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Review

From the Field to the Bottle—An Integrated Strategy for Wine Authenticity

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Abstract: The wine sector is one of the most economically important agro-food businesses. The wine market value is largely associated to terroir, in some cases resulting in highly expensive wines that attract fraudulent practices. The existent wine traceability system has some limitations that can be overcome with the development of new technological approaches that can tackle this problem with several means. This review aims to call attention to the problem and to present several strategies that can assure a more reliable and authentic wine system, identifying existent technologies developed for the sector, which can be incorporated into the current traceability system.

Keywords: wine authenticity; geographical origin; grapevine varietal identification and discrimination; bio-geochemical strategy

1. Wine Authenticity

For all food and beverage production, it is fundamental to employ procedures that control the quality, safety, and authenticity of products. Authenticity in the food industry, in particular in added-value food products, such as wine, has been a major concern that has challenged researchers to develop reliable and feasible technologies for such a purpose [1,2]. The wine sector is a billion-euro business, where highly quoted wines are the preferential target for fraudulent practices. Their quality is known to be deeply influenced by many factors, and amongst them the grapevine varieties used, origins, and growing conditions play a major role [3,4]. The quality of the final product is also strongly influenced by the physical, chemical, and molecular biological transformations involved in the process of winemaking. These transformations are a result of the action of various enzymes, mainly from yeasts, and specific bacteria, which are responsible for many fermentation steps occurring during winemaking [5]. These parameters, related to the history and provenance of wines, strongly set its commercial value; therefore, a set of rigorous legal guidelines and a strong organizational culture towards quality control are required to guarantee the safety and quality of wines [2].

Nowadays, both consumers and winemakers show an increasing interest in finding different ways to assess the authenticity of their products. In this direction, traceability systems can be used as a risk management tool, utilized to easily trace the origin and the overall vinification process [6].

In Europe, traceability systems are applied to promote and protect certain denominations, such as the Protected Designation of Origin (PDO), Protected Geographical Indication (PGI), and Traditional Specialties Guaranteed (TSG). These designations of origin are conferred to high quality agricultural products, like wine, which are strictly linked to their origin area and specific viticulture and oenological practices [7,8].

A specific case of geographical indication concerning wine is its terroir, a term that relates the origin of a certain wine to a very specific area and includes specific geologic and geomorphologic boundaries (e.g., soil, topography, climate, landscape characteristics, and biodiversity features). In vitivinicultural, terroir consists of an area, in which the interactions between the identifiable physical and biological environment and the vitivinicultural practices used will provide distinctive characteristics of the wine originated from that area in particular [9].

The International Organization of Vine and Wine (OIV) has set clearly the definitions of Recognized Geographical Indication and the Recognized Appellation of Origin [10]. Both have the name of the country, region, or place in the label, which requires previous recognition of the authorities of the country concerned, and consist of products of quality and/or characteristics linked to the geographical milieu (natural and human factors), requiring that grapes are harvested in the defined denomination. However, the Recognized Appellation of Origin entails that the products' characteristics are due exclusively or essentially to the geographical location and that the grape transformation is performed in the defined area.

The denominations of origin have been established for quite a long time in Europe, being the first regions defined in the 18th century: Chianti, an Italian denomination, was established in 1716; Tokaj, a Hungarian denomination, was established in 1757; and the Douro Wine Region, a Portuguese denomination, was created in 1756 as the first wine appellation in the world that had, apart from the definition of the wine producing area, regulations on producing methodologies and trade rules. Nowadays, the Appellations of Origin are spread throughout the world in all continents, making it necessary to develop tools that are more sensitive in sensing the differences among regions. Some of the Appellations of Origin are represented in Figure 1.

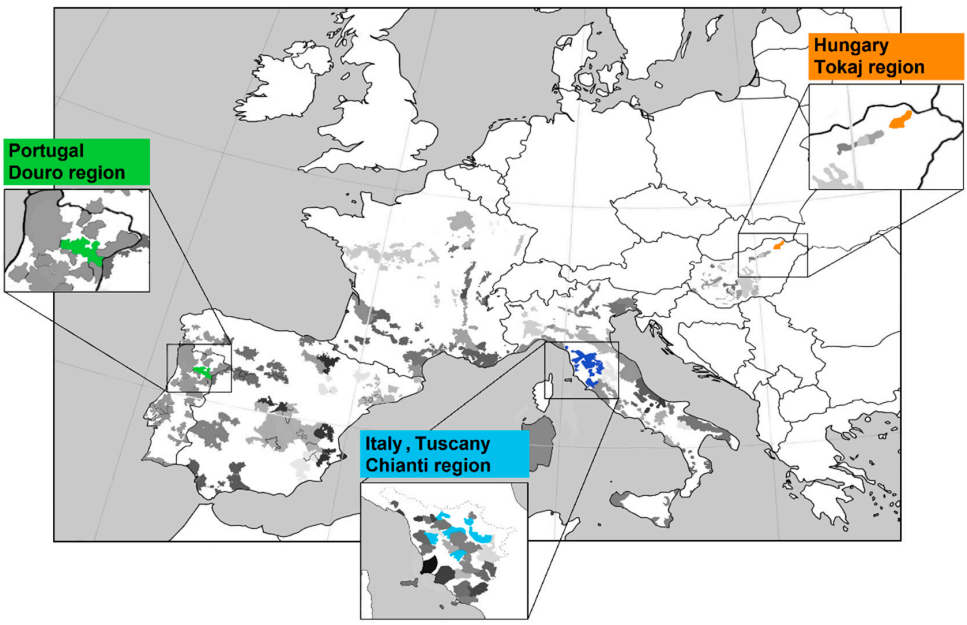

Figure 1. Appellations of Origin in the Old World. In grey are some of the Appellations of Origin in several wine producing areas and highlighted in color are the first Appellations (in blue the Chianti region; in green the Douro region; and in orange the Tokaj region). Adapted from Vineyards © [11] (accessed at 31 July 2018).

The main grapevine varieties from the old world have also been taken to new wine producing countries, giving rise to a diversity of wines commercially available in the market that have the same genetic origin. The most widely spread grapevine variety used for wine production is Cabernet Sauvignon, covering an area of 341,000 ha, mainly grown in China, France, Chile, the United States, Australia, Spain, Argentina, Italy, and South Africa [12]. Thus, the geographical origin identification is crucial under a well-established authenticity scheme.

The label present in wine bottles can also contain further information, such as the grapevine varietal composition. Nevertheless, as for geographical origin, the varietal composition is also regulated by the OIV. The label can state the variety if at least 75% of the grapes belong to such a grapevine variety and is listed in the denomination and if it attributes a specific characteristic to the wine [10]. Wines mentioning two varieties must comprehend exclusively these varieties and they should contain more than 15% of the listed varieties, which must be indicated by decreasing order of importance [10]. When more than two varieties are listed, the label must contain their respective percentages [10].

When production is carried out according to the standardized procedures, it normally results in final products with a high quality, which translates to higher prices at the sale point. Unfortunately, these financial benefits attract the production of counterfeit products and illegal food trades [13]. The dilution of wines with water, addition of alcohol or coloring and flavoring substances, blending with a wine of a lesser quality, and the mislabeling and misrepresentation of grapevine varieties and geographical origin are different kinds of frauds that can be referred to as examples of wine adulteration [14]. With the increasing occurrence of fraudulent practices, fast, reliable, and competent methods are needed to tackle authentication challenges and ensure products' quality. These strategies should guarantee the consumer's protection against mislabeling information of the purchased products, and the honest producers' defense from prejudicial competitors [1,15].

Two of the main requirements for the assessment of wine authenticity are the determination of its geographical origin, since the area of production is associated with the originality and quality of the characteristics of products, and the grapevine varietal identification, with compositional and sensory parameters being highly dependent on the variety (or varieties) used to produce a certain wine [3].

Several methods based on the analysis of metabolites, such as volatile compounds [16], amino acids and proteins [17,18], phenolic compounds [14], anthocyanins [19], mineral composition, and isotope identification [20], have been developed for the assessment of wine authenticity. These can be used for grapevine varietal identification, and some can also be used for geographical origin determination. Promising results have been obtained, however, the metabolic composition of grapes and wines is influenced not only by environmental conditions, cultural practices, and climate changes, but also by the production systems and processing methods used. On the other hand, these variables do not affect the grapevine genotype. Therefore, varietal identification and discrimination might be more accurate and efficient when DNA-based methodologies are used [21,22].

Regarding DNA methodologies, grapevine varietal identification is currently easily guaranteed with the use of simple sequence repeat (SSR) markers, approved and supported by the OIV [23]. Once developed, they are easy and inexpensive to use, and data can be readily compared among laboratories. Nevertheless, drawbacks have been reported for the application of these markers in wine samples due to the low amount of DNA isolated from this type of matrix [7]. The development of single nucleotide polymorphism (SNP) markers is also being considered as an alternative to SSRs. SNP markers have proven to be highly stable and repeatable, with a high discriminating power for grapevine varieties [24].

Although a large number of potential methods/technologies have been developed throughout the years that aim to target wine authenticity, the main system used is still mainly based on traceability systems. These traceability systems are mandatory by Reg. 178/2002 [25] for all agri-food products, including wine. However, the traceability systems are mainly based on registrations that can be adulterated, therefore, constituting a fragility of the system. Therefore, it is important that

multidisciplinary strategies that can assist and control traceability systems are developed so the entire chain can be better protected.

2. The Importance of an Integrated Strategy

The need to develop multidisciplinary strategies in wine authenticity is the only reliable way of guaranteeing that all different terroir levels are contemplated in the analysis. As previously mentioned, the terroir is a result of multidimensional parameters, including soil, climate, and biodiversity features. The use of a unique technology capable of evaluating all these dimensions has so far not been accomplished. Nonetheless, several technologies have proven to be efficient to evaluate one of the components required to design the authenticity plan (Figure 2).

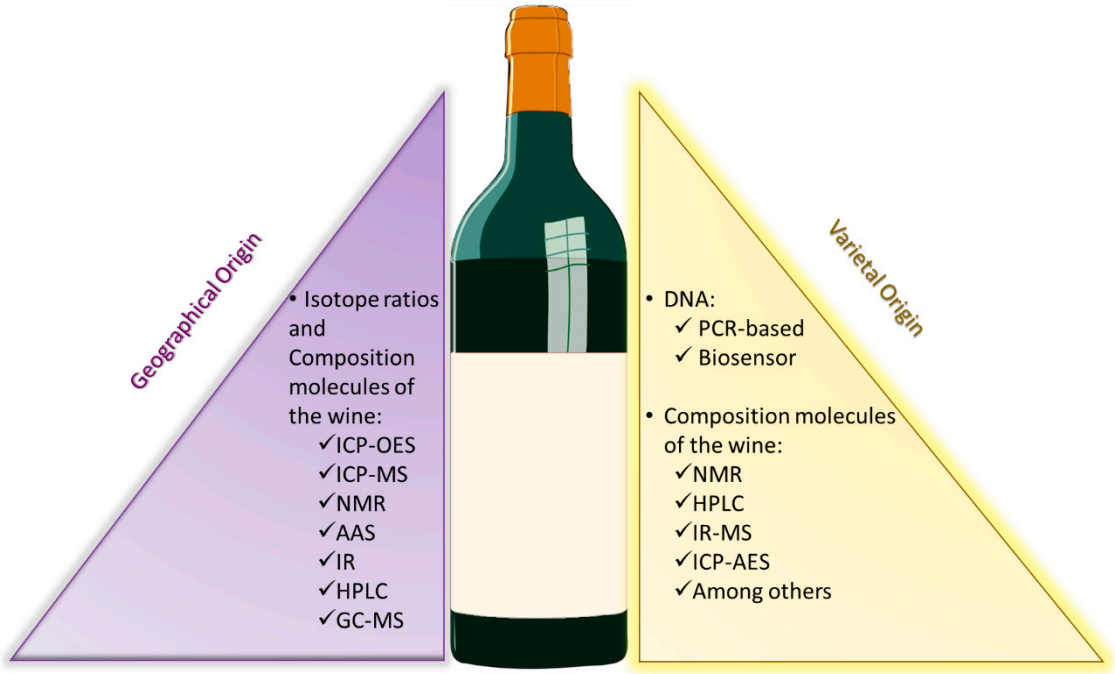

Figure 2. Technologies suitable to assess Geographical Origin and Varietal Identification.

The use of DNA-based detection systems has been extended to a wide variety of food products, including wine, as this remains the most reliable methods for varietal identification purposes. Among the different DNA-based systems, the use of biosensors has emerged as an attractive and alternative method for food authenticity. An optical biosensor system has been used for such a purpose, presenting several advantages, such as low cost, real-time measurement, and label free detection [26,27].

Even though varietal identification is possible when molecular markers are applied to wine samples, the grape origin cannot be detected by these means. Geographical origin can be achieved through chemical and isotopic techniques. Soil related fingerprinting plays a primary role in the determination of the geographical provenience since there is a direct correlation between the chemical composition of the wine and the soil composition, particularly the $^{87}Sr/^{86}Sr$ ratio, which can be used for the definition of the different denominations of origin [28]. Additionally, the mostly widely applied methods that intend to combine both botanical and geographical origin in wine samples are based on spectroscopic and/or spectrometric approaches [29]. These techniques are high-throughput approaches that are based on big datasets, with a previous collection of data considering several varieties and production years for each particular region associated with a statistical treatment. However, these approaches are not always efficient considering varietal identification [30].

Therefore, instead of aiming to define a unique technology, the integration of two-dimensional strategies, one for geographical origin determination and another for varietal identification and quantification, can be a more convenient and reliable way of tackling this issue. This type of approach has been already suggested by Fernandes et al. [31] as a bio-geochemical strategy, considering the grapevine composition through a biological method, and the definition of provenance based on geochemical determination.

3. Determination of the Region of Provenience

Wine is one of the main food products commercialized worldwide with a close and distinct relationship with its geographical place of origin. Some of the most famous wines, because of their high market value, have been a target of fraudulent admixtures, which have been reported through several media sources (newspaper, television, and internet). Considering that wines with commercial value are associated to a production region having distinctive autochthonous properties, this type of fraudulent practice is particularly important [32–34]. Nowadays, some renewed sophisticated consumers are interested in high quality wines, strongly linked to their region of origin [13]. This has led to a challenging topic regarding wine authenticity, which aims to obtain a provenience of origin signature for such wines. The establishment of wine production and geographic provenance limits, related to the wine terroir, is one of the most important issues in wine quality control. The use of geographical indications allows producers to obtain market recognition and often a premium price [35]. The development of sophisticated analytical techniques that are suitable for determining the geographical origin is highly desirable to guarantee the authenticity and geographical traceability of wines.

The presence and concentration of certain trace elements reflect the geochemistry and geomorphology of the different ecosystems. Recent studies have established that the content of selected volatiles (e.g., alcohols, esters, aldehydes, and ketones), elements (e.g., $^{87}Sr/^{86}Sr$, $^{13}C/^{12}C$, $^{18}O/^{16}O$, ME, $(D/H)_1$, $(D/H)_2$, $^{207}Pb/^{206}Pb$, $^{2}H/^{1}H$), and classical parameters (e.g., % ethanol, pH, total acidity, volatile acidity, malic acid, fructose, tartaric acid, lactic acid, succinate, citric acid, glycerol, 2,3-butandiol, dry matter, and relative density) in wines reflect the soil type, the environmental growing conditions, and the manufacturing processes, allowing wine regionality discrimination [36]. Factors, such as the amount of rainfall immediately prior to grape harvest (fermentation), and winery equipment, were shown to have a significant effect on the multi-element and multi-isotopic ratio and, consequently, were specific to the geographical origin of the wine [37].

Nowadays, the most established analytical methods are based on the profiling of trace elements (widely used for geographical discrimination), volatile compounds (used for varieties characterization), phenolic compounds (used for both varietal and geographical characterization, such as: Gallic, protocatechuic, vanillic, syringic, caffeic, p-coumaric and ferulic acids, catechin, epicatechin, quercetin, quercitrin, myricetin, kaempferol, and syringic and protocatechuic aldehydes) [38], organic constituents, mineral contents or composition, and light- or heavy-element isotope ratios using different chromatographic and spectroscopic methods [2]. In the last few years, there has been growing interest in developing analytical methods for wine-growing region authentication (Table 1).

Some of the most widely applied methods to assess the botanical and geographical origin of wine are spectroscopic and/or spectrometric, such as ultra-performance liquid chromatography (UPLC), Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), nuclear magnetic resonance (NMR), ultraviolet-visible spectrophotometry (UV-vis), near-infrared (NIR), mid-infrared (MIR), high-performance liquid chromatography (HPLC), gas chromatography mass spectrometry (GC-MS), inductively coupled plasma optical emission spectrometry (ICP-OES), and inductively coupled plasma mass spectrometry (ICP-MS), among others. All these are high-throughput approaches requiring the use of somewhat complex statistical analyses and, most of the time, a big data set from the defined region considering several production years and varieties to develop a reliable database [31].

The relative abundance of stable isotope ratios of individual elements can act as fingerprints that enable the tracing of the origin of elements in a substance [39]. Because the stable isotope ratios within environmental substances have strong regional variations that are commonly controlled by the underlying geology, this means that these elements can be used as traceability indexes to determine their origins [37,39]. Consequently, stable isotopic ratio analysis of wine can allow its geographical origin to be authenticated thanks to the existence of an official European database (EU-Wine DB) [7]. Strontium isotopes reflect the local geological conditions of the wine terroir and may therefore be linked to the origin of the grapes used for wine production. The use of the $^{87}Sr/^{86}Sr$ isotope ratio as a geographical tracer of food origins is related to the constancy of its value in transferring from the soil to the plant and then into the final product. The Sr isotope ratios can be used to track the geographical origin of wines after analyzing soil, grape, and wine samples from producing areas [28,33]. A recent study involving several Cypriot wines was designed to monitor variations in isotopes and elements' content, aiming to relate them to the grapevine variety, environmental factors, and provenance [20]. The study was able to set a serious of elements (Na, Cu, B, Mn, K, Mg, P, $(D/H)_{II}$, R, and $\delta^{18}O$) that could somehow access varietal identification, since they were also dependent on the geo-climatic conditions [20]. Several studies, using multi-isotopic analysis, have been conducted on the provenance of wines. Day et al. [40] combined $(D/H)_2$ data with multi-element data from 165 authentic grape samples for differentiation of the principal wine production zones in France for the 1990 vintage. These studies were quite promising, however, this method requires the establishment of quite big data-sets to be implemented and on validated independent models. Other authors reported a clear discrimination between wine production regions, reinforcing the importance of Sr isotopes' signature to characterize wine terroirs, and as a robust fingerprint to trace the geographic authenticity of wine [41,42]. Microbial terroir likely involves multiple interactions and have demonstrated that grape and wine microbiota exhibit regional patterns that correlate with wine chemical composition, suggesting that the grape microbiome may influence terroir in aspects, such as microbial distribution, strain diversity, and plant-microbial interactions [32].

Nevertheless, the combination of different methods able to analyze different types of wine compounds seems to be the most promising approach to establish a wine's geographical origin ([35]; Table 1).

Table 1. Overview of analytical techniques for tracing the geographic provenance of wines.

Samples	Analytical Technique	Data Analyzed/Analyte	Purpose of Analysis	References
Mass Spectrometry				
Grape, wine, and soil	IR-MS	$^{87}SR/^{86}SR$	Geographic origin of wine from Canada	[33]
Rocks, soils, and wine	IR-MS	$^{87}SR/^{86}SR$	Geologic and pedologic traceability of Italian wines	[41]
Red wines, musts grape juices, soils, and rocks	IR-MS	$^{87}SR/^{86}SR$	Fingerprinting wine geographic provenance.	[42]
Musts, soils, and grape components (skin, seeds, must, and stem)	TIMS and XRD spectra	$^{87}SR/^{86}SR$	Geographic traceability study of Italian white wine labelled with the Controlled Designation of Origin (DOC)	[36]
Sparkling wines	IR-MS	$\delta^{13}C$	The $\delta^{13}C$ evaluation in the sparkling wines to detect adulteration—wines chaptalization	[43]
Wines and rocks	TIMS	$^{87}SR/^{86}SR$	Radiogenic isotopic evaluation for tracing geographic provenance of wines	[44]
Soils, grapes, and wines	AAS, IR-MS, MC-ICP-MS	$\delta^{18}O$, $(D/H)_I$, $(D/H)_{II}$, $\delta^{13}C$, $\delta^{15}N$, and $^{87}Sr/^{86}Sr$	Development of a geographical traceability model	[45]
Vineyard soils	ICP-MS	$^{87}SR/^{86}SR$	Evaluation of $^{87}Sr/^{86}Sr$ ratio in vineyard soils from Portuguese Denominations of Origin and its potential for origin authentication	[28]
Wines	ICP−MS and multi-element analysis	Li, B, Mg, Al, Si, Cl, Sc, Mn, Ni, Ga, Se, Rb, Sr, Nb, Cs, Ba, La, W, Tl, and U	South African wines classification according to geographical origin	[46]
Red, white, and palhete amphora wines	ICP-MS	Mineral content	Elemental composition characterization of Alentejo wines to establish the geographic origin	[47]
Wine	ICP-MS, ICP-OES and IRMS	Elemental profile (Ca, Al, Mg, B, Fe, K, Rb, Mn, Na, P, Co, Ga, As, Sr), and Isotope ratio ($\delta^{18}O$)	Geographical origin of Chinese wines	[48]
Soils, grapes, and wines	ICP-MS	Cr, Co, Ni, Ga, Se, Y, Zr, Nb, Mo, Pd, In, La, Pr, Sm, Eu, Gd, Tm, Yb, Au, Tl, Th, U	Elemental patterns of wines, grapes, and vineyard soils from Chinese wine-producing regions and their origin association	[49]
Monovarietal wines	ICP-MS	Ba, As, Pb, Mo, and Co	Geographical origin differentiation of Argentinean white wines by their elemental profile	[50]
Spectroscopy				
Wines	SNIF-NMR	Isotopic and trace elements	Characterization of the geographic origin of Bordeaux wines	[51]
Wines	IRMS and SNIF-NMR	Isotopic ratios hydrogen ($^{2}H/^{1}H$), carbon ($^{13}C/^{12}C$), nitrogen ($^{15}N/^{14}N$), oxygen ($^{18}O/^{16}O$)	Regional origin discrimination of Slovenian Wines	[52]
Wines	NMR and MS	Cd, Cr, Cs, Er, Ga, Mn, and Sr	Wine adulterations	[53]
Wines	SNIF-NMR and IRMS in combination with chemometric	Multielement analysis	Geographical origin	[54]

Table 1. *Cont.*

Samples	Analytical Technique	Data Analyzed/Analyte	Purpose of Analysis	References
	Spectroscopy			
Wines authentication	^{1}H NMR, ICP-AES, HPIC	^{1}H and ^{13}C	Classification of wines from Slovenia and from Apulia	[55]
Red wines	MIR	Multielement analysis	Discrimination of wines based on their geographical origin and vintage year	[56]
Red wines	NIR combined with multivariate analysis (PCA, PLS-DA, LDA)	Chemometrics	Geographic classification of Spanish and Australian tempranillo wines	[57]
Sweet wines	F-AAS	Metallic content (Na, K, Ca, Mg, Fe, and Cu)	Classification and geographical differentiation of wines from Canary Islands (Spain)	[58]
	Separation			
Red wines	HPLC, UV, and fluorescence detection	Polyphenol content	Polyphenolic compounds quantification to typify wines according to their geographical origin	[38]
Red wines	HPLC-DAD	Polyphenolic components	Red wines differentiation based on cultivar and geographical origin with application of chemometrics of principal polyphenolic constituents	[59]
Monovarietal wines	HPLC	Non-flavonoid phenolic compounds: hydroxybenzoic acids, hydroxycinnamates, and Stilbenes	Czech Republic wines authentication: Wine discrimination according to the geographical origin	[60]
Red wines	RP-HPLC-DAD-F	Chromatographic profiles and chemometric data analysis	Classification and characterization of Spanish wines according to their appellation of origin.	[61]
Monovarietal red and white wines	SPME-MS and SPME-GC/MS	Volatiles compounds	Differentiation of wines according to grape variety and geographical origin	[62]
Red wines	HPLC	Organic acids (Shikimic and galacturonic acids); plenolic compounds (e.g., alkanes, aldehydes, alcohols, acids).	Varietal and geographic classification of wines according to their geographical origin	[63]
	HS-SPME GC×GC-TOFMS	Volatile compounds		[64]
Red wines	CE	Metals content (Na, K, Ca, Mg, Mn, and Li)	Wines classification according to their geographical origin.	[65]
	Others			
Must and grapes microbiota	DNA	High-throughput sequencing, molecular markers (SSR)	Biogeographical wines characteristics	[66]
Grapevines' fungal communities	DNA	Pyrosequencing of the 26S rRNA gene region	Vine fungi biogeography	[67]
Grape varieties	DNA	Ribosomal ITS region	Geographical region and grape varieties are drivers of population structures of fermentative vineyard-associated *S. cerevisiae* strains	[68]

Table 1. *Cont.*

Samples	Analytical Technique	Data Analyzed/Analyte	Purpose of Analysis	References
	Others			
Grape yeast biota	DNA	RFLP and DNA sequencing	Azorean geographical indications wines: Grape-associated microbial biogeography from five islands of Azores Archipelago	[69]
	Sensory			
Wine	Electronic nose and amperometric electronic tongue	Aroma	Characterization and classification of Italian Barbera wines	[70]
Wine	Electronic nose (fast gas chromatograph)	Aroma profile	Geographical classification of Chilean wines	[71]

IRMS—Isotope Ratio Mass Spectrometry; **ICP-MS**—Inductively coupled plasma mass spectrometry; **ICP-OES**—Inductively coupled plasma optical emission spectroscopy; **NMR**—Nuclear Magnetic Resonance spectroscopy; **SNIF-NMR**—Site-specific Natural Isotopic Fractionation; **FTIR**—Fourier transform Infrared; **MIR**—Mid-infrared spectroscopy; **NIR**—Near-infrared spectroscopy; **IR**—Infrared Spectroscopy; **HPLC-DAD**—High Performance Liquid Chromatography-Diode array detection; **GC**—Gas chromatography; **CE**—Capillary electrophoresis; **PCR-DNA**—Polymerase Chain Reaction based on DNA molecule; **RFLP**—Restriction Fragment Length Polymorphism Analysis; **ITS**—internal transcribed spacer; **SSR-SPME-GC-MS**—solid-phase microextraction-coupled to a gas chromatography-mass spectrometry; **GC-MS**—Gas chromatography mass spectrometry; **UV-VIS**—Ultraviolet and visible spectroscopy; **PCA**—Principal component analysis; **PLS-DA**—discriminant partial least-squares discriminant analysis; **LDA**—linear discriminant analysis; **F-AAS**—Flame-Atomic absorption spectroscopy; **TIMS**—thermal ionization mass spectrometry; **XRD** -ray powder diffraction.

4. DNA Fingerprinting for Varietal Identification

The assessment of a wine traceability and authenticity system embraces a huge and complex DNA-based techniques network (Figure 3) and requires a multidisciplinary analysis, including analytical and molecular validations. Unfortunately, the inconsistencies of the results obtained by analytical assays (e.g., protein, metabolite), due to environmental conditions and processing procedures, makes molecular DNA-based methods the preferred choice when dealing with grapevine varietal identification. In a food authentication molecular approach, there are several critical and important associated research areas, such as sampling and DNA extraction methodology, the development of specific molecular markers, and the sensitivity and suitableness of the detection method, that need to be considered.

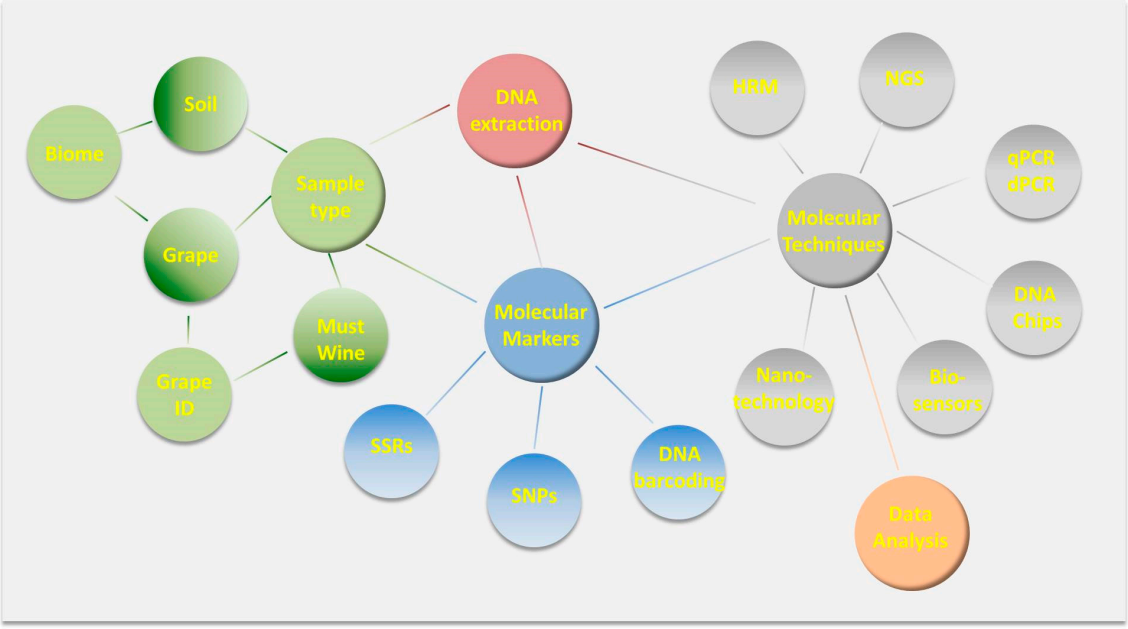

Figure 3. Wine authenticity networks. Diagram highlighting the critical and most important DNA based research areas in wine authentication HRM (high resolution melting), NGS—(next generation sequencing), qPCR/dPCR—quantitative/digital polymerase chain reaction, SSRs (simple sequence repeats), SNPs (single nucleotide polymorphism).

As previously mentioned, wine authenticity relies essentially on the determination of geographical origins and grapevine varietal composition by analytical and molecular methods, with the purpose of confirming the statements in the labels to assure the quality, typicality, and authenticity of the wine. It is well known that climate and biological factors (soil, grapevine variety, and fauna), as well as viticulture and enological procedures, are required to establish the concept of wine terroir. In this context, sampling plays a fundamental role in the wine authenticity network (Figure 3). The determination of the geographical origin is associated with the vineyard soil's isotopic profile by correlating trace elements values in wine and soil samples [28] and microbiome composition. Recent studies highlight the contribution of the autochthonous properties of vineyard microbiota in the winemaking process of a wine from a particular region [32,72].

The microflora of grapes is highly variable, mostly due to the influence of external factors, such as environmental parameters, geographical location, grapevine varieties, and the application of phytochemicals on the vineyards [73]. All these factors are responsible for the final characteristics of the wine, affecting the flavor and aroma attributes and, consequently, its final quality and value. In this case, sampling leaves or berries will provide identification of the grapevine varieties and the native microbial environment for wine authentication [74]. When must and wine samples are used in the authentication process, the recovery of grapevine DNA from such samples is a challenging procedure.

Nevertheless, due to recent technological advancements, it is possible to detect and discriminate the grapevine variety present in such sample types [4,8,75]. In an authenticity system, it is crucial to establish a framework concerning the most suitable sampling and storage conditions regarding the subsequent steps and the final defined purpose (Figure 3). Therefore, it is imperative that all the procedures are well established, producing reproducible results.

Soil, grape, and wine are complex samples that contain many interfering agents for molecular analysis, such as impurities, phenols, acids, metal ions, and salts. Therefore, choosing the best DNA extraction protocols is essential. There are several commercial kits that could be used for DNA extraction from soils, however, the extraction procedure must be adapted to the sample type to produce the best results [76]. DNA extraction from grapevines is well established from any part of the plant [77], and, currently, DNA extraction from the complex must/wine matrices has also been achieved [4,21,78,79]. The DNA extraction efficiency is highly dependent on the wine (e.g., type, age, alcohol percentage, winemaking process) used and therefore the DNA extraction protocol needs to be appropriately designed. In this process, wine properties must be considered, since the presence of natural compounds (e.g., phenolic, polysaccharides) and organic solvents (e.g., phenol/chloroform) used in the DNA extraction protocol may become problematic when proceeding with DNA analysis, as they can interfere with polymerase chain reaction (PCR) amplification. Thus, DNA extraction is one of the main and limiting steps, requiring the establishment of efficient DNA extraction protocols to ensure sufficient DNA yield, which will enable subsequent fingerprint analysis.

High throughput sequencing platforms have recently emerged and have been widely applied in the development of genomic markers. These methods vary in their applicability in terms of the research demands and molecular resources required. DNA markers offer an unequivocal and powerful tool towards a consistent wine authenticity system (Figure 3). The main drawback of DNA based technologies is the DNA degradation/fragmentation observed in processed food-samples and beverages, such as wine [21]. In this context, the quality and length of the DNA fragments retrieved are crucial to establish a proper fingerprint methodology. DNA recovered from wine samples is normally composed of very short length DNA molecules that result from DNA degradation caused by wine fermentation, aging, and storage. Usually, targeted fragments above 200–400 bp, using DNA extracted from must and wine samples as templates, are more difficult to amplify; however, they are still feasible in some regions [80]. When authentication is based on the use of SSR markers, a precise selection of SSR loci must be previously undertaken, considering the discriminative power of the marker (must be high), the molecular weight size range (must be low), the robustness, and the reproducibility. Furthermore, the establishment of a worldwide SSR database, allowing comparison of the results, will reduce the chance of ambiguous varietal identification.

Currently, advancements on sequencing technologies have allowed a wider SNP marker identification between and among different grapevine varieties [24]. SNPs' high abundance and wide distribution through the genome enable the amplification of very small fragments, thus being compatible with DNA recovered from must and wine samples. However, SNPs are, in most cases, biallelic, therefore, to have a reasonable discriminative power among the different profiles, a larger number of SNP markers are required in comparison to SSR markers. For grapevine discrimination, a set of 48 SNPs have been successfully applied to leaf samples [24].

DNA barcoding is useful in certifying both the origin and quality of raw materials, and to detect adulterations in the industrial food chain. In general, DNA barcoding is based on the amplification of short DNA fragments belonging to the mitochondrial (animal foodstuffs) or chloroplast (plant foodstuffs) genomes, which are conserved at the species levels and preserved in most of the processed food products, therefore, being advantageous when compared to other DNA fingerprinting and genotyping approaches. However, DNA barcoding has as its main limit low intraspecific polymorphism, compromising its capacity in distinguishing closely related species [81]. Therefore, the barcode has evolved through the reduction of long barcode regions to short subregions, allowing the

species to still show enough divergence. DNA barcoding represents a well-proven molecular approach to assess the authenticity of food items, although its use is hampered by analytical constraints [82].

Nowadays, technologies based on genomics and bioinformatics approaches are considered the most efficient tools for assessing the genetic authenticity of food products, and, therefore, their incorporation in traceability systems is highly advantageous (Figure 3). Among the DNA-based technologies, High Resolution Melting (HRM) has been shown to be an interesting technology for food authenticity purposes [8]. The recent advances on the instrumentation utilized, as well as on specialized and more efficient fluorescent DNA-binding dyes, have allowed this technique to become a high-throughput screening assay for grapevine varietal identification and wine analysis [8,80,83].

Traditional methods are based on PCR amplification designed for a small number of targets. Usually, this type of approach requires prior knowledge of the target species. The results obtained by direct PCR detection produce presence/absence results for the targeted species, however, no additional information is obtained, such as the presence of other species in the sample. Next generation sequencing (NGS) appears to overcome this drawback. NGS analysis enables the identification of different species in complex food matrices based on the result of a single and unique DNA sequence. Currently, NGS is the only test method that ensures the correct identification of species in complex food matrices by comparison with databases (containing several thousands of species) [84]. Additionally, NGS techniques can also overcome the issue of DNA fragmentation caused during the food processing. An NGS approach can be optimized to target short fragments, thus avoiding false negative results. However, DNA sequences must be informative, resulting in a DNA barcode, since it is a unique identifier. For these reasons, the use of NGS technologies on degraded DNA for authentication purposes would be especially interesting in food analysis [85] and possibly in wine authenticity.

Real-time PCR is still the prime method for food analysis, including pathogen detection, allergens identification, and detection and quantification of different species. High sensitivity, specificity, and reproducibility, and low levels of cross-contamination and reduced analysis time makes real-time PCR an attractive and alternative method to conventional PCR [86]. However, the most important advantage of real-time PCR is its capacity to quantify the starting amount of a specific DNA target. Real-time PCR chemistries are classified into two main groups: Double stranded DNA intercalating molecules or binding dyes, such as SYBR green I and EvaGreen; and fluorophore-labeled oligonucleotides, such as TaqMan probes [86]. Real Time-PCR is being continuously improved on through its instrumentation and chemistry generating better signals, increased sensitivity, short detection times, and excellent stability without causing PCR inhibition. However, there are many challenges yet to be addressed. Recent progresses in RT-PCR analyses includes a new range of fluorescent probe chemistries and nanoparticles owing to their higher sensitivity and short detection times and microfluidic integrations, giving a promising outlook for gene-based point-of-care food analysis at a much lower cost [87]. In the wine sector, quantitative real-time PCR (qPCR) is being applied not only to identify and quantify total yeast population during fermentation and in wine samples to support the terroir concept [88], but also for genetic varietal discrimination and relative quantification in wine samples [22].

Future trends in food analysis will include digital PCR (dPCR). dPCR is an end-point technique that allows absolute quantification without the construction of standard curves. Briefly, the dPCR technique involves sub-dividing the DNA sample (with master-mix) into hundreds to thousands of individual units run concurrently with each other. The individual units are then treated either as negative reactions (no DNA target present) or positive reactions (DNA target presence) [87]. The fraction of negative reactions is used for absolute quantification of the initial DNA concentration of the sample as it follows the Poisson distributions. It is one of the most precise methods when dealing with DNA/RNA quantification. However, since it is a recent technique, further validation is required before it can become a viable replacement of RT-PCR as a standard method for the detection and quantification of DNA/RNA for food analysis.

DNA chips (DNA microarrays) may also be a valuable technique by proving to be a fast, reusable, continuous, selective, and sensitive detection system for fraudulent food products. DNA microarrays involve multiple species-specific oligonucleotide probes to produce distinct fluorescent patterns for the identification of different species providing a unique barcode fluorescent pattern for each species, enabling an effective food product authentication [89]. In wine research, DNA microarrays have been used is several studies, namely for the screening of wine yeast strains [90]. DNA chips are a promising technology that could enable the identification of yeast or bacteria strains linked to a specific region and winemaking practices. Furthermore, microarray technique has been applied to olive cultivars to assure olive oil authenticity and other food matrices [89,91].

DNA nanotechnology emerges as a powerful and growing research area in several fields, including food authenticity [92]. This new and promising technology functionally integrates DNA molecule and/or other nucleic acids with nanoparticles in different physicochemical forms to produce a range of composites with unique properties. These capabilities are attracting attention from food control research communities in pursuit of new applications, including (bio) sensing and labeling tools for the food sector, especially concerning safety and authenticity purposes [82,93]. The development of biosensors in response to this demand is seemingly promising [94]. Recent studies report the potential of a DNA-based biosensor for grapevine discrimination purposes [26,27,95]. This specific type of biosensor uses DNA strands as probes for sensing DNA targets and was developed based on the ability of single-strand DNA molecules to recognize and bind to their complementary strands in a sample. Using it as a transducer functionalized with the single-stranded DNA molecules, the biosensor can respond to alterations in the refractive index of the fiber's surrounding medium generated by analyte binding, and it will detect these interactions [96]. The biosensor proved to be able to distinguish specific grapevine varieties through the detection of small variations in a certain region of their genome using not only synthetic oligonucleotides, but also genomic DNA extracted from leaf, must, and wine samples [27]. The results are promising and show the potential of this technology to be applied to grapevine varietal fingerprinting throughout the wine-chain, analyzing DNA with different levels of contamination from matrices subjected to different processing levels, without the requirement of any labelling or PCR step [26,27].

The resulting data acquired from the above-mentioned DNA-based technologies require the application of various data analysis methods so the several datasets can be made understandable. The acquired data are complex and, therefore, to have a more comprehensive analysis, a multi-disciplinary approach using bioinformatics and data mining resources is required.

All scientific DNA methodologies/techniques presented herein offer a wide range of possibilities for the establishment of an accurate wine authentication system (Table 2). The analytical/molecular analysis, supported by scientific knowledge, current regulations, and by internationally documented quality standards, is required to protect consumers against fraudulent practices and ensure brand fair trade. Nevertheless, a continuous research effort is essential to address emerging wine origin/quality issues.

Table 2. Summary of the pros and cons of the DNA based techniques applied to wine authentication.

Method	Pros	Cons
HRM	• closed-tube method avoiding contaminations • high sensitivity • PCR products are analyzed without gels and hazardous chemicals • fast • data analysis can be performed automatically in a few minutes • allows a good species identification and differentiation • allow a high number of samples	• need for high-quality DNA extracts • dependent on the extraction method (presence of PCR inhibitors) • the results only produce presence/absence results for the targeted species • no quantification of nucleic acids occurs • no DNA quantification is performed • careful primer design required • specific software required
qPCR	• enables quantification of target DNA • measures PCR amplification (quantification of nucleic acids) as it occurs • no post-PCR processing	• is not used to identify the geographical origin of products, type of processing, or addition of chemical adulterant • Specific equipment required • Specific types of chemistries required (Taqman, SYBR green)
dPCR	• provides an absolute quantification of nucleic acids • more accurate and sensitive measurement of the number of copies of target DNA, especially for low concentration and mixed samples • ability to analyze samples containing species mixtures with high sensitivity and in a single trial, performing multiple reactions in parallel • can be performed in microarray format, which can potentially increase the sensitivity • efficient even if the copy number of the target is low and/or PCR inhibitors are present • no need to rely on references or standards	• low equipment offer • specific and expensive equipment required
NGS	• ensures the correct and unambiguous detection and identification of species • allows untargeted detection of thousands of organisms with no requirement for previous knowledge of the sample	• databases required • damage DNA requires a unique identifier (DNA barcode) • only provides relative information on the abundance of each species • specific equipment and software for data analysis required • technically challenging
Biosensors **Nanotechnology** **DNA chips**	• These items are being continuous developed for authenticity purposes. Future devices must link high performance (particularly high sensitivity and selectivity), higher number of samples, sequencing-free, faster detection, miniaturization, portability, and low cost. The design of such powerful devices requires innovative efforts, combining fundamental biological, chemical, and material sciences.	
DNA markers	• allow species identification and differentiation • stability under environmental conditions and production procedures • reliable and accurate for botanical and geographical origin	

Table 2. *Cont.*

Method	Pros	Cons
SSR	• high specificity allowing unequivocal species identification and differentiation • high reproducibility • highly informative	• labelled primers required • large consumable requirement • sequencer required • limited targets • databases required
SNP	• highly informative • high frequency of occurrence • highly reproducible • the analysis can be automated • allow species identification and differentiation according to the target DNA	• primer design required • relatively expensive • specific equipment required • databases required
DNA barcoding	• highly informative • allow species identification and differentiation • use short DNA sequences from the standard part of the genome for species identification overcoming DNA fragmentation	• careful primer design required • only provide insights into species-level • databases required
Data analysis	• integrate a huge amount of biological data through data mining approaches and exploit such information by identifying statistically informative annotations	• specialized laboratories and equipment's required • skilled personnel required • databases required

5. Conclusions and Future Trends

The general food industry is searching for alternative methods applied to food monitoring and authentication. The implemented wine traceability system is not capable of efficiently controlling the production chain, and therefore requires urgent measures to reassure producers, retailers, and consumers against fraudulent practice. The development of alternative technological solutions supporting this have emerged throughout the years, giving a new insight into the sector. However, none of the developed technologies can tackle the authentication of the wine terroir in all its dimensions. Nonetheless, a multidisciplinary approach can be developed, aiming to tackle the main features of the terroir (geographical origin and grapevine varietal origin). Some of the possible technological approaches have been presented and should be considered in the future so that a robust traceability system may be designed for the wine sector.

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References

1. Arvanitoyannis, I.S. Wine authenticity, traceability and safety monitoring. In *Managing Wine Quality*; Reynolds, A., Ed.; Woodhead: New York, NY, USA, 2010; pp. 218–270, ISBN 978-1-84569-484-5.
2. Versari, A.; Laurie, V.F.; Ricci, A.; Laghi, L.; Parpinello, G.P. Progress in authentication, typification and traceability of grapes and wines by chemometric approaches. *Food Res. Int.* **2014**, *60*, 2–18. [CrossRef]
3. Palade, M.; Popa, M.-E. Wine traceability and authenticity—A literature review. *Sci. Bull. Ser. F Biotechnol.* **2014**, *18*, 226–233.
4. Pereira, L.; Martins-Lopes, P.; Batista, C.; Zanol, G.C.; Clímaco, P.; Brazão, J.; Eiras-Dias, J.E.; Guedes-Pinto, H. Molecular markers for assessing must varietal origin. *Food Anal. Methods* **2012**, *5*, 1252–1259. [CrossRef]
5. Moreno-Arribas, M.V.; Polo, M.C. Winemaking biochemistry and microbiology: Current knowledge and future trends. *Crit. Rev. Food Sci. Nutr.* **2005**, *45*, 265–286. [CrossRef] [PubMed]
6. Vukatana, K.; Sevrani, K.; Hoxha, E. Wine traceability: A data model and prototype in albanian context. *Foods* **2016**, *5*, 11. [CrossRef] [PubMed]
7. Villano, C.; Lisanti, M.T.; Gambuti, A.; Vecchio, R.; Moio, L.; Frusciante, L.; Aversano, R.; Carputo, D. Wine varietal authentication based on phenolics, volatiles and DNA markers: State of the art, perspectives and drawbacks. *Food Control* **2017**, *80*, 1–10. [CrossRef]
8. Pereira, L.; Gomes, S.; Barrias, S.; Fernandes, J.R.; Martins-Lopes, P. Applying high-resolution melting (HRM) technology to olive oil and wine authenticity. *Food Res. Int.* **2018**, *103*, 170–181. [CrossRef] [PubMed]
9. Sotirchos, D.G.; Danezis, G.P.; Georgiou, C.A. Introduction, Definitions and Legislation. In *Food Authentication: Management, Analysis and Regulation*; Georgiou, C.A., Danezis, G.P., Eds.; Wiley-Blackwell: West Sussex, UK, 2017; pp. 3–18, ISBN 978-1-118-81026-2.
10. OIV. *International Standard for the Labelling of Wines*; Organisation Internationale de la Vigne et du Vin: Paris, France, 2015.
11. Vineyards. Available online: https://vineyards.com (accessed on 31 July 2018).
12. OIV. *Distribution of the World's Grapevine Varieties*; Organisation Internationale de la Vigne et du Vin: Paris, France, 2017.
13. Danezis, G.P.; Tsagkaris, A.S.; Camin, F.; Brusic, V.; Georgiou, C.A. Food authentication: Techniques, trends & emerging approaches. *Trends Anal. Chem.* **2016**, *85*, 123–132. [CrossRef]
14. Basalekou, M.; Stratidaki, A.; Pappas, C.; Tarantilis, P.; Kotseridis, Y.; Kallithraka, S. Authenticity determination of greek-cretan mono-varietal white and red wines based on their phenolic content using Attenuated Total Reflectance Fourier Transform Infrared spectroscopy and chemometrics. *Curr. Res. Nutr. Food Sci. J.* **2016**, *4*, 54–62. [CrossRef]

15. Lohumi, S.; Lee, S.; Lee, H.; Cho, B.-K. A review of vibrational spectroscopic techniques for the detection of food authenticity and adulteration. *Trends Food Sci. Technol.* **2015**, *46*, 85–98. [CrossRef]
16. Perestrelo, R.; Silva, C.; Câmara, J.S. A useful approach for the differentiation of wines according to geographical origin based on global volatile patterns. *J. Sep. Sci.* **2014**, *37*, 1974–1981. [CrossRef] [PubMed]
17. Duchowicz, P.R.; Giraudo, M.A.; Castro, E.A.; Pomilio, A.B. Amino acid profiles and quantitative structure-property relationship models as markers for Merlot and Torrontés wines. *Food Chem.* **2013**, *140*, 210–216. [CrossRef] [PubMed]
18. Rešetar, D.; Marchetti-Deschmann, M.; Allmaier, G.; Katalinić, J.P.; Kraljević Pavelić, S. Matrix assisted laser desorption ionization mass spectrometry linear time-of-flight method for white wine fingerprinting and classification. *Food Control* **2016**, *64*, 157–164. [CrossRef]
19. González-Neves, G.; Favre, G.; Piccardo, D.; Gil, G. Anthocyanin profile of young red wines of Tannat, Syrah and Merlot made using maceration enzymes and cold soak. *Int. J. Food Sci. Technol.* **2016**, *51*, 260–267. [CrossRef]
20. Kokkinofta, R.; Fotakis, C.; Zervou, M.; Zoumpoulakis, P.; Savvidou, C.; Poulli, K.; Louka, C.; Economidou, N.; Tzioni, E.; Damianou, K.; et al. Isotopic and elemental authenticity markers: A case study on cypriot wines. *Food Anal. Methods* **2017**, *10*, 3902–3913. [CrossRef]
21. Pereira, L.; Guedes-Pinto, H.; Martins-Lopes, P. An enhanced method for *Vitis vinifera* L. DNA extraction from wines. *Am. J. Enol. Vitic.* **2011**, *62*, 547–552. [CrossRef]
22. Catalano, V.; Moreno-Sanz, P.; Lorenzi, S.; Grando, M.S. Experimental review of DNA-based methods for wine traceability and development of a single-nucleotide polymorphism (SNP) genotyping assay for quantitative varietal authentication. *J. Agric. Food Chem.* **2016**, *64*, 6969–6984. [CrossRef] [PubMed]
23. OIV. *2nd Edition of the OIV Descriptor List for Grape Varieties and Vitis Species*; OIV: Paris, France, 2007.
24. Cabezas, J.A.; Ibáñez, J.; Lijavetzky, D.; Vélez, D.; Bravo, G.; Rodríguez, V.; Carreño, I.; Jermakow, A.M.; Carreño, J.; Ruiz-García, L.; et al. A 48 SNP set for grapevine cultivar identification. *BMC Plant Biol.* **2011**, *11*, 153. [CrossRef] [PubMed]
25. REGULATION (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. *Off. J. Eur. Commun.* **2002**, *31*, 1–24.
26. Gomes, S.; Castro, C.; Barrias, S.; Pereira, L.; Jorge, P.; Fernandes, J.R.; Martins-Lopes, P. Alternative SNP detection platforms, HRM and biosensors, for varietal identification in *Vitis vinifera* L. using F3H and LDOX genes. *Sci. Rep.* **2018**, *8*, 5850. [CrossRef] [PubMed]
27. Barrias, S.; Fernandes, J.R.; Eiras-Dias, J.E.; Brazão, J.; Martins-Lopes, P. Label free DNA-based optical biosensor as a potential system for wine authenticity. *Food Chem.* **2019**, *270*, 299–304. [CrossRef] [PubMed]
28. Martins, P.; Madeira, M.; Monteiro, F.; Bruno de Sousa, R.; Curvelo-Garcia, A.S.; Catarino, S. $^{87}Sr/^{86}Sr$ ratio in vineyard soils from Portuguese denominations of origin and its potential for origin authentication. *J. Int. Sci. Vigne Vin* **2014**, *48*, 21–29. [CrossRef]
29. Esslinger, S.; Riedl, J.; Fauhl-Hassek, C. Potential and limitations of non-targeted fingerprinting for authentication of food in official control. *Food Res. Int.* **2014**, *60*, 189–204. [CrossRef]
30. Fang, F.; Li, J.-M.; Zhang, P.; Tang, K.; Wang, W.; Pan, Q.-H.; Huang, W.-D. Effects of grape variety, harvest date, fermentation vessel and wine ageing on flavonoid concentration in red wines. *Food Res. Int.* **2008**, *41*, 53–60. [CrossRef]
31. Fernandes, J.R.; Pereira, L.; Jorge, P.; Moreira, L.; Gonçalves, H.; Coelho, L.; Alexandre, D.; Eiras-Dias, J.; Brazão, J.; Clímaco, P.; et al. Wine fingerprinting using a bio-geochemical approach. *BIO Web Conf.* **2015**, *5*, 02021. [CrossRef]
32. Bokulich, N.A.; Collins, T.S.; Masarweh, C.; Allen, G.; Heymann, H.; Ebeler, S.E.; Mills, D.A. Associations among wine grape microbiome, metabolome, and fermentation behavior suggest microbial contribution to regional wine characteristics. *MBio* **2016**, *7*, e00631-16. [CrossRef] [PubMed]
33. Vinciguerra, V.; Stevenson, R.; Pedneault, K.; Poirier, A.; Hélie, J.-F.; Widory, D. Strontium isotope characterization of wines from Quebec, Canada. *Food Chem.* **2016**, *210*, 121–128. [CrossRef] [PubMed]
34. Belda, I.; Zarraonaindia, I.; Perisin, M.; Palacios, A.; Acedo, A. From vineyard soil to wine fermentation: microbiome approximations to explain the "terroir" concept. *Front. Microbiol.* **2017**, *8*, 821. [CrossRef] [PubMed]

35. Luykx, D.M.A.M.; van Ruth, S.M. An overview of analytical methods for determining the geographical origin of food products. *Food Chem.* **2008**, *107*, 897–911. [CrossRef]
36. Petrini, R.; Sansone, L.; Slejko, F.F.; Buccianti, A.; Marcuzzo, P.; Tomasi, D. The $^{87}Sr/^{86}Sr$ strontium isotopic systematics applied to Glera vineyards: A tracer for the geographical origin of the Prosecco. *Food Chem.* **2015**, *170*, 138–144. [CrossRef] [PubMed]
37. Kelly, S.; Heaton, K.; Hoogewerff, J. Tracing the geographical origin of food: The application of multi-element and multi-isotope analysis. *Trends Food Sci. Technol.* **2005**, *16*, 555–567. [CrossRef]
38. Rodríguez-Delgado, M.-Á.; González-Hernández, G.; Conde-González, J.-E.; Pérez-Trujillo, J.-P. Principal component analysis of the polyphenol content in young red wines. *Food Chem.* **2002**, *78*, 523–532. [CrossRef]
39. Nakano, T. Potential uses of stable isotope ratios of Sr, Nd, and Pb in geological materials for environmental studies. *Proc. Jpn. Acad. Ser. B* **2016**, *92*, 167–184. [CrossRef] [PubMed]
40. Day, M.P.; Zhang, B.; Martin, G.J. Determination of the geographical origin of wine using joint analysis of elemental and isotopic composition. II—Differentiation of the principal production zones in france for the 1990 vintage. *J. Sci. Food Agric.* **1995**, *67*, 113–123. [CrossRef]
41. Braschi, E.; Marchionni, S.; Priori, S.; Casalini, M.; Tommasini, S.; Natarelli, L.; Buccianti, A.; Bucelli, P.; Costantini, E.A.C.; Conticelli, S. Tracing the $^{87}Sr/^{86}Sr$ from rocks and soils to vine and wine: An experimental study on geologic and pedologic characterisation of vineyards using radiogenic isotope of heavy elements. *Sci. Total Environ.* **2018**, *628–629*, 1317–1327. [CrossRef] [PubMed]
42. Marchionni, S.; Buccianti, A.; Bollati, A.; Braschi, E.; Cifelli, F.; Molin, P.; Parotto, M.; Mattei, M.; Tommasini, S.; Conticelli, S. Conservation of $^{87}Sr/^{86}Sr$ isotopic ratios during the winemaking processes of "Red" wines to validate their use as geographic tracer. *Food Chem.* **2016**, *190*, 777–785. [CrossRef] [PubMed]
43. Martinelli, L.A.; Moreira, M.Z.; Ometto, J.P.H.B.; Alcarde, A.R.; Rizzon, L.A.; Stange, E.; Ehleringer, J.R. Stable carbon isotopic composition of the wine and CO_2 bubbles of sparkling wines: Detecting C_4 sugar additions. *J. Agric. Food Chem.* **2003**, *51*, 2625–2631. [CrossRef] [PubMed]
44. Marchionni, S.; Braschi, E.; Tommasini, S.; Bollati, A.; Cifelli, F.; Mulinacci, N.; Mattei, M.; Conticelli, S. High-precision $^{87}Sr/^{86}Sr$ analyses in wines and their use as a geological fingerprint for tracing geographic provenance. *J. Agric. Food Chem.* **2013**, *61*, 6822–6831. [CrossRef] [PubMed]
45. Durante, C.; Baschieri, C.; Bertacchini, L.; Bertelli, D.; Cocchi, M.; Marchetti, A.; Manzini, D.; Papotti, G.; Sighinolfi, S. An analytical approach to Sr isotope ratio determination in Lambrusco wines for geographical traceability purposes. *Food Chem.* **2015**, *173*, 557–563. [CrossRef] [PubMed]
46. Coetzee, P.P.; Steffens, F.E.; Eiselen, R.J.; Augustyn, O.P.; Balcaen, L.; Vanhaecke, F. Multi-element analysis of South African wines by ICP−MS and their classification according to geographical origin. *J. Agric. Food Chem.* **2005**, *53*, 5060–5066. [CrossRef] [PubMed]
47. Cabrita, M.J.; Martins, N.; Barrulas, P.; Garcia, R.; Dias, C.B.; Pérez-Álvarez, E.P.; Costa Freitas, A.M.; Garde-Cerdán, T. Multi-element composition of red, white and palhete amphora wines from Alentejo by ICPMS. *Food Control* **2018**, *92*, 80–85. [CrossRef]
48. Fan, S.; Zhong, Q.; Gao, H.; Wang, D.; Li, G.; Huang, Z. Elemental profile and oxygen isotope ratio ($\delta^{18}O$) for verifying the geographical origin of Chinese wines. *J. Food Drug Anal.* **2018**, *26*, 1033–1044. [CrossRef] [PubMed]
49. Zou, J.-F.; Peng, Z.-X.; Du, H.-J.; Duan, C.-Q.; Reeves, M.J.; Pan, Q.-H. Elemental patterns of wines, grapes, and vineyard soils from Chinese wine-producing regions and their association. *Am. J. Enol. Vitic.* **2012**, *63*, 232–240. [CrossRef]
50. Azcarate, S.M.; Martinez, L.D.; Savio, M.; Camiña, J.M.; Gil, R.A. Classification of monovarietal Argentinean white wines by their elemental profile. *Food Control* **2015**, *57*, 268–274. [CrossRef]
51. Martin, G.J.; Mazure, M.; Jouitteau, C.; Martin, Y.-L.; Aguile, L.; Allain, P. Characterization of the geographic origin of Bordeaux wines by a combined use of isotopic and trace element Measurements. *Am. J. Enol. Vitic.* **1999**, *50*, 409–417.
52. Ogrinc, N.; Košir, I.J.; Kocjančič, M.; Kidrič, J. Determination of authenticy, regional origin, and vintage of Slovenian wines using a combination of IRMS and SNIF-NMR analyses. *J. Agric. Food Chem.* **2001**, *49*, 1432–1440. [CrossRef] [PubMed]
53. Ogrinc, N.; Košir, I.J.; Spangenberg, J.E.; Kidrič, J. The application of NMR and MS methods for detection of adulteration of wine, fruit juices, and olive oil. A review. *Anal. Bioanal. Chem.* **2003**, *376*, 424–430. [CrossRef] [PubMed]

54. Košir, I.J.; Kocjančič, M.; Ogrinc, N.; Kidrič, J. Use of SNIF-NMR and IRMS in combination with chemometric methods for the determination of chaptalisation and geographical origin of wines (the example of Slovenian wines). *Anal. Chim. Acta* **2001**, *429*, 195–206. [CrossRef]
55. Brescia, M.A.; Košir, I.J.; Caldarola, V.; Kidrič, J.; Sacco, A. Chemometric classification of apulian and slovenian wines using ^{1}H NMR and ICP-OES together with HPICE data. *J. Agric. Food Chem.* **2003**, *51*, 21–26. [CrossRef] [PubMed]
56. Picque, D.; Cattenoz, T.; Corrieu, G.; Berger, J. Discrimination of red wines according to their geographical origin and vintage year by the use of mid-infrared spectroscopy. *Sci. Aliments* **2005**, *25*, 207–220. [CrossRef]
57. Liu, L.; Cozzolino, D.; Cynkar, W.U.; Gishen, M.; Colby, C.B. Geographic classification of spanish and australian Tempranillo red wines by visible and near-infrared spectroscopy combined with multivariate analysis. *J. Agric. Food Chem.* **2006**, *54*, 6754–6759. [CrossRef] [PubMed]
58. Frías, S.; Trujillo, J.P.; Peña, E.; Conde, J.E. Classification and differentiation of bottled sweet wines of Canary Islands (Spain) by their metallic content. *Eur. Food Res. Technol.* **2001**, *213*, 145–149. [CrossRef]
59. Makris, D.P.; Kallithraka, S.; Mamalos, A. Differentiation of young red wines based on cultivar and geographical origin with application of chemometrics of principal polyphenolic constituents. *Talanta* **2006**, *70*, 1143–1152. [CrossRef] [PubMed]
60. Pavloušek, P.; Kumšta, M. Authentication of Riesling wines from the Czech Republic on the basis of the non-flavonoid phenolic compounds. *Czech J. Food Sci.* **2013**, *31*, 474–482. [CrossRef]
61. Serrano-Lourido, D.; Saurina, J.; Hernández-Cassou, S.; Checa, A. Classification and characterisation of Spanish red wines according to their appellation of origin based on chromatographic profiles and chemometric data analysis. *Food Chem.* **2012**, *135*, 1425–1431. [CrossRef] [PubMed]
62. Ziółkowska, A.; Wąsowicz, E.; Jeleń, H.H. Differentiation of wines according to grape variety and geographical origin based on volatiles profiling using SPME-MS and SPME-GC/MS methods. *Food Chem.* **2016**, *213*, 714–720. [CrossRef] [PubMed]
63. Etièvant, P.; Schlich, P.; Cantagrel, R.; Bertrand, M.; Bouvier, J.-C. Varietal and geographic classification of french red wines in terms of major acids. *J. Sci. Food Agric.* **1989**, *46*, 421–438. [CrossRef]
64. Robinson, A.L.; Adams, D.O.; Boss, P.K.; Heymann, H.; Solomon, P.S.; Trengove, R.D. Influence of geographic origin on the sensory characteristics and wine composition of *Vitis vinifera* cv. Cabernet Sauvignon wines from Australia. *Am. J. Enol. Vitic.* **2012**, *63*, 467–476. [CrossRef]
65. Peng, Y.; Liu, F.; Ye, J. Quantitative and qualitative analysis of flavonoid markers in Frucus aurantii of different geographical origin by capillary electrophoresis with electrochemical detection. *J. Chromatogr. B* **2006**, *830*, 224–230. [CrossRef] [PubMed]
66. Bokulich, N.A.; Ohta, M.; Richardson, P.M.; Mills, D.A. Monitoring seasonal changes in winery-resident microbiota. *PLoS ONE* **2013**, *8*, e66437. [CrossRef] [PubMed]
67. Taylor, M.W.; Tsai, P.; Anfang, N.; Ross, H.A.; Goddard, M.R. Pyrosequencing reveals regional differences in fruit-associated fungal communities. *Environ. Microbiol.* **2014**, *16*, 2848–2858. [CrossRef] [PubMed]
68. Schuller, D.; Cardoso, F.; Sousa, S.; Gomes, P.; Gomes, A.C.; Santos, M.A.S.; Casal, M. Genetic diversity and population structure of Saccharomyces cerevisiae strains isolated from different grape varieties and winemaking regions. *PLoS ONE* **2012**, *7*, e32507. [CrossRef] [PubMed]
69. Drumonde-Neves, J.; Franco-Duarte, R.; Lima, T.; Schuller, D.; Pais, C. Association between grape yeast communities and the vineyard ecosystems. *PLoS ONE* **2017**, *12*, e0169883. [CrossRef] [PubMed]
70. Buratti, S.; Benedetti, S.; Scampicchio, M.; Pangerod, E.C. Characterization and classification of Italian Barbera wines by using an electronic nose and an amperometric electronic tongue. *Anal. Chim. Acta* **2004**, *525*, 133–139. [CrossRef]
71. Beltrán, N.H.; Duarte-Mermoud, M.A.; Muñoz, R.E. Geographical classification of Chilean wines by an electronic nose. *Int. J. Wine Res.* **2009**, *2009*, 209–219. [CrossRef]
72. Knight, S.; Klaere, S.; Fedrizzi, B.; Goddard, M.R. Regional microbial signatures positively correlate with differential wine phenotypes: Evidence for a microbial aspect to terroir. *Sci. Rep.* **2015**, *5*, 14233. [CrossRef] [PubMed]
73. Pinto, C.; Pinho, D.; Cardoso, R.; Custódio, V.; Fernandes, J.; Sousa, S.; Pinheiro, M.; Egas, C.; Gomes, A.C. Wine fermentation microbiome: A landscape from different Portuguese wine appellations. *Front. Microbiol.* **2015**, *6*, 905. [CrossRef] [PubMed]

74. Mezzasalma, V.; Ganopoulos, I.; Galimberti, A.; Cornara, L.; Ferri, E.; Labra, M. Poisonous or non-poisonous plants? DNA-based tools and applications for accurate identification. *Int. J. Leg. Med.* **2017**, *131*, 1–19. [CrossRef] [PubMed]
75. Bigliazzi, J.; Scali, M.; Paolucci, E.; Cresti, M.; Vignani, R. DNA extracted with optimized protocols can be genotyped to reconstruct the varietal composition of monovarietal wines. *Am. J. Enol. Vitic.* **2012**, *63*, 568–573. [CrossRef]
76. Fatima, F.; Pathak, N.; Rastogi Verma, S. An improved method for soil DNA extraction to study the microbial assortment within rhizospheric region. *Mol. Biol. Int.* **2014**, *2014*. [CrossRef] [PubMed]
77. Lodhi, M.A.; Ye, G.-N.; Weeden, N.F.; Reisch, B.I. A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. *Plant Mol. Biol. Rep.* **1994**, *12*, 6–13. [CrossRef]
78. Baleiras-Couto, M.M.; Eiras-Dias, J.E. Detection and identification of grape varieties in must and wine using nuclear and chloroplast microsatellite markers. *Anal. Chim. Acta* **2006**, *563*, 283–291. [CrossRef]
79. Işçi, B.; Kalkan Yildirim, H.; Altindisli, A. Evaluation of methods for DNA extraction from must and wine. *J. Inst. Brew.* **2014**, *120*, 238–243. [CrossRef]
80. Pereira, L.; Gomes, S.; Castro, C.; Eiras-Dias, J.E.; Brazão, J.; Graça, A.; Fernandes, J.R.; Martins-Lopes, P. High Resolution Melting (HRM) applied to wine authenticity. *Food Chem.* **2017**, *216*, 80–86. [CrossRef] [PubMed]
81. Barcaccia, G.; Lucchin, M.; Cassandro, M. DNA barcoding as a molecular tool to track down mislabeling and food piracy. *Diversity* **2015**, *8*, 2. [CrossRef]
82. Valentini, P.; Galimberti, A.; Mezzasalma, V.; De Mattia, F.; Casiraghi, M.; Labra, M.; Pompa, P.P. DNA barcoding meets nanotechnology: Development of a universal colorimetric test for food authentication. *Angew. Chem. Int. Ed.* **2017**, *56*, 8094–8098. [CrossRef] [PubMed]
83. Pereira, L.; Martins-Lopes, P. *Vitis vinifera* L. Single-nucleotide polymorphism detection with high-resolution melting analysis based on the UDP-glucose:Flavonoid 3-O-Glucosyltransferase gene. *J. Agric. Food Chem.* **2015**, *63*, 9165–9174. [CrossRef] [PubMed]
84. Giusti, A.; Armani, A.; Sotelo, C.G. Advances in the analysis of complex food matrices: Species identification in surimi-based products using next generation sequencing technologies. *PLoS ONE* **2017**, *12*, e0185586. [CrossRef] [PubMed]
85. Tillmar, A.O.; Dell'Amico, B.; Welander, J.; Holmlund, G. A universal method for species identification of mammals utilizing next generation sequencing for the analysis of DNA mixtures. *PLoS ONE* **2013**, *8*, e83761. [CrossRef] [PubMed]
86. Navarro, E.; Serrano-Heras, G.; Castaño, M.J.; Solera, J. Real-time PCR detection chemistry. *Clin. Chim. Acta* **2015**, *439*, 231–250. [CrossRef] [PubMed]
87. Salihah, N.T.; Hossain, M.M.; Lubis, H.; Ahmed, M.U. Trends and advances in food analysis by real-time polymerase chain reaction. *J. Food Sci. Technol.* **2016**, *53*, 2196–2209. [CrossRef] [PubMed]
88. Wang, C.; García-Fernández, D.; Mas, A.; Esteve-Zarzoso, B. Fungal diversity in grape must and wine fermentation assessed by massive sequencing, quantitative PCR and DGGE. *Front. Microbiol.* **2015**, *6*, 1156. [CrossRef] [PubMed]
89. Scarano, D.; Rao, R. DNA markers for food products authentication. *Diversity* **2014**, *6*, 579–596. [CrossRef]
90. Mendes-Ferreira, A.; lí del Olmo, M.; García-Martínez, J.; Pérez-Ortín, J.E. Functional genomics in wine yeast: DNA arrays and next generation sequencing. In *Biology of Microorganisms on Grapes, in Must and in Wine*; König, H., Unden, G., Fröhlich, J., Eds.; Springer International Publishing: Cham, Switzerland, 2017; pp. 573–604, ISBN 978-3-319-60021-5. [CrossRef]
91. Consolandi, C.; Palmieri, L.; Severgnini, M.; Maestri, E.; Marmiroli, N.; Agrimonti, C.; Baldoni, L.; Donini, P.; De Bellis, G.; Castiglioni, B. A procedure for olive oil traceability and authenticity: DNA extraction, multiplex PCR and LDR–universal array analysis. *Eur. Food Res. Technol.* **2008**, *227*, 1429–1438. [CrossRef]
92. Nummelin, S.; Kommeri, J.; Kostiainen, M.A.; Linko, V. Evolution of structural DNA nanotechnology. *Adv. Mater.* **2018**, *30*, 1703721. [CrossRef] [PubMed]
93. Stephen Inbaraj, B.; Chen, B.H. Nanomaterial-based sensors for detection of foodborne bacterial pathogens and toxins as well as pork adulteration in meat products. *J. Food Drug Anal.* **2016**, *24*, 15–28. [CrossRef] [PubMed]
94. Mehrotra, P. Biosensors and their applications—A review. *J. Oral Biol. Craniofacial Res.* **2016**, *6*, 153–159. [CrossRef] [PubMed]

95. Gonçalves, H.M.R.; Moreira, L.; Pereira, L.; Jorge, P.; Gouveia, C.; Martins-Lopes, P.; Fernandes, J.R.A. Biosensor for label-free DNA quantification based on functionalized LPGs. *Biosens. Bioelectron.* **2016**, *84*, 30–36. [CrossRef] [PubMed]
96. Moreira, L.; Gonçalves, H.M.R.; Pereira, L.; Castro, C.; Jorge, P.; Gouveia, C.; Fernandes, J.R.; Martins-Lopes, P. Label-free optical biosensor for direct complex DNA detection using *Vitis vinifera* L. *Sens. Actuators B Chem.* **2016**, *234*, 92–97. [CrossRef]