Recent advances in NMR-based metabolomics of alcoholic beverages

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ARTICLE INFO

Keywords:
Wine
Alcoholic beverage
Asia
Metabolomics
Nuclear magnetic resonance
Authentication

ABSTRACT

Alcoholic beverages have a complex chemistry that can be influenced by their alcoholic content, origin, fermentation process, additives, and contaminants. The complex composition of these beverages leave them susceptible to fraud, potentially compromising their authenticity, quality, and market value, thus increasing risks to consumers' health. In recent years, intensive studies have been carried out on alcoholic beverages using different analytical techniques to evaluate the authenticity, variety, age, and fermentation processes that were used. Among these techniques, NMR-based metabolomics holds promise in profiling the chemistry of alcoholic beverages, especially in Asia where metabolomics studies on alcoholic beverages remain limited.

1. Introduction

Asia has a long history of local production of alcoholic beverages dating back hundreds of years (Wiwanitkit, 2019). Despite the variety of alcoholic beverages in terms of taste, odor, and appearance, global production of alcohol is increasingly dominated by a shrinking number of companies (Day & McSweeney, 2016; Luo et al., 2020; Wardencki, 2018; Jernigan & Ross, 2020). In addition to being a substantial part of the food sector worldwide, production and consumption of alcoholic beverages play an important role in food security and health (Agnoli et al., 2018; Rugani, 2018).

The flavor and aroma of alcoholic beverages are attributed to their complex composition of chemical compounds such as esters, alcohols, acids, lactones, carbonyl compounds, acetals, phenols, and sulfur- and nitrogen-containing volatile compounds (Charapitsa et al., 2021; Uzhel et al., 2020; Zhou et al., 2015). This complexity means that alco-
holic beverages are susceptible to adulteration, creating the challenging need for authentication. Falsification of labels or fraud in a commodity is considered a criminal offense (Neufeld & Rehm, 2018; Patra et al., 2019). Thus, proper labelling must accurately reflect a product's information in order to fulfill the expectations of consumers. The adulteration of alcoholic beverages is performed to increase the alcoholic strength of a product with cheaper sources of sugar and starch besides grapes or other fruits (Newman et al., 2018). Another adulteration practice involves watering down beverages to lessen the alcohol content of alcoholic beverages (Lachenmeier, 2016a, 2016b).

Adulterations have been reported all over the world including incidents of unrecorded alcohol samples from China exceeding the specified alcohol content (Newman et al., 2018) and deaths in the Philippines due to suspected poisoning reportedly caused by lambanog, a Filipino alcoholic drink (Crisostomo, 2018; FDA, 2018a, 2018b; Lim, 2019).

Authenticity methods are in increasing demand for commercially fermented food and beverages, and much progress has been reported such as the verification of alcoholic beverage authenticity and detection of adulteration (Gilszczynska-Świło & Chmielewski, 2017; Pereira et al., 2018). Traditional methods such as sensory analysis for food authentication are still being used as an integral part of product standards and quality control (O’Sullivan, 2017; “Uses and good practices of sensory evaluation in the alcoholic beverage industry,” 2017). One of the most recent and advanced analytical techniques that could improve the expensive and time-consuming methodologies in metabolomics—the comprehensive qualitative and quantitative analysis of the distribution of all metabolites present in a given biological system (Kueger et al., 2012; López-Ruiz et al., 2019). This type of omics is employed to study the impact of agricultural practices, processing, and storage on the global chemical composition of food (García-Cañas & Simó, 2019; Zhang et al., 2020). In addition, this method is important for identifying novel bioactive compounds, authenticating product, and classifying region-of-origin (Johanningsmeier et al., 2016). Using the omics studies, foodomics has been developed for investigating traditional and new issues in food analysis through the use of epigenomics, genomics, transcriptomics, proteomics, and metabolomics tools (Braconi et al., 2018; Cifuentes, 2012). Moreover, metabolomics has been successfully applied to display the metabolite profiles in various alcoholic beverages to assess safety, authenticity, quality, and traceability (Añón et al., 2015).

NMR-based metabolomics provides structural and quantitative information, which can be advantageous in the identification of unknown metabolites (Brennan, 2014). In general, the use of metabolomics in alcoholic beverages has created new opportunities to help monitor the production process for product safety, quality, and traceability (Hirst & Richter, 2016; Rizzo et al., 2018; Singh et al., 2017). While alcoholic beverages have a high rate of adulteration amongst commodities, limited studies have been conducted for these beverages in Asia. This review focuses on the role of metabolomics applications in the profiling of alcoholic beverages in Asia, focusing on recent advances in NMR-based metabolomics approaches to ensure quality and safety in alcoholic beverages.

2. Metabolomics in wine and alcoholic beverages

Recent studies describing NMR-based metabolomics in alcoholic beverages show its growing field of applications. A brief comparison in terms of advantages, disadvantages, and compounds/properties analyzed using different analytical techniques are presented in Table 1. Significant emphasis is given to the implementation of the NMR techniques in the following section, and relevant and up-to-date examples of applications on alcoholic beverages are presented.

Nuclear magnetic resonance (NMR) spectroscopy allows identification of compounds without the need for complicated sample preparation. This spectroscopic technique can discriminate compounds that have identical masses and can be used to elucidate metabolic mechanisms and pathways using stable isotope labeling (Markley, 2017). In addition to NMR spectroscopy, analytical techniques such as gas chromatography (GC) or liquid chromatography (LC) and Fourier transform (FT) methods are also employed in wine metabolites studies. Some of the separation techniques are used in tandem with mass spectrometry (MS) for its high selectivity and sensitivity as well as the potential to identify metabolites (Abd Ghafar et al., 2020; Karamanou & Aliferis, 2020). Combining a separation technique such as GC or LC with MS provides isobar separation, reduces the complexity of the mass spectra through metabolite separation in the time dimension, and delivers additional information on the physicochemi-

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**Table 1**

Selected Representative Analytical Techniques used in Alcoholic Beverage Metabolomics.

| Technique | Advantages | Disadvantages | Compounds/Properties Analyzed | References |
|-----------|------------|---------------|------------------------------|------------|
| LC–MS     | High throughput and good coverage of metabolites | Data may have biological or analytical variability due to sample preparation, instrument condition, or operating environment | Polyphenols, amino acids, biogenic amines, and ammonium ions, trans-resveratrol, allergic residues, characterization of whiskey and beer | Donato et al., 2016; Lu, Liao, & Chen, 2018; Redruello et al., 2017; Spinelli et al., 2019; Collins et al., 2014; Hughey et al., 2016 |
| GC–MS     | High sensitivity, peak resolution, and reproducibility | Requires sample derivatization or pre-treatment due to differences in polarity of the analytes and to enhance the volatility and thermal stability of the metabolites | Ethanol, biogenic amines, semi-volatile and volatile constituents | Cai, Rice, Koziel, & Dharmadhikari, 2017 |
| FT–IR     | Can analyze most samples | Difficulty in analyzing molecules that do not vibrate, Trouble differentiating compositions in a mixture | Alcohol, grape quality, total antioxidant capacity, wine poly saccharides, quality control | Barnaba, Bellincostro, & Mencarelli, 2014; Boulet, Williams, & Doco, 2007; Lachenmeier, Richling, López, Frank, & Schreier, 2005; Versari, Parpinello, Scanzina, & Rio, 2010 |
| NMR       | Nondestructive | Difficulty in detecting metabolites at very low concentrations, thus requiring solvent suppression methods to improve resolution for better metabolite identification | Structural characterization, classification of variety and vintage, authentication, quality control | Fan et al., 2018; Geona et al., 2016; Kew, Goodall, & Ubrín, 2019; Monakhova, Kuballa, & Lachenmeier, 2012; Fotakis & Zervou, 2016; Cassino et al., 2019; Belmonte-Sánchez et al., 2020; Munn et al., 2020; Gougnon et al., 2019; Sánchez-Esteban et al., 2018; Dzhamak et al., 2017; Giepiekloiski et al., 2019 |
cal properties of the metabolites (Dettmer et al., 2007; Zeki et al., 2020). Only a brief discussion of non-NMR techniques is presented in this paper. We refer the readers to more comprehensive reviews that cover the role of MS, gas or liquid chromatography, and FT methods in metabolomics (Aszyk et al., 2018; Beale et al., 2018; Esteki et al., 2018; Hird et al., 2014).

2.1. Mass spectrometry (MS)

Mass spectrometry is one of the common analytical techniques for the analysis of metabolites in complex biological samples. Its high sensitivity and fast data acquisition enable MS to be one of the most widely used analytical tools in the field of metabolomics (Brunius et al., 2016). Liquid chromatography and gas chromatography are separation methods typically combined with MS. Each of these methods provides solutions to complex mixture analyses and have been used extensively in metabolomics. LC coupled with electrospray-ionization MS (LC–ESI–MS) is commonly applied for detecting metabolites in complex biological samples and in profiling unknown metabolites. This “soft” ionization method forms intact molecular ions and aids in initial identification (Aszyk et al., 2018; Bowen & Northen, 2010), allowing for a wider range of metabolite detection than GC–MS because metabolite volatility is not required (Vinaixa et al., 2016). In addition, good chromatographic separation will result in higher quality MS data with reduced background noise.

Recent applications of LC–MS in alcoholic beverages include analysis of polyphenolic compounds, biogenic amines, resveratrol, and allergenic residues (Donato et al., 2016; Lu et al., 2018; Redruello et al., 2017; Spinnelli et al., 2019). Successful identification of metabolites requires accurate mass measurements and high-quality mass spectra. By obtaining the mass spectra, the structures of the metabolites of interest can be further confirmed, as fragmentation helps in deducing possible structures of metabolites during spectra interpretation (Tada et al., 2019). Because fragmentation patterns may contain a wide variety of possible metabolite structures, it is difficult to identify metabolites through MS alone. To solve this problem, the detected ions can be identified using a spectral library match or mass-based search using the retention index derived from retention time (Matyushin et al., 2020). However, no such index currently exists for LC–MS experiments, and the comparison of retention times can only be performed under identical experimental conditions (Chaleckis et al., 2019). On the other hand, use of collisional cross section (CCS) values from LC–MS coupled with ion mobility spectrometry (IMS) can give additional confidence in identifying detected ions (D’Atri et al., 2018; Nichols et al., 2018).

Similar to LC–MS, GC–MS has been widely used in the food sector and in wine metabolomics studies due to its high sensitivity, peak resolution, and reproducibility (Kang et al., 2016; Korban et al., 2021; Stupak et al., 2017). However, one drawback of GC–MS is the complex sample preparation prior to analysis, which aims to produce extracts that are compatible with the GC technique. Pre-treatment and extraction of the sample are sometimes required to enhance the volatility and thermal stability of the metabolites. Sample derivatization is one of the pretreatment procedures commonly used in GC–MS analysis of metabolites to modify polarity and facilitate chromatographic separation on a column of low polarity (Moros et al., 2017). One-step reactions are preferred, but sometimes, two-step derivatizations are more desirable when the obtained derivatives show better chromatographic separation or more adequate mass fragmentation (Miyagawa & Bamba, 2019).

GC–MS analysis also requires an extraction process in order to separate the metabolites and enhance their concentration. Extraction of metabolites from the sample should be as comprehensive as possible, but care should be observed to prevent metabolite degradation or modification (Desbrosses et al., 2005; Pinu et al., 2017). Recent methods have focused on improving the extraction of metabolites from alcoholic beverages using solid phase microextraction (SPME) (Cai et al., 2017; Papageorgiou et al., 2018; Riu–Aumatell et al., 2014; Sagandykova et al., 2017), or stir bar sorptive extraction (SBSE) (Hevia et al., 2016; Takase et al., 2015; Zhou et al., 2015) methods. Most analyses using GC–MS involve determination of volatile aromatic compounds, organic acids, amino acids, carbohydrates, and polyphenols in wines and alcoholic beverages (Cai et al., 2017; Hevia et al., 2016; Papageorgiou et al., 2018; Takase et al., 2015).

2.2. Fourier transform infrared (FT–IR) spectroscopy

In contrast with the chromatographic techniques previously discussed, FT–IR spectroscopy provides rapid and nondestructive analysis of alcoholic beverages with little sample preparation required. It is a valuable metabolic fingerprinting tool that has the ability to simultaneously analyze compounds such as carbohydrates, polyphenols, amino acids, proteins, and polysaccharides (Anjos et al., 2020; Garcia–Hernandez et al., 2020; Puzeu et al., 2019; Teixeira dos Santos et al., 2016) and has been applied for quality control and authentication (Anjos et al., 2016; Basalekou et al., 2020; Yadav & Sharma, 2019). However, its main drawback is its inability in the differentiation of the chemical constituents present in a mixture (Kumar et al., 2019; Lerma–Garcia et al., 2018). Nevertheless, combining FT–IR spectroscopy with different analytical techniques provides information on elemental composition, molecular structure, and chemical speciation. This enables accurate identification of the main compounds found in alcoholic beverages such as ethanol, sugars (e.g., glucose and fructose), and organic acids (Li et al., 2014; Wang, Shao, Chen, & Li, 2019; Wang et al., 2017).

2.3. NMR spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is a widely used analytical tool in metabolomics due to its non-destructive nature and rapid analysis (Wishart, 2019), providing a high-throughput method with minimal sample preparation, an advantage over chromatographic techniques. Unlike FT–IR spectroscopy, NMR-based techniques enable simultaneous identification and absolute quantification of compounds; prediction of authenticity parameters such as variety, geographical origin and vintage; and the potential for the detection of unexpected and unknown fraud or infection (Trignano et al., 2015). NMR is widely used today for alcoholic beverage analysis, with continuous novel research for additional uses. (Diamantidou et al., 2018; Fan et al., 2018; Geana et al., 2016; Kew et al., 2019; Kuballa et al., 2018).

Due to proton abundance in metabolites, proton (1H) NMR spectroscopy is often used for profiling an extensive range of metabolites such as amino acids, polyphenols, sugars, and other organic compounds present in alcoholic beverage samples (Bordiga et al., 2016; Lu et al., 2018; Peterson & Waterhouse, 2016; Zerbib et al., 2018). In particular, 1H NMR methodologies have been applied to determine chemical constituents (phenolics, sugars, and organic acids) and to measure the alcohol content for authenticating the quality of alcoholic beverages (Belmonte-Sánchez et al., 2020; Polak & Bartoszek, 2015). The chemical composition obtained from NMR spectroscopic data constitutes the so-called alcohol beverage metabolome, which can vary significantly depending on the geographic origin, variety, aging, fermentation process, and quality of alcoholic beverages (Alañón et al., 2015; Bokulich et al., 2016; Dzhimak et al., 2017; Spraul et al., 2015). Because metabolites from a sample may produce complex 1D NMR spectra with poorly resolved proton resonances, high-field NMR instruments and two-dimensional NMR (2D NMR) spectroscopy are typically employed to improve resolution and provide additional NMR spectral data to aid in the interpretation of NMR data (Bo et al., 2019; Moser et al., 2017).

High-resolution NMR spectroscopy has become the most suitable technique for analyzing beverages at the molecular level following the development of high-field NMR spectrometers. Multivariate statis-
tical analysis or chemometrics in combination with NMR spectroscopy for data processing have also improved the sample analysis by reducing analysis time and providing more informative results (Medina et al., 2019). Chemometric methods such as partial least squares (PLS), principal component analysis (PCA), and partial least squares-discriminant analysis (PLS-DA) can determine characteristic patterns of compounds or parameters related to a geographical origin, the adulteration of samples, or certain specific conditions such as processes, storage, climate, and variety (Borràs et al., 2015; Magdas et al., 2019).

While the ultimate goal of metabolomics is the identification and quantitation of all small molecules in an alcoholic beverage sample, a single method allowing complete metabolome analysis does not exist. Several complementary analytical techniques should be applied to improve the scope and identification ability in metabolite analysis.

3. Recent applications of NMR-based metabolomics

During the last few years, NMR-based metabolomics has been applied to characterize complex food matrices and has been widely used in profiling food samples for quality and authentication assessment (Rocha & Sheen, 2018). Authenticity of alcoholic beverages has been extensively investigated because it is a product that can be easily adulterated due to its signature chemical properties (high alcohol content, low pH) and its availability throughout the world (Ahmad et al., 2018; Cioch-Skoneczny et al., 2020). In addition to authenticity, traceability has an important role in the quality assurance of alcoholic beverages. A traceability protocol must be established on the supply chain to track and manage all representative events which may arise from raw material production to selling the final product (Kamiloglu, 2019). Alcoholic beverages are valuable commodities that are recognized for their geographical region, variety, and age. These factors are the main determinant for determining consumer preference and market value (Anderson et al., 2019). Therefore, the use of a metabolomics platform adds value by providing fingerprints to classify samples of alcoholic beverages by variety, age, fermentation processes, and geographic origin. The following section discusses how NMR-based metabolomics have been applied to evaluating different alcoholic beverages in Asia.

3.1. Variety effect

Different kinds of plant and fruit sources are needed to create an alcoholic beverage. The final product is both complex and information-intensive, so it is important to understand the behaviors and characteristics of different varieties (Chay et al., 2017; Li & Sun, 2019) from the plant source to the finished product.

The characteristic metabolites of Cabernet Sauvignon and Shiraz from Shanxi province, China were analyzed by $^1$H NMR spectroscopy.
(Zhu et al., 2018), and the biomarkers for the differentiation between wine samples were identified using pattern recognition analysis. The NMR spectra (Fig. 1a) showed the proton signals of the major metabolites of Cabernet Sauvignon and Shiraz red wines. The PCA score plot (Fig. 1b, left) from the 1H NMR data of Cabernet Sauvignon and Shiraz dry red wines suggested significant differences in metabolites from these two wines. Data from the PLS-DA score plot (Fig. 1b, right) showed more pronounced differences in metabolites present between Cabernet Sauvignon and Shiraz dry red wines compared to the one illustrated in PCA score plot. The main contributors to these metabolite differences as identified from the PLS-DA loading plot (Fig. 1c) were proline, tartaric acid, glycerin, lactic acid, choline, succinic acid, and gallic acid. Proline in particular had largest difference in content (0.268 g/L difference) between the two wine samples. The proline content had a direct impact on the depth and complexity of wine taste and flavor, affecting both the sweetness and bitterness in the wine (Zhu et al., 2018). Because proline in wines is not consumed by fermentation and maturation, the difference in proline content in the study is primarily attributed to the different wine varietals (Zhu et al., 2018). The PLS-DA score plot also revealed significant difference in tartaric acid content (0.022 g/L) between the two wine samples. This nonvolatile acid could impart dark red color to wines, and its content depends on the type of grape that is used in making wine (Zhu et al., 2018). Finally, the difference in succinic acid content (4 × 10⁻³ g/L) between the two samples was also found to be significant. Succinic acid content is dependent on yeast nitrogen metabolism and had an influence on the sweetness and body thickness of wines (Zhu et al., 2018). Overall, these significant differences may be used for discrimination between Cabernet Sauvignon and Shiraz dry red wine samples. Further, the significant differences in metabolite content between these wines could be utilized for establishing an evaluation system for assessing the quality and authenticity of these wines.

Another study investigated the metabolites present in different varieties of dry red wine (Cabernet Sauvignon, Merlot, and Cabernet Gernisch) vinified in Changli, Hebei province, China, using 1H-based techniques (Hu et al., 2020). NMR data was processed by integrating the chemical shift between 0 and 10 ppm followed by removal of the internal standard (4,4-dimethyl-4-silapentanesulfonate, DSS), residual ethanol, and residual water peaks. The integral data was subsequently normalized and subjected to multivariate statistical analyses. While most of the metabolites (mainly amino acids, organic acids, sugar, phenolic compounds, etc.) that were identified from these wine samples are similar, their concentrations are different. PLS-DA loading plots of the three dry red wines revealed content differences in proline, ethyl acetate, valine, 2,3-butanediol, succinic acid, glycerol, lactic acid, β-glucose, malic acid, tartaric acid, α-D-glucuronic acid, choline, alanine, and gallic acid. Relative contents of these metabolites were determined by comparing the peak area of their proton resonances to that of DSS. Among these metabolites, succinic acid and glycerol contents were highest in Merlot. On the other hand, gallic acid content was highest for Cabernet Sauvignon. Lactic acid, alanine, proline, and malic acid contents were highest in Cabernet Gernisch. These main metabolites were closely related to the flavor, taste, and functional activity of wine. For example, glycerol contributes to mouth-feel perception because of its sweet taste and viscous nature while succinic acid makes wines rich in flavor and mellow in taste. Succinic acid content is influenced by malic acid levels and yeast species (Hu et al., 2020). Because the three dry red wines underwent the same brewing process, the differences in their glycerol and succinic acid contents are most likely due to the wine varietals. The other main metabolites namely gallic and malic acids have reported health benefits. This study provided an effective and feasible method for establishing metabolic content profiles for dry red wines that can be used for the identification of dry red wine varieties and could offer reasonable advice for consumers to choose suitable dry red wines according to their preferences (i.e., flavor, taste, and health).

### 3.2. Aging/vintage effect

The changes in alcoholic beverages after fermentation and before bottling are also essential in their metabolomics. Aging is a result of oxidation and non-oxidative processes (Li et al., 2017). To ensure consistent aging before bottling, beer, wine, and spirits undergo “matur-ation” – (Miller & Miller, 2019) a long and expensive process in which the beverage acquires a distinct flavor due to factors such as type of alcohol and wood matrix (González-Robles & Cook, 2016; Morishima et al., 2019). The effect of aging on alcoholic beverages is monitored by the changes in their composition such as their anthocyanin content (Li et al., 2017), yeast interactions (Legras et al., 2016), and aging techniques (Santos et al., 2019).

1H NMR analysis coupled with multivariate statistical analyses (PCA and PLS-DA) and hierarchical cluster analysis (HCA) was used to investigate the genetic relatedness of grapevine (V. vinifera) cultivars grown on the same site and vinified through similar processes from Hebei, China (Hu, Yue, Zhu, Wen, & Zhang, 2015). NMR data was acquired using 1H and 1H–1H correlation spectroscopy (COSY) NMR techniques and processed by integrating the chemical shift between 0 and 10 ppm followed by removal of DSS, residual ethanol, and residual water peaks. PCA and PLS-DA revealed discriminant grape metabolites such as proline, valine, tartaric acid, glucose, and total phenols (mostly gallic acid). Content of phenolic compounds is influenced by several factors such as grape cultivar, fermentation processes, maturation with wood contact, and wine age. While NMR assignments for these compounds are typically confounded by their complex structures, the total content of phenolic compounds can be a useful biomarker to discriminate wine in terms of grape cultivars. Hu et al. identified gallic acid as the main phenolic compound present at different levels in the tested wine samples: Ruby Cabernet > Cabernet Sauvignon > Merlot > Syrah > Zinfandel. PCA and PLS-DA also revealed several discriminating compounds arising from alcoholic fermentation: 2,3-butanediol, glycerol, valine and succinic acid. Overall, the NMR data coupled with multivariate and hierarchical cluster analyses presented groupings that reflect the genetic heritage of the cultivars and revealed important biomarkers for fermentation processes and cultivars discrimination.

Metabolite differences in 2009, 2010, 2011, and 2012 vintages of Cabernet Sauvignon wines from Ningxia was explored using 1H NMR techniques coupled with pattern recognition methods (PCA and PLS-DA) (Hu et al., 2016). The variation in metabolite compositions was found to be small while the differences in metabolite levels were found to be significant among the vintages (Fig. 2 showing discrimination of 2011 and 2012 vintages). The metabolites responsible for the differentiation were identified as 2,3-butanediol, ethyl acetate, valine, proline, succinic acid, lactate, acetic acid, glycerol, gallic acid and choline. Because fermentation technique, yeast, and grape varieties were similar for these wine vintages, varying concentrations of most of the discriminating metabolites were likely influenced by climatic factors such as average temperature, rainfall, and evaporation. For example, the higher-than-average temperature (17.65 °C) and evaporation (1562.1 mm) and less rainfall (243.5 mm) in 2009 were favorable climatic conditions for grapes to reach optimum ripeness, thus likely affecting their sugar content. This climatic condition in 2009 may have influenced the higher valine, 2,3-butanediol, gallic acid, and proline content in 2009 vintage relative to 2011 vintage, when the climatic conditions were characterized by lower-than-average temperature (15.24 °C) and evaporation (1423.6 mm) and high rainfall (262.2 mm). Taken together, this study opens new opportunities for exploring other discriminants that can be useful for classifying alcoholic beverages according to their vintage, especially in regions with the most variable climates.

### 3.3. Fermentation process

Alcoholic beverages are obtained through the process of fermentation of a sugar to ethyl alcohol and other minor products such as amino
acids and carbohydrates (Yıldırım, 2021). Most fermentations of alcoholic beverages are accomplished using strains of the yeast *Saccharomyces cerevisiae* (Walker & Stewart, 2016). The fermentation process is one of the most important steps in producing wine and other alcoholic beverages, and the process must be monitored in order to achieve a high quality product.

NMR-based metabolomics studies have also been applied in recent years to monitor the fermentation process of alcoholic beverages. For example, selected bacterial and yeast strains (*Bacillus licheniformis*, *Pediococcus pentosaceus*, *Lactobacillus plantarum*, *Pichia kudriavzevii*, *Wickerhamomyces anomalus*, *Saccharomyces cerevisiae*, and *Saccharomyces fibuligera*) were investigated for their amylolytic activity, ethanol tolerance, and metabolite production during sorghum-based laboratory-scale alcoholic fermentation using $^1$H NMR spectroscopy coupled with multivariate statistical analysis (Li et al., 2018). Principal component analysis showed variations in the metabolites produced by different mixtures of pure cultures. *S. cerevisiae* was found to be associated with significant production of lactic acid (17.7 mmol/g with *S. cerevisiae* vs 0.3 mmol/g without *S. cerevisiae*). However, *S. cerevisiae* cannot convert starch into ethanol whereas *S. fibuligera* and *B. licheniformis* can convert starch or polysaccharides to soluble sugars such as maltose, maltotriose, and dextrin (Li et al., 2018). Therefore, *S. fibuligera* is introduced with *S. cerevisiae* in the alcoholic samples during fermentation. Lactic acid bacteria had high amylolytic and proteolytic activities, thus contributing to increased saccharification and protein degradation. Finally, *W. anomalus* was found to have a positive effect on the flavor of the Daqu-derived product as this species produces acetate esters such as ethyl acetate.

In recent years, inactive yeasts or deactivated yeasts are introduced in wine fermentation as they are reported to increase the rate of alcohol fermentation and influence the aroma components in wine. The effect of five inactive yeasts (Optim-White®, Noblesse®, Opti-LEES, Boster Blanche®, and Mannostab®) on the metabolites of Chardonnay dry white wines vinified in 2016 in Hebei province, China was investigated using $^1$H NMR spectroscopy coupled with multivariate analysis (PCA/PLS-DA) (Hu et al., 2019). The main metabolites of the wine
samples were identified from NMR data (Fig. 3a), and the differences of metabolites between the wines with inactive yeast and the original wine were analyzed using PCA (Fig. 3b) and PLS-DA (Fig. 3c). PCA and PLS-DA plots revealed significant influence of the added inactive yeasts on the levels of polyols, organic acids, amino acids, and other metabolites in the dry white wine samples. The main metabolites were found to be glycerol, 2,3-butanediol, lactic acid, malic acid, tartaric acid, succinic acid, gallic acid, proline, alanine, valine, ethyl acetate and choline. The higher glycerol levels in Opti-LEES, Boster Blanc®, and Noblesse® relative to the original wine suggest yeast-induced enhancement in activity and fermentation efficiency. Malic acid content is related to malolactic fermentation in which lactic acid bacteria convert maleate and citrate into lactate and other components (Hu et al., 2019). Higher levels of this organic acid in Optim-White®, Noblesse®, and Mannostab® relative to the original wine suggest yeast-induced enhancement in activity and fermentation efficiency. The contents of main amino acids valine, proline, and alanine in Noblesse® and Mannostab® were higher than that of the original wine. Other distinct metabolites such as ethyl acetate were also found to be higher in Opti-LEES, Boster Blanc®, Noblesse®, and Mannostab® compared to that of the original dry white wine. In summary, this study provided a theoretical basis for using NMR-based metabolomics in monitoring composition and content differences among original dry white wine samples to investigate the effect of inactive yeast to fermentation process.

3.4. Quality control/authentication

Quality assessment and authentication are crucial issues to the food and beverage industry, especially with alcoholic beverages. The quality of alcoholic beverages is often assessed by physicochemical and sensory tests (Ickes & Cadwallader, 2017; Palczak et al., 2019). Physicochemical laboratory tests include routine analysis of density, alcohol, or pH values, while sensory evaluation relies on human experts. There is an interrelated role of sensory, emotional, and cognitive responses, which affects the panelists’ issues of personal taste (Palczak et al., 2019; Stelick & Dando, 2018).

In recent years, integrated approaches using metabolomics in addition to sensory properties to assess the quality of alcoholic beverages have shown promise to improve upon sensory analysis (Nikolantonaki et al., 2018; Taniguchi et al., 2019). The assessment of alcoholic beverage authenticity is receiving increased attention.
since alcoholic beverages are often subject to fraudulent practices (Lachenmeier, 2016a, 2016b; Rodgers, 2020). The adulteration of such commodities consists of the addition of any substance to the natural wine to change its composition. The most common forms of adulteration include addition of water and sugar, mixing with lower quality wines, and fabricating labels (Lachenmeier, 2016a, 2016b; Zhang & Xue, 2016). Adulteration of alcoholic beverages is especially concerning due to the potential to harm customer health. For example, recent samples of lambanog, a popular alcoholic beverage from the Philippines, tested positive for high levels of methanol. The lambanog products tested by the Food and Drug Administration of the Philippines (FDA) were found to have methanol levels ranging from 6.5 percent to 21.8 percent. These levels greatly exceeded the Philippine National Standard and is suspected to have caused the deaths of some individuals (Críosóstomo, 2018; FDA, 2018a, 2018b; Lim, 2019). Thorough chemical analysis of the metabolic composition of such products could prevent these frauds. However, the association between chemical composition and wine origin is a challenge due to variability in the chemical composition and the chemical changes produced during the winemaking and fermentation stages. These issues highlight the need for modern analytical techniques and advanced data analysis to obtain reliable information to ensure the authenticity of wines.

4. Conclusion

The complex chemistry of alcoholic beverages and the factors influencing its chemical composition make it a difficult task to characterize such samples. Alcoholic beverage quality and authenticity need to be monitored from the plant source to the finished product, making profiling every step in the production a challenging task. Metabolomics is an emerging field ofomics research. NMR spectroscopy contributes greatly to metabolomics by producing information-rich, reliable, and reproducible data sets in both non-targeted or global and multivariate statistical analysis. Recent studies have demonstrated the promising potential of NMR-based metabolomics in understanding wine chemistry and profiling metabolites in alcoholic beverages.

New developments in NMR hardware and techniques, especially in combination with chemometric methods, have advanced detection of various metabolites in terms of geographical origin, variety, vintage, fermentation, and quality assessment in wines and alcoholic beverages. NMR-based metabolomics studies in Asia have exhibited promising opportunities for determining biomarkers and for establishing metabolomic “fingerprint” that may have important implications for assessing the quality, authenticity, and safety of alcoholic beverages. For example, phenolic compounds may be used as biomarkers for determining wine varietals. However, assignment of proton signals is limited by their complex structures; therefore, the use of other NMR-based techniques such as DOSY 1H NMR may help in identifying this class of compounds in alcoholic beverages. DOSY 1H NMR is also useful for providing information on the relative molecular sizes of the metabolites (e.g., maltose and maltotriose versus glucose and fructose) present.

Further research should be developed using multidimensional or orthogonal techniques, such as GC × NMR, or LC × NMR in metabolomics studies in the near future. Multidimensional methods should also be employed to enhance resolution of complex mixtures, achieve a higher peak capacity, and increase the sensitivity and selectivity. Investigation into novel applications using portable analysis may yield advantages such as decreased consumption of reagents and samples and fast automated analysis that can generate results in real time at a low cost (Spyros, 2016). Studies involving other alcoholic beverages aside from wine are seldom researched, especially in Asian countries; thus, it is recommended to turn these methods to locally-produced alcoholic beverages such as beer, whiskey, and those produced from other plant sources such as rice and coconut wine. In summary, research in metabolomics has enhanced our understanding of alcoholic beverages, but there is great potential for further advances both in methods used and their application to unraveling the components of complex matrices such as pharmaceuticals, dietary supplements, foods, and biological extracts.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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