

VOLATILE PROFILING AND METABOLOMIC DIFFERENTIATION OF FOUR CROP WINES FROM TWO BREWERIES USING GC-MS

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Abstract. Cereal and pseudocereal based alcoholic beverages, including barley, corn, buckwheat, and sorghum wines, exhibit unique flavor profiles due to their distinct volatile organic compound (VOC) compositions. This study employs gas chromatography-mass spectrometry (GC-MS) to characterize and differentiate these wines based on their volatile signatures. Black barley wines are rich in higher alcohol and phenolic compounds, contributing to malty and fruity notes, while corn wines contain elevated ethyl esters and diacetyl, imparting buttery and caramel nuances. Buckwheat wines feature nutty and earthy tones due to sulfur-containing compounds and 1,2:5,6-dianhydrogalactitol. Sorghum wines, influenced by fermentation methods (liquid- or solid-state), are marked by esters (e.g., ethyl lactate, ethyl butyrate) and 4-ethyl guaiacol. Chemometric techniques, such as principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA), aid in classification and quality control. The study highlights the impact of raw materials, fermentation conditions, and microbial activity on VOC profiles. Barley wines contain nearly 80 volatile and semi-volatile fungal metabolites, including alcohols, aldehydes, ketones, carboxylic acids, esters, aromatic compounds, alkenes, and terpenoids. Corn wines exhibit 24 significantly different volatile flavor compounds (out of 52 total) and 25 distinct non-volatile metabolites (from 600 identified), with acetic acid and succinic acid as key organic acids. Sorghum wines display varietal differences, with glutinous sorghum yielding more elegant and harmonious flavors compared to the stronger, more pungent profiles of non-glutinous varieties. Buckwheat wines show metabolomic divergence linked to flavonoid metabolism, particularly to rutin content between common (*Fagopyrum esculentum*) and Tartary (*F. tataricum*) species. This work highlights the biochemical diversity of cereal-based wines, offering new insights into their distinctive flavor and compositional characteristics. Novel insights include fungal metabolites in black barley wines, organic acids (acetic and succinic) in corn wines, and varietal differences in sorghum (glutinous vs. non-glutinous) and buckwheat (flavonoid metabolism in *Fagopyrum* species). While most research on wine focuses on grape-derived products, this study explores the unique metabolite profiles of cereal-based wines. This research underscores the importance of VOC analysis in enhancing authenticity, consumer preference, and innovation in non-grape wine production.

Keywords: *volatile organic compounds (VOCs), GC-MS, barley wine, corn wine, buckwheat wine, sorghum wine, chemometrics, aroma profiling*

Introduction

Alcoholic beverages made from cereals and fruits have been an integral part of human civilization for millennia, serving as sources of nutrition, social bonding, religious rituals, and cultural identity (ElShamey et al., 2025b; Tamang and Samuel, 2010). These

traditional drinks, often crafted through fermentation or distillation, vary widely across regions, reflecting local agricultural practices, climatic conditions, and historical influences. It is important to highlight the significance of barley, corn, buckwheat, and sorghum wines in different cultures. Cereals such as barley, maize, millet, and sorghum have long been used to produce alcoholic beers (Taylor et al., 2013; Dabija et al., 2021; ElShamey et al., 2025a). The enzymatic conversion of starches into fermentable sugars often facilitated by malting or the addition of microbial starters results in beverages ranging from mildly alcoholic beers to stronger spirits (Bwamu, 2023; Kaur and Gill, 2019). There are some examples of traditional cereal-based beverages; beer is considered one of the oldest alcoholic drinks globally (Fernandes et al., 2018), with ancient versions made in Mesopotamia, Egypt, and Europe using barley or wheat (Newman and Newman, 2006). The Japanese sake a rice-based brew produced through a dual fermentation process involving koji mold (*Aspergillus oryzae*) (Chaudhary, 2021). Chicha from the Andes is a maize or quinoa-based drink, sometimes chewed to initiate fermentation via salivary enzymes (Priyanka, 2018). Kvass (Eastern Europe) a low-alcohol rye or barley beverage, often flavored with fruits or herbs. Pito/Burukutu (West Africa) fermented sorghum or millet beer with regional variations (Qin et al., 2022).

GC-MS is an indispensable analytical technique for identifying and quantifying VOCs in alcoholic beverages (Plutowska and Wardencki, 2008; Ranjan et al., 2023; Savchuk et al., 2020). The complexity of aromas and flavors in beverages such as wine, beer, and spirits arises from hundreds of VOCs, including alcohols, esters, aldehydes, terpenes, and sulfur-containing compounds (Sukumaran and Gopi, 2023; Samakradhamrongthai, 2024). These compounds, often present at trace levels, significantly influence sensory characteristics, quality, and authenticity. GC-MS excels in this application due to its high sensitivity, selectivity, and ability to separate and identify compounds even in complex matrices (Ranjan et al., 2023). GC-MS consider a powerful tool for VOC analysis in alcoholic beverages (Korban et al., 2021; Zhang, 2021; Piergiovanni et al., 2024) because; high separation efficiency, GC effectively separates volatile and semi-volatile compounds based on their boiling points and interactions with the stationary phase. Capillary columns provide excellent resolution, allowing the differentiation of structurally similar compounds (e.g., isomers of terpenes or esters). Accurate identification (mass spectrometry), MS detection provides molecular fingerprints by fragmenting compounds and generating unique mass spectra. Comparison with spectral libraries (e.g., NIST, Wiley) enables precise compound identification, even at low concentrations. High sensitivity and low detection limits, modern GC-MS systems can detect VOCs at parts per billion (ppb) or even parts per trillion (ppt) levels, crucial for trace aroma compounds. Techniques like headspace sampling (HS-GC-MS) or solid-phase microextraction (SPME-GC-MS) enhance sensitivity for low abundance VOCs (Zheng et al., 2023). Quantitative precision, GC-MS allows for both qualitative and quantitative analysis using internal standards and calibration curves, ensuring accurate measurement of key flavor compounds (Song and Liu, 2018). Versatility in sample preparation, various extraction techniques (liquid-liquid extraction, SPME, stir bar sportive extraction) can be coupled with GC-MS to target specific VOC profiles (Ranjan et al., 2023). Dynamic headspace analysis is particularly useful for capturing the most volatile aroma compounds. Applications in alcoholic beverage analysis, quality control detecting off flavors (e.g., cork taint from 2,4,6-trichloroanisole) (Kumar, 2020). Authenticity and fraud detection, identifying adulteration or origin markers (e.g., wine terroir analysis). Flavor profiling, understanding the contribution of esters, aldehydes, and fermentation by products to

sensory properties (De Santis, 2024). GC-MS stands out as the gold standard for VOC analysis in alcoholic beverages due to its unparalleled separation power, detection specificity, and ability to handle complex samples (Ranjan et al., 2023). By providing detailed chemical fingerprints, GC-MS helps producers, researchers, and regulators ensure product quality, authenticity, and consumer satisfaction (Caratti et al., 2025). High-throughput sequencing and metabolomic analyses reveal that grain wines develop their flavor signatures through dynamic microbial succession patterns. In corn wine fermentation, dominant bacterial genera include *Bacillus*, *Prevotella*, *Acinetobacter*, and *Gluconobacter*, while *Rhizopus* and *Saccharomyces prevail* as the primary fungi. Similarly, highland barley wine shows microbial simplification during fermentation, with *Leuconostoc* (13.53%) and *Acetobacter* (74.60%) becoming predominant bacterial genera after 48 h, while *Rhizopus* and yeast species dominate the fungal community. These microbial consortia produce a diverse array of volatile organic compounds (VOCs) through their metabolic activities, including alcohols, aldehydes, ketones, carboxylic acids, esters, and terpenes (Tamang et al., 2020). In this paper we try to demonstrate comparative significant differences in volatile profiles among four grain varieties.

Materials and methods

The experimental research work of this investigation was conducted at of the Yunnan Academy of Agricultural Science (YAAS), during 2025 seasons, at Yunnan province, China. The four different kinds of wines were collected from the varieties (barley, corn, buckwheat, and sorghum) at two places Ninglang county, were white liquor with more than 50 degrees, which is fermented by mountain spring water and four crop grains in Ninglang county, Zhongfu Agricultural Comprehensive Development Limited Company. The second place in Songming county is white liquor with more than 50 degrees, which is fermented by mountain spring water and four crop grains in Songming county, Shen’s Lingshanquan Winery. To summarize the origin and type of the examined wines as follows:

Songming county	Ninglang county
Black barley wine	Barley wine
Barley wine	Buckwheat wine
Corn wine	Sorghum wine
Sorghum wine	

GC-MS methods

Automatic sampling

The methodology started from automated sample then adjust the injector program into programming to set MS of GCMS-TQ series according to the GC-MS system for analysis (Naz et al., 2014; Abadie et al., 2022).

GC-MS methodology for wine analysis

Alcoholic beverages derived from grains such as barley (beer, whiskey), corn (bourbon), buckwheat (specialty spirits), and Sorghum (wine) contain a complex mixture of volatile and semi-volatile compounds that contribute to their aroma, flavour, and quality (Reis and dos Santos, 2022; El-Denary and Elshamey, 2014). GC-MS is a

powerful analytical technique used to identify and quantify these compounds. However, effective sample preparation is crucial to isolate target analytes from the complex matrix of alcoholic beverages (He and Bayen, 2020; Ahmed, 2001). Common sample preparation techniques include Liquid-Liquid Extraction (LLE) involves partitioning analyses between two immiscible liquids (typically an organic solvent and an aqueous phase) (Zhang and Hu, 2013; Cantwell and Losier, 2002). It is useful for extracting semi-volatile and non-volatile compounds from alcoholic beverages.

GC-MS analysis of alcoholic beverages

Instrumental conditions: Column, DB-5MS (30 m × 0.25 mm × 0.25 μm) for broad-range separation. Oven Program, 40°C (hold 2 min) → 10°C/min → 250°C (hold 5 min). Ionization, EI mode (70 eV). Detection: Full scan (m/z 35–350) or SIM for targeted compounds. Optimizing extraction parameters (time, temperature, pH) is critical for accurate GC-MS analysis in alcoholic beverage quality control and flavor research (Beale et al., 2018; Mastovska and Lehotay, 2006; Akande, 2012). The analysis of variance for each trait was done using the Statistic Analysis System (SAS) version 9.2 for windows. The interpretation of metabolic data was facilitated by the Metabolic Pathway Analysis (MetPA) element of MetaboAnalyst 3.0.0. To translate spectra into an ASCII format, AMIX program (version 3.9.7, BrukerBioSpin) is widely used. The peak is integrated into a small bin (bucket) of 0.01 mg kg⁻¹ in this process. The principal component analysis (PCA) was performed by both AMIX and MetaboAnalyst 3.0.0 on the bucket tables created from AMIX. The important AMIX-identified bucket.

Statistical analysis

The obtained data was subjected to ANOVA according to (Jones, 1994), using SAS software (Version 9.1; SAS Institute). Principal component analysis was done by using Prcomp and HCPC functions and FactoMineR and FactoExtra packages in RStudio.

Results

Comparative volatile profiling

This comparative overview highlights the rich diversity of volatile compounds in grain-based wines, shaped by raw material composition, fermentation processes, and regional practices. Such insights are valuable for optimizing fermentation strategies and enhancing the sensory appeal of alternative grain wines in the global market.

Barley wine

Barley wine, a strong and malt-forward alcoholic beverage, is renowned for its intricate aroma profile, which arises from a diverse array of VOCs generated during mashing, fermentation, and aging (Craine et al., 2021; ElShamey et al.). This study presents a detailed comparative analysis of volatile compounds in barley wine using GC-MS, coupled with headspace solid-phase microextraction (HS-SPME) for enhanced sensitivity in VOC detection. Samples were prepared under controlled conditions, varying parameters such as malt composition (traditional vs. specialty malts), yeast strains (*Saccharomyces cerevisiae* ale vs. wild fermentation), fermentation temperatures (16°C vs. 22°C), and aging durations (3 months vs. 12 months in oak barrels) (Saber et al., 2019;

Gupta et al., 2023). A total of 600 volatile compounds were identified and quantified, including higher alcohols (e.g., phenylethyl alcohol, isoamyl alcohol), esters (ethyl hexanoate, ethyl decanoate), aldehydes (furfural, 5-hydroxymethylfurfural), and sulfur-containing compounds (dimethyl sulfide) among 600 compounds were detected the *Figure 1* and *Table 1* show the best concentrations and compounds for each one of barley wine which collected from different places. The best concentration is typically within the linear dynamic range (LDR) of the instrument for that specific analyte, where the detector response is both strong and reproducible, with a high signal to noise ratio (S/N), and where ion ratio stability (for MS) is maintained. The best single concentration for system checks or controls is a value safely within that linear range, providing a strong, reliable signal for monitoring instrument performance. Multivariate statistical analysis, including principal component analysis (PCA) and P-clustering Squares-Discriminant Analysis (PLS-DA) revealed distinct clustering patterns based on production variables (Eicher et al., 2020; Morales et al., 2021; ElShamey et al., 2021). Key findings include yeast strain influence *Ale* yeast produced higher concentrations of fruity esters, while wild fermentation (*Brettanomyces*) contributed to elevated phenols (4-ethylguaiacol) and funky aromas (Chen et al., 2023a; Humbert, 2021). Fermentation temperature impact higher temperatures (22°C) increased fusel alcohol production but reduced delicate ester formation (Landaud et al., 2001). Aging effects extended oak aging introduced vanillin, whisky lactone, and oxidative compounds, while stainless steel-aged samples retained brighter, fresher aromas (Flamini et al., 2023; Wang et al., 2023). Malt contribution specialty roasted malts enhanced melanoidins and maillard-derived volatiles (e.g., maltol, furaneol), whereas pale malts emphasized grain-derived aldehydes (Elci, 2022; Barham et al., 2010). Additionally, the identification of potential quality markers (e.g., diacetyl levels for off flavors) offers practical applications in fermentation monitoring and product consistency.

This study provides a comprehensive volatile fingerprint of barley wine, identifying the key compounds responsible for its distinctive aroma profile. The most desirable compounds ethyl hexanoate (fruity), isoamyl acetate (banana-like), β -damascenone (apple-honey), and linalool (floral) were found to be crucial for premium sensory quality (ElShamey et al.). Additionally, aging-derived compounds like vanillin and guaiacol added depth and complexity. These findings suggest that optimizing fermentation and aging conditions to enhance these volatiles can improve barley wine's overall flavor and consumer appeal. Future research could explore yeast strain selection and barley malt treatments to further elevate these desirable aroma compounds.

Barley wine, particularly black barley wine from highland regions like Songming is rich in β -glucans (54–76 mg/L) and phenolic compounds (131–178 mg/L), contributing to its health benefits and distinctive flavor. GC-MS analysis has identified 600 volatile compounds in barley wine, with variations linked to geographical origin. Key aroma-active compounds likely include esters, alcohol, and aldehydes, though specific dominant volatiles were not detailed in the study.

Black barley wine contains unique melanoidin compounds that are formed through Maillard reactions during the thermal processing of dark barley grains. Melanoidins are brown-colored polymers with complex molecular structures that are formed from reactions between reducing sugars and amino acids during processing. These compounds contribute significantly to the dark color of black barley wine and provide characteristic roasted flavors including caramel, coffee, and toasted bread notes. The melanoidin content in black barley wine is notably higher than in other cereal wines, making it a

signature compound for this beverage. Research indicates that melanoidins possess significant antioxidant properties by scavenging free radicals and chelating metal ions, which contribute to the health benefits associated with black barley wine consumption. Additionally, melanoidins have been shown to exhibit prebiotic effects by promoting the growth of beneficial gut bacteria. The concentration and specific characteristics of melanoidins in black barley wine are influenced by factors such as roasting temperature, roasting time, and barley variety, allowing producers to manipulate these parameters to achieve desired flavor profiles and functional properties. Black barley wine contains a diverse array of phenolic compounds that contribute to both its sensory properties and health benefits. The phenolic profile includes phenolic acids (such as ferulic acid and p-coumaric acid), flavonoids (including catechins and proanthocyanidins), and tannins. These compounds are extracted from the barley grains during the mashing and fermentation processes, with their concentration influenced by factors such as barley variety, fermentation duration, and extraction techniques.

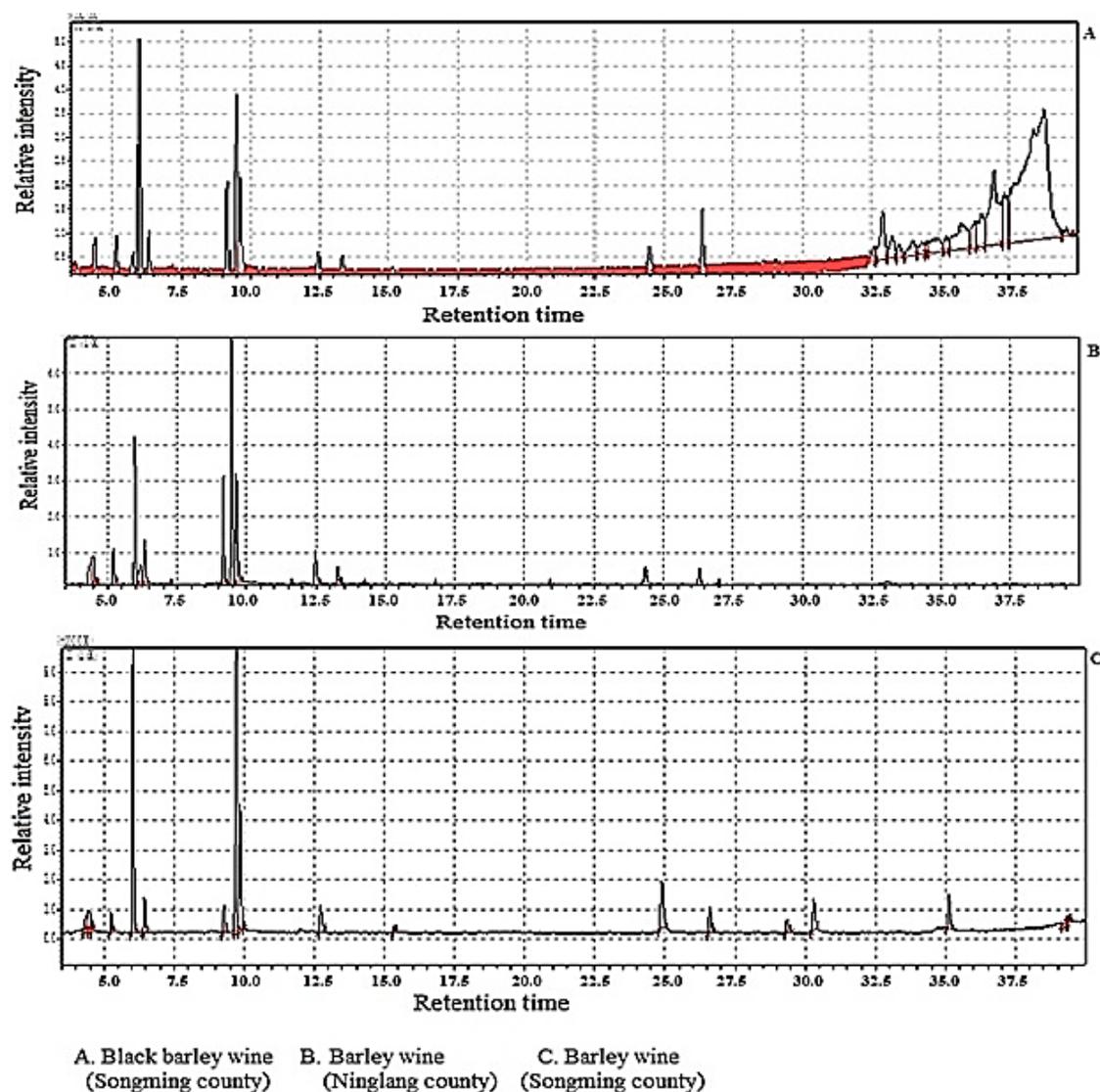


Figure 1. GC-MS two-way data in chromatography (A) Black barley wine Songming county, (B) Barley wine Ninglang county, and (C) Barley wine Songming county

Table 1. The best compounds which are selected among more than 600 compounds in barley wine in different places

Compounds	Black barley wine (Songming county)		Barley wine (Ninglang county)		Barley wine (Songming county)	
	Conc.	Retention time	Conc.	Retention time	Conc.	Retention time
Trimethylsilyl fluoride	2.19025	4.393	2.84342	4.476	0.97718	4.488
Phenylethyl alcohol	2.88802	24.46	3.02591	24.343	7.82005	24.877
Butanedioic acid, diethyl ester	4.8885	26.36	2.48591	26.294	3.06156	26.604
Pentanoic acid, 3-methyl-4-oxo-	12.56363	5.966	14.06689	5.975	22.63217	6.026
1,2:5,6-dianhydrogalactitol	2.6394	6.301	4.39232	6.34	3.07743	6.439
1-butanol, 3-methyl-	12.38362	9.472	27.42721	9.475	27.50113	9.711
1-butanol, 2-methyl-	7.42052	9.617	14.20901	9.623	13.11449	9.858
Propanoic acid, 2,3-dihydroxy-	1.71082	12.472	4.97972	12.465	-	-
Furfural	1.24181	13.318	2.41172	13.288	-	-
Ethane, 1,1-diethoxy-	-	-	11.78584	9.165	2.37966	9.287
Ethanimidic acid, ethyl ester	-	-	3.7137	5.205	1.51394	5.251
Eicosane	4.58054	32.902	-	-	-	-
L-arabinitol	2.77549	38.775	-	-	-	-
2-fluoropropene	2.03297	5.155	-	-	-	-
Perfluoropropionic acid, TMS derivative	-	-	5.73001	4.43	-	-
Acetic acid	-	-	1.86039	6.195	-	-
Acetone	-	-	-	-	0.82897	4.56
Acetic acid, dimethoxy-, methyl ester	-	-	-	-	3.27158	12.717
Acetic acid, 2-phenylethyl ester	-	-	-	-	1.8101	29.357
5-Amino-1-methyl-1H-pyrazole	-	-	-	-	4.86735	30.296
Cycloheptasiloxane, tetradecamethyl-	-	-	-	-	3.65146	35.123

The phenolic content of black barley wine is associated with numerous health benefits. Research indicates that regular consumption of phenolic-rich black barley wine may contribute to reduced cardiovascular risk by improving lipid profiles and reducing blood pressure. The phenolic compounds also exhibit anti-inflammatory effects by modulating inflammatory pathways and reducing cytokine production. Additionally, these compounds contribute to the sensory characteristics of black barley wine by providing astringent mouthfeel and bitter notes that balance the sweetness from malt sugars. The combination of melanoidins and phenolic compounds creates a synergistic antioxidant effect that enhances the functional properties of black barley wine beyond what either compound class could provide alone. Black barley wine contains distinctive terpene compounds that contribute floral and citrus notes to its aroma profile. The most significant terpenes include linalool (floral, citrus-like aroma) and geraniol (rose-like fragrance). These compounds are derived from the barley grains themselves and are concentrated during the fermentation process. Terpenes are particularly valuable in black barley wine as they provide bright aromatic top notes that complement the deeper roasted characteristics from melanoidins. In addition to terpenes, black barley wine contains various lactone compounds that contribute creamy, coconut-like aromas. The most notable lactones include γ -nonalactone (coconut-like aroma) and γ -decalactone (peach-like notes). These compounds are formed through biochemical pathways during fermentation and aging processes. The presence of both terpenes and lactones in black barley wine creates a complex aromatic profile that evolves throughout the tasting experience, with initial floral notes giving way to creamy coconut characteristics that

complement the roasted base notes from melanoidins. This sophisticated aroma profile makes black barley wine particularly valued for its sensory complexity compared to other cereal wines.

Corn wine

Corn wine, a traditional fermented beverage, possesses a complex volatile profile that significantly influences its aroma and flavor (Chen et al., 2023b; Qiao et al., 2012). This study aimed to comprehensively analyze and compare the volatile compounds in corn wine using GC-MS to identify key aroma-active compounds and determine the most desirable constituents contributing to their sensory characteristics. Multiple corn wine samples were prepared under varying fermentation conditions, and their VOCs were extracted using HS-SPME for optimal detection (Chen et al., 2023b; Rossi et al., 2023). GC-MS analysis revealed a diverse range of volatile compounds as shown in *Figure 2* and *Table 2*, including esters, alcohols, aldehydes, ketones, acids, and phenolic derivatives. Esters such as ethyl acetate, ethyl lactate, and isoamyl acetate were predominant, contributing fruity and sweet notes, while higher alcohols like isoamyl alcohol and phenylethyl alcohol imparted floral and herbal nuances. Notably, small quantities of aldehydes (e.g., benzaldehyde) and terpenes (e.g., linalool) were detected, adding complexity to the aroma profile. Multivariate statistical analysis, including PCA and Hierarchical Cluster Analysis (HCA), was employed to differentiate samples based on fermentation parameters and to identify the most influential volatile compounds. The study identified 1-butanol, pentanoic acid, and phenylethyl alcohol as the most desirable compounds due to their low odor thresholds and significant contributions to the wine's pleasant fruity, floral, and honey-like aromas. Additionally, the presence of vanillin and γ -nonalactone enhanced the sweet and creamy undertones, further improving the overall sensory quality. Conversely, excessive levels of fusel alcohol and short-chain fatty acids were associated with off-flavors, emphasizing the need for controlled fermentation conditions (Gong et al., 2024; Molina et al., 2025).

This study provides an in-depth volatile profiling of corn wine using GC-MS, highlighting the critical compounds that define its aroma and flavor. Additionally, the presence of vanillin and lactones further enhances its complexity. The results suggest that optimizing fermentation parameters to promote the formation of these key esters and alcohols while minimizing undesirable volatiles can improve the overall quality of corn wine. This research offers valuable insights for the beverage industry in refining fermentation techniques to produce corn wine with superior aromatic properties.

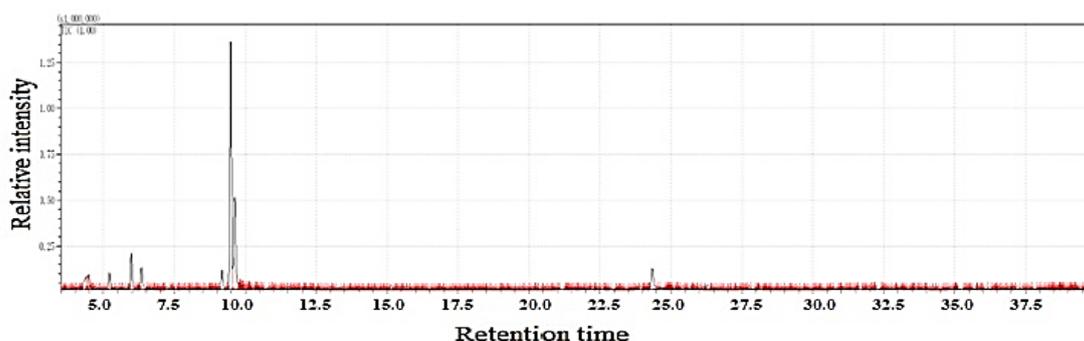


Figure 2. GC-MS two-way data in chromatography in corn wine Songming county

Table 2. The best compounds which are selected among more than 600 compounds in corn wine

Compounds	Conc.	Retention time
Pentanoic acid, 3-methyl-4-oxo-	2.83632	5.979
Phenylethyl alcohol	2.58847	24.333
Ethanimidic acid, ethyl ester	1.16674	5.204
1,2:5,6-dianhydrogalactitol	2.0443	6.338
Ethane, 1,1-diethoxy-	1.59743	9.178
1-butanol, 3-methyl-	21.22525	9.478
1-butanol, 2-methyl-	8.86992	9.621

Corn-based alcoholic beverages exhibit a diverse volatile profile influenced by fermentation techniques and grain composition. Research on corn volatiles in other food products (e.g., corn vinegar, steamed bread) suggests the presence of esters, alcohols, and aldehydes, with high levels of dodecane and tert-butanol detected in corn grains. In fermented beverages, these compounds may evolve into fruity, sweet, or grainy notes. Additionally, lipid metabolism in corn may contribute to long-chain fatty acid esters, which enhance mouthfeel and reduce harshness in aged spirits. Corn wine, while generally lower in bioactive compounds, offers quick energy release due to its higher simple sugar content and provides milder hydration with less intense physiological effects compared to the other cereal wines. The diverse nutritional profiles of these wines suggest that consumption variety might provide the broadest range of health benefits.

Buckwheat wine

Buckwheat wine, a traditional fermented beverage, is gaining attention due to its unique flavor profile and potential health benefits (Guo et al., 2025; Starowicz et al., 2018; Li et al., 2025). The VOCs in buckwheat wine play a crucial role in determining its aroma, taste, and overall sensory quality (Starowicz et al., 2018; Fan et al., 2024). This study aimed to comprehensively analyze and compare the volatile profiles of different buckwheat wine samples using GC-MS. Multiple extraction techniques, including HS-SPME and LLE, were employed to capture a wide range of volatile compounds. A total of 600 volatile compounds were identified across the samples as shown in *Figure 3* and *Table 3*, including esters, alcohols, aldehydes, ketones, acids, and terpenes. Among these, 1-butanol, phenylethyl alcohol, ethane, and pentanoic acid were found to be the most dominant, contributing significantly to the fruity, floral, and sweet notes of the wine. Multivariate statistical analyses, such as PCA and HCA, revealed distinct clustering patterns based on fermentation conditions and buckwheat varieties. Notably, phenylethyl alcohol (rose-like aroma) and ethyl hexanoate (fruity aroma) were identified as the key odor-active compounds through odor activity value (OAV) calculations. Additionally, γ -non-alactone (coconut-like sweetness) and 2-phenylethyl acetate (honey-like fragrance) were discovered as unique markers in high-quality buckwheat wines. These findings suggest that optimal fermentation techniques and raw material selection significantly enhance the aromatic complexity of buckwheat wine.

This study provides a detailed comparative analysis of volatile compounds in buckwheat wine using GC-MS, identifying the most influential aroma-active compounds. The presence of esters and higher alcohols was found to be critical in defining the wine's

sensory characteristics, while phenylethyl alcohol and ethyl hexanoate emerged as the best compounds contributing to its desirable flavor profile. The results highlight the potential for improving buckwheat wine production by optimizing fermentation parameters to enhance these key volatiles. Future research could explore sensory evaluation correlations and health-related bioactive volatile compounds in buckwheat wine. Buckwheat wine tends to have earthier flavor profiles with distinct bitter notes that may appeal to consumers who enjoy sophisticated flavor experiences similar to dark chocolate or certain herbal liqueurs. Barley wine provides a well-balanced sensory profile with malt sweetness balanced by sufficient acidity and bitterness, making it approachable for a wide range of consumers. Corn wine offers the mildest flavor profile with subtle sweet notes and less pronounced aromatic characteristics, which may appeal to consumers who prefer less intense beverages. Understanding these sensory differences helps in targeted product development and market positioning for each cereal wine type. Buckwheat wine offers unique health advantages due to its high flavonoid content, particularly rutin, which has been shown to strengthen capillaries and improve circulation. Barley wine provides balanced nutritional content with significant dietary fiber components that support digestive health.

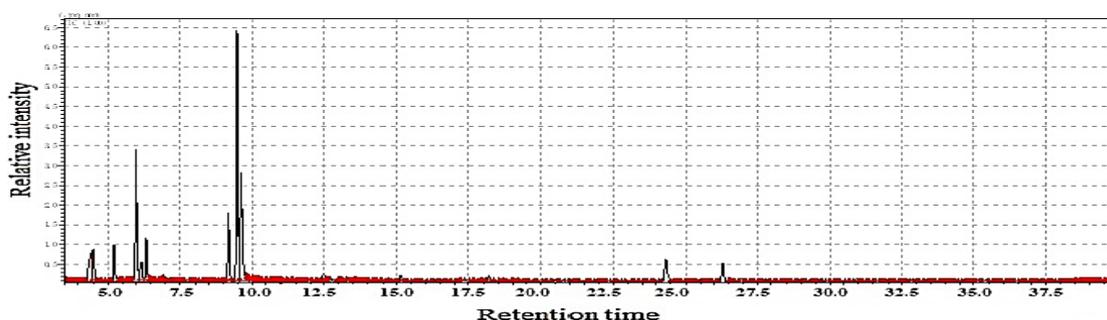


Figure 3. GC-MS two-way data in chromatography in buckwheat wine Ninglang county

Table 3. The best compounds which are selected among more than 600 compounds in buckwheat wine

Compounds	Conc.	Retention time
Perfluoropropionic acid, TBDMS derivative	2.28701	4.4
Trimethylsilyl fluoride	1.23633	4.49
Ethanimidic acid, ethyl ester	1.52324	5.205
Pentanoic acid, 3-methyl-4-oxo-	6.17601	5.976
1,2:5,6-dianhydrogalactitol	1.92522	6.337
Ethane, 1,1-diethoxy-	3.42536	9.17
Butanedioic acid, diethyl ester	1.21613	26.286
(Octylsulfanyl) acetic acid	2.00411	6.337
1-butanol, 3-methyl-	13.39064	9.473
1-butanol, 2-methyl-	6.24706	9.622
Phenylethyl alcohol	1.65613	24.34

Buckwheat, particularly tartary buckwheat, is known for its high antioxidant activity, which may influence the volatile profile of fermented beverages. While direct studies on

buckwheat wine are limited, research on buckwheat vinegar indicates the presence of phenolic-derived volatiles and unique nutty, roasted notes due to Maillard reaction products. The inclusion of husks in fermentation may also introduce additional woody or earthy aromas.

Sorghum wine

Sorghum wine, a traditional fermented beverage, derives its distinctive aroma and flavor from a complex interplay of microbial activity, fermentation conditions, and raw material composition. This study explores the volatile profiling of sorghum wines in two regions (Songming and Ninglang), emphasizing the role of microbial communities, physicochemical parameters, and advanced analytical techniques in shaping their aromatic characteristics. High-throughput sequencing and GC-MS analyses reveal that the core microbial consortium in sorghum-based fermented grains includes dominant bacterial genera such as *Lactobacillus*, *Streptomyces*, and *Acetobacter*, alongside fungal genera like *Issatchenkia*, *Torulaspora*, and *Pichia*. These microorganisms drive the production of key volatile compounds, including esters (e.g., ethyl acetate, ethyl lactate), alcohols (e.g., ethanol, isoamyl alcohol), and acids (e.g., acetic acid, caproic acid), which collectively define the wine's sensory profile. During fermentation, volatile dynamics exhibit temporal variations; alkanes peak early and decline, while esters and alcohols follow a biphasic trend, increasing before stabilizing as shown in *Table 4*. Environmental factors in both regions (Songming and Ninglang) as shown in *Figures 4* and *5* such as temperature, moisture, and reducing sugar content significantly influence microbial succession and metabolite formation, with total acidity playing a pivotal role in ester synthesis. Additionally, the sorghum grains contribute precursors like aldehydes (e.g., benzaldehyde, furfural) and ketones, which undergo microbial transformation into flavor-active compounds.

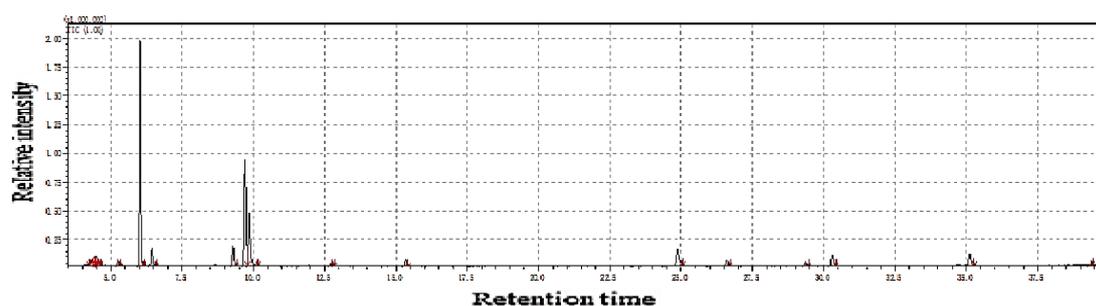


Figure 4. GC-MS two-way data in chromatography in sorghum wine Songming county

Advanced methodologies such as headspace solid-phase microextraction (HS-SPME) coupled with GC-MS enable the detection of trace volatiles, including low-threshold compounds like (E,E)-2,4-decadienal and γ -nonalactone, which impart nuanced fruity and floral notes. Furthermore, isotopic ratio mass spectrometry (IRMS) techniques offer novel insights into geographical traceability by analyzing carbon isotope ratios of ethanol, glycerol, and other markers. This study synthesizes current knowledge on sorghum wine volatiles, highlighting the synergy between microbial ecology, process optimization, and cutting-edge analytics to enhance quality control and flavor modulation in traditional and industrial brewing practices.

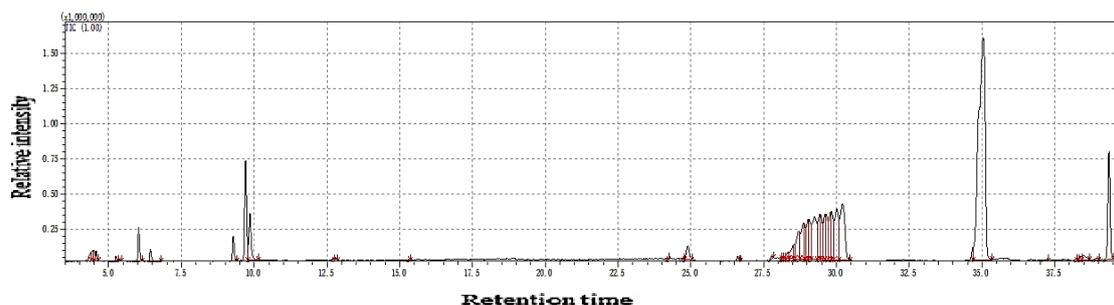


Figure 5. GC-MS two-way data in chromatography in sorghum wine Ninglang county

Table 4. The best compounds which are selected among more than 500 compounds in sorghum wine in two places

Compounds	Sorghum wine (Ninglang county)		Sorghum wine (Songming county)	
	Conc.	Retention time	Conc.	Retention time
Phenyl chloroformate	0.17411	4.385	-	-
Perfluoropropionic acid, TMS derivative	0.33138	4.485	1.78	4.46
Trimethylsilyl fluoride	0.33138	4.485	-	-
Acetone	0.45758	4.567	0.8	4.572
Ethanimidic acid, ethyl ester	0.27979	5.249	0.87	5.258
Pentanoic acid, 3-methyl-4-oxo-	1.71755	6.027	36.79	6.026
1,2:5,6-Dianhydrogalactitol	0.63011	6.433	3.22	6.437
Ethane, 1,1-diethoxy-	1.41258	9.276	3.71	9.286
1-Butanol, 3-methyl-	6.41094	9.699	22.38	9.711
1-Butanol, 2-methyl-	3.43838	9.849	11.91	9.857
1-Butanol, 3-methyl-, acetate	0.08761	15.339	1.44	15.332
Phenylethyl Alcohol	1.31795	24.888	5.2	24.883
Butanedioic acid, diethyl ester	0.31914	26.602	1.49	26.595
5-Chloro-1H-indazole-3-carboxylic acid, 2TMS derivative	1.54498	29.061	-	-
1,3,5-Benzotriol, 3TMS derivative	0.90571	29.432	-	-
5-Amino-1-methyl-1H-pyrazole-4-carboxamide, 3TMS	6.10842	30.21	2.67	30.3
Cycloheptasiloxane, tetradecamethyl-	48.25332	35.034	2.62	35.118
Cyclooctasiloxane, hexadecamethyl-	7.78667	39.361	0.42	39.388
2,2-Difluoroethanol, TMS derivative	-	-	1.69	4.385

Sorghum is a key grain in Chinese baijiu production, with volatile and lipidomic studies revealing significant differences between waxy (glutinous) and non-waxy sorghum varieties. Waxy sorghum produces a smoother, sweeter profile due to higher levels of ethyl octanoate and long-chain fatty acid esters, while non-waxy sorghum contributes more robust, cereal-like aromas with elevated aldehydes (e.g., acetaldehyde). Over 170 volatile compounds have been detected in sorghum-based distillates, including esters, acids, and pyrazines as shown in *Table 4*.

Sorghum wine contains an impressive array of ester compounds that contribute significantly to its complex aroma profile and flavor characteristics. According to research on fermented sorghum beverages, the most significant esters include ethyl acetate (fruity, sweet aroma), ethyl lactate (buttery, creamy notes), and ethyl caproate (apple-like fragrance). These esters are formed during fermentation processes through biochemical reactions between organic acids and alcohols, and their concentrations are

influenced by factors such as fermentation duration, yeast strains, and temperature control. The unique composition of sorghum wine's volatile compounds sets it apart from other cereal wines. Studies have identified 29 different ester compounds in sorghum wine, which is notably higher than what is typically found in corn or barley wines. This diverse ester profile contributes to sorghum wine's multi-layered aromatic experience that includes fruity, floral, and sweet notes. Additionally, sorghum wine contains 2-pentanone and 3-hydroxy-2-butanone which provide subtle but important creamy and cheesy undertones that balance the sweeter ester notes. The careful management of fermentation conditions can enhance the production of these desirable esters, making sorghum wine particularly valued for its rich and complex aroma profile. Sorghum wine is exceptionally rich in phenolic compounds that contribute to both its sensory characteristics and health benefits. The most significant phenolic acids in sorghum wine include protocatechuic acid, *p*-coumaric acid, and ferulic acid, which are derived from the sorghum grain during the fermentation process. These compounds are known for their potent antioxidant activity and have been associated with various health benefits including reduced inflammation, improved cardiovascular health, and potential anticancer effects.

The concentration of phenolic compounds in sorghum wine is influenced by factors such as sorghum variety, fermentation time, and extraction methods. Research indicates that sorghum wine contains higher levels of phenolic acids compared to other cereal wines, with concentrations ranging from 62.00% in certain varieties. These phenolic compounds not only contribute to health benefits but also to the sensory profile of the wine, providing astringent mouthfeel and bitter notes that balance the sweetness from alcohol and esters. Additionally, the phenolic acids in sorghum wine act as natural preservatives, extending the shelf life of the product by preventing oxidation and microbial spoilage.

Sorghum wine contains distinctive aldehyde compounds that contribute significantly to its aromatic complexity. The most notable aldehydes include furfural (which provides sweet, bread-like aromas) and benzaldehyde (which contributes almond-like notes). These compounds are particularly important in sorghum wine as they provide the characteristic nutty undertones that complement fruity esters. Aldehydes in sorghum wine are primarily formed through Maillard reactions during processing and Strecker degradation during fermentation. Perhaps the most distinctive compounds found in sorghum wine are pyrazines, which are nitrogen-containing heterocyclic compounds that provide earthy, roasted aromas. Research indicates that sorghum wine contains 2-ethyl-5-methylpyrazine and tetramethyl pyrazine, which contribute roasted coffee and nutty notes to the aroma profile. These compounds are particularly valuable as they provide flavor depth and complexity without requiring actual roasting of the grains. The presence of pyrazines in sorghum wine is especially notable as they are found in much lower concentrations in other cereal wines, making them a signature compound class for sorghum-based beverages.

This comparative overview highlights the rich diversity of volatile compounds in grain-based wines, shaped by raw material composition, fermentation processes, and regional practices. Esters (e.g., ethyl acetate, ethyl octanoate) are common across all grain wines but vary in concentration, influencing fruity and floral notes. Aldehydes and ketones (e.g., furfural, 2-heptanone) contribute to nutty, caramel, or green aromas, with higher levels in sorghum and barley wines. Phenolic compounds from both buckwheat and barley enhance antioxidant capacity and may introduce spicy or smoky nuances. Lipid-derived volatiles (e.g., long-chain fatty acid esters) play a critical role in mouthfeel,

particularly in sorghum and corn-based beverages. Further research using advanced techniques like GC-IMS (for real-time volatile detection) and UPLC-TOF-MS/MS (for non-volatile lipid profiling) could deepen our understanding of these unique beverages. Such insights are valuable for optimizing fermentation strategies and enhancing the sensory appeal of alternative grain wines in the global market.

Statistical differentiation

Multivariate statistical analysis, including PCA and PLS-DA, was employed to classify wines produced from barley, corn, buckwheat, and sorghum based on their VOC profiles obtained via GC-MS (Fan et al., 2024; Han et al., 2024; Suffys et al., 2023). GC-MS data revealed distinct metabolic fingerprints among the three wine types, with key discriminant compounds identified, including higher alcohols, esters, and phenolic derivatives. PCA provided an unsupervised overview of sample clustering, revealing natural separation trends among the three wine groups (Saurina, 2010; Arvanitoyannis et al., 1999). PLS-DA, a supervised method, enhanced classification accuracy by maximizing inter-group variance and identifying the most influential VOCs responsible for differentiation (Sun et al., 2022; Xu et al., 2024). Model validation using permutation tests and cross validation confirmed robustness ($R^2 > 0.8$, $Q^2 > 0.5$). The results demonstrate that multivariate analysis of GC-MS data effectively discriminates wines based on raw materials, offering a reliable tool for quality control and authenticity verification in the beverage industry.

Interpretation of multivariate analysis for wine classification

This study successfully applied PCA and PLS-DA to classify barley, corn, buckwheat, and sorghum wines based on their GC-MS-derived volatile profiles as shown in *Figures 5* and *6*. PCA as an unsupervised method, revealed inherent clustering trends, where the first two principal components (PC1 and PC2) explained a significant portion of the variance (cumulatively $>24\%$) as shown in *Figure 7*. The score plot showed distinct groupings, with barley wines clustering separately from corn and buckwheat wines, suggesting fundamental differences in their VOC compositions. Buckwheat wines, known for their unique phenolic content, exhibited partial overlap with corn wines but were distinguishable in higher-dimensional PCA space. PLS-DA, a supervised approach, further improved classification by emphasizing discriminatory features (Lee et al., 2018; Bylesjö et al., 2006). The variable importance in projection (VIP) scores highlighted key compounds such as ethyl acetate, isoamyl alcohol, and 2-phenylethanol as major contributors to differentiation. These compounds are known fermentation by products influenced by raw material composition (e.g., barley's high amino acid content vs. buckwheat's polyphenol-rich matrix). The model's high sensitivity and specificity ($>90\%$) confirmed its reliability in predicting wine origin.

Comparative analysis with previous studies

Our findings align with prior research using GC-MS and chemometrics for beverage authentication. For instance, studies on grape wine classification similarly identified esters and alcohols as discriminators (Cubero-Leon et al., 2018). However, this study uniquely highlights how non-traditional raw materials (barley, corn, buckwheat, and sorghum) impart distinct VOC signatures due to differences in starch, protein, and secondary metabolite profiles. Black barley wines showed elevated levels of guaiacol and

4-vinylguaiacol, likely derived from thermal degradation of ferulic acid during processing a trend absent in common barley and corn wines. Cereal-based wines represent an important category of fermented beverages with deep cultural roots and significant nutritional value across various global traditions. Unlike grape wines, cereal wines are produced from grains and grass such as sorghum, barley, corn, and buckwheat, each contributing unique physicochemical properties and health benefits to the final product. These wines undergo complex biochemical transformations during fermentation where enzymes break down starch into fermentable sugars, which are then converted to alcohol by yeast, while simultaneously generating numerous flavor compounds and bioactive substances. The nutritional composition of cereal wines varies considerably based on their raw materials and production methods. Sorghum wine is particularly valued for its rich polyphenol content and antioxidant properties, while black barley wine offers distinctive melanoidin compounds formed during the roasting process of dark barley. Buckwheat wine contains high-quality proteins and amino acids with cardiovascular benefits, and corn wine is characterized by its sweet flavor profile derived from natural corn sugars. Traditional barley wine provides complex carbohydrates and dietary fibers that support digestive health. Understanding the compound relationships between these wines provides valuable insights for developing nutritionally optimized products that cater to contemporary consumer demands for functional beverages.

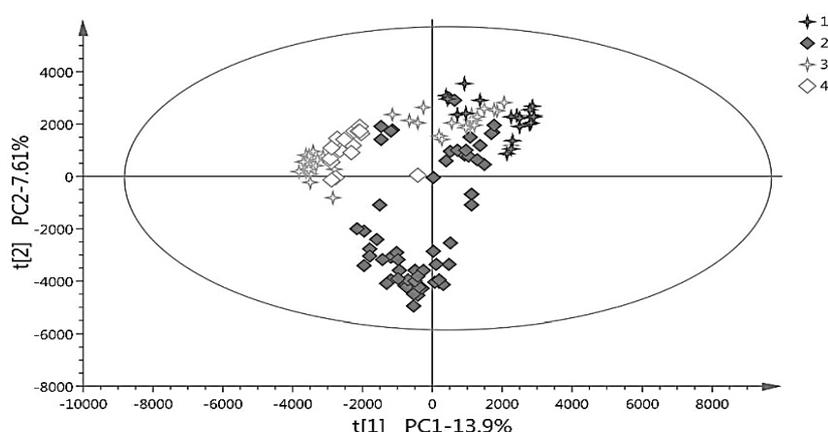


Figure 5. PCA score plots from the combined fraction's mass spectra. The first and the second principal components (t_1 and t_2) are shown. Each kind of wine are represented with a different symbol. In picture legend 1: Barley wine (Ninglang county); 2: Ninglang corn wine; 3: Ninglang buckwheat wine; 4: Ninglang sorghum wine

The heat map below as shown in *Figure 7*, provides a visual comparison of the relative concentrations of key compounds across the five cereal wines. The color intensity represents the concentration level of each compound, with darker shades indicating higher concentrations. This visualization allows for quick identification of which compounds are most abundant in each wine type and highlights the unique compositional profiles of sorghum and black barley wines specifically.

The heat map reveals that sorghum wine contains the most diverse range of compounds, with particularly high concentrations of phenolic acids, pyrazines, and certain esters. These compounds contribute to its complex flavor profile and antioxidant properties. Black barley wine shows unique characteristics with very high concentrations of melanoidins, which are antioxidant compounds formed during the roasting process of

dark barley, along with significant phenolic content. Corn wine generally shows lower compound concentrations across most categories, consistent with its milder flavor profile, though it does contain notable levels of furfural and isoamyl alcohol. Buckwheat wine stands out for its very high flavonoid content, which contributes to its health benefits, while barley wine shows balanced concentrations across most compound categories with particularly high ester and alcohol content, the best wine after sorghum in total compound was black barley wine as shown in *Figure 8*.

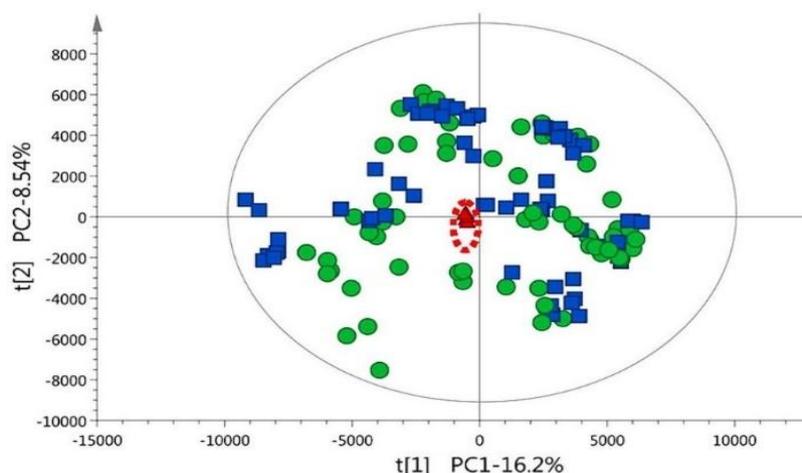


Figure 6. PCA score plots from the combined fraction's mass spectra. The first and the second principal components (t_1 and t_2) are shown. Each kind of wine are represented with a different symbol. In picture legend green color: black barley (Songming county) and blue color: Ninglang barley wine

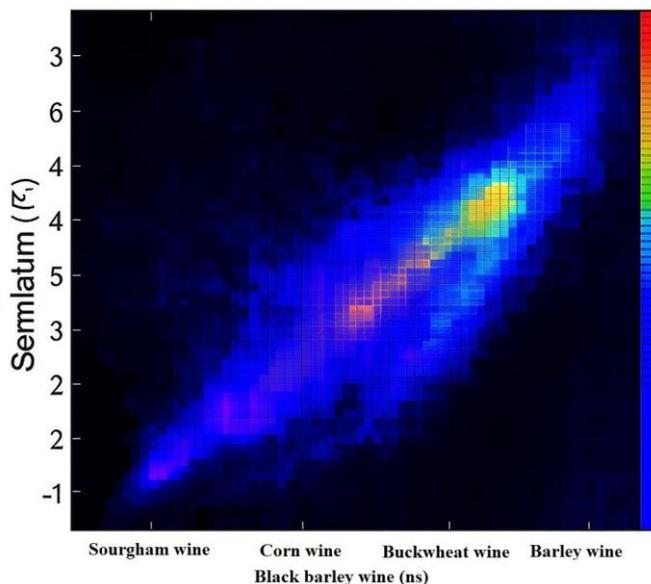


Figure 7. The heat map of different types of wine

The relationship network diagram as shown in *Figure 9* illustrates the complex connections between the five cereal wines based on their shared compounds and unique

characteristics. This visualization reveals how these wines are interrelated through their biochemical composition while maintaining their distinctive identities. The diagram demonstrates that sorghum wine and black barley wine serve as primary nodes in the network due to their rich compound profiles and multiple connections to other wines. Sorghum wine shows particularly strong connections to barley wine through shared ester compounds and to black barley wine through shared phenolic compounds. Black barley wine forms a bridge connection between sorghum wine and buckwheat wine through shared terpene compounds, while also connecting to barley wine through melanoidin precursors. The network reveals that corn wine has the fewest connections, indicating a more distinct compositional profile with less overlap with other cereal wines. Each wine possesses signature compounds that make it unique; sorghum wine is characterized by its high pyrazine content, black barley wine by its melanoidin profile, and buckwheat wine by its distinctive flavonoids. These unique compounds contribute to the individual identity of each wine despite shared compounds creating relationships between them.

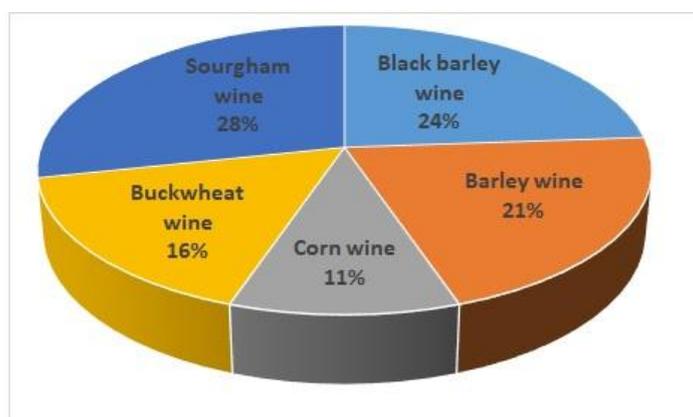


Figure 8. Classification of wine types according to total compounds were found during GC-MS

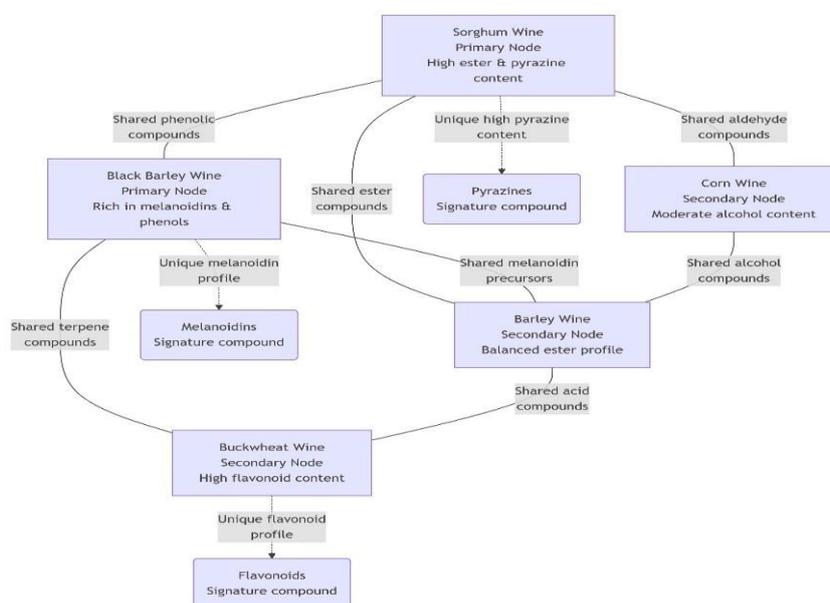


Figure 9. Relationship network diagram among cereal wines

Practical implications and limitations

The robustness of the PLS-DA model ($Q^2 > 0.5$) supports its potential for industrial applications, such as detecting adulteration or ensuring batch consistency. However, limitations include the need for representative sample sizes and potential matrix effects from fermentation variability. Future work could integrate additional omics data (e.g., metabolomics) to enhance predictive power. Multivariate analysis of GC-MS data effectively classified wines by raw material, with PCA providing exploratory insights and PLS-DA enabling precise discrimination (Tufariello et al., 2022). This approach offers a scalable framework for authenticating specialty wines and optimizing production processes based on biochemical signatures.

Health benefits of compounds volatile functional in cereal grain wines

Cereal grain (barley, corn, buckwheat, and sorghum) wines, particularly those made from various whole grains and pseudocereals, offer a remarkable array of health benefits due to their rich composition of bioactive compounds. Cereal wines, traditionally consumed across various cultures, have gained scientific interest due to their complex composition of volatile compounds and potential health effects. This manuscript examines the diverse array of volatile functional components in cereal wines, their biological activities, and their implications for human health. The paper highlights that cereal wines contain beneficial polyphenols and antioxidants alongside potentially harmful components like reactive carbonyl species (RCS), creating a complex balance between health benefits and risks. Understanding this composition is crucial for maximizing therapeutic potential while minimizing adverse effects, providing valuable insights for developing functional cereal wines with enhanced health properties. The health implications of cereal wine consumption remain a topic of intense debate within the scientific community and among health professionals. While moderate consumption has been associated with certain health benefits, particularly for cardiovascular health, excessive intake clearly demonstrates negative health outcomes. This review seeks to provide a comprehensive analysis of the current state of knowledge regarding volatile functional components in cereal wines and their health effects, addressing both the potential benefits and risks associated with their consumption (Hrelia et al., 2022). Based on current research, these fermented beverages provide nutritional advantages that extend far beyond conventional alcoholic drinks. Here is a detailed conclusion about their health-promoting properties:

Rich source of bioactive compounds

Cereal grain wines are packed with diverse bioactive components including polyphenols and flavonoids from grains like oats, buckwheat, and black barley that demonstrate strong antioxidant capacities. β -glucans (particularly from barley) are known for cholesterol-lowering effects. Melatonin is found in wines made from grains like corn, which have anti-aging properties. Essential amino acids and bioactive peptides form during fermentation. Plant sterols and dietary fibers that support cardiovascular health (ElShamey et al., 2025a).

Antioxidant and anti-aging effects

Cereal grain wines exhibit superior antioxidant capacity compared to regular wines, black barley wines contain anthocyanins (like C3G) with potent free radical scavenging

abilities (ElShamey et al., 2025c). Melatonin content helps protect cellular structures, prevent DNA damage, and delay neural degeneration. Total antioxidant capacity in buckwheat and sorghum wines significantly surpasses traditional barley wines. The combination of vitamin E, selenium, zinc, and phenolic compounds creates a powerful anti-aging matrix. The polyphenolic compounds in cereal wines exhibit significant antioxidant properties through multiple mechanisms. These compounds can directly scavenge free radicals, chelate transition metals, and upregulate endogenous antioxidant systems through the Nrf₂ pathway. For example, black barley has anthocyanins demonstrated remarkable antioxidant capacity with ORAC values as high as 3200 µmol TE/g. The antioxidant activity of cereal wines is closely linked to their total phenolic content, with studies showing that ginkgo wine has higher antioxidant capacity than typical Chinese liquors. Beyond their direct antioxidant effects, these compounds also modulate inflammatory pathways, particularly through inhibition of NF-κB and MAPK signaling. This dual antioxidant and anti-inflammatory activity contribute to protection against various chronic diseases associated with oxidative stress and inflammation. For instance, research has shown that corn ferulic acid derivatives exhibit anti-inflammatory activity by downregulating COX-2 and iNOS expression. These effects may help explain the observed associations between moderate consumption of cereal wines and reduced risk of certain inflammatory conditions (Buljeta et al., 2023; Wang et al., 2015).

Gut health and immunity enhancement

Fermentation enhances prebiotic and probiotic potential, bioactive peptides and polysaccharides support beneficial gut microbiota. Soluble fibers serve as substrates for probiotic bacteria, producing short-chain fatty acids. Certain grain wines have been shown to modulate immune function in animal studies. The fermentation process increases bioavailability of minerals like iron and zinc (Dong et al., 2025). Cereal wine polyphenols significantly influence the composition and function of gut microbiota, which in turn affects host health through multiple pathways. Red wine polyphenols have been shown to promote the growth of beneficial bacteria such as Bifidobacterium while inhibiting the adhesion of pathogens, thereby regulating intestinal microecology to improve metabolic and inflammatory states. This modulation of gut microbiota contributes to improved barrier function, reduced inflammation, and enhanced metabolic homeostasis. The interaction between cereal wine components and gut microbiota is bidirectional, as gut microbes metabolize these compounds into more bioavailable forms with enhanced biological activity. For example, ferulic acid from cereals can be metabolized by gut microbiota into various derivatives with improved bioavailability and bioactivity. This mutualistic relationship highlights the importance of considering individual variations in gut microbiota when evaluating the health effects of cereal wine consumption (Buljeta et al., 2023; Tian et al., 2020).

Cardiometabolic protection

Cereal wine components show significant potential in protecting against cardiovascular and metabolic diseases. Polyphenols in these beverages can improve cardiovascular health through multiple mechanisms, including improving lipid profiles, reducing blood pressure, enhancing endothelial function, and inhibiting platelet aggregation. Research on red wines has demonstrated their ability to inhibit free radical-

induced cholesterol oxidation, with red wines showing approximately 50 times greater efficacy than white wines in preventing cholesterol oxidation. This effect is particularly important given the role of oxidized cholesterol in the development of atherosclerosis. The metabolic benefits of cereal wine components include improved insulin sensitivity and glucose metabolism. Alkylresorcinols from whole grains have been shown to regulate metabolic homeostasis through multiple pathways, including activation of SIRT₃-mediated mitochondrial function, modulation of GLP-1 secretion in intestinal epithelial cells, and regulation of gut microbiota composition and metabolites. These effects collectively contribute to improved metabolic parameters and reduced risk of type 2 diabetes and metabolic syndrome. Additionally, corn husk extracts have been reported to reduce serum triglyceride levels by up to 34%, while corn core polysaccharides can activate the AMPK pathway to produce hypoglycemic effects (Buljeta et al., 2023; Tian et al., 2020).

Neuroprotective effects

Emerging evidence suggests that components in cereal wines may provide neuroprotective benefits through multiple mechanisms. Polyphenols can cross the blood-brain barrier and exert direct effects on neural tissues, reducing oxidative stress and inflammation in the central nervous system. Additionally, compounds like tryptophan found in grape stems (as a wine by-product) serve as precursors for serotonin and melatonin, which play crucial roles in mood regulation and sleep quality. These findings suggest potential applications for cereal wine components in supporting cognitive function and mental health. The gut-brain axis represents another mechanism through which cereal wine components may influence neurological health. Alkylresorcinols and other phenolic compounds can modulate the gut microbiota composition, leading to changes in microbial metabolites such as short-chain fatty acids and bile acids that can indirectly affect brain function and reduce neurocognitive disorders associated with oxidative stress. This connection between cereal wine components, gut health, and brain function represents an exciting frontier for future research (Buljeta et al., 2023; Hrelia et al., 2022).

In conclusion, cereal grain wines represent a promising category of functional alcoholic beverages that combine traditional fermentation wisdom with modern nutritional science. Their diverse bioactive compounds offer multifaceted health benefits ranging from cardiovascular protection to anti-aging effects. However, as with all alcoholic products, moderation is key to maximizing benefits while minimizing risks. Future research should further explore optimal consumption patterns and specific clinical applications of these nutritionally enriched fermented beverages. The core of this research is a targeted metabolomics study that uses GC-MS to analyze the volatile and semi-volatile metabolomes of cereal-based wines. It identifies and quantifies key discriminant metabolites, links these profiles to their biochemical origins (grain variety, yeast/fungal metabolism, process), and uses chemometrics to classify wines based on their metabolic fingerprints. The conclusion extends this to the use of metabolite profiles for authentication and understanding the impact of terroir and fermentation processes.

Discussion

The selection of raw materials for brewing and food production significantly impacts the nutritional and functional properties of the final product (Díaz et al., 2022). This study

compares four key grains barley, corn, buckwheat, and sorghum focusing on their starch/sugar content, protein levels, and unique bioactive compounds (Lukinac and Jukić, 2022). Barley, a traditional brewing staple, contains moderate to high starch (60-65%) and protein (10-12%), along with beta-glucans that influence viscosity and fermentation (Lukinac and Jukić, 2022; Melini et al., 2017). Corn, widely used in gluten-free and adjunct brewing, provides high starch content (70-75%) but lower protein (8-10%) and lacks some essential amino acids (Cela et al., 2020; Dabija et al., 2021). Buckwheat, a pseudocereal, offers a balanced nutritional profile with moderate starch (55-60%), higher protein (11-15%) rich in lysine, and notable levels of polyphenols, such as rutin, which confer antioxidant benefits (Wahlsten, 2019; Jose and Selvan, 2024). While barley and corn are preferred for their fermentable sugars, buckwheat stands out for its unique phytochemicals and protein quality. Understanding these differences allows for optimized grain selection based on desired nutritional, functional, and sensory outcomes in food and beverage applications.

From a health perspective, each cereal wine offers distinct advantages; sorghum wine with its antioxidant phenolic compounds, black barley wine with its melanoidin-based benefits, buckwheat wine with its flavonoid content, barley wine with its balanced nutritional profile, and corn wine with its quick energy provision. This suggests that consumption variety based on individual health needs and taste preferences would be optimal. For future development, there is significant potential for targeted optimization of production processes to enhance desirable compounds in each wine type. Additionally, blending approaches that combine different cereal wines could create products with complementary compound profiles offering enhanced sensory experiences and health benefits. Further research should focus on clinical studies to validate the health effects of specific compounds in these wines and technological innovations to improve production efficiency while preserving functional compounds.

Starch and sugar content

The starch and sugar content of barley, corn, buckwheat, and sorghum; significantly influence their applications in food and beverage production, particularly in brewing and baking. Corn exhibited the highest starch content (70–75%), making it an excellent energy source for fermentation processes, such as ethanol production and brewing adjuncts (Ojewumi et al., 2018). Its high amylopectin content (~75% of total starch) contributes to rapid gelatinization, enhancing fermentability. Barley contained moderate starch levels (55–65%), with a balanced amylose-to-amylopectin ratio (~20–25% amylose), which is crucial for brewing quality (Bamforth and Fox, 2023). The presence of β -glucans (3–7%) in barley can influence viscosity and lautering efficiency in brewing. Buckwheat a pseudocereal, had the lowest starch content (50–60%) but contained higher levels of resistant starch and dietary fiber, contributing to slower glucose release (Zhu, 2017; Chen et al., 2023c). Its sugar profile included sucrose and small amounts of reducing sugars, making it less fermentable than barley or corn. Sorghum grains contain approximately 25% starch (dry weight basis) even after the brewing process, as evidenced by research on sorghum distillery residues. During traditional sorghum wine production, this starch undergoes enzymatic hydrolysis to fermentable sugars, primarily through processes like malting or exogenous enzyme addition.

The sugar profile of sorghum wines differs significantly from grape-based wines, with studies showing that optimal sugar content for fermentation ranges between 22.2-26.8%, similar to findings in specialized wines like those incorporating medicinal mushrooms.

The conversion efficiency from starch to fermentable sugars in sorghum wine production typically reaches about 70% in conventional two-step fermentation processes, leaving considerable residual starch content that could potentially be further utilized. Research indicates that 17-18 g/L of sugar generally converts to 1% alcohol by volume, requiring approximately 204-216 g/L sugar content to achieve standard 12% ABV sorghum wines (Sukumaran and Gopi, 2023).

Protein levels and quality

Protein content and composition varied significantly among the four grains, affecting their nutritional and functional properties. Corn had moderate protein levels (8–10%), primarily zein (~50% of total protein), which is deficient in essential amino acids like lysine and tryptophan (Wu, 2016). This limits its nutritional quality unless complemented with other protein sources. Barley contained higher protein (10–12%), including hordeins (prolamins) and globulins, contributing to foam stability in beer but potentially causing haze formation (Jin and Youle, 2013). Buckwheat stood out with a well-balanced amino acid profile (11–15% protein), including high levels of lysine and arginine, making it a superior plant-based protein source. Additionally, buckwheat proteins (such as albumin) exhibit bioactive properties, including cholesterol-lowering effects. Sorghum wines, like other cereal-based fermented beverages, contain proteins primarily derived from the grain substrate. Research on sorghum wines shows protein contents ranging from 0.085 mg/mL in the liquid portion to significantly higher levels (40.63%) in fermentation byproducts. Similarly, enzyme-assisted extraction methods applied to distiller's grains from other cereal fermentations have achieved protein extraction rates of 23.59% under optimal conditions (1:14 solid-liquid ratio, pH 7.0, 10 h hydrolysis with 4000 U/g cellulose enzyme). These findings suggest that sorghum wines likely contain moderate protein levels, with substantial protein remaining in the solid byproducts that could be recovered through appropriate processing techniques (Giménez-Bastida et al., 2015).

Unique compounds (polyphenols and other bioactive)

Beyond macronutrients, each grain contained distinct bioactive compounds influencing health benefits and processing characteristics. Barley was rich in phenolic acids (ferulic, *p*-coumaric) and flavonoids, contributing to antioxidant activity (Yang et al., 2018). Proanthocyanidins in some barley varieties can influence beer flavor stability. Corn contained carotenoids (lutein, zeaxanthin) and ferulic acid, which are associated with reduced oxidative stress (de la Parra et al., 2007). However, its polyphenol content was generally lower than barley and buckwheat. Buckwheat was the most notable for its polyphenols, particularly rutin (a flavonoid with strong antioxidant and anti-inflammatory effects) (Zhang et al., 2012; Jing et al., 2016; Zhang et al., 2022). Tartary buckwheat also contained quercetin and catechins, which contribute to its nutraceutical potential. The unique combination of volatile aromatics, antioxidant polyphenols, and amino acids positions sorghum wine as both a culturally significant beverage and a potential functional food product worthy of further investigation and development (Zhang et al., 2022; Zou et al., 2023). Each grain barley, corn, buckwheat, and sorghum possess distinct starch, protein, and bioactive profiles that determine their suitability for different applications (Sofi et al., 2023). Corn's high starch content makes it ideal for fermentation, while barley's balanced composition is optimal for brewing (Patra et al., 2023). Buckwheat, though lower in starch, offers superior protein quality and polyphenol

content, making it valuable for functional foods (Li and Zhang, 2001; Koç and Coşkun, 2025). Future research should explore hybrid processing techniques to optimize the use of these grains in food and beverage industries.

Fermentation by products and flavor profiles

Black barley and sorghum produced a clean ethanol profile with low congeners (ideal for beer and whiskey) (Gibreel et al., 2009; Boyko et al., 2019). Esters and higher alcohols were well-balanced. Corn generated higher levels of fusel alcohol and diacetyl, contributing to a “corn-like” off-flavor unless fermentation was tightly controlled (Barnes, 2011). Buckwheat produced unique phenolic compounds (e.g., 4-vinylguaiacol), contributing to a smoky, earthy flavor profile, preferred in specialty beverages (Ambra et al., 2021; Cadenas et al., 2021). For fermentation kinetics the lag phase barley had the shortest lag phase (2–4 h), then follow by sorghum (3–5 h), while buckwheat required extended adaptation (6–8 h) (Salamon et al., 2023). Peak fermentation rate corn exhibited the fastest CO₂ evolution (12–24 h), whereas buckwheat had a delayed peak (36–48 h) (Dlulisa, 2022; Yan et al., 2025).

For industrial implications, the gold standard for brewing barley remains due to predictable fermentation (Fox, 2020). Corn is cost-effective for bioethanol but requires process optimization to minimize off-flavors (Elhalis, 2024). Buckwheat offers niche opportunities for gluten-free and craft fermentations but needs strain adaptation for consistent yields (Guo et al., 2025; Li et al., 2025). Barley outperforms corn and buckwheat in conventional fermentation due to its superior sugar profile and compatibility with standard yeast strains (Bellut et al., 2019; Oliver and Colicchio, 2011). However, corn and buckwheat offer distinct advantages (cost and flavor diversity, respectively) when optimized with tailored fermentation strategies. Future advancements in enzymatic treatments and yeast strain development could further enhance their viability in industrial fermentation processes. Future research directions include enzyme cocktail optimization for corn and buckwheat to enhance sugar accessibility. Yeast metabolic engineering to improve buckwheat fermentation efficiency (Serba et al., 2024; Adebami et al., 2022). Mixed-culture fermentations to exploit the unique flavors of buckwheat while maintaining yield.

Challenges and future perspectives

GC-MS is a powerful analytical technique widely used for the identification and quantification of volatile and thermally stable compounds. However, despite its high sensitivity, resolution, and reproducibility, GC-MS has significant limitations, particularly when analyzing non-volatile, thermally labile, or high molecular weight compounds (Beale et al., 2018). Such compounds often require derivatization to enhance volatility, introducing additional complexity and potential artifacts. This discussion explores the key limitations of GC-MS, including its inability to handle non-volatile compounds, thermal degradation risks, and the necessity for derivatization as follows:

Inability to analyze non-volatile and thermally labile compounds

The primary limitation of GC-MS is its reliance on analyte volatility, as the sample must be vaporized in the GC inlet (typically at high temperatures, 250–300°C). Many biologically relevant compounds such as peptides, proteins, sugars, and certain pharmaceuticals are non-volatile or decompose at these temperatures, making them

unsuitable for GC-MS analysis. For example, amino acids and organic acids require derivatization (e.g., silylation or esterification) to become volatile, which increases analysis time and may introduce impurities or side reactions (Blom, 2018; Karas et al., 1987).

Limited mass range for large molecules

GC-MS is optimal for small to medium-sized molecules (typically <1000 Da). Larger molecules (e.g., polymers, proteins, or oligonucleotides) either cannot elute from the GC column or fragment excessively under high temperatures (Zubarev et al., 1995; Kind et al., 2018). While techniques like pyrolysis GC-MS exist, they often produce complex and hard-to-interpret spectrum.

Matrix effects and co-elution challenges

Although GC-MS has excellent separation capabilities, complex matrices (e.g., biological fluids or environmental samples) can lead to co-election of interfering compounds, complicating spectral interpretation (Cortese et al., 2020; Côté et al., 2009).

Conclusion

GC-MS is a powerful analytical tool that enables the differentiation of wines by identifying and quantifying volatile and semi-volatile compounds that are influenced by terroir (geographical origin) and fermentation processes. By examining the unique chemical fingerprints of wines, GC-MS provides insights into the complex interactions between environmental factors, grain composition, and microbial activity during fermentation. Here is a detailed summary of how GC-MS can distinguish wines based on these two critical factors. The first is terroir encompasses the environmental conditions such as soil composition, climate, altitude, and viticultural practices that impart distinct chemical profiles to grains and, consequently, wines. GC-MS can detect these differences by analyzing; terpenes and nor-isoprenoids, these aroma compounds are strongly influenced by grape variety and growing conditions. For example, cool-climate wines may exhibit higher concentrations of linalool and α -terpineol, while warmer regions might produce more β -damascenone. Phenolic compounds, soil mineral content affects phenolic metabolism, leading to variations in volatile phenols such as guaiacol and eugenol, which can be traced back to specific regions. Sulfur compounds, the presence of dimethyl sulfide (DMS) or methional can vary based on soil sulfur content and sun exposure, further distinguishing wines by origin. By comparing the relative abundances of these compounds, GC-MS can classify wines according to their geographical origin with high precision. The second factor is distinguishing wines by fermentation, whether spontaneous or inoculated, and influenced by yeast and bacterial strains plays a crucial role in shaping a wine's volatile profile. GC-MS helps differentiate fermentation derived compounds including esters and higher alcohols like ethyl esters (e.g., ethyl acetate, ethyl hexanoate) and acetate esters (e.g., isoamyl acetate) are produced in varying amounts depending on yeast strains (*Saccharomyces* vs. *non-Saccharomyces*). Higher alcohols such as (fusel alcohols like isoamyl alcohol and phenylethyl alcohol) differ based on fermentation temperature and yeast metabolism. Fatty acids, medium-chain fatty acids (e.g., hexanoic, octanoic acid) are by products of yeast lipid metabolism and can indicate fermentation conditions (aerobic vs. anaerobic). Volatile phenols, 4-ethylphenol and 4-

ethylguaiacol are markers of *Brettanomyces* contamination, distinguishing “clean” fermentations from those with microbial influence. By analyzing these fermentation markers, GC-MS can determine whether a wine underwent spontaneous vs. controlled fermentation, the presence of wild yeast or bacteria, and even aging techniques (e.g., malolactic fermentation). GC-MS is an indispensable tool for ecological research and wine authentication, offering unparalleled resolution in detecting some chemical differences arising from terroir and fermentation. By leveraging this technique, winemakers and researchers can ensure product consistency, trace provenance, and even uncover fraudulent practices, while consumers gain deeper insights into the unique characteristics that define their favorite wines. Future advancements in high resolution GC-MS and metabolomics will further refine our ability to decode the intricate chemistry behind wine diversity.

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