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Authenticity and chemometrics of wine and beer

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Abstract

In these days, the agro-food economy is focused on the consumer demands regarding safety, quality and security of food and especially of wine products. Authenticity of wine has been extensively examined as wine is an easily adulterated product as it has high alcohol content and low pH and also is available worldwide. For these reasons, continuous controls are required to maintain the quality of wine. Usually, volatile compounds are used to characterize varieties, minerals are used for geographical differentiation whereas amino acids as well as phenolic compounds evaluation are used for both. There is a necessity to be developed advanced techniques for wines authentication and this is the challenge for the researchers, who currently are given a special focus. Wine adulteration for both variety and origin has been very extensive. Therefore, apart from novel experimental techniques (GC-MS, ICP-MS, ¹³C NMR and FT-IR among others) it is also used Multivariate Analysis comprising Principal Component Analysis (PCA), Discriminant Analysis (DA), Canonical Analysis (CA), Cluster Analysis (CLA).

Beer is a very common alcoholic beverage which comes in different types. Its authenticity has always received a lot attention of the scientific literature. This resulted in numerous studies focused on the classification of beers, according to different types, brands and factories, based on the determination of characteristic compounds or chemical, physical fingerprint in beers by various analytical instruments such as vibrational spectroscopy, fluorescence, mass

spectroscopy nuclear magnetic resonance etc. In this review, the beer authenticity & traceability studies are categorised according to their objective, from less challenging to more challenging, the determination of type and brewing process, the determination of brand identity, the determination of origin and ultimately, the determination of the style of beer (craft vs conventional).

Beer authenticity is a multivariate problem that needs advanced numerical (chemometrics). The literature review identified that the number of samples and the study design need to be improved, the quality of the chemometrics methods needs to be elevated. More work needs to be in getting the chemometric models out of the lab and into the real world, with academia and industry working closely in larger studies and big data. The areas that are still under investigation together with other proposals are also discussed thoroughly in this review.

1. Authenticity & Chemometrics of Wine

1.1 Introduction

The authenticity of wines is a critical issue of great international interest. In many countries, specific quality control programs are mandatory to protect consumers from unintentional errors occurring during wine production, as well as to reduce counterfeiting. For this reason, advanced analytical techniques have been developed, for the measurement of trace elements or the simultaneous identification of a wide range of analyzers, for better data analysis and improved means for the traceability of wine, and the distinction and classification of grapes and wines (mainly in terms of grape varieties, geographical origin and processing conditions) (Versari et al., 2014).

The general term "fraud" refers to different types of fraud, such as diluting wines with water, adding alcohol, dyes and flavourings, mixing or substituting lower quality wines, affixing a false label (variety and geographical origin) and the addition of exogenous sugars to increase the alcoholic strength (Holmberg, 2010). However, this is a complex issue, as wine legislation varies by country.

Unfortunately, regulations, good winemaking practices and traceability procedures are not always sufficient to guarantee the authenticity of a given product, so one of the main challenges to wine authenticity is the need for accurate reference materials and standards that can be used to prove the identity of the wine (Gary et al., 2011). For this reason, it is necessary to develop appropriate analytical approaches for the analysis of the chemical composition and its changes related to adulteration in wines. The most common analytical methods are based on the creation of trace element profiles, phenolic compounds, volatile compounds and isotopes, using different chromatographic and spectroscopic techniques (Vaclavik et al., 2011). In particular, volatile compounds are used to characterize varieties, trace elements are used to distinguish wines geographically, and phenolic compounds are used for both (variety and origin) (Cuadros-Inostroza et al., 2010), (Figure 1.1).

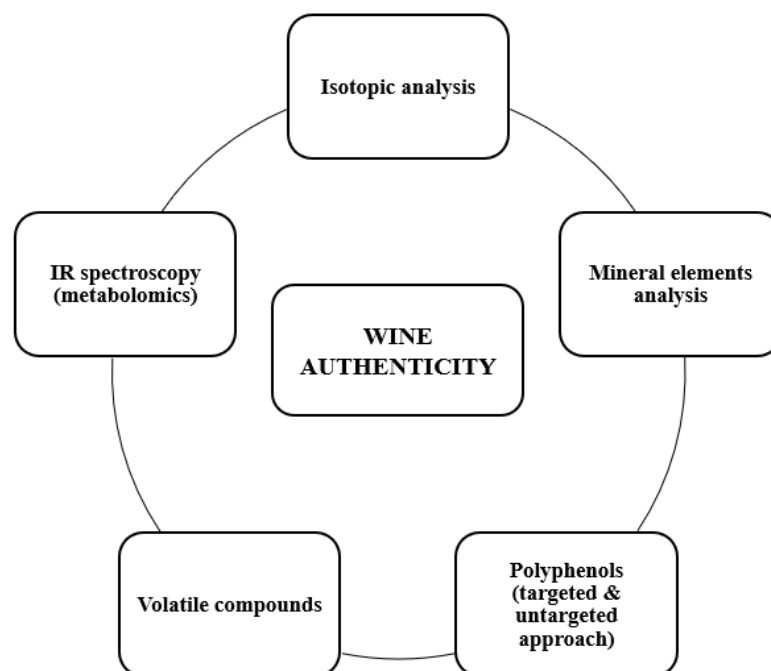


Figure 1.1: Analytical methods used to prove wine authenticity.

The successful classification of various wine products according to their variety, geographical origin, and certain aspects of the winemaking process, have been made through the combination of instrumental analyses with multivariate statistics. Multivariate statistical methods are a useful tool for the classification of grape and wines, based on complex sets of chemical or sensory data. Especially, Principal Component Analysis (PCA) is very often used to establish the relationships and differentiation among the variables, Cluster Analysis (CA) is employed to discover natural groupings of samples, Linear Discriminant Analysis (LDA) is applied to select the most important and significant variables and Canonical Discriminant Analysis (CDA) is applied to identify similarities between samples (Pasvanka et al., 2019).

1.2 Analytical methods on wine authenticity

1.2.1 Mineral elements

Recently, studies focused on food authenticity are in successive increase for preserving costumers against fraud and commercial disputes. For this purpose, the geographical origins of various food products have been verified developing quantitative methods with subsequent multivariate analysis (Pasvanka et al., 2015).

It has been observed that during the winemaking process, mineral elements are not transformed or metabolized, but they remain unaltered in the final product. For that reason, the classification of wines, as it concerns their geographical origin and variety, can be rely on the element profile (Cozzolino et al., 2015). The analysis of inorganic compounds has been employed as one of the most promising methods, either on its own or in conjunction with other methods- that are referred below- for detection of wine authenticity for both their variety and geographical origin. The analyses of inorganic compounds in wines are usually carried out by atomic absorption spectroscopy (AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES) or ICP-mass spectrometry (ICP-MS) (Versari et al., 2014).

The wine contains 2-4 g/L of inorganic ingredients, which include mainly inorganic acids and some organic ones. The mineral elements are formed in the roots of the vine and transferred to the grapes, from where they finally pass to the must and then to the wine (Soufleros, 1997).

Mineral elements are used as indicators of the origin of wines, however as the composition of wines in inorganic components depends on many factors, the data should be studied carefully. Many of these factors, which affect the metal content of wines, are natural sources (such as the geochemistry of the vineyard soil), the external contamination of the vine during its development, the various contaminations during the vinification process, as well as grape variety, plant maturity and climatic conditions (Likar et al., 2015). Other possible sources that affect the mineral content of wines are the air pollution, pesticides and fertilizers that are used, irrigation water, aging processes, oenological practices and various additives during vinification (Vlope et al., 2009). For this reason, the classification of wines based on their mineral profile, is influenced not only by geographical origin, but also by various exogenous factors.

Potassium (K) and calcium (Ca) are the natural ingredients of grapes and their concentration in wine reflects the condition of the vine in the final stages of grape ripening. However, K is also added to the wines as a metasilphite or carbonate and phosphorus (P) as a calcium phosphate or ammonia, thus increasing its final concentration in wines (Catarino et al., 2018).

The concentration of aluminum (Al) in both must and wine could be increased by the use of bentonite and, to a lesser extent, by contact with aluminum surfaces. However, winemaking equipment is now usually made of stainless steel and these contaminants are rare. The elements manganese (Mn), magnesium (Mg), strontium (Sr), barium (Ba) and several rare earths are also reported as useful elements, even if Mn and Mg can be introduced through viticultural practices, such as the use of fertilizers and pesticides, while the concentrations of rare earths contained in wines may also increase due to the use of bentonites (Pasvanka, 2021).

Copper (Cu) can be transferred to the must by the use of various chemicals, however, the main source of copper in the final product comes from the addition of CuSO_4 , in order to remove hydrogen sulfide. Residual copper can contribute to oxidative deterioration, which eventually leads to tanning of wine, especially the white ones. This phenomenon is one of the main oenological problems for winemakers and some metal ions (Cu, Fe, Mn) are the activators of this process. This affects the organoleptic characteristics of the wines, such as loss of aromatic freshness and the appearance of sediments in the wine. To minimize the incidence of these problems, it is generally recommended to keep the copper concentration below 0.3 - 0.5 mg/L. Moreover, some metal ions such as copper (Cu), iron (Fe), aluminum (Al), zinc (Zn) and nickel (Ni), contribute to the development of turbidity and alterations in aroma and taste (Pérez-Álvarez et al., 2019).

Elements, the content of which depends on the geographical origin, due to the direct relationship with the composition of the soil in which the vine is grown, are Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, Sr and Zn. Other minerals, such as Fe, Zn, Cu, Cr, Se, Ca, Co and Ni, which are essential for the human body, are an integral part of one or more enzymes involved in metabolic or biochemical processes. These elements are also important for the successful completion of alcoholic fermentation and for the activation of yeast enzymes (Catarino et al., 2015). Alkaline elements, rubidium (Rb), lithium (Li) and cesium (Cs), are good indicators of geographical origin, as they are not included in the group of elements resulting from contamination in the various stages of wine making, while boron (B) and strontium (Sr) are natural elements derived from their presence in the soil (Paneque et al., 2017). Knowledge of the metal content of wines is also essential for both producers and consumers, as some of them carry toxicological risks, such as cadmium (Cd) and lead (Pb) (Kruzlicova et al., 2013).

Concluding, the main factors influencing the mineral content in wines, are the soil in which the vine is grown, including its pollution, the vine's ability to absorb minerals and all the procedures

during the winemaking processes. Finally, the division of the elements into groups according to their origin moves in three axes (Figure 1.2).

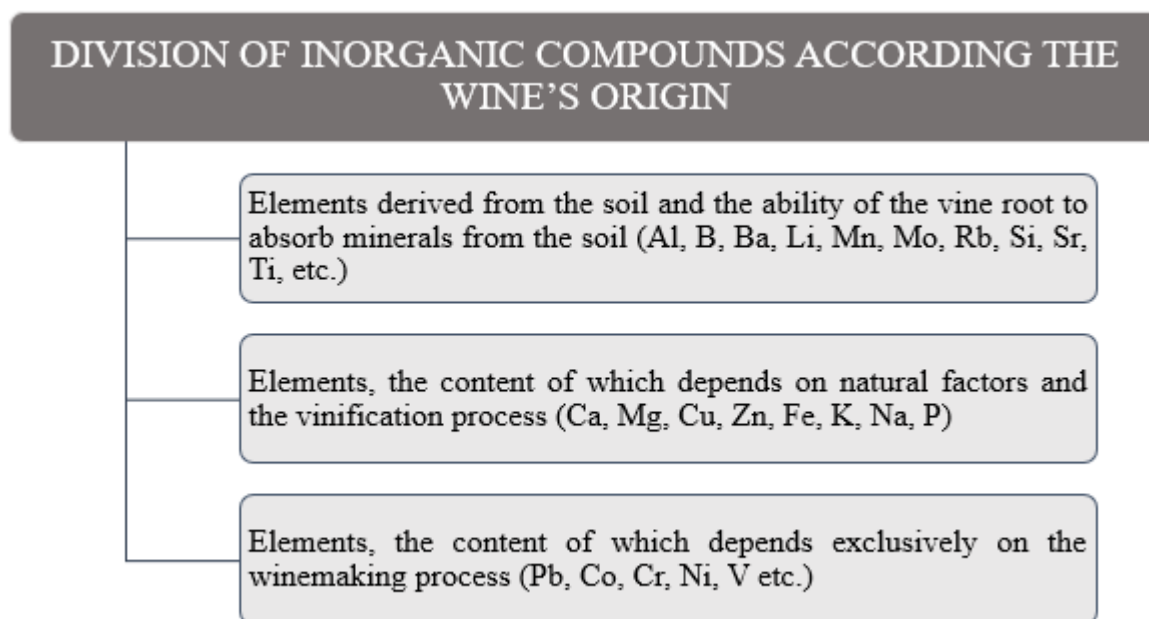


Figure 1.2: Classification of inorganic compounds in wines according their provenance.

Many countries have imposed rules as a way to control element profile in wines. These rules must be followed by the producers to obtain the right to export their wine products to the markets.

In a study carried out with 44 red and white wines from different regions in Poland and different types of grapes, was made an attempt to reveal specific relationships between the wine samples or between the chemical variables in order to classify the wines according to their metal content by application of chemometric analysis. For this discrimination, two techniques, inductively coupled plasma and mass spectrometry (ICP-MS) and inductively coupled plasma and optical emission spectrometry (ICP-OES), were applied. The chemometric methods used were cluster analysis (hierarchical and non-hierarchical with K-means algorithm) and principal components analysis (PCA), which revealed that two major factors are responsible for the wine quality and especially soil toxic metals content and soil natural composition. The wine samples could be

readily separated by geographic objectives, and for each region some discriminating metal variables could be defined (Płotka-Wasyłka et al., 2018).

In another study (Pérez-Álvarez et al., 2019), excellent classification results according to viticultural and oenological factors were obtained by application of inductively coupled plasma mass spectrometry (ICP-MS). The discriminant functions classify 100% of the 34 red and white wines, from different grape varieties, geographical zones, soil types, foliar N application, SO₂ addition and oak ageing, with the exception of grape variety (97.0%) and oak ageing (95.8%). A statistical analysis on mineral elements (major, minor, and trace) of red, white and all wines together was performed using variance analysis (one-way ANOVA). Differences between averages were compared using the Duncan test at 0.05 probability level. Discriminant analyses were carried out on data showing the concentration of elements found in the wines in order to classify them according to the aforementioned factors.

In another investigation, 639 Italian red wines from 18 different geographical regions were classified with Partial Least Squares-Discriminant Analysis (PLS-DA). 31 chemical elements -Rare Earth Elements (REE), major and trace elements- all contributed to the discrimination of samples (Bronzi et al., 2020). In this study, the combination of ICP-MS with chemometrics was demonstrated to be useful for distinguish Chianti Italian red wines from samples of other Italian regions.

Rocha and collaborators investigated whether the wines from different Portuguese Protected Geographical Indications – PGI could be differentiated based on their elemental profile. For this purpose, 60 red and white wine samples, from 6 different PGI were analysed for a wide range of inorganic compounds (53) by FAAS and ICP-MS. Discriminant analysis (DA) was used to find the elements with discriminant power. The results showed that the concentration of several elements (e. g. Ca, Mn, Co, Zn, Br, Sr, Cd, W, Tl) was significantly different among

wines and could reliably discriminate the wines from the different Portuguese PGI regions (Rocha et al., 2019).

A stepwise approach combined with Kruskal-Wallis non-parametric test to remove non-significant features, and Linear Discriminant Analysis (LDA) to derive a feature importance index was used to classify wines samples according to place of origin. Aiming at improving categorization accuracy, different classification techniques were tested. When applied to a wine dataset comprised of 53 samples from four South America countries and 45 chemical elements concentrations reported by ICP-OES and ICP-MS, the proposed framework yielded 99.9% accurate classifications in the testing set (Soares et al., 2018).

The ICP-MS method also allowed to fully discriminate 180 samples of red and white wines from various regions in Greece, different vinification techniques and tow contagious cultivation years (Pasvanka, 2021). The inorganic compounds were classified correctly up to 99% for both variety and region. In this study, the creation of the element "identity" of the wines and the creation of prediction models were achieved, in order to distinguish them in terms of varieties and winemaking regions. Through chemometrics (ANOVA, PCA, CA) it was confirmed that the elemental fingerprints, and especially the rare earths, are reliable indicators of authenticity, especially for the determination of the geographical origin.

1.2.2 Polyphenol Imprint

High performance liquid chromatography (HPLC) is widely used in the determination of wine polyphenols. However, the development of HRMS or NMR techniques combined with the advanced chemometrics, has been proven as a useful tool in order to not only identify but also recognize numerous wine and grape types (Diamantidou et al., 2018).

Metabolomic studies are divided into two main categories, the targeted and untargeted approaches (Figure 1.3). Targeted metabolomics is hypothesis testing approaches that aim to identify, detect and quantify a specific group of compounds, while untargeted are hypothesis-

generating approaches that focus on the comprehensive detection of multiple endogenous and exogenous molecular with low weight (<1 kDa) metabolites. Suspect screening metabolomics is a midway approach (Flamini et al., 2013) where specific mass to charge ratios are targeted without the use of commercial standards but only based on literature or mass spectral bases.

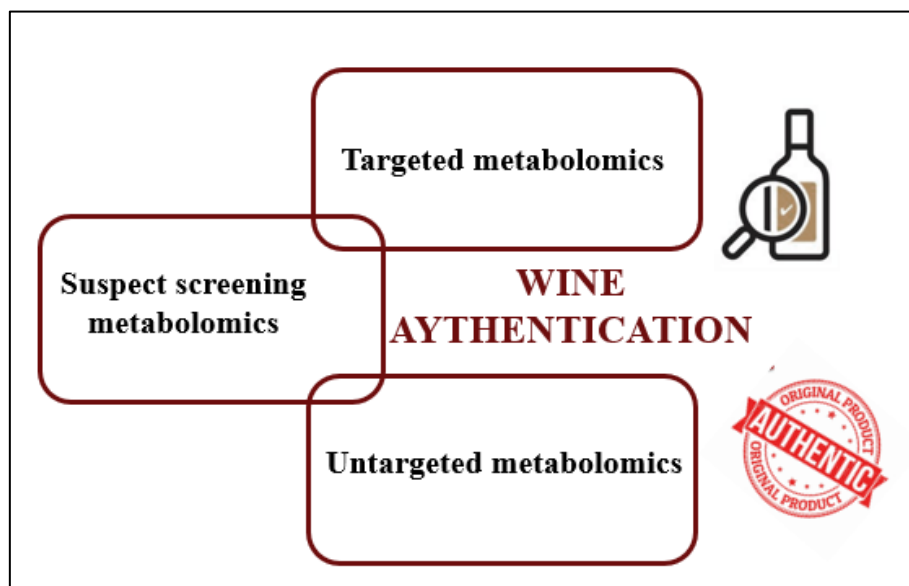


Figure 1.3: Categories of wine metabolomics.

Several studies have been conducted worldwide contributing to wine authenticity and especially based on the polyphenol imprint to discriminate wine variety and geographical origin. Characteristic example is the recent study that Arapitsas et al. (2020) conducted, in which, the characterization of the metabolome of 11 single-cultivar, Italian red wines with the use of untargeted HRMS metabolomics was provided. In this study, quercetin was found more abundant in Sangiovese wines followed by Nebiolo and Nerello, isorhamnetin was more abundant in Nebiolo wines, while anthocyanin content was found higher in Teroldego wines. A good example of the discriminating abilities of polyphenols were reported in 2020 (Stój et al., 2020) with wine samples from Poland. In this study, were analyzed phenolic compounds in red monovarietal wines produced from Zweigelt (*Vitis vinifera*) and Rondo (non-*Vitis vinifera*) varieties while using the Ultra Performance Liquid Chromatography (UPLC) with a

PDA detector, coupled to mass spectrometry method. Fifty-five phenolic compounds belonging to five classes (anthocyanins, flavonols, flavan-3-ols, phenolic acids, stilbenes) were identified in Zweigelt and Rondo wines. The wines of the Zweigelt variety had lower concentrations of anthocyanin monoglucosides than those of the Rondo variety. Also, wines of the Zweigelt variety contained the highest concentrations of flavan-3-ols, and wines of the Rondo variety contained the highest concentrations of anthocyanin diglucosides. As it concerns data processing, hierarchical cluster analysis (HCA) revealed that Zweigelt wines and Rondo wines formed two separate groups.

In another study, determined the anthocyanin and proanthocyanidin profiles of ninety samples from Greek red grape varieties by HPLC-UV and MS detection. The authors reported significant differences in the anthocyanin and proanthocyanidin profiles of skin extracts of these varieties, while this was not observed in the seeds (Kyraleou et al., 2020). Data statistical analysis was conducted with variance analysis (ANOVA) while Principal Component Analysis (PCA) was used to examine any possible grouping of samples.

Even though most reports on phenolic compounds are referred to red wines, there are also many in rose or white wines. A specific statistical analysis involving ion ratios, discriminant analysis, and genetic algorithm (GA) was then applied to study the classification of rose wines according to their geographical origin (Gil et al., 2020). They focused on polyphenols and distinguished that the compounds used for discrimination were vanillic acid, peonidin 3-O-acetyl-Glucoside-(epi) catechin, peonidin 3-O-Glucoside, and (epi)catechin-ethyl-(epi) catechin isomers.

As it concerns the statistical analysis of the datasets, tools such as the Hierarchical Cluster Analysis (HCA) and the Principal Component Analysis (PCA) have been used to depict differential or common trends among wine samples (Tzachristas et al., 2020). However, these approaches are less used when searching for discriminative markers among the groups. For that reason, various supervised methods including partial least squares (PLS) and orthogonal partial

least squares (OPLS), artificial neural networks (ANN), canonical variate analysis (CVA), support vector machine (SVM) have been previously employed (Alañón et al., 2015). A typical example is the study conducted from Portinale et al. (2017). In this survey presented a data analysis approach, based on machine learning techniques for the authenticity of 158 Italian samples. After analyzing, the data derived from spectrophotometric as well as chromatographic characterization of the wine phenolic fraction in both unsupervised and supervised approaches. And the selected anthocyanins, phenolic acids, stilbenes, and flavonoids were used for the authenticity assessment.

1.2.3 Volatile compounds

Minor constituents in foods, especially volatile compounds, are inherently associated with the global quality and positive sensory attributes of various wines (Tahir et al., 2019). It is very important the role of flavor compounds in human health due to their antioxidant, anti-cancer, anti-inflammatory, and anti-obesity properties (Ayseli et al., 2016). The aroma of wines can be influenced by many factors, including grape variety, climate, fermentation condition, yeast strains, winemaking process and aging and storage conditions (Versari et al., 2014, Weldegergis et al., 2011) therefore, there is a need of appropriate sample size to validate the authenticity of wines based on volatiles compounds. Several attempts have been made to establish highly sensitive and selective approaches for measuring of aroma components where comprehensive research have been performed on the various extraction and separation techniques such as SPE, SPME as well as enhancing the chromatographic used such as HPLC and GC-MS (Marín-San Román et al., 2020). However, these techniques have many drawbacks like time-consuming, expensive, and complex methods while extraction process that are used may affect the chemical composition of their aroma compounds (Boido et al., 2013). Therefore, scientists continue to make significant attempts to find alternative rapid, with no or minimal sample preparation, and cost-effective methods that might complement or replace conventional

analytical tools in terms of measurement of aroma compounds. For these reasons, vibrational spectroscopies including infrared (e.g., FT-IR, NIR and MIR), Raman, and hyperspectral imaging (HSI) coupled with chemometric techniques could be used as alternatives for quantitative analysis of various aroma compounds in wines (Tahir et al., 2019).

Certain volatiles are characteristics for particular wine varieties (usually referred to as impact odorants) (Figure 1.4). Especially, norisoprenoid compounds contribute to the varietal character of Chardonnay wines, methoxypyrazines contribute to distinctive Sauvignon Blanc and Cabernet Sauvignon aroma, and isoamyl acetate was reported as an important aroma constituent of young Pinotage wines. Also, furaneol (4-hydroxy-2,5-dimethylfuran-3(2H)-one) has been reported as a caramel odour contributor to Merlot aroma, while a sesquiterpene, rotundone, has recently been shown to be responsible for the pepper aroma in Shiraz wines. Aside from these impact odorants, the common and universal pattern of wine volatiles is dominated by the major fermentation products such as alcohols, esters and fatty acids (Weldegergis et al., 2011).

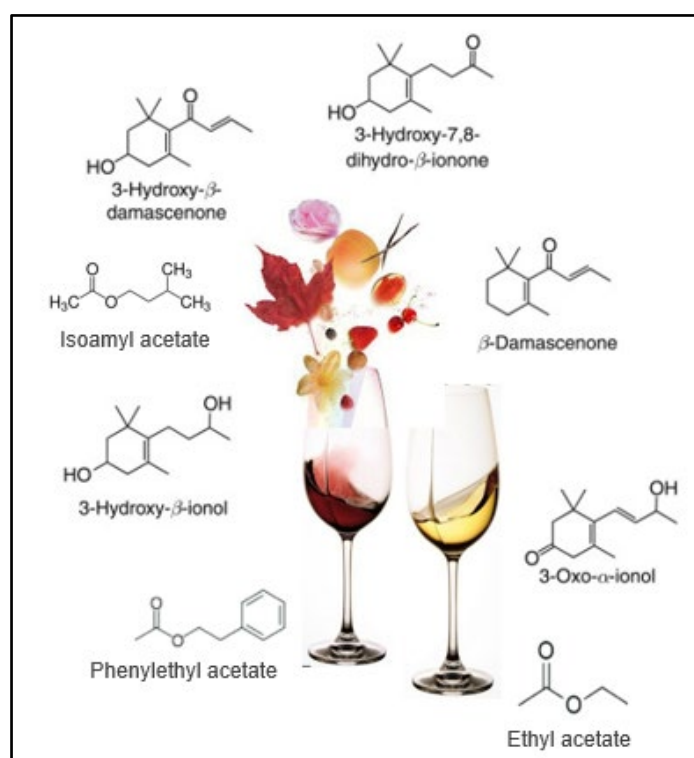


Figure 1.4: Indicative volatile compounds that give aroma to the wine.

An overview of the main extraction techniques used for the analysis of volatile compounds in grape and wine samples were reported in the study of Marín-San Román et al. (2020). In their study they notice that the sample preparation stage is necessary before doing the chromatographic analysis of the volatile compounds as the range of concentrations in which many of these compounds are found is quite low (ng/L). The main analytical techniques that presented in this review are LLE (liquid-liquid extraction), SPE (solid phase extraction), SPME (solid phase microextraction), SBSE (stir bar sorptive extraction) and TF-SPME (thin film solid phase microextraction) with the last two providing better results and advantages in comparison with the other techniques.

Another example of the discriminating abilities of volatile compounds were reported in 2011 (Weldegergis et al., 2011) in 334 wine samples of six different cultivars from South Africa. In this study, thirty-nine volatile components partially responsible for the flavour of wine were quantified. In order to investigate possible correlation between volatile content and grape variety and geographical origin were used chemometrics techniques. Factor analysis (FA), principal component analysis (PCA) and linear discriminant analysis (LDA) demonstrated significant differences in the levels of certain volatiles as a function of region and cultivar, with the latter factor proving to be more influential. A few volatile compounds were identified as potential predictors of the white wine cultivars while prediction for red wine cultivars was poor. Isoamyl acetate and ethyl octanoate were seen as influential in the authentication of Pinotage wines.

In another investigation, the headspace solid phase microextraction (HS-SPME) methodology combined with gas chromatography-ion trap/ mass spectrometry (GC-IT-MS) and flame ionization detector (GC-FID), applied to the extraction of volatile compounds in wines. The qualitative method allowed the extraction and identification of 60 volatile compounds in the sample studied, notably the classes of alcohols, esters, and fatty acids and the validation for the

quantification of 55 volatile compounds of importance in wines and applied to twelve samples of Merlot red wine from South of Brazil. The calculation of the odor activity value (OAV) showed the most important components of the sample's aroma with ethyl isovalerate, ethyl hexanoate, octanoic acid, 1-hexanol and ethyl cinnamate had the greatest contribution to the aroma of the wines analyzed, which is predominantly fruity with the presence of fatty and herbal odors (Arcari et al., 2017).

The availability of chemical markers of wine aging is of great importance in winemaking as it detects frauds and also ensure the wine authenticity. In this view, the GC-GC-ToFMS has been used for the characterization of twenty-three monovarietal Madeira wines from five *Vitis vinifera* L. grape varieties (Marsala, Madeira, Porto and Jerez), aged from 3 to 20 years old and matured in oak casks. Independently of the variety and the type of wine, the chemical groups that could be used as potential age markers were predominantly acetals -1,1 diethoxyethane, diethoxymethane, 1,1-diethoxy-2-methylpropane, 1-(1-ethoxyethoxy)-pentane, 2-propyl-1,3-dioxolane and trans-dioxane- that are formed during the fermentation step and its content increases remarkably during the aging process most likely due to the oxidative condition that occurred during this step (Perestrelo et al., 2011).

1.2.4 Isotopic analysis

In recent years, it has been observed that a powerful tool for geographical origin traceability and authenticity of food products is stable isotopes ratios at natural abundance (Francois et al., 2020). The isotopic ratio allows the identification of molecules that are present in food, that have the same chemical structure, but come from different raw materials and also allows the detection of molecules that are processed by different processes such as biological or industrial composition.

Isotopic methods have provided a powerful tool for detecting fraud, since it does not have to be done during the vinification of the product, but can also in the final product, even after many

years. The novelty of the isotopic analysis is the ability to identify molecules that have the same chemical structure but come from different raw materials and also to detect processed molecules. Furthermore, taking advantage of the different physicochemical properties of the isotopes of the same element, can distinguish any mixtures, certify whether the alcohol in the wines comes from fermentation or from additives, to investigate and identify whether sugars or other raw materials have been added, to determine the geographical origin and certainly to detect the isotopic imprint at all stages from the raw material to the final product, isotopic identity and quality always with the aim of protecting consumers (Raco et al., 2015).

Table 2.1 depicts the verification of authenticity / adulteration of wines using fixed isotopes.

Table 2.1: Presentation of the isotopic ratios tested to determine the factors affected and the corresponding types of fraud in authenticity of wines.

ISOTOPIC RATIO	AFFECTED BY	TYPE OF FRAUD
$^{13}\text{C}/^{12}\text{C}$	Photosynthetic cycle, Water	It separates only the plants of different synthetic cycles
$^{18}\text{O}/^{16}\text{O}$, $^2\text{H}/^1\text{H}$	The Origin of Water	Add water (dilution)
$^{13}\text{C}/^{12}\text{C}$, D/H	Metabolism Photosynthetic cycle (C3, C4, CAM)	Illegal addition of alcohol from cane and / or sugar beet, cereals Illegal addition of sugar. Mixtures of sugars and alcohols of different origins.
$^{13}\text{C}/^{12}\text{C}$, D/H,	The chemical composition	Add synthetic alcohol
$^{13}\text{C}/^{12}\text{C}$, $^{18}\text{O}/^{16}\text{O}$, $^2\text{H}/^1\text{H}$, D/H	All the above and the distillation time in relation to the temperature	Mixtures of different distillation fractions

Stable isotopes ratios are of a great interest for verifying the authenticity of wines for several reasons. The non-homogeneous repartition of oxygen-18 ($\delta^{18}\text{O}$) and deuterium ($\delta^2\text{H}$) over the globe linked to the hydrological cycle provides information on plant growth localization and meteorological conditions and plant metabolism has a decisive impact on carbon-13 ($\delta^{13}\text{C}$) and deuterium (D/H) stable isotope ratios in the molecules metabolized by plants. (Karalis et al., 2020).

A study carried out in Cyprus (Kokkinofita et al., 2017) a parallel implementation of SNIF-NMR, IRMS and ICP-AES facilitated the investigation of the provenance, variety, vineyard, and vintage year in relation to 76 red and white Cypriot wines' classification. It has to be noted that the facilitation of both elements and stable isotope ratios and the combination with chemometrics (PCA) led to their classification and contributed to authenticity assurance of wines. Specifically, unsupervised method (principal component analysis) illustrated the importance of grape variety and origin and supervised analysis mentioned the vineyard effect and the contribution of the vintage year.

Wu et al. (2021) presented a classification of 240 red wine samples from France with an accuracy of 98.2% using multivariate analyses of the isotopic and elemental data. Inductively Coupled Plasma Optical Emission Spectroscopy (ICPOES) was used to analyses five major elements (Ca, K, Mg, Na and Fe) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) employed to determine the trace elements. The $\delta^{13}\text{C}$ values of wine ethanol and glycerol and the $\delta^{18}\text{O}$ values of the wine water were analyzed by GC-C-IRMS. By PLS-DA confirmed the wine origin and potentially detected the fake Bordeaux wines from other wine regions in France. Finally, ANN was used to carry out discriminant analysis of the above samples to investigate whether a different chemometric traceability model could improve on the accuracy found with PLS-DA.

Another example of light isotope's observations for geographical origin characterization studied 51 authentic red wines from Argentina produced in two consecutive harvest years, 2007 and 2008 (Di Paola- Naranjo et al., 2011). The $\delta^{13}\text{C}$ showed the lowest values for the region with higher mean annual precipitation, but the effects were also connected to soil inputs (i.e., the content of organic matter from C3 and C4 plants). Analysis of variance (ANOVA) was performed with each sample while discriminant analysis (DA) was used to evaluate whether wine and soil samples could be distinguished according to their geographical origin or grape variety. Furthermore, generalized procrustes analysis (GPA) was applied in order to assess the relationship between wine and soil.

1.2.5 Spectroscopy

It has been observed that spectroscopic techniques are a more friendly and economical alternative to the traditional methodologies as they involve more complex instrumentation that may not readily be developable in an industry setting. Spectroscopic techniques (e.g., near-infrared, mid-infrared, NMR, UV-visible) provide a great solution as they are simple and can afford speed of analysis, simple sample preparation, environmental friendliness. It is not accidental that spectroscopic techniques have been utilized for different grape and wine studies, such as for targeted and non-targeted chemical analyses, prediction of sensory attributes, and wine traceability and authenticity. Chemometrics is frequently used in conjunction with spectroscopy for sample classification and discrimination (Ranaweera et al., 2021).

UV-Vis spectroscopy is a fast, low-cost, and reliable analytical technique that has been used in wine analysis (Aleixandre-Tudo et al., 2017). Spectra recorded at UV and visible wavelengths (typically 190–800 nm) provide information about compounds in wine containing a chromophore, such as hydroxybenzoic (280 nm) and hydroxycinnamic (320 nm) acids, flavan-3-ols (280 nm), flavonols (370 nm), and anthocyanin glucosides (520 nm) (Ranaweera et al., a, 2021). Azcarate et al. (2013) were able to correctly classify Argentinian Sauvignon

blanc wine samples with 100% accuracy according to their geographical origin by means of a non-targeted method combined with appropriate chemometric methods such as Linear Discriminant Analysis (LDA) and Partial Least Squares Discriminant Analysis (PLS-DA). Another example with a high achievable percent (97.5% correct classification of grape variety) in four monovarietal Cretan wines from Greece showed that the latent variables resulting from Orthogonal Projections to Latent Structures-Discriminant Analysis (OPLS-DA) could be related to the absorption of aromatic compounds such as flavonols and phenolic acids (Philippidis et al., 2021). However, Tan et al. (2016) proposes the combination of UV-Vis spectroscopy with other spectroscopic methods (IR, fluorescence) to improve the classification models and provide suitable results for wine authentication.

IR spectroscopy is the most frequently applied spectroscopic method in comparison to other methods as it is a user-friendly and rapid technique that provides various information on many components in wines and can be used for the determination of parameters such as alcohol content, pH, volatile compounds, organic acids, sugars, and polyphenols (Aleixandre-Tudó et al., 2020). Two main IR-based techniques are applied according to the range in the spectral region: near-infrared-NIR ($14000\text{-}4000\text{ cm}^{-1}$) and mid-infrared-MIR ($4000\text{ to }400\text{ cm}^{-1}$). Near-infrared spectra contain less intense bands than mid-infrared and it is not applicable to assign chemical groups specifically with NIR due to overlapping signals with ethanol and water around 1950 nm. On the other hand, in mid-infrared spectroscopy, there is a fingerprint region from $1500\text{ to }400\text{ cm}^{-1}$ that includes unique absorption patterns of compounds in wines such as phenolics, and signals associated with various functional groups can be assigned, such as C=O related to organic acids at 1700 cm^{-1} , combinations of C-H vibrations and information related to ethanol and sugars at around $2300\text{ to }2100\text{ cm}^{-1}$ (Geana et al., 2019). The introduction of techniques such as Fourier transform (FT) and attenuated total reflectance (ATR) increased the applicability of IR methods to wine authenticity. FT has improved data collection speed and

reproducibility (Rodriguez-Saona et al., 2011) and ATR simplifies the sample handling process and is advantageous in routine analysis (Cozzolino et al., 2015). Roullier-Gall et al. (2019) combined ultra-high-resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) and electrochemical oxidation strategies to characterize the antioxidant property of Chardonnay wines from France only differing in SO₂ added after prior pressing. They concluded that sulfur-containing compounds appeared to decrease with the electrochemical oxidation, whereas nitrogen-containing compounds were mostly formed. In another study (Hu et al., 2019) developed database models based on FT-MIR and FT-NIR coupled with chemometrics (PCA, SIMCA, and DA) and proposed that it could be applied as a reference for geographical origin traceability of Cabernet Sauvignon wines (Australia, Chile, and China) in a more comprehensive, effective and rapid manner. Especially, they achieved correct classification using: SIMCA, 97%, 97%, and 92% for Australian, Chilean, and Chinese wines; and DA, 86%, 85%, and 77%, respectively.

Raman Spectroscopy involves detecting the inelastic scattered light emitted from molecular vibrations of a sample, approximately in the range of 200 to 3600 nm (Ranaweera et al., a, 2021). The Raman technique produces a weak signal, but the development of optimized detection capability provides the opportunity to obtain enough information regarding the chemical composition of the wine sample. There are two different regions in Raman spectroscopy, with Stokes Raman scattering having more dominant water, sucrose and ethanol peaks, and anti-Stokes Raman scattering from minor components such as aromatic compounds, including many phenolics (Magdas et al., 2018). Also, there are two types of Raman techniques that are applied in food analysis, FT-Raman spectroscopy and surface-enhanced Raman spectroscopy (SERS). The advantage of the latter over FT-Raman is the selectivity afforded by specific molecules being adsorbed to metal nanostructures which enhances the intensity of Raman signals. A characteristic study that depicts the effectiveness of FT-Raman spectroscopy

was Magdas's et al. (2019) survey. They manage to discriminate 126 white wines from Romania and France according to their variety (84%), provenance (100%), and harvest year (95%) in conjunction with LDA chemometrics. Surface-enhanced Raman spectroscopy (SERS) was chosen from Zanuttin et al. (2019) in order to discriminate wines according to variety and producer combined with SIMCA chemometrics. Major metabolites were identified (purines, carboxylic acids, glutathione) with an overall correct classification of 87%. Although these methods show high rates of discrimination, there are some drawbacks as it is necessary to prepare a colloidal dispersion of Ag nanoparticles to add to the sample and also requires spectral pre-processing such as multiplicative scatter correction to avoid the effect of fluorescence that can obscure Raman scattering, especially when analyzing wine (Xu et al., 2020).

Fluorescence Spectroscopy is an analytical technique very useful due to its sensitivity, selectivity, and rapidity (Airado-Rodríguez et al., 2011). It is based on the emission of longer wavelength light from a substance after absorption of energy in the UV or visible range. This analytical method can be well applied to wine analysis, with common fluorophores being a variety of phenolic compounds, vitamins, and aromatic amino acids. According to the fluorophore's molecular and macromolecular constituents in the wine sample, a three-dimensional excitation-emission matrix (EEM) recorded over multiple excitations and emission wavelengths can be obtained and considered to be the molecular fingerprint of the sample (Coelho et al., 2015). Therefore, this approach in combination with chemometrics can be utilized for authentication of wine and their discrimination according to geographical origin or variety. Indeed, in a survey conducted in 60 Slovakian white wines (Sádecká et al., 2020) synchronous fluorescence spectroscopy was applied to discriminate the samples according to their variety using LDA, achieving an overall rate of 100% correct classification in validation and 93% for prediction. In another newly published study (Ranaweera et al., b, 2021),

researchers used the absorbance-transmission and fluorescence excitation-emission matrix (A-TEEM) approach combined with extreme gradient boosting discriminant analysis (XGBDA) to classify 221 commercially produced Australian red wines by origin and variety with the remarkable percentages of 99.7% and 100% accuracy, respectively.

NMR Spectroscopy can be used for quantitative analysis of analytes such as amino acids, sugars, alcohols, carboxylic acids, and phenolic compounds, for qualitative analysis to determine molecular structures and for compositional profiling of a sample. NMR can be based on the acquisition of ^1H , ^2H , or ^{13}C spectra; however, for wine authentication, ^1H NMR spectroscopy is the most effective analytical method as data acquisition is fast and highly reproducible when compared to the other spectroscopic techniques (Solovyev et al., 2020). A good example of wine authentication by NMR method combined with classical multidisciplinary approaches was reported recently in 2019 by Gougeon et al. (2019). The analyzed samples were separated into three categories, the first was half bottled wines topped up with wine of different origin, the second was suspect wine samples from foreign markets, and the third category was authentic samples. Moreover, Gougeon et al. (2019) developed a similarity score index in order to compare authentic with suspect wine samples and they determined that epicatechin, catechin, gallic acids, and syringic acids were among the phenolic compounds evaluated as possible authenticity markers. In another recent study (Fan et al., 2018), ^1H NMR was chosen as a nontargeted method for wine authentication. Indeed, researchers collected 99 red and 71 white wines from China and applied NMR analysis combined with PCA and LDA for separating red and white wine samples from different varieties. Furthermore, Mascellani et al. (2021) used NMR to classify over 900 wines from the Czech Republic according to type, based on color and residual sweetness, and variety using a random forest (RF) machine learning algorithm and LDA. They approve that metabolomics not only provides a better understanding of wine character but also an easy and rapid tool for

assessing many aspects of wine quality. The application of the RF algorithm for the purpose and scale of wine authentication of this study represents a novel, yet unexplored state-of-the-art approach with promising potential for food fraud.

Concluding, spectroscopic techniques have become a method of choice by many researchers for their routine analysis of wine and generally in food. The advantages of easy to use in analysis, the availability of a wide range of instrumentation and sampling handling options allowed the preference of these methods by the wine and beer industries worldwide. However, some issues related to the poor understanding of the technology and also the high dependence on mathematics, are still some of the few drawbacks of these techniques (Chapman et al., 2019).

Table 1.2: Collection of studies referred to wine authenticity assessment combined with chemometrics.

ANALYTE(S)	CATEGORY OF SAMPLE	NUMBER OF SAMPLES	COUNTRY	ANALYTICAL TECHNIQUE(S)	CHEMOMETRIC METHOD(S)	REFERENCE
Metallic content of wines	Red & white wines	44	Poland	ICP-MS, ICP-OES	PCA, HCA	Płotka-Wasyłka J. et al. (2018)
Categorization of wines in terms of variety, aging, region and the addition or not of sulfur.	Red & white wines	34	Spain	ICP-MS	ANOVA	Pérez-Álvarez E. P et al. (2019)
Geographic characterization of Italian red wines in terms of their metallic content.	Red wines	639	Italy	ICP-MS	PLS-DA	Bronzi B. et al. (2020)

Multi-elemental analysis and differentiation of wines according to the origin.	Red & white wines	60	Portugal	FAAS, ICP-MS	ANOVA, LDA	Rocha S.et al. (2019)
Authenticity of wines from different varieties and winemaking techniques.	Red & white wines	180	Greece	ICP-MS	ANOVA, PCA, CA	Pasvanka K. (2021)
LC-MS metabolomic classification of 11 monocultivar Italian wines-metabolomic study.	Red wines	110	Italy	UPLC-QTOF MS	PCA	Arapitsas et al. (2020)
Classification of wines produced from different grape varieties based on the analysis of phenolic compounds.	Red wines	20	Poland	UPLC-PDA-MS/MS	PCA, HCA	Stój et al. (2020)

Discrimination of wines with a genetic algorithm and MS ion intensity ratios.	Rosé wines	60	France	UPLC-QTOF MS	RF, LDA	Gil et al. (2020)
Discrimination of Greek grape varieties, according to the anthocyanin and proanthocyanidin profiles.	Red wines	90	Greece	HPLC-MS	ANOVA, PCA	Kyraleou et al. (2020)
Chemometric investigation of the volatile content of South African wines	Red & white wines	334	South Africa	GC-MS	FA, PCA, LDA	Weldegergis et al. (2011)
Volatile composition of Merlot red wine and its contribution to the aroma.	Red wines	12	Brazil	GC-IT/MS, GC-FID	ANOVA	Arcari et al. (2017)
The establishment of potential age markers of Madeira wine	Madeira wines	23	Portugal	GC-GC-ToFMS	PCA	Perestrelo et al. (2011)

Elemental profile and classification of wine samples according to the country's origin.	Red wines	53	South America	ICP-OES, ICP-MS	LDA	Soares et al. (2018)
Isotopic and elemental profile of wine authenticity.	Red & white wines	76	Cyprus	SNIF-NMR, IRMS, ICP-AES	PCA, OPLS-DA, PLS, CV-ANOVA	Kokkinofa et al. (2017)
Verification of wine's origin using isotope and elemental analyses coupled with chemometrics	Red wines	240	France	ICP-MS, ICP-OES, GC-C-IRMS	ANOVA, PLS-DA, ANN	Wu et al. (2021)
Fingerprints for main varieties of Argentinean red wines.	Red wines	51	Argentina	Q-ICP-MS, TIMS	ANOVA, DA, GPA, CCA	Di Paola-Naranjo et al. (2011)
Classification and correlation between the Sauvignon blanc wine samples by UV-Vis	White wines	7	Argentina	UV-Vis	PCA, CA, LDA, PLS-DA	Azcarate et al. (2013)

spectroscopy and chemometric methods.						
Discrimination according to grape variety, aging process and barrel/container type.	Red & white wines	4	Greece	UV-Vis	OPLS-DA	Philippidis et al. (2021)
Electrochemical triggering of the Chardonnay wine metabolome.	White wines	3	France	FT-ICR-MS	ANOVA	Roullier-Gall et al. (2019)
Geographical discrimination of C. Sauvignon.	Red wines	540	Australia, Chile, China	NIR, MIR	PCA, SIMCA, DA	Hu et al. (2019)
Discrimination of wines geographically, varetally, and by vintage.	White wines	126	Romania, France	FT-RAMAN	LDA	Magdas et al. (2019)

Discrimination of wines according to variety and producer.	White wines	180	Italy	SERS	PCA, SIMCA	Zanuttin et al. (2019)
Discrimination of wines according to variety.	White wines	60	Slovakia	Fluorescence spectroscopy	PCA, LDA	Sádecká et al. (2020)
Discrimination of wines from different varieties and geographical origin.	Red wines	221	Australia	Fluorescence spectroscopy	XGBDA	Ranaweera et al., b, (2021)
Wine analysis and authenticity using ¹ H-NMR Metabolomics data.	Red wines	19	China	NMR	ANOVA, PCA	Gougeon et al. (2018)
Varietal differentiation of wines produced in different regions.	Red & white wines	170	China	NMR	PCA, LDA	Fan et al. (2018)

Metabolomic approach for wine varietal classification.	Red & white wines	917	Czech Republic	NMR	RF, PCA	Mascellani et al. (2021)
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**PCA, principal component analysis; HCA, hierarchical cluster analysis; ANOVA, analysis of variance; PLS-DA, Partial Least-Squares Discriminant Analysis; LDA, linear discriminant analysis; DA, discriminant analysis; CA, cluster analysis; RF, random forest; FA, factor analysis; MANOVA, multivariate analysis of variance; OPLS-DA, orthogonal Partial Least-Squares Discriminant Analysis; PLS, Partial Least-Squares; CV-ANOVA, cross-validated analysis of variance; XGBDA, extreme gradient boosting discriminant analysis; SIMCA, soft independent modeling of class analogy; GPA, Generalized Procrustean Analysis; ANN, Artificial neural network analysis.*

2. Authenticity & Chemometrics of Beer

2.1 Introduction

2.1.1 Production and types of beer

Beer is the most consumed alcoholic beverage globally with evidence of use since the very first human civilisations. Beer is in increased demand globally fueled by emerging economies such as Brazil, India and China. It is produced from yeast fermentation of cereal malt, usually barley, in the presence of hops which contribute to bitterness and act as preservative agents. Chemically, beer is a matrix of various volatile and non-volatile compounds contributing to flavour and aroma. There are many steps involved in the brewing process that determine the quality and properties of beer. The mashing step represents a fundamental part of the brewing process. This step generates fermentable sugars from the primary cereal carbohydrates from the cereal, commonly barley with the help of hydrolytic enzymes. These sugars are converted into alcohol during fermentation reaching a level between 3-12% depending on the specific origin and production techniques. There are different types of fermentation that can result in different types of beer. The top fermented beers involve the yeast *Saccharomyces cerevisiae* which naturally floats to the top of the fermentation resulting in the production of the ale-type beers. The bottom fermented beers which are the lager-type beers, the yeast *Saccharomyces pastorianus* typically sinks to the bottom of the tank during fermentation (Gresser, 2009).

The most popular type of beer worldwide is lager (90%) followed by ale and pale ale. Multinational multibillion companies such as Heineken, Carlsberg, Budweiser and Guinness, control the market for larger and other beers which are mainstream large-scale beers or industrial beers. American style lager, the most common type of lager, is a characteristically light beer with low alcohol content (3-6%).

2.1.2 The premium or craft beer and the legislation framework

In the last 30 years mainly in Europe and USA, the brewing market has witnessed an enormous development of artisanal microbreweries that produce the premium, craft beer or artisanal beer. The premium American-style lager is significantly more expensive than the standard lager. The major difference between these two types of American lager is the carbohydrate source; while Premium lager use only barley malt, the standard uses other starch sources such as wheat, exclusively, or in conjunction with barley. In general, craft beer production is characterised partially by using of select raw materials, such as barley malt or wheat and hop, but also due to tapping into local and seasonal products such as fruit and spices, in combination with creative and traditional brewing technology. In some cases, such as the trappist beers, the authenticity is connected to a specific place of production, i.e., the monasteries and brewers in specific places in France, Belgium and the Netherlands (Mannina et al., 2014, Engel et al., 2014)

In the EU space alone, the number of microbreweries increased exponentially in the last years (e.g., the UK has passed from 1032 units in 2011 to 1897 in 2020, Brewers of Europe Association, 2022). With the increased consumption of premium and standard beers worldwide, increasing food fraud practices have emerged (Spink et al., 2019). In the beer sector, several cases of beer fraud have been reported, wherein the fraudsters have switched the labels and bottle caps of less expensive brands by the labels and caps of the more expensive market leader brands (Pereira et al. 2016). Although this illegal practice does not solely involve craft/premium beers, higher production cost and retail price, premium beers target counterfeit because there is a considerable economic gain to be made (da Silva et al., 2019).

Different legislation across countries does not help the situation as the term “craft” or “premium beer” is not often defined. According to German law, only malted barley, hops,

yeast, and water may be used to prepare bottom fermented beer. This also applies to the preparation of top-fermented beer. However, amongst others, the use of different malt as well as pure cane, beet, or invert sugar are permitted for the preparation of top-fermented beer, which created an opening for craft beers to operate (Gresser, 2009). In Italy, craft breweries were defined for the first time as “small independent producers whose beer does not undergo to pasteurization and micro-filtration during the production process” (National Law n. 154 of 28/7/2016). In the USA, the market is less regulated. The American Brewer Association (<https://www.brewersassociation.org>) defines craft brewers as small, independent, and traditional.

2.1.3 Research gap and need for innovation

Craft beer samples are selected by consumers due to their unique taste, their origin and sometimes based on the information claimed on the label (e.g., “unfiltered product, unpasteurised”, “craft beer”). In fact, craft beers opened new opportunities for setting prices on the highly competitive beer market, because of the willingness of consumers to pay for this type of premium beer (Kaufmann et al., 2022). Therefore, the authentication and traceability of beer products is an important issue for both beer consumers and producers. Conventional methods for beer analysis focus on their physicochemical characteristics (pH, special gravity, ethanol content etc) and they are not fit-for-purpose for the discrimination of different types of beers, whether these are beers from large scale breweries (industrial beers) and craft beers (both regular craft and speciality beers). An exception to this could be the organoleptic analysis but this is not implemented in the legislation in the same way that it is implemented for example for olive oil. Hence, all stakeholders agree that reliable analytical methods able to identify and discriminate products are needed to protect the beer market against fraud and counterfeit.

In the following few sections, we will review the recent scientific literature for advances in the identification and discrimination of beer from a forensic point of view, as well as include advances in beer physicochemical profiling that would inform Quality Control (QC) and Quality Assurance (QA) in the factory. The common angle is that in both cases, untargeted analytical methods are used couple with advanced multivariate methods, also known as chemometrics or machine learning algorithms to gain insights in dealing with such large amount of information (big data).

2.2 Analytical method for authenticity and traceability

2.2.1 Beer authenticity & traceability

As Figure 2.1 suggests, the classification of beers including distinguishing brands (brand identity), origins (geographical origin) and brewing processes (brewing type, fermentation type), as well as identifying authenticity of the label (craft vs standard beer, the “beer style”) has always attracted the attention of the research community and in some cases the industry. It is mainly achieved by several analytical instruments, such as liquid chromatography-mass spectrometry (LC–MS), gas chromatography-mass spectrometry (GC–MS), proton nuclear magnetic resonance ($^1\text{H-NMR}$) spectroscopy, near or standard infrared spectroscopy (NIR, FT-IR) Ultraviolet –visible spectroscopy, fluorescence spectroscopy.

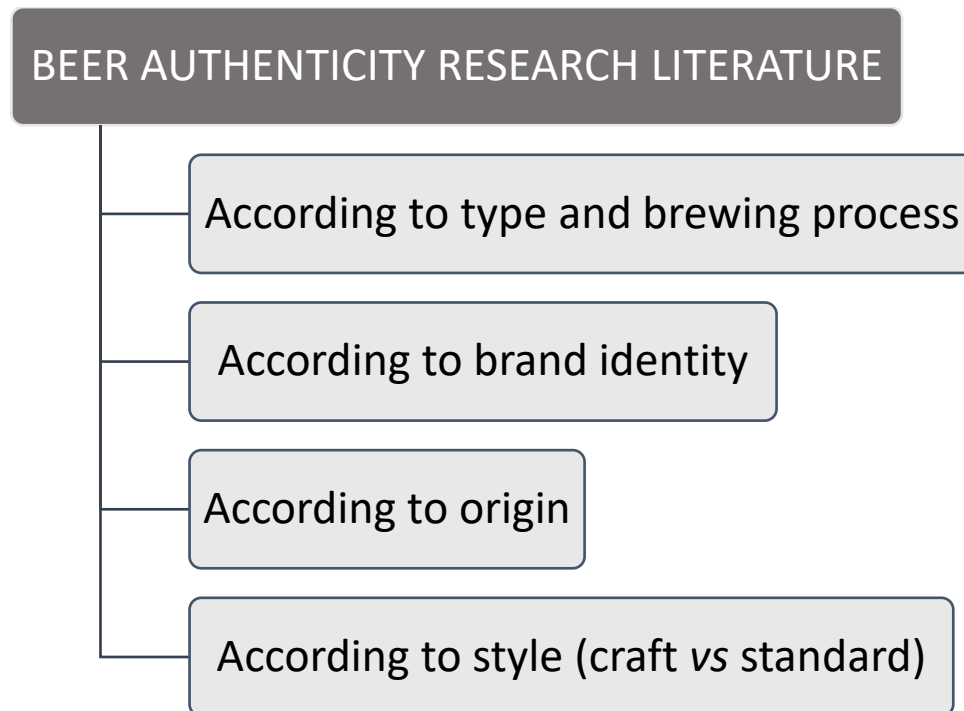


Figure 2.1: Classification of research studies according to their study aims in beer authenticity literature. Note that the size of the box roughly indicates the relative ratio of published papers in each area.

One must keep in mind that the chemical discrimination of beers is genuinely multivariate in nature. Thus, almost all the analytical approaches developed using the techniques mentioned above employed one or more chemometric tools, to perform the multivariate analysis. Examples are LDA, PLS-DA and SIMCA and SVM, ANN for classification and PCA for exploration of the data, identification of clustering, outliers etc.

As in every food product, the chemical composition of beer strongly depends on the raw materials used as well as the brewing production followed. Therefore, the chemical fingerprints and compositional profiles of the beer have a direct impact on the discrimination characteristics observed using the chemometric methods. Specific fundamental differences will be reflected

in the chemical fingerprint and thus, can aid the discrimination. For example, Ale beers are produced at a warmer temperature than lager beers (typically 15-24 °C) which allowing yeasts to generate more volatiles such as esters, hence providing characteristic flavour notes. In parallel, the extraction of components from the raw materials is also favoured by the elevated temperature resulting in generally richer extracts in ale beers compared to lager (Pérez-Ràfols & Saurina, 2015). The above would certainly help the discrimination in relation to the type or brewing process (ale *vs* lager). Table 1 summarises the research studies in the area by scope, analytes, type of beer used, number of samples in the study, production country when available as well the analytical techniques and chemometric methods used.

Table 2.2: Collection of studies referred to beer authenticity assessment combined with chemometrics.

SCOPE	ANALYTE(S)	CATEGORY OF SAMPLE	NUMBER SAMPLES	COUNTRY	ANALYTICAL TECHNIQUE(S)	CHEMOMETRIC METHODS	REFERENCE
Discrimination by type	Phenolic profile	lager vs ale	63	Spain	C18 HPLC/DAD	PLSDA, SIMCA	Perez-Rafalos & Saurina (2015)
Discrimination by type	fingerprints	lager vs ale	40	USA	¹ H NMR – 800MHz	OPLS-DA	Vasas et al. (2021)
Discrimination by type	metabolomic fingerprint	lager vs ale	24	Germany Belgium	UHPLC-HRMS	PLS-DA, VIP and p-value	Gallart-Ayala et al. (2016)
Discrimination by type	Polyphenol profile as markers	lager vs ale	63	Spain and other European	HPLC-DAD	PLS-DA	Perez-Rafols et al. (2015)

Discrimination by type	Spectral fingerprint	beer types and styles	100	European	¹ H-NMR	peak-by-peak processing	Cavalini et al. (2021)
Classification per manufacturer	Volatile fingerprint	Trappist and Rochefort specialty beers	124	European	HS-SPME-GC-TOF-MS	PLS-DA, LDA and ANN-MLP	Cajka et al. (2010).
Classification per manufacturer	Multiple fingerprints	American style lagers	135	China	Fluorescence, UV-Vis spectroscopy	LDA, data fusion	Tan et al. (2015)
Classification per manufacturer	Spectral fingerprint	pale lagers	108	China	EEM Fluorescence Spectroscopy	PARAFAC, LDA, PLS-DA, k-NN	Fang et al. (2021)
Premium beer authenticity (brand identity)	Spectral Fingerprinting	Trappist and Rochefort specialty beers	233	Belgium, Netherlands, Czech Republic	NMR-600MHz	SIMCA, PLS-DA	Mannina et al. (2016)

Premium beer authenticity (brand identity)	Multi-method fingerprint	Different craft beer (pale ale)	60	Italy vs other European	thermogravimetry, MIR, NIR UV VIS	SIMCA, PLS-DA and data fusion	Biancolillo et al. (2014)
Premium beer authenticity (brand identity)	Spectral fingerprint	Trappist and speciality (Abbey)	267	Belgium, Netherlands, Czech Republic	MIR (FT-IR)	ECVA and classifiers LDA, k-NN, QDA	Engel et al. (2012)
Classification per production site	Spectral fingerprints	lager	67	Spain	HS-MS e-nose	LDA	Vera et al. (2011)
Classification by origin	Multi-method fingerprint	Not specified	68	Germany, Portugal, Spain	Elemental analysis, UV-VIS, pH etc	SIMCA, ANN, SVM, LDA	Alcázar et al. (2012)
Discriminating by style and origin	Spectral Fingerprinting	craft beers vs industrial	31	Germany, Belgium,	¹ H NMR 500 MHz (1D, 2D)	PCA, OPLS-DA	Palmioli et al. (2020)

				Italy, Netherlands, Denmark			
Discriminating by style	Volatile fingerprint	Pilsner-style Lager craft vs industrial	79	Italy	HS-SPME/GC– MS	PLS-DA	Giannetti et al. (2019)
Discrimination by style	Spectral fingerprint	different beer types and styles	61	Germany	¹ H NMR 600 MHz	PCA, PLS-R	Kaufmann et al. (2022)
Discrimination by style	Spectral fingerprint	Premium vs standard lager	40	Brazil	¹ H-NMR	SIMCA, PLS-DA	da Silva et al. (2019)
Discrimination by style	Electrochemical fingerprint	craft beer of different types	60	China	Potentiometric measurements	LDA, LS-SVM	Yin et al. (2021)

Discrimination by style	Electrochemical fingerprint	Craft lager	113	USA	(potentiostat/ galvanostat) e(nose)	SIMCA, PLS-DA	Mutz et al. (2021)
Forensic discrimination	Spectral fingerprint	lager beers (high/low price)	141	USA	Direct Mass Spectrometry	PLSDA	Pereira et al. (2016)
Monitor craft beer manufacturing (PAT)	Spectral fingerprint	Pale Ale	7	Belgium	NIR	PCA PLS, MSPC	Franca et al. (2021)

Other

predicting palate fullness	Multi-method fingerprint	Lager beer	41	Germany	Physicochemical & Sensory descriptors	Correlation HCA and PLS-R	Krebs et al. (2021)
classify beers according to their ethanol content and bitterness	Multi-method fingerprint	Lager and ales of different styles	29	Brazil and world beers	Physicochemical, Phenolics, HPLC	PLS-R , PLS-DA	Moura-Nunes et al. (2016)

assess aroma profiles using e-nose	e-nose multi- signal	beer of different types and styles	20	Belgium, Czech, NL, PL, Australia, USA, Mexico	MOS e-nose 9 sensors	ANN	Gonzalez Viejo et al. (2020)
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MSPC, Multivariate Statistical Process Control; LS-SVM, least-squares support vector machine.

2.2.1.1 According to the type and brewing process

More studies have focused on differentiating beers according to type and brewing process and fewer on the origin and production style (craft vs standard) which is arguably harder to do. To discriminate a unique target from other types of beers, for example, a Trappist beer from non-Trappist beers, is a much more defined analytical problem. In this example, UV spectroscopy (Weeranantanaphan & Downey, 2010) and FT-IR (Engel et al., 2012) were used to successfully confirm claimed identity for samples of a single beer brand based on their spectral profiles.

More sophisticated untargeted methods such as the high resolution mass spectrometry (UHPLC-HRMS) provide more insights and sometimes can lead to identification of biomarkers. In the study of Gallant-Ayala et al. (2016), UHPLC-HRMS metabolomic fingerprint was used to distinguish between different beer types a Pilsner lager and Belgian ales beer types. The analysis benefits from the fact that different types of yeast are involved in each type (top-fermenting ales vs. bottom-fermenting Pilsners) and fermentation temperatures (higher for ales) which in turn results in different molecular profiles produced. Using the MS-based biomarker discovery approach (Variable-in-Projection or VIP and p-value from the Analysis of Variance statistical test), p-coumaric and ferulic acids, and 5-methylthioadenosine were tentatively identified as characteristic descriptors of Pilsner and Belgian ale beers, respectively. Other researchers have done something similar e.g., Trappist vs non-Trappist, (Mattarucchi et al., 2010) and Rocheford vs non-Rocheford (Cajka et al., 2011). More recently, Giannetti et al. (2019) applied HS-SPME/GC-MS and chemometric modelling to characterise Pilsner-style Lager craft beers by their volatile fraction while Inui et al. (2013) explored the aroma profiling of different types of beers using a very advanced GC setup (GC×GC/Time of Flight-MS).

One of the most widely applied methods in classification of type or brewing processing is $^1\text{H-NMR}$. The molecular fingerprint of NMR is also capable of discriminating beer types because it informs about the structural characteristics of the beer metabolome. Earlier studies such as the work of Duarte et al. (2002) paved the way to show that such discrimination differing in type (ale or lager) is possible even using 1D and 2D-NMR and exploratory and non-classifying methods such as PCA. However, the authors admitted that high-resolution NMR alone is insufficient to enable the entire assignment of the beer spectra, which is however not needed for the chemometric approach. A more systematic study with robust multivariate analysis framework was followed by Cavalini et al. (2022). This study used 100 industrial production beer samples from diverse origins, brewery, beer style and fermentation type. Through innovative use of chemometrics that utilises peak-by-peak processing which systematically calculates interval-specific signals, they were able to show discrimination according to type and in some cases according to style (IPA vs ale beer) within the same class of top-fermented beers. Other attempts for the discrimination of ale and lager beers were also demonstrated by Vasas et al. (2021). Although the sample size of some of the studies is generally small, the combination of a simpler analytical problem and a sophisticated technique (UHPLC-HRMS, GC-MS, or $^1\text{H-NMR}$) indicates that discrimination of beer type is quite established at this point.

Many studies go as far as using the metabolomic approach to identify actual markers for the discrimination (Mannina et al., 2014; Cavalini et al., 2022). Flavonoids, such as certain phenolic acids (syringic and coumaric acids) can influence the flavour and the nutritional content of the beer. The phenolic profile can be easily resolved by liquid chromatography. Relatively new in the literature is their potential role as chemotaxonomical descriptors of foods and beverages. Perez-Rafalos & Saurina (2015) used a straightforward C18 HPLC/DAD method to quantify the polyphenol profile and applied simple chemometric techniques to successfully discriminate lager and ale beers, showcasing the polyphenol profile as another of

the potential markers for beer type identity. Their research indicated that coumaric and ferulic acids were more abundant in lager while syringic and gentisic acids were more present in ale beer (Perez-Rafalos et al., 2015).

More experimental or niche methods have also been applied with generally good results. Early efforts in the chemometrics techniques can be traced back to the study of Bellido-Milla et al. (2000) who used flame atomic spectrometry and molecular absorption spectrometry coupled with PCA to showcase the differences. Through electro and potentiometry, Yin et al. (2021) were able to generally discriminate between beers of different types and flavours (Weiss beer, draft beer, stout beer, craft 1903, and Pilsner beer). Ghasemi-Varnamkhasti et al. (2014) was able to discriminate between alcoholic and non-alcoholic beer using the signal of a metal-oxide semiconductor sensors (type MOS) e-nose and relatively standard chemometric techniques (SIMCA, PLS-DA, LDA, SVM). Recently, an interesting custom off-the-shelf MOS e-nose 9-sensor array was employed to successfully classify beers from different styles and types of fermentation with the help of advanced multivariate methods (ANN ML frameworks) (Gonzalez Viejo et al., 2020).

2.2.1.2 According to brand identity

Although there meant to be some overlap between this and the category above (identification of types of beer) there are several very interesting studies explicitly dealing with identifying certain brands of beer even if they belong to the same type.

Whether it is a specific brand, or a beer with a designation of origin (like Trappist Beer) involved, consumers and the regulators feel very strongly towards potential fraud, which can have dire consequences for the industry at large. Using a very large dataset from the EU TRACE project, Mannina et al. (2014) classified 238 beers in Trappist vs non-Trappist, Rochefort vs non-Rochefort categories with high accuracy (95 and 90% respectively) using ¹H-

NMR and relatively conventional chemometric techniques (PLS-DA and SIMCA). On the same sample set, a high end approach (headspace solid-phase microextraction gas chromatography coupled to gas chromatography–time-of-flight mass spectrometer, HS-SPME GC–TOFMS) could recognise beer brands based on their volatile fingerprint (Cajka et al., 2010). It is notable that the researchers used advanced multivariate calibration methods in the form of artificial neural networks with multilayer perceptrons (ANN-MLP) in parallel with the conventional methods (PLS-DA/ SIMCA). In fact, the ANN-MLP classifier is one of the earliest uses in the literature of beer authenticity and paved the way for the follow up studies.

More recently, excitation-emission matrix fluorescence spectroscopy coupled with multi-way chemometric techniques has been proposed for the non-invasive classification of Chinese pale lager beers produced by four different manufacturers (Fang et al., 2021).

Apart from single instrument techniques, the researchers in the area recognised the benefit of using signals from multiple analyses, techniques, and instruments, which is also compatible with the concept of chemometrics.

The work of Biancolillo et al. (2014), who reported the fusion of responses from several instrumental techniques to enhance the discrimination power among two high quality Italian beers, has been influential. The techniques involved were heterogenous: various spectroscopic (UV, Visible, MIR, and NIR) techniques and a thermogravimetric (TG) measurement; they were ingeniously selected to unambiguously characterise the samples. The different analytical signals and numerical parameters were fused to create revised PLS-DA and SIMCA calibration models and ultimately achieved better results than the models of the standalone techniques. Tan et al. (2015) also showed that chemometric classification of Chinese lager beers by manufacturer is possible using only a trio of spectroscopy techniques (fluorescence, UV, and visible spectroscopies) coupled with a simple LDA.

The argument against the data fusion strategy using so many techniques is laborious and time consuming. However, the truth is that the chemical information obtained is more comprehensive and generally this facilitates classification or discrimination, and not only an academic exercise.

2.2.1.3 According to origin

Until now research where beers were differentiated according to type and brewing was presented. The problem becomes exponentially more challenging when a beer of the same type can derive from different raw materials or production methods solely due to its geographical origin. Fewer examples of geographical discrimination of beers can be found in the literature.

In its simplest form, geographical identification could mean identifying a production site within the same country or within the same production company (brand). Such identification is usually linked with the presence of certain biomarker profile that is either deriving from the secondary analysis or is a key measuring parameter. Vera et al. (2011) demonstrated that Head Space Mass Spectroscopy (HS-MS) e-nose spectral fingerprints could differentiate the aroma of Spanish lager beers from different factories. Although the study used simple chemometrics (LDA), it was possible to discriminate between 3 or 4 production sites based on 111 discreet volatiles that were tentatively identified. Czech and non-Czech brands could be identified by their free amino acid content (Kabelová et al., 2008). Similarly, three Spanish production areas were identified according to their mineral profile, which links soil to food origin through the bioaccumulation of soil markers in the food from the plant nutrition pathway.

In the global market, however, beer origin detection is challenging. In a follow up study, Alcazar et al. (2012) were able to identify the country of production (only among three countries, Germany, Portugal, and Spain), where a plethora of chemical and physical descriptors were implored to aid with discrimination. These included multiple minerals as well

as chemical indexes (total amino acids, total polyphenols, pH, real extract, and absorbance at 430 nm). Although a proper data fusion strategy was not followed, the level 1 SVM chemometric models showed very good accuracy (>99%).

2.2.1.4 According to style (craft vs standard)

Style in beer refers to the production style and it can vary usually between craft (both conventional and special beers) and industrial from well-known large breweries. The lines have been blurred because the large-scale manufacturers of industrial beers are now also producing craft beers to compete in price and in quality with the small-scale producers and the microbreweries that produce premium craft beers.

The identification of style is inherently a more difficult analytical problem. The distinction is generally impossible with the traditionally used beer analytical methods (apart from the organoleptical examination in special cases). In view of this, the question arises as to whether and to what extent it is possible to distinguish the different types of brewing based on untargeted analytical techniques and chemometrics. The beer style however is interconnected with origin and production methods. For example, the only thing that could differentiate a craft from an industrial beer could be the aroma, when the type (e.g., lager) and origin (e.g., Italy) are the same. In other words, the production method could result in different volatile and non-volatile chemical profile, without this necessary coming from the addition of adjuncts (fruits and different cereals) that are often used in craft beers. In this analytical problem, the research requires a higher level of innovation both in the techniques used and in the chemometric analysis to result in a tentative method. The most crucial element is the study design and how the samples are differentiated. Some researchers use the information exactly as supplied by the manufacturer on the label (e.g., premium vs standard) whereas others use the retail price as the differentiator between the two classes.

With the application of NMR spectroscopy, it has been found that aromatic signals are more appropriate to discriminate among brewing styles. Silva et al. (2019) employed $^1\text{H-NMR}$ spectroscopy combined with chemometrics to discriminate lager beer samples from two different classes, according to their style and information provided on the label. Using the same technique and different chemometric models (OPLS-DA), craft beers were well discriminated from industrials (Palmioli et al., 2020).

“E-tongue” is a new term for an emerging technique based on electrochemical fingerprints generated from potentiostat or galvanostat sensors in custom configurations. Mutz et al. (2021) proposed a testing procedure using commercially available screen-printed electrodes with promising results for the rapid differentiation of Brazilian lager beer (premium and standard American-style lager). E-nose is a similar technique based either on electrochemical fingerprints from their MOS sensors or, in its most sophisticated variant, mass fragment fingerprints from a mass spectrometer. In the implementation of Pereira et al. (2016), the e-nose system was based on paper spray mass spectrometry (PS-MS) and applied in a forensic context for the differentiation of beers. In this study the researchers let the market price define their class boundaries (premium vs standard lager) and were still able to achieve almost 100% of reliability rate, without any false negative or false positive prediction, using partial least squares discriminant analysis (PLS-DA) and Ordered Predictors Selection (OPS) for variable selection.

2.2.2 Beer rapid analysis in Quality Control

The general composition of beer consists of water, carbon dioxide (CO_2), alcohol, and extract mainly of organic acids, alcohols, amino acids, and carbohydrates. Quality Control (QC) is an essential element of beer production. For the industry, quality is defined in terms of maintaining batch-to-batch consistency and safety of the final products. In principle, QC can occur in any

or all of the three major biochemical processes of beer production (malting, mashing and fermentation) and innovative ways to automate this process is always beneficial for the industry.

The concept of QC is first described in the influential work of Duarte et al. (2004), where FT-IR and the ^1H NMR spectra of beers of different types (ale, lager, alcohol-free) were used to extract spectral information about factors affecting beer production. Although the chemometrics used are limited to PCA (which doesn't really offer classification, only exploration of data) and the methods were not rapid (30 mins for an NMR measurement), the researchers identified specific spectral regions that informed follow up research. Specifically, they identified that the $1200\text{-}800\text{ cm}^{-1}$ of the FT-IR and the aromatic region (6.0-10.0 ppm) of the NMR were able to give direct information about ethanol content (separating beers with more than 6.0% alcohol from the rest) and the carbohydrate source, i.e., different sugars indicating different cereals (FT-IR) and the type of beers in general (ale, lager, and alcohol-free) (NMR). The seminal work of Lachenmeier et al. (2005) elevated the concept of QC by linking the ^1H -NMR signals to QC reference values that are used in the beer industry (original gravity, ethanol and lactic acid to MEBAK-guidelines) using PLS- regression. Rodrigues et al. (2011) offered insights into beer quality and beer ageing, specifically, by monitoring and modelling the storage with ^1H -NMR. Interestingly, when NMR spectra were classified as a function of their age, a clear aging trend was observed using PLS-DA, and a potential marker was identified (5-hydroxymethylfurfural).

In the framework of QA/QC, all previously mentioned works used a ^1H -NMR, a tool that is not possible to have industrial applications, at least in the production line (in-line) or in QC or new product development laboratory.

Lachenmeier (2007) explored the feasibility of FT-IR for rapid evaluation of quality and authenticity of beers and found that the majority (85%) of all samples tested were classified (PLS-R) as being in conformance with predetermined legal and quality requirements (relative density, ethanol, lactic acid and original gravity). About 10% of all samples exceeded these quality limits and were referred for confirmatory analysis. This is a big improvement because both the cost and time of FT-IR analysis are very low compared to NMR. Very recently, Franca et al. (2021) introduced advanced monitoring concepts for craft beer manufacturing using Multivariate Statistical Process Control as part of Process Analytical Technology (PAT). This study demonstrated the potential of rapid non-invasive remote analytical sensors (here, the NIR) and the power of large dataset chemometrics in a real-life scenario in the industry (7 batches of Belgian Pale Ale). NIR spectroscopy is certainly more applicable in an industrial setting than FT-IR on NMR and generally more suited for proper in-line QC, with many examples in other food and drink industries (REF).

Lastly, as sensory acceptability is the important element of the beer quality for the industry, some works have been done towards linking sensory profiles with analytical fingerprints and subsequently predicting positive or negative sensory attributes of a beer sample from those fingerprints deriving from untargeted analysis and modelling with multivariate algorithms. An example is the palate fullness prediction using a battery of physicochemical parameters (original gravity, viscosity, indices of macromolecular distribution, total nitrogen and β -glucan) that are usually recorded for QC purposes (Krebs et al., 2021). A good correlation between palate fullness and other sensory characteristics (as assessed by a proper sensory panel) and the analytical parameters was established, and a regression model was built using PLS-R to predict the palate fullness intensity in beers showing decent performance ($R^2 = 0.7993$).

3. Conclusion

3.1 Authenticity & Chemometrics of Wine. Wine authentication is of major concern to the whole food industry and also has great importance in quality control and consumer information. The capabilities of various analytical methods, used in combination with complicated chemometrics, on the discrimination and classification of wines, have been widely proven in many surveys the last decades. For that reasons, further developments should aim at improving the classification power of the proposed methods and design certification tools to allow the accurate classification of all the wine samples analyzed to develop a much larger database.

3.2 Authenticity & Chemometrics of Beer. The literature agrees the beer authenticity is a multivariate problem that needs multivariate statistics (chemometrics in this case). However, the quality of the chemometrics methods, however, needs to be elevated as many of the research studies still perform linear classification using PLS-DA, LDA and SIMCA and non-linear, next generation classifiers such as the ANN or SNV are rarely used. Regarding the samples, it is noted that the researchers could not have access to the vast samples (and therefore untapped data) that the industry has. Naturally, the research studies show the potential and prove the concept so that these methods can be adopted, improved and scaled for industry applications. It is in this step that more work needs to be done, with academia and industry working closely in larger studies and big data. In terms of characterisation of beer, resolution of the beer volatile and non-volatile profile, the research community has used the most modern and the most sophisticated equipment to analyse beers and identify chemical markers that could aid discrimination. Rapid methods have also been employed including novel technologies such as the e-nose and the e-tongue. This literature review indicates that areas under investigation where robust solutions have still not been proposed are the rapid identification of beer style and geographical origin. But this is not an issue only found in beer; numerous other products,

such as cheese, wine, milk, also face the same problem and it is something that would require significantly more innovation in analytics and algorithms to achieve in the years to come.

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