as type of distillation method used were used in the PLS-DA. The VIP approach is mainly used in a PLS-based context, where the influence of each instrumental variable in defining the variable space is determined. When a variable has a VIP index higher than 1, it is considered relevant for the classification [1,26].

3. Results and Discussion

3.1. ATR-FTIR Spectral Interpretation of Spirits

ATR-FTIR spectra (Table 2, Figure 1) were interpretated in order to ascertain the chemical profile of the tsipouro/tsikoudia products. Table 2 showcases the FTIR spectral bands spanning from 4000 to 499 cm^{-1}. In this spectral data table, each row represents a different spirit, while the columns denote specific wavelength ranges. The values in each cell correspond to the intensity of spectral response for the respective spirit at the given wavelength range. This dataset provides insights into the chemical compositions and structural properties of the analyzed spirits. The bands ranging from 3500 to 3200 and 1642 to 1648 cm^{-1} signify the stretching and bending vibrations of hydroxyl groups found in water and organic acids [6,27]. Furthermore, the bands at 2986 and 2901 cm^{-1} are corresponded to the C-H stretching vibrations of methyl and methylene groups in alcohols and organic acids, especially ethanol and tartaric acid [28]. The intensity ratio of 2986:2901 cm^{-1} ranges from 4.53 to 6.33, for all tsipouro/tsikoudia samples examined. The band at 1420 cm^{-1} denotes the C-H rocking vibration [27] and at 1450–2460 cm^{-1} is associated with O-H bending deformation [6], while the band at 1385 cm^{-1} is attributed to the C-O stretching vibrations of tartaric acid [28].

Table 2. The spectral absorbance bands (intensities) of thirty-nine (39) grape marc spirits (tsipouro/tsikoudia) after a duplicate analysis.

Spirit Code	590–700	877–879	1043–1045	1083–1085	1275	1350–1310	1384–1385	1419–1420	1450–1460	1643–1646	2901–2904	2980–2986	3500–3200
T1	0.001	0.053	0.223	0.061	0.008	0.003	0.010	0.003	0.019	0.158	0.022	0.124	0.006
T2	0.005	0.049	0.211	0.057	0.008	0.003	0.010	0.003	0.019	0.005	0.019	0.119	0.004
T3	0.003	0.046	0.204	0.056	0.007	0.002	0.011	0.003	0.018	0.003	0.018	0.114	0.003
T4	0.000	0.043	0.195	0.053	0.007	0.003	0.009	0.008	0.016	0.003	0.018	0.109	0.006
T5	0.003	0.050	0.222	0.060	0.008	0.011	0.012	0.009	0.018	0.166	0.021	0.124	0.004
T6	0.002	0.053	0.226	0.062	0.008	0.011	0.012	0.009	0.018	0.169	0.022	0.126	0.006
T7	0.000	0.045	0.202	0.054	0.007	0.003	0.011	0.008	0.017	0.004	0.019	0.110	0.003
T8	0.000	0.051	0.222	0.060	0.008	0.010	0.012	0.003	0.019	0.159	0.021	0.122	0.006
T9	0.001	0.050	0.215	0.058	0.008	0.010	0.009	0.008	0.018	0.004	0.023	0.121	0.014
T10	0.002	0.053	0.226	0.062	0.009	0.011	0.012	0.009	0.018	0.003	0.021	0.128	0.014
T11	0.001	0.050	0.222	0.060	0.008	0.009	0.012	0.009	0.018	0.009	0.020	0.123	0.019
T12	0.000	0.049	0.216	0.059	0.008	0.003	0.012	0.003	0.019	0.157	0.019	0.120	0.003
T13	0.002	0.056	0.237	0.064	0.009	0.011	0.012	0.010	0.019	0.010	0.026	0.132	0.013
T14	0.004	0.047	0.205	0.056	0.008	0.000	0.008	0.003	0.018	0.159	0.019	0.115	0.006
T15	0.000	0.048	0.213	0.058	0.008	0.010	0.012	0.008	0.017	0.170	0.019	0.120	0.005
T16	0.002	0.049	0.215	0.059	0.008	0.010	0.011	0.008	0.017	0.170	0.023	0.120	0.007
T17	0.003	0.052	0.216	0.056	0.009	0.011	0.010	0.009	0.019	0.008	0.021	0.119	0.013
T18	0.004	0.044	0.202	0.056	0.007	0.002	0.008	0.007	0.017	0.004	0.020	0.114	0.003
T19	0.002	0.052	0.222	0.062	0.008	0.002	0.010	0.009	0.018	0.004	0.028	0.127	0.003
T20	0.003	0.049	0.208	0.056	0.007	0.003	0.009	0.008	0.017	0.004	0.020	0.118	0.007
T21	0.002	0.049	0.214	0.058	0.007	0.010	0.010	0.008	0.018	0.003	0.023	0.121	0.006
T22	0.004	0.051	0.222	0.061	0.008	0.010	0.010	0.009	0.019	0.166	0.022	0.124	0.004
T23	0.003	0.048	0.209	0.057	0.008	0.003	0.012	0.002	0.018	0.003	0.021	0.116	0.012
T24	0.000	0.050	0.220	0.060	0.009	0.010	0.012	0.009	0.017	0.169	0.021	0.121	0.002
T25	0.001	0.051	0.223	0.061	0.008	0.009	0.011	0.009	0.018	0.008	0.021	0.125	0.007
T26	0.002	0.048	0.211	0.058	0.008	0.011	0.011	0.009	0.017	0.174	0.022	0.119	0.006
T27	0.004	0.051	0.223	0.060	0.008	0.010	0.011	0.009	0.019	0.175	0.022	0.123	0.013
T28	0.002	0.047	0.210	0.057	0.008	0.002	0.011	0.002	0.018	0.003	0.019	0.115	0.013
T29	0.003	0.045	0.198	0.053	0.007	0.003	0.010	0.007	0.016	0.003	0.022	0.110	0.005
T30	0.002	0.079	0.312	0.087	0.012	0.015	0.019	0.010	0.023	0.164	0.034	0.178	0.007
T31	0.002	0.077	0.304	0.085	0.012	0.014	0.018	0.011	0.022	0.008	0.032	0.173	0.013
T32	0.003	0.081	0.314	0.087	0.012	0.014	0.019	0.011	0.023	0.009	0.032	0.180	0.020
T33	0.002	0.108	0.394	0.114	0.014	0.019	0.024	0.012	0.027	0.009	0.044	0.231	0.008
T34	0.002	0.100	0.375	0.107	0.014	0.017	0.023	0.012	0.024	0.008	0.040	0.219	0.007
T35	0.003	0.069	0.274	0.077	0.011	0.004	0.016	0.010	0.023	0.158	0.028	0.155	0.004
T36	0.000	0.050	0.217	0.059	0.008	0.010	0.012	0.008	0.018	0.164	0.020	0.119	0.013
T37	0.002	0.051	0.221	0.060	0.008	0.009	0.012	0.008	0.017	0.003	0.020	0.123	0.008
T38	0.001	0.045	0.199	0.054	0.007	0.002	0.009	0.008	0.017	0.003	0.019	0.109	0.006
T39	0.003	0.043	0.192	0.052	0.007	0.002	0.008	0.008	0.016	0.004	0.017	0.105	0.013

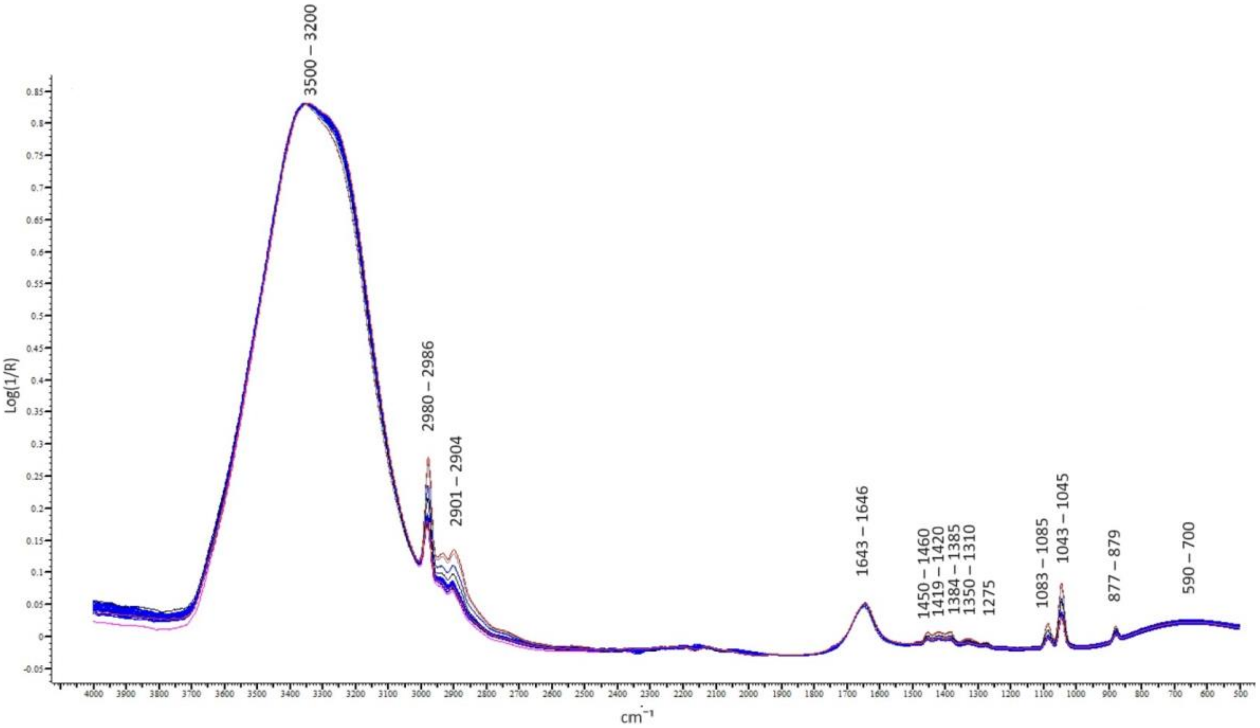


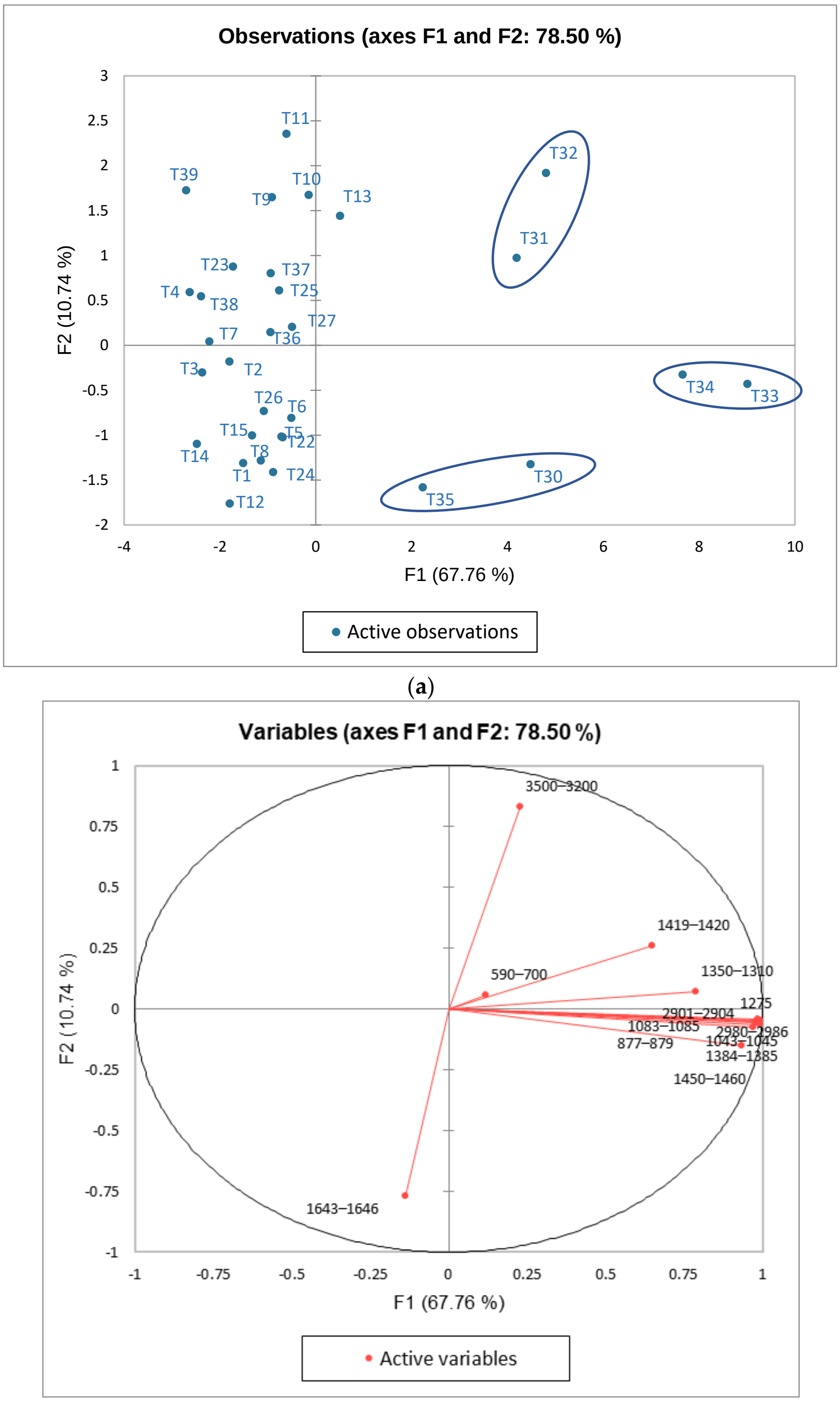
Figure 1. Overlay MIR spectra of thirty-nine (39) samples of tsipouro.

Moreover, the band from 1350 to 1310 cm^{-1} signifies the O-H bending vibration (out of plane) of the phenols or/and alcohols [27], and the band at 1275 cm^{-1} is assigned to the C-O stretching vibrations of organic acids [29]. Additionally, the bands at 1083–1085 cm^{-1} and 1043–1045 cm^{-1} are corresponded to the C-O stretching vibrations of primary alcohols and are very important regions for ethanol and methanol quantification [6,27]. Coldea et al. (2013) [30] noted that ethanol exhibits distinct peaks at 1047 and 1087 cm^{-1}, with the former being the major signal and the latter the minor signal. On the other hand, methanol displays characteristic vibrations at 1020 and 1112 cm^{-1}, with the former being the major signal and the latter the minor signal. Interestingly, regarding ethanol, the intensity ratio of major/minor signals (1043–1045:1083–1085) ranges from 3.46 to 3.85, for all tsipouro samples examined. Moreover, Yadav and Sharma (2019) [11] reported that the bands at 1047 and 1087 cm^{-1} could be attributed to the C-O stretching vibration in fermentation products such as carbohydrates, phenolics, esters, and lactones. Furthermore, the band at 877–879 cm^{-1} is related to the C-H out of plane bending vibrations of aromatic compounds [11,27,29], while the region from 590 to 700 cm^{-1} represents the O-H out of plane bending vibrations of various alcohols [27]. The presence of outliers in the spectral data highlights the diversity and complexity of tsipouro/tsikoudia compositions. According to Table 2 the samples that show the greatest variation in terms of their values in almost all bands are those made from the homemade distillation process [1].

3.2. Classification of the Spirits through Chemometrics

Initially, Principal Component Analysis (PCA) was performed on the averaged values of the duplicate measurements from all acquired ATR-FTIR data directly and 78.50% of the total variance in the data was explained in two principal components (PC1: 66.76%, PC2: 10.74%) with a p-value (two-tailed) < 0.0001 (Figure 1).

Graphical representation of the results is shown in Figure 1, where PC1 is plotted against PC2. In that representation, the main separation of products is driven by some of the distillates from Tyrnavos being clearly differentiated from all other samples with very high scores in PC1. Those were the samples T30–32 and T34, with the spirit T32 showing the highest PC1 score and clearly separated from all the rest. Products T33–35 and T30 were all traditional homemade tsipouro samples from the PDO of Tyrnavos, but so were products T31 and T32, which were largely clustered in first and fourth quadrant all with positive PC1 values but at the same time with variation in their PC1 scores. These results are in agreement with the results from the HS-SPME GC/MS analyses of the same samples [1]. Samples T36–T39 were also traditional homemade spirits from different geographical areas and were all clustered in the second quadrant (Figure 2). The grape varieties used for making the samples did not appear significant in the classification of the spirits from ATR-FTIR data.



(**a**)

(**b**)

Figure 2. Principal Component Analysis (PCA) run on the FT-IR data of 39 Greek grape marc spirit samples: (**a**) PCA biplot and (**b**) the correlation circle of the initial variables used.

3.2.1. Type of Distillation Effect

The clear clusters of homemade spirits that appear in Figure 2 led to the need for further analysis to investigate the factor of the distillation method. Partial least squares–discriminant analysis (PLS-DA) is one of the most widely used classification techniques in chemometrics [1,31,32]. Therefore, a partial least squares–discriminant analysis (PLS-DA) was used to run the same data matrix in order to evaluate the effect of distillation process on the acquired spectral data (Figure 3). Distillation process could be classified in three techniques of copper alempic and fractional distillation among the spirits, namely that of: one distillation (CAD1), two distillations (CAD2), and homemade distillation (HD). The outcome of the PLS-DA approach was a product space with clear clusters according to the distillation technique used to make the spirits at 74.36% correct classification (accuracy) (Figure 3). The sensitivity and selectivity of classification of the PLS-DA method were calculated at 95.40% and 71.43%, respectively. Specifically, the initial classification values were 44.44%, 95.00% and 60.00% for the single distillation (CAD1), double distillation (CAD2), and homemade products (HD), respectively (Figure 2).

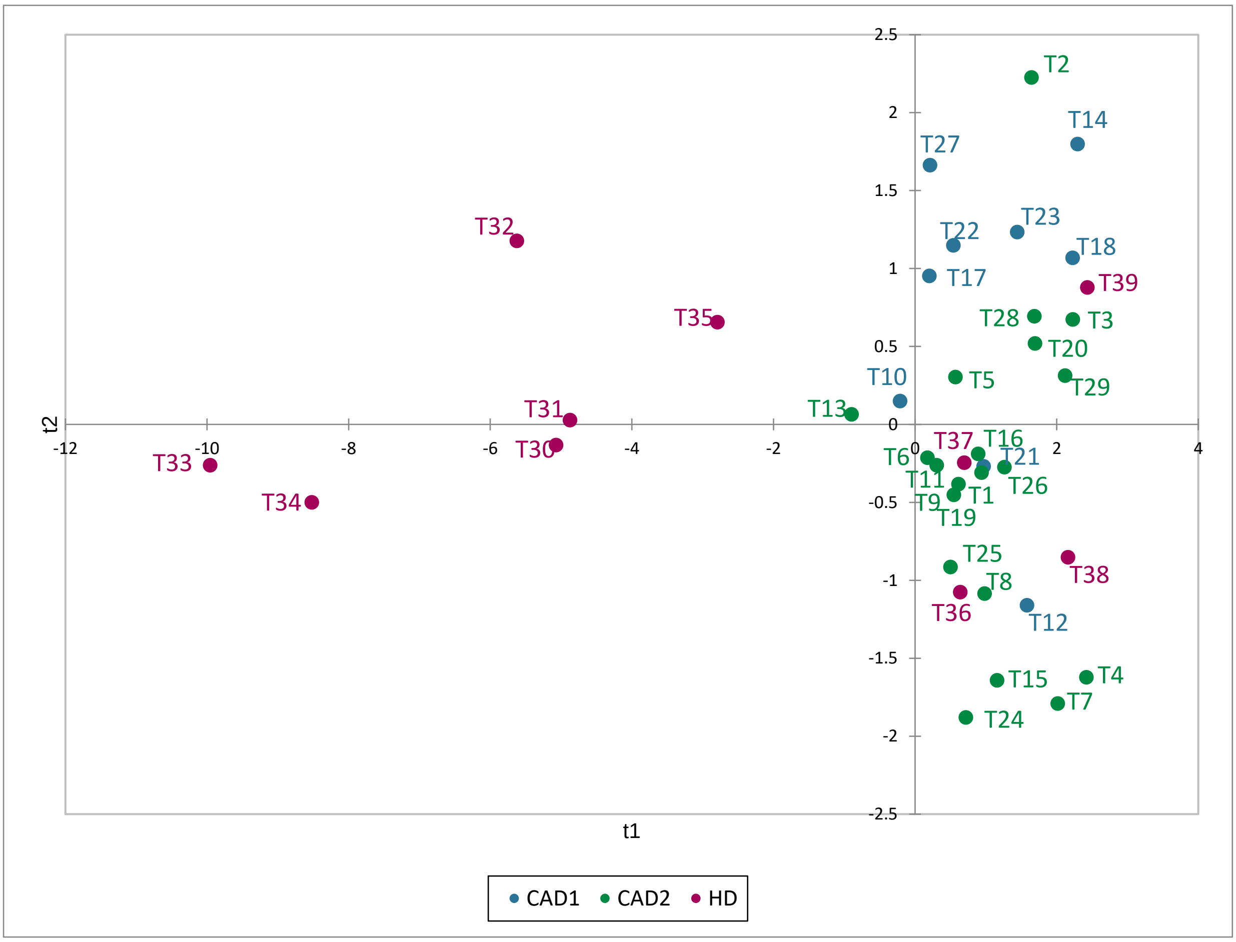


Figure 3. Partial least squares–discriminant analysis (PLS-DA) regression of ATR-FTIR data of the spirits when coded according to the distillation technique used for production. CAD1: spirits made by single distillation; CAD2: spirits made by double distillation; and HD: homemade traditional spirits.

The most important marker for the classification of double-distilled products (CAD2), differentiating them from products of the other distillation techniques, was in the range of 590–700 cm^{-1}, which corresponds to the O-H out of plane bend of alcohols with a variable importance in projection (VIP) value of 1805. The other significant bends in the classification with VIP > 1 were at 1384–1385 cm^{-1}, 1275 cm^{-1}, 877–879 cm^{-1}, 1083–1085 cm^{-1}, 1043–1045 cm^{-1}, and 2980–2986 cm^{-1}. Those markers indicate the C-O and C-H stretching vibrations of alcohols and organic acids (Table 3). In the research of Kokoti et al. (2023), a similar discrimination of distillation process was carried out in tsipouro samples from fractional distillation, copper alembic, and homemade distillation using the volatile profile of the products, measured with SPME-GC/MS analysis. In the main findings of the aforementioned research, the authors demonstrated that the most significant variables in product classification were ethanol, higher alcohols, esters, and terpenes. These results show great relevance to those of our study [24]. Extensive research has underscored the pivotal role of distillation techniques in shaping the distinct qualities of grape marc spirits. Investigation into this domain has unveiled a fundamental divergence in alcohol concentration between traditionally homemade Orujo spirits and their industrially manufactured counterparts in the research of Cortes et al. (2005) [33], who also analyzed their volatile profile, indicating the importance of fraction collection through the distillation process. In spirit production, irrespective of the raw materials utilized, increased distillation iterations are commonly associated with elevated quality attributes of the resulting spirit. Additionally, in the grape marc spirit market, a spirit made with double distillation is considered of higher quality and is priced more expensively. ATR-FTIR analysis, and specifically the aforementioned marker, could play a significant role in the quality control of the category in general, and specifically in the detection of the distillation process used.

Table 3. Variable Importance in Projection in the classification of the spirits based on: (1) number of distillations, (2) major region classification (north–south), and (3) the differentiation of spirits from Crete from those of Thessaly, Tyrnavos, Central Greece, and Peloponnese, respectively.

Classification		Variable	VIP
Number of distillations		590–700	1.805
		877–879	1.238
		1275	1.230
		1083–1085	1.227
		1043–1045	1.226
		1384–1385	1.213
		2980–2986	1.202
North–South		1384–1385	1.409
		1083–1085	1.219
		1043–1045	1.213
		1275	1.255
Crete	Thessaly	1350–1310	1.987
		1419–1420	1.746
	Tyrnavos	1350–1310	1.648
		1419–1420	1.399
		1384–1385	1.302
	Central Greece	1350–1310	1.454
		1384–1385	1.202
	Peloponnese	1350–1310	1.846
		1419–1420	1.823
		1450–1460	1.606

3.2.2. Geographical Origin Effect

Further in the investigation of the important variables from the ATR-FTIR analysis, a differentiation was observed in terms of the geographical compartment of the spirit origin. In particular, samples were divided into two main groups, namely, the northern geographical divisions (Thrace, Macedonia, Epirus, Thessaly, Tyrnavos, and Central Greece) and the southern geographical divisions (Peloponnesus and Crete) (Figure 4). Outlier samples of the initial PCA were not included in the above classification. Those two groups consisted of nineteen and twelve samples for the north and south of Greece, respectively. PLS-DA was again used, and the results are displayed in Figure 4 with a total correct classification of the method of 87.10% (sensitivity and selectivity) and specifically 89.5% correct classification for the northern and 83.3% for the southern-origin spirits (Figure 4). Markers with VIP > 1, which were more important for that classification, were at 1275 cm^{-1} for the C-O stretch of organic acids, at 1384–1385 cm^{-1} for the C-O stretching vibration of tartaric acid, and at 1043–1045 cm^{-1} and 1083–1085 cm^{-1} for ethanol (Table 3).

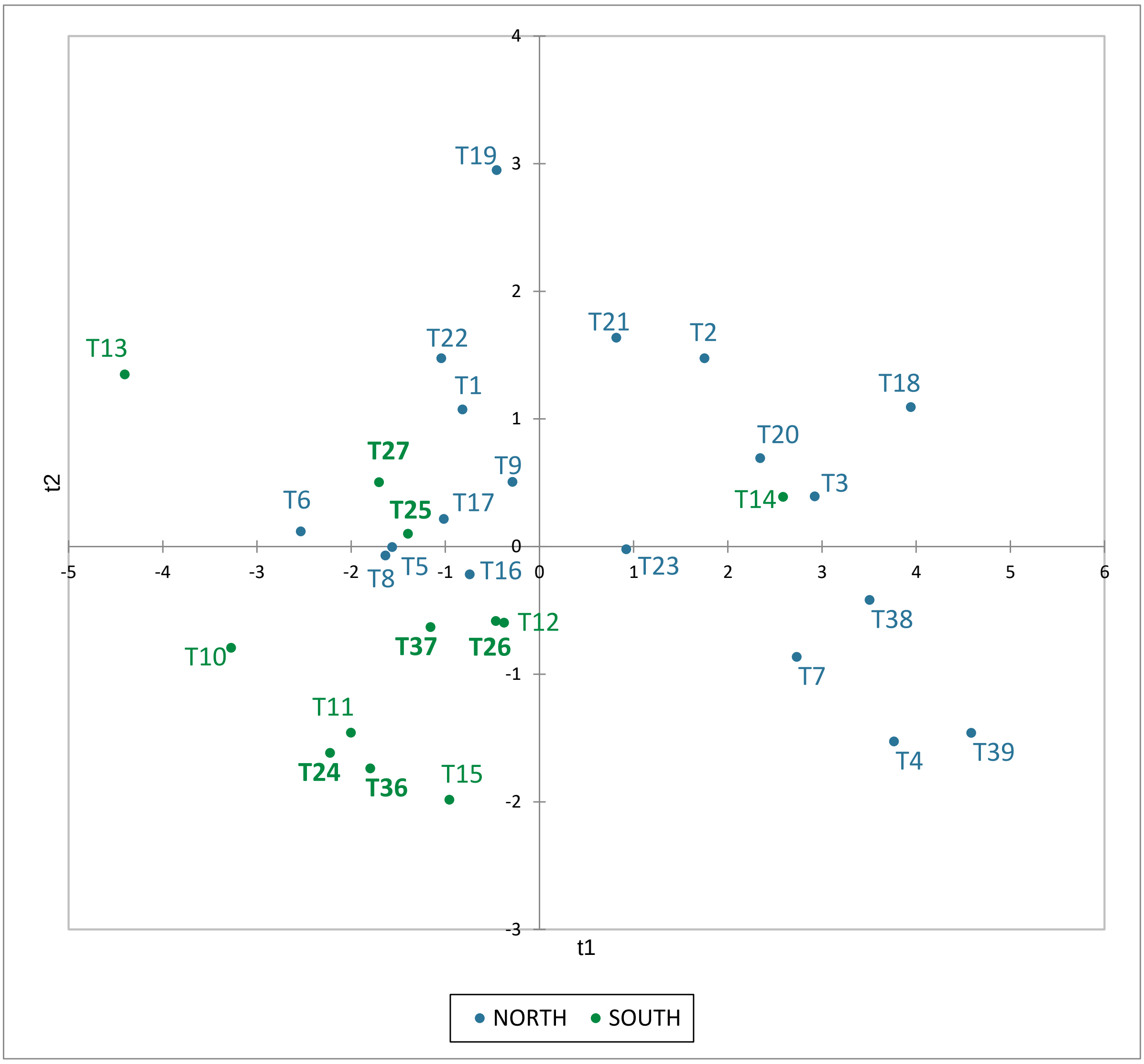


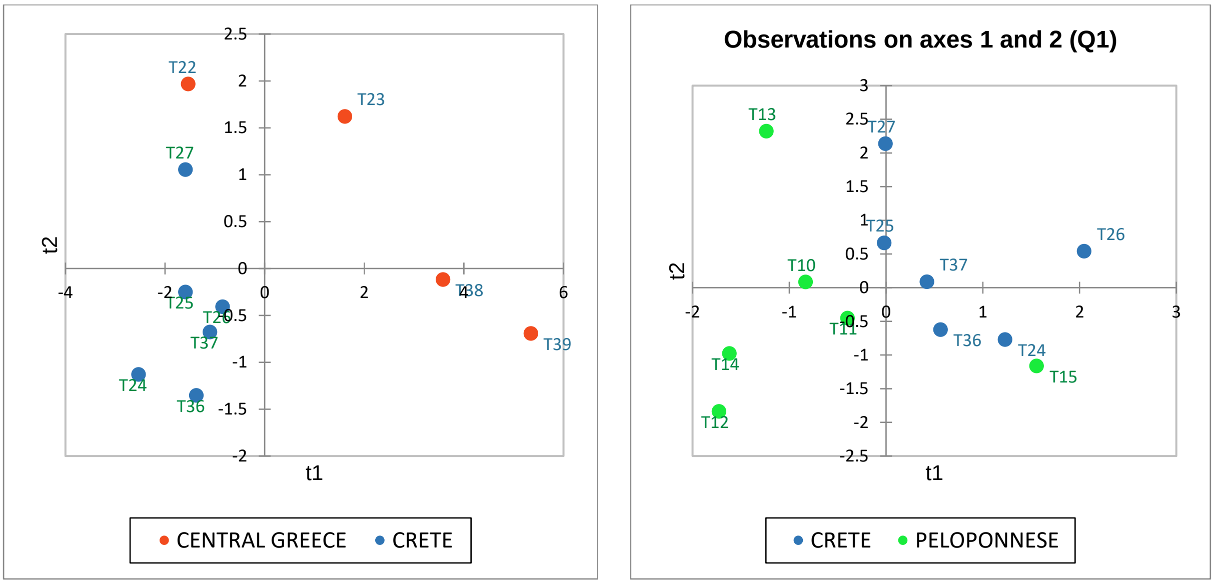
Figure 4. Partial least squares–discriminant analysis (PLS-DA) regression of ATR-FTIR data of the spirits when coded according to major geographical division. Samples coded for north represent samples originating from Thrace, Macedonia, Epirus, Thessaly, Tyrnavos, and Central Greece. Samples coded for south represent samples originating from Peloponnese and Crete.

ATR-FTIR is a method that has been previously applied to other spirits with the aim of categorizing them based on their geographical origin. Specifically, Yadav and Sharma in 2019 used ATR-FTIR in their research to classify illicit liquor from seven different regions of Punjab state of Northern India and achieved an overall rate of 93% of correct discrimination. Also, ATR-FTIR analysis has been previously employed for the classification of unifloral honeys sourced from Sardinia, Italy [34]. This analytical technique yielded promising results in discerning the unique chemical fingerprints of each honey variety, facilitating the identification of their respective origins. Furthermore, in the review research of Basalekou et al. (2020) [22], FT-IR spectroscopy is presented as a successful tool in wine authentication and traceability and can provide classifications based on a large number of parameters such as origin, grape variety, maturation, vintage, and adulteration. Additionally, in the research of Ioannou et al. (2023) [25], Greek honey samples from different botanical and geographical origins were investigated and the results predicted with high overall accuracy the geographical (93.55%) and botanical (96.77%) origin of the honey samples. Moreover, ATR-FTIR spectroscopy holds considerable promise for the classification of natural resins. Research has demonstrated its efficacy in providing a valuable approach to categorize resins into distinct families, leveraging a set of band positions for precise classification [35].

According to Figure 5, the region with the clearest separation from the rest was Crete, whose samples appear to have much stronger clustering compared to the rest of the geographical divisions and can be viewed in that figure by bold writing. Interestingly, the traditional name given for marc spirits from Crete differs from all other Greek grape marc spirits. Namely, grape marc spirits from Crete are called "tsikoudia", whereas marc spirits from all other areas in Greece are called: "tsipouro" (Table 1). Subsequently, we proceeded to a more detailed classification of the spirits by comparing them in groups of two; we used each one of the four represented geographical regions with the largest number of samples, namely: Thessaly, Tyrnavos, Central Greece, and Peloponnese. All above regions were compared to that of Crete with respect to product representation (Figure 5a–c). The aim of this classification was the detection of the most significant FTIR markers for the separation of the Cretan products from those from the rest of the geographical origins. Results are presented in Figure 5a–d from a PLS-DA analysis and in the two principal components a total of 44.78%, 95.34%, 81.84%, and 44.03% of the total of variance was explained for the comparison with Thessaly, Tyrnavos, Central Greece, and Peloponnese, respectively. When a variable has a VIP index higher than one, it is considered relevant for the classification [26]. The most important marker for the classification of Crete from the other regions was in the band 1350–1310 cm^{-1} at all four plots (Table 3). According to the results of Tsapou et al. (2023) [1], where HS-SPME GC/MS analysis was conducted, the region of Crete was also classified mainly because of phenols. Also, as noted in prior studies, materials enriched with phenolic compounds demonstrate a propensity for superior classification in FTIR. FTIR analysis is effective for detecting phenolic compounds due to their unique chemical structures and corresponding infrared absorption spectra. Phenolic compounds contain one or more hydroxyl groups (-OH) attached to an aromatic ring, which gives rise to characteristic absorption bands in the infrared region [36–38].

(**a**) (**b**)

Figure 5. *Cont.*



(**c**) (**d**)

Figure 5. Partial least squares–discriminant analysis (PLS-DA) to compare (**a**) Crete–Thesaly; (**b**) Crete–Tyrnavos; (**c**) Crete–Central Greece; and (**d**) Crete—Peloponnese.

4. Conclusions

Spectroscopic techniques offer comparable accuracy to traditional chemical composition or sensory methods, with the added advantages of being cost-effective and less time-intensive. Although the integration of multivariate data analysis and spectroscopy primarily yields qualitative insights, it is important to note potential inaccuracies stemming from similar environmental conditions, raw materials, preparation techniques, and limited sample sizes for calibration models. The original hypothesis in this study was that we could employ FTIR analysis combined with chemometrics to quickly and accurately classify a relatively large set of grape marc spirits from Greece. Despite the above-mentioned limitations, the combination of ATR–FTIR spectroscopy and chemometric tools proved in our case to be an efficient and accurate approach for classifying the spirit samples. Specifically, according to our findings, the combination of ATR–FTIR with chemometric methods like PCA and PLS-DA led to highly accurate classification of grape marc spirits with 87.1% sensitivity and selectivity of the method based on their geographical origin. From all four designated viticultural regions for the production of PDO grape marc spirits represented in the sample set, the region of Crete was the one most profoundly distinguished. Additionally, when we attempted to classify those spirits based on the distillation technique used, we achieved an accuracy of 74.36% with specific accuracy of 95% for the products resulting from double distillations. Therefore, ATR-FTIR spectroscopy combined with chemometrics could be a useful tool for a fast but efficient classification of complex food products such as the grape marc spirits from Greece.

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