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Nontargeted ^{1}H NMR fingerprinting and multivariate statistical analysis for traceability of Greek PDO Vostizza currants

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Abstract: In this study, non-targeted ^{1}H NMR fingerprinting was used in combination with multivariate statistical analyses for the classification of Greek currants based on their geographical origins (Aeghion, Nemea, Kalamata, Zante, and Amaliada). As classification techniques, Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) were carried out. To elucidate different components according to PDO (Protected Designation of Origin), products from Aeghion (Vostizza) were statistically compared with each one of the four other regions. PLS-DA plots ensure that currants from Kalamata, Nemea, Zante, and Amaliada are well classified with respect to the PDO currants, according to differences observed in metabolites. Results suggest that composition differences in carbohydrates, amino, and organic acids of currants are sufficient to discriminate them in correlation to their geographical origin. In conclusion, currants metabolites which mostly contribute to classification performance of such discriminant analysis model present a suitable alternative technique for currants traceability. The study results contribute information to the currants' metabolite fingerprinting by NMR spectroscopy and their geographical origin.

Practical Application: This study presents an analytical approach for a high nutritional value Greek PDO product, Vostizza currant. A further research and implementation of this method in food industry, can be the key to food fraud incidents. Thus, application of this work opens up posibilities to "farm to table" mission.

KEYWORDS
currants, food traceability, geographical origin, metabolomics, NMR fingerprinting

1 | INTRODUCTION

Dried grapes are considered to be a popular vine product around the world, with high nutritional and medical value, known as raisins or currants (Langová et al., 2020). Currants are dry red grapes originally from Greece, while raisins are dried white grapes. Vine products from grapes cultivar "Black Corinth" and "Sultana" constitute 80% of the global dried grapes production. Corinthian currant (*Vitis vinifera L.*, var. Apyrena) Vostizza, from Black Corinth variety, holds a Protected Destination of Origin (PDO) name due to the cultivation area's microclimate and the incline of the vineyard, placed in the town of Aeghion. Vostizza is cultivated as an arid crop and their site are

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preferred not to exceed 750 m altitude (Council Directive 96/23/EC, 2010). In Greece, hybrid *V. berlandieri X. V. rupestris* is commonly used, as its genotype is considered to be well adapted in water-stress conditions and it is more efficient regarding photosynthesis, growth, and water-use ability in comparison with other genotypes (Flexas et al., 2009; Pou et al., 2008). Currants are considered to be an excellent source of fibers, carbohydrates, minerals, vitamins, amino acids, triterpenes, fatty acids, and phenolic compounds (Carughi, 2008; Chiou et al., 2014). In comparison with other Corinthian currants, Vostizza is characterized by high quality, nutritional value and high percentage of anthocyanins (Chiou et al., 2014; Panagopoulou et al., 2019; Vasilopoulou & Trichopoulou, 2014). Besides the anthocyanins, Vostizza currants present lower total phenolics content and antiradical activity (Chiou et al., 2014).

In order to prevent food frauds, the European Union has established quality certifications in food products such as PDO and PGI. The latest regulation for these quality labels came into force in December 2012—Regulation (EU) No 1151/2012. PDO products refer to products of a particular geographical area, where their production, process and preparation take place (Chiou et al., 2014; Grunert et al., 2016; Vasilopoulou & Trichopoulou, 2014). Thus, in recent years, many techniques have been developed for food provenance certification (El Sheikha, 2018). Among the variety of new analytical techniques for food traceability, nuclear magnetic resonance (NMR) has been considered a powerful and ever expanding tool for that purpose, by identifying and qualitatively and/or quantitatively determining a broad range of food product metabolites (Sobolev et al., 2019).

Food product metabolites are generally characterized by their complexity. Moreover, molecular composition of food is affected by many factors such as environmental conditions, type of farming or genetic and geographical origin, and so forth. (Bernillon et al., 2013; Cong et al., 2015; Tang et al., 2019). Characterization of the full metabolic profile or determination of a specific (food) compound such as amino acids, fatty acids, phenolics, antioxidants, carotenoids, and so forth, may provide significant information for food traceability, authentication, safety and quality (Bergantin et al., 2018; Consonni et al., 2019; Cuevas et al., 2017; Kalogiouri et al., 2016; Mannina, 2017; Oms-Oliu et al., 2013; Ritota et al., 2010).

There are numerous studies that have been focused on food geographical origin by means of NMR methodologies (Chiou et al., 2014, Vasilopoulou& Trichopoulou, 2014, Grunert et al., 2016, Consonni et al., 2019, Mannina et al., 2011) (Hatzakis, 2019) (Mannu et al., 2020) (Spiteri et al., 2020) (Viski, 2021). Target and nontargeted approaches coupled with chemometrics are already used for food traceability. NMR based metabolomics focusing on polar metabolites of sesame seeds, or nontargeted metabolomics for honey or lentils provenance determination, represent some of the most indicative examples of NMR in food analysis (Gerginova et al., 2020; Kim et al., 2020; Longobardi et al., 2017; Spiteri et al., 2020). Food products such as cocoa beans, beef, wheat, cherries, rice, tomatoes, and cabbage have also been analyzed and classified according to their geographical origin by NMR spectroscopy (Huo et al., 2017; Jung et al., 2010; J. Kim et al., 2013; Lamanna et al., 2011; Longobardi et al., 2013; Marseglia et al., 2016; Masetti et al., 2017). Moreover, application of nontargeted NMR analysis for geographical origin characterization has been reported for grape and wine varieties (Amargianitaki et al., 2017; Fotakis et al., 2016; Godelmann et al., 2013; Mannu et al., 2020; Papotti et al., 2013; Petrakis et al., 2008). However, this is the first time that NMR fingerprinting is being exploited for currants cultivars differentiation.

In the present study, ^{1}H NMR nontargeted fingerprinting in combination with pattern recognition techniques such as principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA), was used to highlight Vostizza PDO currant differentiation among currants from other geographical origins. Samples collected from four Greek areas (Zante, Kalamata, Nemea, and Amaliada) were examined in addition to PDO product.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Currants harvested in 2020 originated from Aeghion, Zante, Kalamata, Nemea, and Amaliada cultivars (Figure 1). The vines, including all geographical origins from where the samples collected, are 15 years old, grafted in the Grape phylloxera resistant rootstock Richter No. 110 (*V. berlandieri* × *V. rupestris*). The vast majority of the vineyards are cultivated under rainfed conditions. As far as fertilization is concerned, 11-15-15+TE (60 kg/ha) and 20-19-19 (250 g/ha) were used in all cultivars. Information about climate conditions of last year from May to October for these locations was collected from the National Observatory of Athens and is presented in Table 1. The period of year, which was decided to be included in Table 1, refers to cultivars' blooming stage, fruit setting, cell division, cell expansion, and finally ripening stage (Koufos et al., 2018). Furthermore, Figure 2 depicts the flow diagram of Vostizza PDO production process. PDO currant samples from Aeghion and from four other geographical origins were collected on same days just before the packaging and transferred under aseptic conditions to laboratory.

FIGURE 1 Geographical distribution of samples

TABLE 1 Climatic conditions of each location from May to October

Geographical region	Average temperature (°C)	Total precipitation (mm)	Average wind speed (km/h)
PDO Vostizza Aeghion	24.1	39.3	5.5
Zante	22.8	3.4	3.9
Kalamata	24.5	32.2	5.3
Amaliada	23.2	27.5	5.6
Nemea	21.6	28.7	5

TABLE 2 Number of samples of each geographical area that were concluded in the statistical analysis

Geographical region	Number of samples
Nemea (Corinthia)	6
Kalamata (Messinia)	4
PDO Vostizza (Achaia)	6
Zante (Ionian Islands)	6
Amaliada (Ilia)	4

FIGURE 2 Flow diagram of PDO Vostizza currants' production

Currant samples were stored at room temperature until analysis.

2.2 | Sample preparation

Metabolites that are responsible for differentiation of grapevine cultivars are organic acids, amino acids, carbohydrates, phenylpropanoids, and flavonoids. Therefore, the protocol for extraction of metabolites from currants was according to Kashif et al., 2009. Fresh currants samples of 0.75 g were mixed with 1050 μl KH_2PO_4 buffer (pH = 6.0) in 100% D_2O including 0.005% DSS (sodium trimethylsilylpropanesulfonate) and 450 μl Methanol HPLC grade (Sigma-Aldrich) in ratio water/methanol 7:3 (v/v) (Ali et al., 2011; Kashif et al., 2009). Samples were homogenized with Tissue Raptor II (Qiagen) for 10 min. The samples were ultra-sonicated for 40 min at 80 kHz at room temperature and centrifuged at 14,000 rpm at 25°C for 15 min. The supernatant was collected in 1.5 ml vial and stored at −80°C for 3 hr. Then, samples were placed on freeze dryer for lyophilization for 16 h. NMR experiments were conducted in a total of 26 freeze dried currants samples, including 6 samples of each one of the three examined geographical regions and 4 samples of the other two (Table 2). 55 mg of each sample were added in the vial and solubilized in the NMR solvent (550 μl D_2O). The pH was not readjusted before the NMR experiments as the p[D^+] of neutral heavy water at 25°C is 7.44. The final samples were vortexed for 1 min and 550 μl were transferred in 5 mm NMR tubes (Bruker BioSpin srl.).

2.3 | ^{1}H NMR experiments

The samples were analyzed in a 700 MHz NMR spectrometer (Bruker Avance III HD) equipped with a 5 mm cryogenically cooled TCI gradient probe at 298 K and TopSpin 4.0.7 (Bruker BioSpin srl.). One-dimensional ^{1}H NOESY experiment with presaturation routine for water suppression, with 64 scans of 98.3 K data points, 14,005.60 Hz spectral with recorded for each one of the 26 samples and mixing time 0.01 s. Two-dimensional homonuclear ^{1}H-^{1}H *J*-res spectra were also recorded using 4 scans per 128 increments and 78.13 Hz spectral width for F1 (spin–spin coupling constant axis), and 12.3 K data points with 11,627.91 Hz spectral width for F2 (chemical shift axis).

2.4 | Data analysis and statistics

Before the chemometric analysis, the recorded NMR spectra were calibrated on the singlet peak of DSS in 0.00 ppm while zero- and first-order phase correction of spectra were also applied manually using the TopSpin 4.0.7 by Bruker BioSpin. AMIX software (Bruker) was used for the conversion of spectral data into bucket tables. The total spectral region (0.00–10.00 ppm) of the 26 ^{1}H 1D NMR spectra were divided into 172 integrated spectral bins of equal width 0.04 ppm excluding the water signal (4.70–5.19 ppm), DSS resonances (0.51–0.74 ppm and 1.69–1.80 ppm). SIMCA 16.0.1 (Umetrics, Sweden) and the programming language R (Rstudio 3.5.2) were used for the multivariate and univariate statistical analysis of the spectral data (bucket tables) (Saccenti et al., 2014). PCA and PLS-DA were selected for the investigation of the categorization of the 5 regions currant samples using 6 PCs and LVs, respectively. The cumulative values of R^2 and Q^2 were calculated via sevenfold cross validation, and a permutation test with 200 random arrangements of *y*-variables evaluated the statistical significance of the PLS-DA model. Autoscaling was selected as the most suitable scaling method for the current dataset. Univariate analysis was based on the nonparametric statistical test Kruskal–Wallis by ranks (H test) for independent samples (level of significance, $a = 0.01$). The box plots derived by univariate analysis reflect groups' variability demonstrated as relative intensities (values given in arbitrary units) (Benjamini & Hochberg, 2000). The false discovery rate (FDR) correction was applied according to the Benjamini and Hochberg method (Benjamini & Hochberg, 2000).

3 | RESULTS AND DISCUSSION

3.1 | Metabolites identification

Thirty (30) metabolites were identified in the polar phase of the PDO samples (Table 3). Nonautomated assignment conducted using the free evaluation version of Chenomx NMR Suite 8.3, the online available database Biological Magnetic Resonance Data Bank (BMRB), the specified in food chemistry and biology open-access databases FooDB (foodb.ca) and FoodComEx (foodcomex.org), and finally the available bibliography concerned on NMR studies was applied on vine-derived products (Pereira et al., 2005) (Ulrich et al., 2008) (Son et al., 2009b) (Ali et al., 2011) (Mulas et al., 2011) (Gallo et al., 2014) (Bhouri et al., 2016).

Three spectral regions can be distinguished in the ^{1}H NMR spectrum including the region of aryl (phenolics) (6.00–8.50 ppm), sugars (3.20–5.50 ppm), and region of amino acids and organic compounds (0.85–3.20 ppm). The saccharides glucose and fructose are the most abundant sugars in the examined samples. Additionally, the detected ethanol is a natural component of vine-derived products (Gallo et al., 2014; Ali et al., 2010). A plethora of metabolites are detected in the upfield region of the spectrum where an intense variability is also observed in the chemical shifts of malate and citrate (Table 3).

3.2 | Multivariate and univariate statistical analysis

Statistical analysis of currants' ^{1}H NMR data was performed following previously described NMR metabolomics' methodology (Chasapi et al., 2019). PCA (R^2X (cum) = 85.3% and Q^2 (cum) = 63.3%) was selected to obtain an initial overview of the samples' distribution and a satisfying clustering was achieved based on 56.3% of the total data variance (Figure 3). Loadings of PC1 and PC2 reveal that aryl (phenolics) region, the γ-αminobutyric acid (GABA), and the unassigned metabolites of the spectral region 2.94–2.98 ppm are responsible for the distribution in multivariate sample space (Figure S1 and Table S1, Supporting Information). The supervised PLS-DA model led to a better categorization of the 5 geographical regions based on 54.3% of the total variance that is explained by the first two latent variables (LV1: 30.7%, LV2: 23.6%) (Figure 4). The model was constructed using 6 LVs and evaluated by sevenfold cross validation (R^2Y (cum) = 84.6% and Q^2 (cum) = 51.6%). The harvest from Kalamata (blue), Zante (yellow), and Vostizza (red) are classified in separate groups in the 3D PLS-DA plot. Currant from Nemea (purple) and Amaliada (green) seem to form sparser clusters but they still define their own metabolic space (Figure 4). VIP scores of PLS-DA with values above 1.00 reveal that the amino acids glutamate, glutamine, proline, threonine, isoleucine, and the organic compounds malate, acetate, formate, and tartrate are statistically significant for the classification of the 26 currant samples into their subcategories/clusters (Figure S2 and Table S2, Supporting Information).

Univariate statistical analysis also performed to investigate each variable separately for its significance in characterization of each currant composition (data distribution). This approach provides supplemental information about the relative intensities of the metabolites between the different classes (Figure S7, Supporting Information). Proline, malate, tartrate, and alanine were revealed statistically significant, after the FDR correction ($a = 0.01$), for the five-group categorization with *p*-values 0.0003, 0.0051, 0.0013, and 0.0019, respectively (Figure 5).

A better visualization is achieved after the statistical comparison of the Vostizza PDO currants with the cur-

TABLE 3 Metabolites identification in the PDO currant through 1D ^{1}H NMR and 2D *J*-res spectra

Chemical class	Metabolites	δ ^{1}H/^{1}H multiplicity	Chemical group
Carbohydrates	α-glucose	3.40/t	C4H
		3.52/dd	C2H
		3.67/t	C3H
		3.75/dd	C6H′
		3.82/m	C5H
		3.88/m	C6H
		5.22/d	C1H
	β-glucose	3.23/dd	C2H
		3.39/m	C4H
		3.45/m	C5H
		3.47/t	C3H
		3.71/dd	C6H′
		3.83/m	C6H
		4.631/d	C1H
	Fructose	3.56/m	$C1H_2$
		3.68/m	$C1H_2$ & $C6H_2$
		3.80/m	C3H, C5H & $C6H_2$
		3.88/dd	C4H
		3.98/m	C5H
		4.01/dd	$C6H_2$
		4.10/m	C3H & C4H
	Sucrose	4.20/d	Fructofuranosyl-C3H
		5.40/d	Glucopyranosyl-C1H
Amino acids	4-aminobutyrate (GABA)	1.92/m	$C3H_2$
		3.03/t	$C4H_2$
	Alanine	1.47/d	β-CH_3
	Arginine	1.68/m	γ-CH_2
		1.90/m	β-CH_2
	Glutamate	2.15/m	β-CH_2
		2.29–2.39/m	γ-CH_2
	Glutamine	2.14/m	β-CH_2
		2.39–2.47/m	γ-CH_2
	Isoleucine	0.94/t	δ-CH_3
		1.00/d	γ-CH_3
		1.25 & 1.35/m	γ-CH_2
		1.96/m	β-CH
	Leucine	0.95 & 0.96/d & d	$\delta 1$-CH_3 & $\delta 2$-CH_3
	Methionine	2.14/m	β-CH_2 & SCH_3
	Proline	1.99/m	γ-CH_2
		2.06/m & 2.34/m	β-CH_2
	Threonine	1.32/d	γ-CH_3
		4.27/m	β-CH
	Valine	0.98/d & 1.03/d	γ-CH_3
		2.26/m	β-CH
Organic acids	Acetate	2.06/s	CH_3
	Citrate	2.76/d	C2Ha & C4Ha
		2.88/d	C2Hb & C4Hb
	Formate	8.36/s	CH
	Fumarate	6.65/s	$(CH=)_2$
	Lactate	1.321/d	CH_3
	Malate	2.67/dd & 2.84/dd	CH_2
		4.41/dd	CH
	Tartrate	4.46/s	C2H & C3H

(Continues)

TABLE 3 (Continued)

Chemical class	Metabolites	$\delta^1H/^1H$ multiplicity	Chemical group
Ketones	Acetoacetate	2.26/s	CH_3
	Acetone	2.22/s	$(CH_3)_2$
	Hydroxyacetone	2.14/s	CH_3
		4.38/s	CH
Phenols	Substituted phenolic compound	6.44/d/J = 15.90 Hz	H8
		6.95/d/J = 8.40 Hz	H5
		7.14–7.16/dd/J = 8.30, 1.95 Hz	H6
		7.23/d/J = 1.83 Hz	H2
		7.67/d/J = 15.90 Hz	H7
Alcohols & Polyols	2,3-butanediol	1.13/d	$C1H_3$ & $C4H_3$
	Choline	3.19/s	$N^+(CH_3)_3$
		4.05/m	CH_2
	Ethanol	1.22/t	CH_3
		3.65/q	CH_2

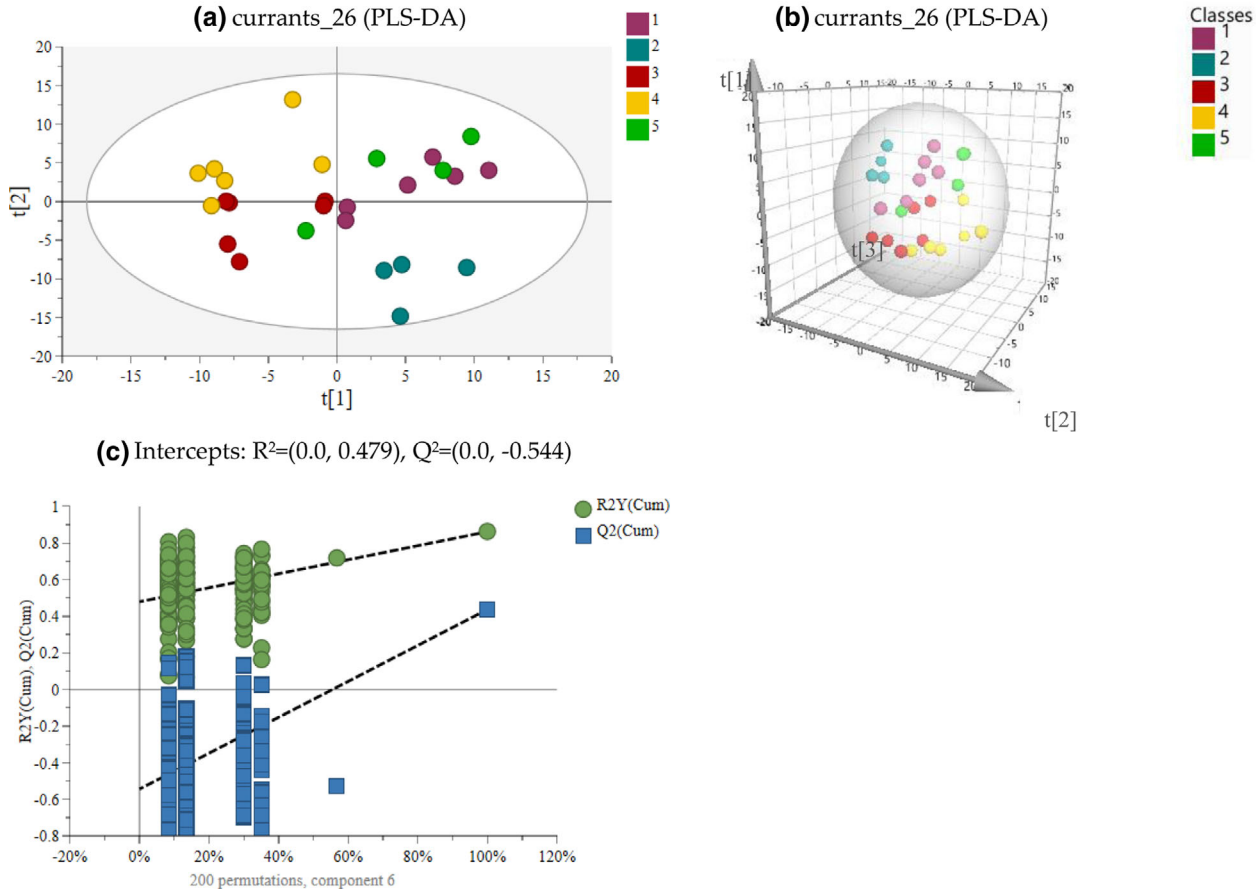

FIGURE 3 (a,b) 2D and 3D PLS-DA plots of the five examined currant regions (purple: Nemea, blue: Kalamata, red: PDO Vostizza, yellow: Zante, green: Amaliada). The model was constructed using 6 LVs and was evaluated by sevenfold cross validation (R^2Y (cum) = 84.6% and Q^2 (cum) = 51.6%); (c) statistical validation test was performed with 200 random permutations in the PLS-DA model. The permutation plot validates the statistical significance of the model as the permuted R^2 (green circles) and Q^2 (blue boxes) (bottom left) are significantly lower than the original values of R^2 and Q^2 (top right)

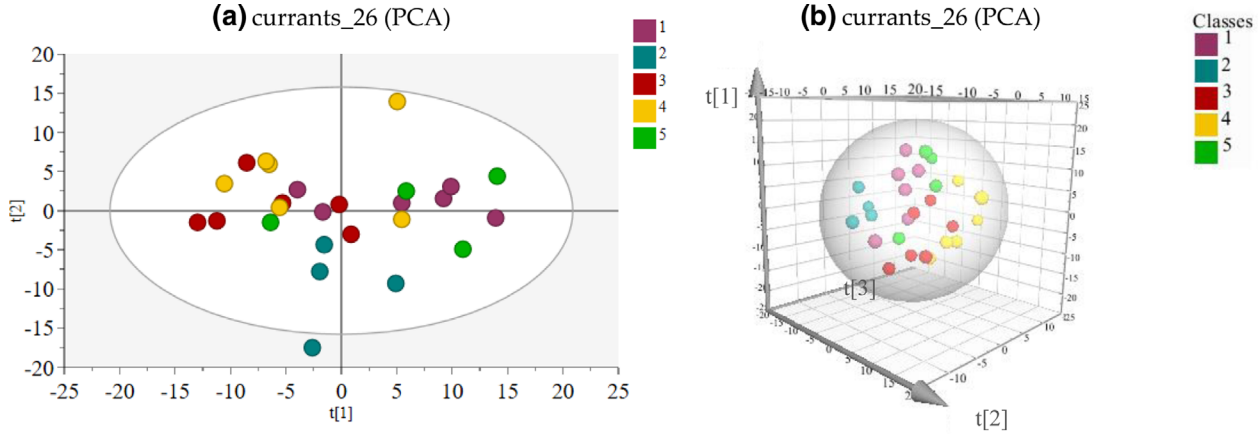

FIGURE 4 (a,b) 2D and 3D PCA plots of the five examined currant regions (purple: Nemea, blue: Kalamata, red: PDO Vostizza, yellow: Zante, green: Amaliada). The model was constructed using 6 PCs that is explained by the first three principal components (PC1: 35.8%, PC2: 20.5%, PC3: 16.2%)

rants (products) of each one of the four other geographical regions. PLS-DA classification models ensure that currants from Kalamata, Nemea, Zante, and Amaliada when compared directly with Vostizza PDO currants display a reliable classification by geographical origin. However, the responsible metabolites characterizing the unique currant composition of each geographical origin differ. More specifically, differences of the four examined currants ^{1}H NMR profile regarding the Vostizza's PDO currants are presented below.

3.2.1 | Nemea versus Vostizza PDO

PDO currants in their comparison with Nemea's seem to be constituted by higher concentrations of malate, glutamate, and glutamine, while they observed lower levels of threonine (Figure S3, Supporting Information).

3.2.2 | Kalamata versus Vostizza PDO

Glutamate, glutamine, and formate are elevated in Vostizza's samples compared to Kalamata's and malate, fumarate, citrate, GABA, and threonine are decreased (Figure S4, Supporting Information).

3.2.3 | Zante versus Vostizza PDO

The statistically significant metabolites for the separation of Zante's and Vostizza's harvests are formate, fumarate, glutamate, and glutamine and they are decreased in the PDO currant samples (Figure S5, Supporting Information).

3.2.4 | Amaliada versus Vostizza PDO

The amino acids proline and methionine are increased in PDO's currants but alanine, fumarate and formate seem decreased when they are statistically compared with Amaliada's currants (Figure S6, Supporting Information).

3.3 | Discussion

Currants contain sugars, consisting mainly of glucose and fructose, but the percentage varies from sample to sample (Gul et al., 2016; Qureshi et al., 2020). Besides these, currants contain minerals, vitamins (ascorbic acid, pyridoxine, riboflavin, thiamin), aryl, and phenolic compounds, fibers, tartaric acid, and so forth. (Schuster et al., 2017).

Several publications report the identification of specific metabolites in vine products, concluding carbohydrates (glucose, fructose and sucrose), organic acids (malic and tartaric acids), and amino acids (proline, glutamine, threonine, etc.), that are in agreement with our findings (Fotakis et al., 2013; Nikolidaki et al., 2017; Teixeira et al., 2014). Geographical origin has already been identified as a factor that affects the total amino acid and organic acid content of the currant sample (Pereira et al., 2006; Teixeira et al., 2014). Likewise, differences in phytochemical and sugar content could be partially explained by a variety of factors (Teixeira et al., 2014). Different water percentage or moisture content of berries, environmental factors such as average temperature, sunshine, rainfall, or the drying process can highly contribute to their composition (Breksa et al., 2010; Panagopoulou et al., 2019; Serratosa et al., 2008). Furthermore, carbohydrates concentration in the samples depends on developmental stage, viticultural practices as well as

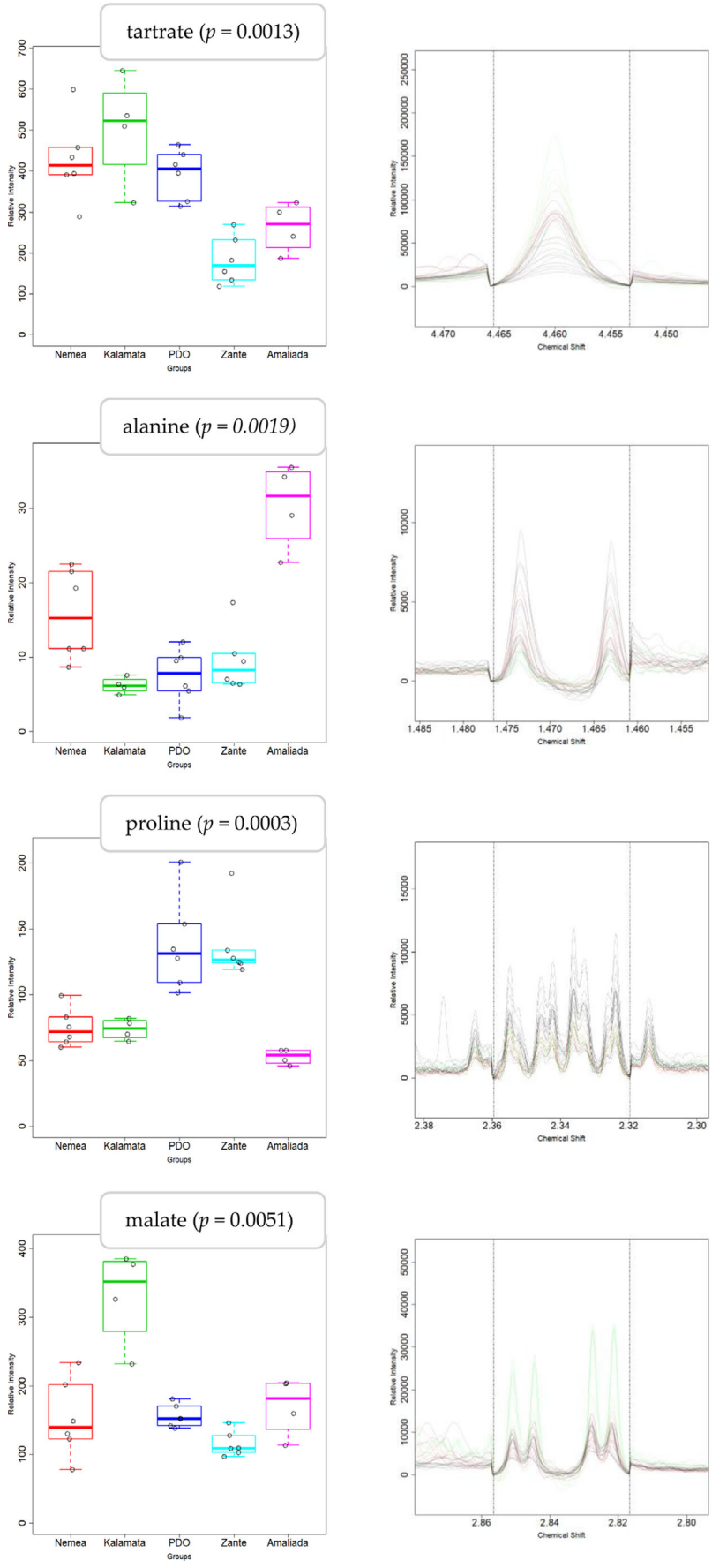

FIGURE 5 Significant metabolites after the FDR correction ($a = 0.01$). The box plots of the univariate statistical analysis represent the distribution of the samples in each group and the median corresponds to the relative intensity of each one

on the genotype (Chiou, 2014; Clingeleffer, 2010; Kliewer et al., 2005; Liu et al., 2007; Shiraishi et al., 2010). Quantitative analysis by ^{1}H NMR spectroscopy of vine-products metabolites such as organic acids, mono- and disaccharides, and amino acids is applied since 1980s. Nowadays, NMR spectroscopy is considered as a user-friendly tool to assess traceability and authentication of these products. (Fotakis et al., 2013).

Fructose and glucose are the dominant sugars in Corinthian currants, although variations have been observed in sugar content over different geographical origin (Nikolidaki et al., 2017; Şimşek et al., 2004; Son et al., 2009b). The NMR data of this study suggest that Vostizza PDO currants contain higher percentage of glutamate and glutamine and lower percentage of threonine in comparison with currants from Kalamata and Nemea. Zante currants are characterized by increased formate, fumarate and glutamate levels related to Vostizza PDO. As regards Amaliada currants, the amino acid alanine and the organic compounds fumarate and formate have elevated concentrations compared to the PDO product. Different levels of malate, γ-aminobutyric acid (GABA) and proline in currants among the different geographical regions are also reported. The percentage of malate concentration depends on temperature scale since high malate levels are observed when berries grow at low temperatures (Smart, 1987). In contrast, berries in warm climates are characterized by elevated proline and GABA (Pereira et al., 2006). Furthermore, amino acid composition of currants is high related to cultivar and geographical location. Variations in amino acid content also reveal differences in genotype among grape cultivars (Shiraishi, 2002; Shiraishi et al., 2010; Soufleros et al., 2003). In general, areas characterized by high temperatures and less rainfalls produce grapes with higher level of sugars and proline and lower percentages of malate, citrate, alanine, and threonine (Son et al., 2009b).

Natural products' flavonoids detection via NMR spectroscopy requires an altered extraction protocol specific for berries. Another common procedure is the selection of an organic NMR solvent (pyridine-*d5*, DMSO-*d6*) suitable for the lipophilic components of wine-products (Ali et al., 2011; Blunder et al., 2017; Feng et al., 2017; Mabry et al., 1970). NMR data of this study refer to the identification of a polar substituted phenolic compound that could be a hydroxycinnamic acid derivative or free hydroxycinnamic acid (Table 3 and Figure S8, Supporting Information) (Makila et al., 2016). Additionally, the chemical shift variability of tartaric acid (in the range 4.35–4.58 ppm), is possibly indicative of its conjugation with caffeic acid and/ or coumaric acid to the formation of the caftaric and coutaric acid, respectively. Because of this conjugation, the chemical environment of tartrate's C2H and C3H protons is altered, leading to a slightly different chemical shift of the ^{1}H NMR singlet peak of tartrate (Kashif et al., 2011). It is known that both caftaric and coutaric acids are natural components of currants (Karadeniz et al., 2000; Williamson & Carughi, 2010).

The current study presents an analytical approach in food analysis to discriminate currants based on their provenance. NMR spectroscopy is a tool useful in quality control applications and it is widely used in pharmaceutical and food industries to prevent fraud incidents (Gouilleux et al., 2018; Hu et al., 2017; Kuballa et al., 2018; Petrakis et al., 2015). However, currant analysis has to face some challenges. The constructed classification models were able to distinguish currants according to their geographical origin, but factors such as year of harvest, environmental or viticultural practices may affect the total metabolite content of currants (Bertram et al., 2010; Fabani et al., 2017; Falasca et al., 2014; Scognamiglio et al., 2015). The classifications reported in this study provide an important insight based on the reported number of currant's samples. However, it is acknowledged that more samples are required for defining robust classification models. This study opens up possibilities to extend the results here obtained to different currants crop years, even using a higher number of currants' samples. A further improvement in the currants traceability and authenticity issue could regard studying relationships occurring between currants metabolites and detailedclimatic parameters using NMR data.

4 | CONCLUSION

The present work demonstrates that nontargeted ^{1}H-NMR profiling of Greek currants and chemometric analysis discriminate successfully Greek PDO Vostizza currants according to their geographical origin. Within the framework of this study, the geographical discrimination of Vostizza PDO, Kalamata, Zante, Amaliada, and Nemea currants, was achieved by multivariate statistical analysis of the ^{1}H NMR fingerprints. Our results strongly suggest that amino acids (glutamate, glutamine, proline, threonine, isoleucine) and organic compounds (malate, acetate, formate, and tartrate) extracted from PDO currants, are distinguishable from metabolites of Amaliada, Zante, Kalamata, and Nemea currants. This study could be considered as a potential exploitation of NMR fingerprinting for currants traceability. In conclusion, further analysis could enhance the sensitivity and accuracy of specific metabolites, important for the determination of currants' geographical origin.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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