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Article

The effect of sterilization treatment on the synthesis of key biomolecules and microbial communities in fruit wine fermentation

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Abstract: Sterilization treatments impact microbial communities and key biomolecule synthesis, influencing the aroma and quality of fruit wine fermentation. However, the roles of microorganisms in various fermentation techniques and the impact of sterilization treatments on aroma formation and key biomolecule synthesis are not well understood. This study aims to elucidate the effects of sterilization treatments on the synthesis of key biomolecules and microbial community dynamics in fruit wine fermentation, focusing on their relation to aroma compounds. Using purple fruit as the primary subject, we analyzed the starting and final microbe populations in controlled test fermentation (CTF) and natural test fermentation (NTF) under different sterilizing treatments employing advanced sequencing strategies. We utilized multivariate analysis and regression analyses, via the SPSS tool to examine relationships between microbial fungal and bacterial genus-level communities, microbiological diversity, permanent substances aromatic substances, and physiological indexes. In NTF, we identified a total of 150 fungal genera, and 140 bacterial genera, with dominant genera including Candida, Burkholderia, Streptococcus, and Oenococcus. In CTF, 400 fungal genera, and 120 bacterial genera were identified, with the dominant genera being Geotrichum, Pichia, Aspergilus, and Saccharomyces, alongside Streptococcus, Paucibacter, Pantoea, Akkermansia, Lactobacillus, and Bifidobacterium. Positive correlations were observed between specific microbial genera and flavor compounds in both fermentation methods. This study provides insights into how sterilization treatments affect microbial dynamics and key biomolecule synthesis, offering valuable resources for enhancing the aromatic profile of fruit wine.

Keywords: sterilization treatment; biomolecule synthesis; fruit wine fermentation; microbial communities

1. Introduction

Tarko and Duda [1] depict the fruit wines are made from various fruits, each with unique flavors and characteristics. Traditional fruits include grapes, apples, berries, plums, cherries, peaches, pineapples, and pears. Pears are commonly used in Perry, a pear-based wine. Purple fruits, such as grapes, blackberries, and elderberries, are a focus in wine production. Apples are used for apple wine and cider, while raspberries are used in plum wine. The wine-making procedures are illustrated in **Figure 1**.

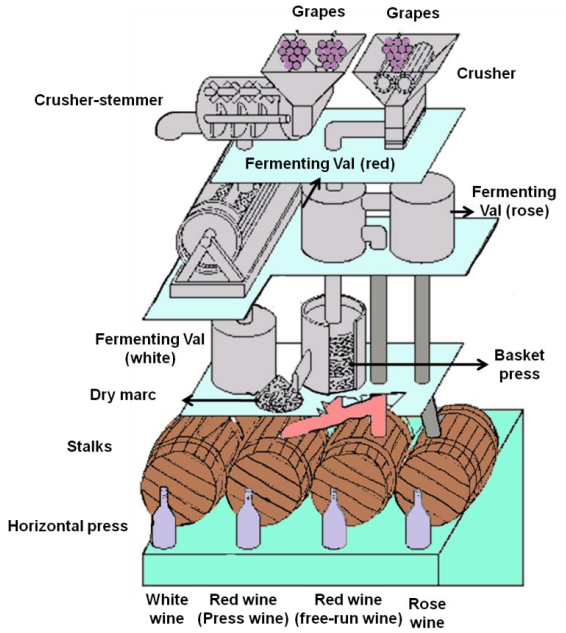

Figure 1. Wine making procedure.

Chen et al. [2] utilize the triploid Summertime Black grapefruit, which has a deep black or purple cover and no seeds, and is highly sought after for its durability, early maturity, high yield, disease resistance, and lack of seeds. It conducts a multitude of physiological roles, such as gut-microbiota modulation, cardioprotective, hypoglycemic, antioxidant, and anticancer effects. However, it currently controls the market for fresh fruit and raisins. Fruit and vegetable consumption juice, a valuable commodity containing bioactive components, is one of the world's most important agricultural enterprises. Lactic acid-microorganisms fermentation (LAF) can enhance the quality of foods, dietary intake, and efficiency by raising flavonoid and phenol levels in fruit juices. A major international fruit manufacturer is dealing with a substantial waste and low-quality fruit problem. To overcome this, biotechnological technologies and the agro-industry can be employed to convert trash into useful products. Fruit juice is an effective way to reduce wastage and increase value. Pineapple, which is an extremely common tropical fruit, has a high agronomic production and can be utilized as a foundation in the wine-making process analyzed by Boondaeng et al. [3]. A pineapple is a great option for use in the beverage industry since it has a strong aroma, a decent taste and acid material, and all other characteristics. Wine, a drink with alcohol derived primarily from matured grape juice, is an example of fruit juice rich in antioxidants and phenolic compounds, substances that help protect cells from destruction caused by oxidation and inflammation utilizing the Yıldırım-Yalçın et al. [4]. Jiang et al. [5] utilize the Dragon fruit, also known as the pitaya or the pitahaya, which is a tropical fruit that has important antioxidant properties due to its violet-red pigment, betalains. It is extremely vulnerable and can be processed into alcohol-based drinks or wine after harvesting preservation and value addition. Dragon fruit includes a high concentration of pectic compounds in its peel and paper pulp, which can be reduced by applying a pectolytic enzyme. This can alter the physical composition of the juice, influencing its flavor, color, and antioxidant capability in fruit wine. By integrating cell wall disintegrating digestive enzymes and

saccharomyces strains, it is feasible to vary the type and aesthetic properties of drinks with alcohol to meet the ever-changing consumer demand. Fermentation has been utilized for food storage from prehistoric times and can be found in several regions by utilizing Jiang et al. [6]. Fermentation is now used all over the world to extend the shelf life of raw products and to create foods with desirable organoleptic and nutritional attributes. Standardized fermentation technologies have been created to improve conventional techniques, consequential in sanitary, safer goods with predictable chemical characteristics. The utilization of specific starting cultures is critical for achieving these outcomes observed by Giuffrè and Giuffrè [7]. The fermentation procedures are depicted in **Figure 2**.

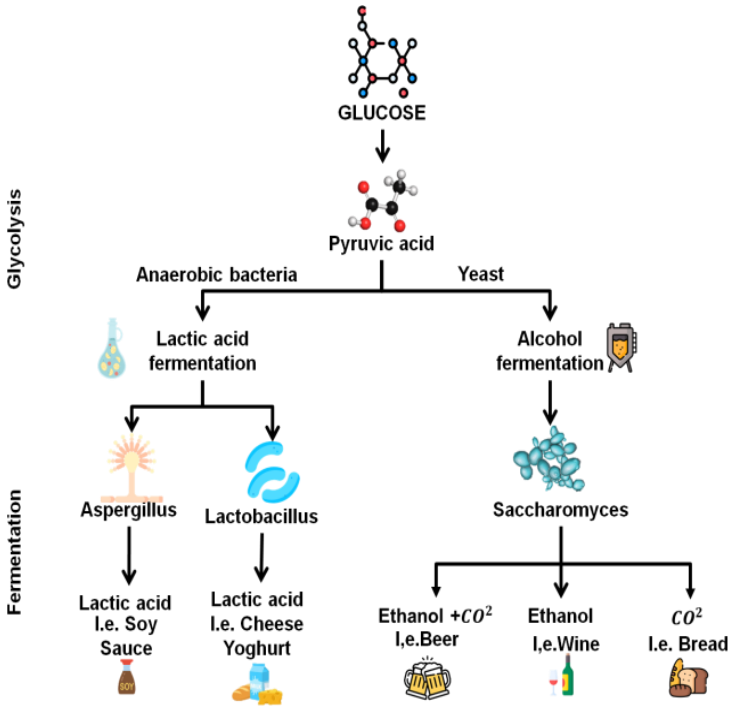

Figure 2. Fermentation procedure.

Yuan et al. [8] utilized the hongqu rice wine, an ancient Chinese fermentation alcohol made from glutinous and red fermentation rice, resulting in a reddish hue and flavor. It is used in medicine and nutritious foods due to cholesterol-lowering properties. However, conventional brewing techniques can produce toxic metabolites, stifling industry growth and internationalization. The flavor consistency of Hongqu wines is influenced by the composition of microbe populations, including molds, bacteria, yeasts, and bacteria, which can reduce sensory quality and raise health issues.

The primary objective of this research is to investigate how sterilizing treatments affect the dynamics of the microbiological society and the mixture of significant biomolecules during the fermentation of fruit wines, with a focus on how these changes impact the generation of odor and the overall quality of the wine. To better understand the relationship between microbial species, aromatic ingredients, permanent substances, and physiological indexes in fruit wine, the study analyzes natural test fermentation (NTF) and controlled test fermentation (CTF) under various

sterilizing circumstances. The goal of this study is to offer practical advice on improving fruit wine's aromatic profile and quality.

1.1. Contributions of this study

The research presented here sheds light on how sterilizing procedures affect microbial dynamics and essential biomolecule synthesis during fruit wine fermentation. Key contributions involve the following.

- Microbial Community Dynamics: The study identifies and compares the microbial diversity in NTF and CTF under different sterilization treatments. It highlights the dominant fungal and bacterial genera in both fermentation techniques, offering a detailed microbial profile.
- Aroma and Flavor Compound Correlations: Through multivariate and correlation analyses, the study demonstrates positive relationships between specific microbial genera and the production of aroma and flavor compounds. This finding provides essential knowledge on how microorganisms contribute to the aromatic profile of fruit wine.
- Impact of Sterilization on Biomolecule Synthesis: The research reveals how different sterilization treatments affect the synthesis of key biomolecules, such as nonvolatile metabolites, which are critical to fruit wine quality.
- High-Throughput Sequencing for Comprehensive Analysis: The application of high-throughput sequencing technology enables a detailed and accurate analysis of microbial communities, enhancing the understanding of fermentation processes at a molecular level.
- Practical Applications for the Wine Industry: The findings offer practical insights for winemakers, suggesting methods to optimize microbial conditions and sterilization processes to enhance wine aroma and quality.

The next portions of this study are the following, Portion 2: the literature reviews based on the wine-making fermentation process in various fruits, Portion 3: material and methods of analyzing the different sterilization treatments, Portion 4: result and discussion of this study, and Portion 5: conclusion of the study.

2. Literature review

Li et al. [9] showed that bacteria-derived cellulose (BC) could be manufactured on-site using hydrolyzed material and grape dung. The BC's morphological and biomechanical characteristics were investigated, with significant purity and greater crystallinity indexes than the high sugar (HS) medium. The FTIR data revealed in-situ polyphenol binding onto the customized BC. Wine pomace-based BC possessed more tightly wound ultrafine fibrils, wider fiber ribbon diameter dispersion, and reduced breakdown by heat temperatures. It also has higher mechanical hardness and greater strength than the HS medium. The BC produced from apple the waste hydrolyzed material exhibited a slower phenolic compound release rate and higher antioxidant activity. Faria et al. [10] investigated ultra-high-pressure sterilization (UH-PS) as a replacement for heat treatment for Xinli No. 7 juice (XL-7). UH-PS successfully eliminates germs in XL7 juice; however, HS treatment reduces nutritional value. UH-PS treatment preserves glycolic acid content, essential elements, and antioxidant

capacity while reducing browning and increasing juice stability. The degradation rate of both UH-PS with HS-treated XL-7 juice rises with storage space hotness. UH-PS as well as HS-treated XL-7 fruit juice had a calculated shelf life of 41–68 days at 4 ℃, depending. Integrating UH-PS pretreatment with temperatures below freezing storage could extend the longevity of the juice. Chawafambira [11] assessed the production of a probiotic beverage cultured by lactic acid bacteria using jujube juice that has undergone four sterilizing procedures. The findings demonstrated that the treatments did not affect L. plantarum's capacity to grow in jujube juice. Following the sterilization and pasteurization processes, the juice's particle size increased and its sugar content decreased. Jujube juice's stability was enhanced by freezing plasma sterilizing (FPS) and continuous ultraviolet sterilization (CUVS), and following FPS treatment, the juice's tartaric acid concentration considerably rose. The sensations and nutrient content of the juice that was CUVS treated and the control juice did not change significantly. Compared to previous treatments, the juice treated with CUVS had a superior color. Yang et al. [12] examined whether fermenting juice from blueberries produced exopolysaccharides. Under ideal circumstances (pH, temperature, amount of inoculation, and addition of glucose), the maximum yield was obtained at 2.2 ± 0.1 g/L. The ideal conditions reduced the amount of phenolics and anthocyanins while increasing the number of live cells and total acids. Zhang et al. [13] utilized the absorbance zones of –OH and –CH were identified using visible near-infrared spectral analysis, and a prediction model based on competitive adaptable reweighted selection and the random forest was created, showing accurate prediction of exopolysaccharide concentration throughout fermentation. Using the cultivar Saccharomyces cerevisiae Microbial Type Culture Collection (MTCC) and Gene Bank 178 and recovered yeast, Patel et al. [14] examined five mango varieties to produce wine. The North Indian native mango variety (Dashehari) displayed higher organoleptic and functional features and could have antioxidants that are anti-cancerous, anti-inflammatory in nature, and antibacterial activities. The inexpensive wine made from the indigenous mango variety had notable physicochemical characteristics, ability to antioxidant, and alcoholic level. Yuan et al. [15] utilized the conditions of fermentation for brewed green jujube fruit wine and examined its quality. It looked at alterations in volatile components, antioxidant capability, and physicochemical indices. The starting sugar, bacteria furthermore, fermentation duration, and sulfur dioxide (SO_2) treatments were all optimized. Results demonstrated that the beverage had significant 2-diphenyl-1-picrylhydrazyl (DPPH) eliminated free radical abilities through the fermentation process of alcohol. Fifty volatile chemicals were also detected; following fermentation, there was a notable decrease in the amount of ketones, aldehydes, heterocyclic, and aromatic compounds. Muñoz et al. [16] assessed the impact of different ultrasound treatments on the microbiota found in wine, such as Lactiplantibacillus plantarum, a yeast called Brett spp., and Omyces cerevisiae. Numerous yeasts and bacteria were subjected to six treatments; Lactiplantibacillus plantarum being the most resistant and the fungus Brett spp. the most sensitive. The vinegar used by Kim et al. [17] was made from apple concentrate by fermenting alcohol and acetic acid in two stages, resulting in a pH of 2.94 and a pH level of 6.20%. The browning index rose dramatically as a result of the fermenting procedure. However, the essential quality metrics of the non-thermal sterilization of raw vinegar from apples and sterilization alcohol weren't

different considerably. Compared to the other samples, the non-thermally pasteurized raw apple vinegar had a substantially greater total organic acid concentration, with malic acid having the greatest level. Fermented apple vinegar has antioxidant activity and a total polyphenol content that have been more than twice as high compared to those of industrial goods. Wu et al. [18] evaluated the consequences of the preprocessing of Huyou juices containing methylene dicarbonate upon its qualitative features, using pasteurization Huyou juices as a comparison. The application of Dimethyl Dicarbonate (DMDC) guarantees Huyou juice's microbiological safety as well as its sensory and physiological qualities. After fermentation, Huyou juice's flavor becomes better and its total polyphenol and total flavonoid levels rise. In a sensory examination, the Huyou juice that has been fermented and sterilized with DMDC has a superior color, odor, and quality in general. When stored at 4 ℃, several physicochemical characteristics are measured, including pH, Total Phenolic Content (TPC), Total Flavonoid Content (TFC), vitamin C, and viable cell count. After four weeks of storage, the viability percentages of LAB in both fermenting pasteurization and DMDC-sterilized Huyou beverages stay at 7.0 log CFU/ml. The impact of several sterilizing techniques on apple juice indicators of quality was assessed by Zhu et al. [19]. The findings indicate that ascorbic acid levels are raised by sonication while accessible pectin and protein levels are decreased by ultra-high pressure sterilization. The juice's flavor changes as the ratio of sugar to acid rises. The greatest loss of volatile substances occurs during microwave sterilization, whereas volatile materials are retained during ultra-high-pressure sterilization. This investigation may aid in the identification of appropriate sterilizing techniques to preserve the quality of hazy apple juice. The application of autonomous lactic acid bacteria, also called from Chinese bayberry (CB) alcohol that spontaneously fermented was studied by Gu et al. [20]. Numerous LAB strains were recovered and identified, including Weissella cibaria, the bacterium Le the stomach, Lactococcus lactis, Lacto plant bacillus plantarum, and Limos lactobacillus fermentum, plantain Zero Fermentable Material (ZFM) 710, 715, and 720 because ZFM722, were the strains that were chosen, and they both answered well to unfavorable fermented conditions. LAB induced a discernible change in the composition of organic acids and aided in the growth of the yeast *S. cerevisiae* throughout fermentation.

3. Methodology

In this study on fruit wine fermentation, purple passion fruits were cleaned and chosen for their uniform dimensions, maturation, and lack of mechanical damage, infections, or pests. The commercially available microbe *cerevisiae* was utilized for NTF and controlled fermentation CTF to speed up the process of fermentation. The fermentation process of purple passion fruit wine is expressed in **Figure 3**.

The following discusses the identification of biophysical indicators, volatile aromatic chemicals, permanent compounds; and the diversity of microorganisms during the passion fruit wine's fermenting procedure.

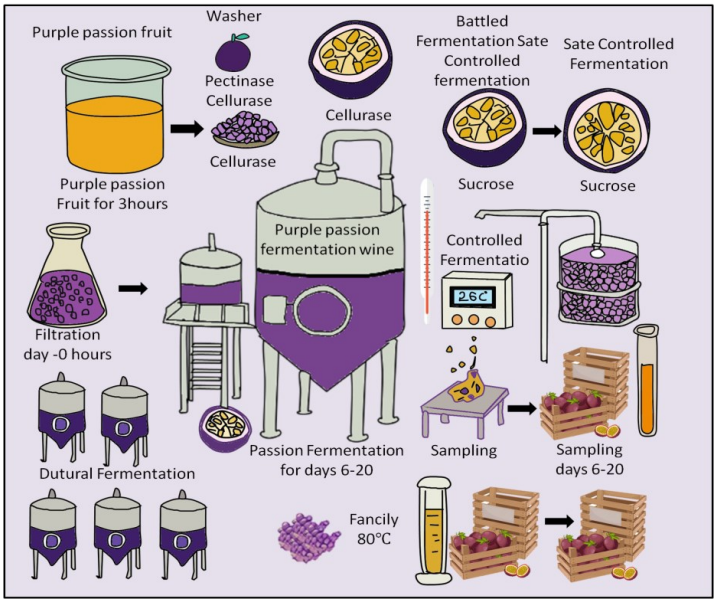

Figure 3. Purple passion fruit wine fermentation.

3.1. Fermentation procedure

The process of purple passion fruit wine fermentation involves washing and breaking the fruit, adding 0.1% pectinase and 0.1% cellulose for 3.5 h to break down cell walls, aiding in juice extraction and clarity. The Enzymatic Treatment procedure steps are depicted in the following Equations (1) and (2).

$$Pectin + Pectinase \longrightarrow Simple\ Sugars \quad (1)$$

$$Cellulose + Cellulase \longrightarrow Glucose \quad (2)$$

After filtration, sucrose is added to increase sugar content and aid in fermentation. The initial Brix (sugar concentration) is controlled at 20.5%.The fermentation setup includes Natural Fermentation (NF) with sucrose and spontaneous fermentation using natural yeasts, or Controlled Fermentation (CF) with 0.04% inoculation of Saccharomyces cerevisiae. The alcoholic fermentation equation is calculated as Equation (3).

$$Glucose(C_6H_{12}O_6) \rightarrow 2\ Ethanol(C_2H_5OH) + 2CO_2 + \text{Flavor Compounds} \quad (3)$$

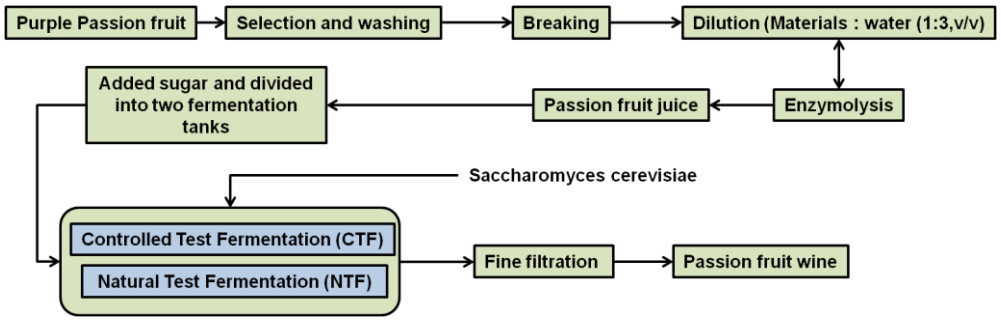

Figure 4. Fermentation procedure.

The wine manufacturing progression takes place at a controlled 28 ℃ temperature, with daily and daily samples taken to monitor progress. The yeast

converts sugars into alcohol and carbon dioxide, producing aromatic compounds that influence the wine's flavor profile. There are two stages of fermentation: primary fermentation (0–6 days) with rapid yeast activity, and secondary fermentation (6–20 days) with slower fermentation as sugar levels decrease, resulting in a wine with specific aromatic and flavor characteristics based on fermentation conditions and microbial activity. **Figure 4** illustrates the step-by-step fermentation chemical procedure workflow.

3.2. Physicochemical analysis

The physicochemical analysis of fruit wine fermentation involves several key chemical methods. These include the determination of soluble solids using a Brix meter, which provides a direct reading of the percentage of dissolved solids in the sample. The total sugars are quantified using the Phenol-Sulfuric Acid Method, which involves the reaction between sugars and sulfuric acid, leading to the formation of furfural or hydroxymethylfurfural, which reacts with phenol to form a yellow-orange color measurable at 490 nm are depicted in Equation (4).

$$Sugars(C_6H_{12}O_6) \xrightarrow{H_2SO_4} C_6H_6O_3 + H_2O \quad (4)$$

$$C_6H_6O_3 + H_2O + C_6H_5OH \longrightarrow Yellow - orangeComplex\ (absorbsat\ 490\ nm)$$

The alcohol content is typically measured by distillation followed by specific gravity or by alcohol-specific meters. Total acids are usually determined by titrating the wine sample with a base until neutralization. The Acid-Base Neutralization Reaction is used to measure high-acids (HAC) and total acids (TACs).

$$Total\ Acids\ (HA) + NaOH \longrightarrow NaA\ (salt) + H_2O \quad (5)$$

A pH meter is used to measure the wine specimen's pH to determine its acidity level. The Folin-Ciocalteu Procedure is used for calculating the total amount of phenols, the substances responsible for the purple, blue, and red colors in wine. Buffers with pH values of 1.0 and 4.5 are utilized to quantify the anthocyanins; the difference in absorbance across both scenarios is used to calculate the anthocyanin concentration.

$$Phenolic\ Compounds + Folin - Ciocalteu\ Reagent + Na_2CO_3 \longrightarrow Blue\ Complex\ (absorbs\ at\ 750\ nm) \quad (6)$$

These techniques yield detailed information about the physicochemical characteristics of fruit spirits, such as their acidity, sugar concentration, alcohol percentage, phenolic substances, and color components like cyan pigments, all of whom are important markers of the quality of the wine.

$$Flavylium\ Cation\ (colored\ form, pH\ 1) \leftrightarrow Hemiketal\ (colorless\ form, pH\ 4.5) \quad (7)$$

3.3. Specimen gathering

Assessing soluble solids (Brix), gas production (bubbles), and microbial growth are all part of the sampling procedure in both controlled and spontaneous fermentation. This shows how fermentation is progressing as well as how sugar content is changing, both of which have an impact on microbial development and the synthesis of fragrance compounds. The Phases are:

- Starting Phase: It's a Starting CTF (SCTF) for controlled fermentation, Starting NTF (SNTF) for natural fermentation Observation, Soluble solids decrease, bubbles are generated, and 20% Brix is observed.
- Final Phase: It's a Final CTF (FCTF) for controlled fermentation and, a Final NTF (FNTF) for natural fermentation Observation: Soluble solids stop decreasing, and no visible bubbles are observed. 6.2% Brix is observed, and the chemical reaction is consistent throughout but occurs at varying rates in each stage, with the middle stage being the most active.

3.4. Microbiological diversity analysis

The extraction and amplification of microbial Deoxyribonucleic Acid (DNA) from passion fruit wine includes numerous processes, including DNA extraction, Polymerase Chain Reaction (PCR) multiplication of particular areas, and sequencing. Total microbial DNA was extracted from the wine using the TGuide S96DNA Separation Kit (Beijing, China) while conducting tests in. The amino acids were measured and evaluated for authenticity using a reader for microplate by amplifying particular sections of the isolated DNA using PCR; the microbial populations can be thoroughly examined. The Internally Transcribed Spacer (ITS) geographical area of fungi is the target of fungal amplifying the DNA, which is followed by cycles of denaturation, annealing, and elongation. The 16S messenger Ribonucleic Acid (RNA) gene sequence of bacteria is the target of bacterial DNA amplification, which is followed by cycles of denaturing, annealing, and stretching. 1.8% electrophoresis on agarose gels is used to examine the PCR results from the bacterial and fungal amplifications to identify DNA fragments and gauge the amplification's effectiveness. For additional examination, suitable samples with distinct bands of the anticipated size are chosen. Sequenced libraries are created utilizing the SMRTbell Pattern Prep Kit (PacBio) (Guangzhou, China), and the accuracy of the final collection is assessed using a Qubit technological fluorometer while conducting tests. The amplified DNA is then subjected to high-throughput DNA sequencing using a Sequencer II Sequencer, which provides information on the microbial diversity present in the passion fruit wine. This laborious process makes it possible to identify and characterize the microbial communities in passion fruit wine using DNA extraction, amplification of specific regions, and arranging of those areas to understand their composition and potential contributions to the fermentation process are depicted in **Figure 5**.

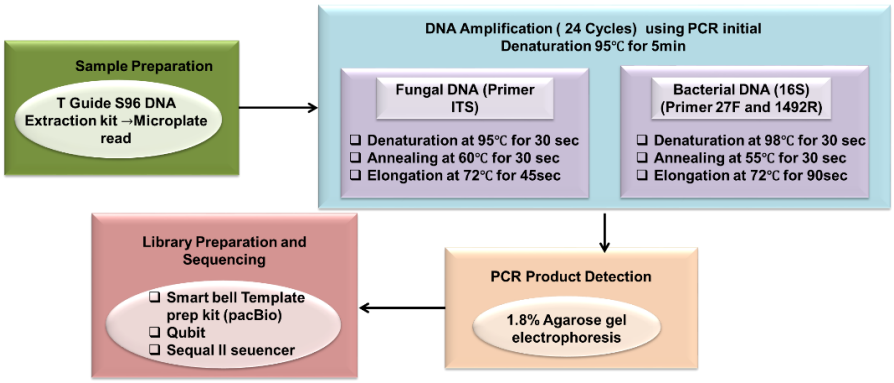

Figure 5. Microbiological diversity analysis.

3.5. Permanent substances identification

Several procedures are involved in the extraction and analysis of metabolites from a sample: sample preparation, extraction, derivatization, and analysis using Gas Chromatography-Time of Flight-Mass Spectrometry (GC-ToF-MS) while conducting tests in (Shanghai, China). The method begins with sample preparation and extraction, which typically entails inserting a 99 µL specimen into a 1.9 mL Eppendorf, Inc. tube. The specimen is mixed with 398 µL of the extracted methanol solvent, using Adonitol as a standard for internal use. The containers are vortexed for a minute to guarantee full mixing. After 14 min and 52 s of ultrasonication in cold water baths, 14 min and 54 s of centrifugation at 4 ℃ and 12,000 revolutions per minute (rpm) are conducted. The samples are subsequently moved to a disposable tube for analysis afterward. To concentrate the metabolites, the concentrate is dried in an automated concentrator at 20 ℃ for 1 h and 57 min. The dried sample is treated with methyl amination, which improves the volatility and stability of the carbonyl compounds for Gas Chromatography (GC) analysis through the transformation of them into their corresponding oximes. Following methoxyamination, BSTFA is added to produce hydroxyl and amine groups, which enhance volatility and identification in mass spectrometry. An Inspiron 8740 gas chromatograph and a Falcon high-temperature kinetic energy mass spectrometer fitted with a Dimethylpolysiloxane 5% Diphenyl Siloxane (DB-5MS) capillaries article are used to perform the GC-ToF-MS analysis. The amount to be injected is 1 µL, and the carrier gas is helium. The starting temperature of the protocol is set at 49 ℃ for 60 sec, before being maintained at 309 ℃ at a rate of 9 ℃/60sec, culminating in a stay at 310 ℃ for 480 sec. The temperatures of the ion source, transfer line, and injection are adjusted to 249 ℃, 279 ℃, and 279 ℃, respectively. The data is collected at a scan speed of 13.7 spectra per second throughout a range of masses of 40–520 atomic weight units (AMU). To avoid causing solvent contamination with the examination of target molecules, a 7.05-min solvent delay is incorporated. The KNApSAcK database is used for qualitative analysis to identify compounds in the sample, and the internal norm harmonization procedure is used for quantitative analysis to ensure the correct quantification of metabolites procedure are depicted in **Figure 6**.

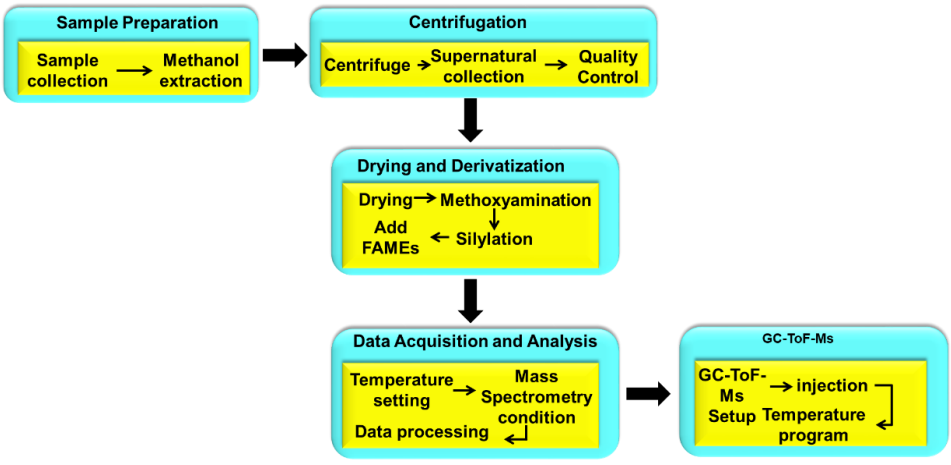

Figure 6. Permanent substances identification.

3.6. Volatile aromatic chemicals recognizing

To prepare specimens and evaluate volatile compounds, headspace solid phase microextraction (HS-SPME) (Wuxi, China) is employed in conjunction with gas chromatography-mass spectrometry (GC-MS) while conducting tests. Sample transfer, which involves moving 0.19 mL of the sample into a 19 mL vacuum vial, starts the procedure. By sustainable phase separation and raising the volatility of some components, salt is added to increase the efficiency of extraction. To account for variations that could occur during the extraction and analysis procedures, an internal standard is added. A 120 μm Divinylbenzene (DVB), Chemical Weathering Resistance (CWR), and Polydimethylsiloxane (PDMS) optic wrapped with a blend of polymers capable of adsorbing a broad spectrum of volatile chemicals is used to create the Solid Phase Microextraction (SPME) fiber. The sample is heated at 60 ℃ for 5 min, allowing more compounds to escape into the headspace. The fiber is then exposed to the headspace for 15 min at 60 ℃, adsorbing volatile compounds in the headspace for analysis. After exposure, the fiber is desorbed for 5 min at 250 ℃, causing the adsorbed compounds to vaporize and enter the gas chromatography system for analysis. Inspiron 8890 gas chromatograph and Agilent 7000D mass spectrometers are used in the GC-MS analysis. A DB-5MS microfluidic column, which is appropriate for distinguishing unstable organic molecules according to their chemical characteristics, is used by the system. Helium is used as a carrying gas in the GC operating environment, with an intake temperature of 249 ℃ and a detector's sensitivity of 280 ℃. The initial temperature for the GC oven temperature program is 39 ℃ for three minutes and fifteen seconds. Thereafter, temperature ramps of 100 ℃ at 12 ℃/min, 180 ℃ at 8 ℃/min, and 278 ℃ at 27 ℃/min are included. The electron efficient ionization mode is set to 70 eV, and the mass spectrometer settings are crucial for efficient ionization and transfer of compounds into the mass spectrometer. Data acquisition involves scanning mass spectra every 1 second in the mass-to-charge ratio range of 50–500 amu. The linear retention index (LRI) is used for compound identification, confirming the identity of compounds based on their retention time relative to standard compounds.

3.7. Statistical analysis

Using the Unified system for the DNA based fungal species identification (UNITE) and Silva databases for taxonomic classification, generating raw data from the PacBio Sequel platform, and applying Statistical Package for the Social Sciences(SPSS) statistics are the steps involved in doing significance analysis and data processing in a study. Quantitative Insights Into Microbial Ecology (QIIME), a potent tool for evaluating high-throughput microbial community sequencing data, is used to process the data. Data import, quality assurance, Operational Taxonomic Unit (OUT) selection, taxonomic assignment, and diversity analysis are all steps in the process. To find associations between variables or microbial taxa and their abundance, and correlation analysis, are uncover important traits or taxa that set one group apart from another, the data are evaluated, offering insights into the underlying biological mechanisms.

4. Result and conclusion

In this investigation, utilizing a 7-day test, we noticed that saccharomyces and microbial growth in the fermentation solution reduced with time, which caused a peak in sugar consumption and an ensuing decrease in alcohol output. Most significantly, the alcohol contents in the CTF samples were higher, the fermenting periods shorter, and increases in dissolved solids and sugars more significant than in NTF samples. Passion fruit acidity during the initial stage had to be raised to a value of 9.54 g/L to ensure a high multiplication of yeast and prevent many detrimental microorganisms, establishing optimal fermentation conditions. It has been remarked that there is a substantial difference between CTF and NTF during the fermentation period regarding pH and total acid content. Both compounds at higher levels in the CTF contribute significantly to wine color stability and general quality. The values of **Table 1** have been determined as mean ± standard deviation (n = 3) for passion fruit wines during fermentation time. Superscripts in all the corresponding values have represented significant variations in CTF and NTF groups of the same fermentation time. This table shows the changes in all the parameters over 7 days, such as the content of anthocyanin, total sugar, alcohol percentage, total acidity, pH, and the total phenolic compounds considered significant in the interpretation of fermentation processes and the quality of wine.

Table 1. Physicochemical indices.

Duration Period (Day)	Measures Physical and chemical properties							
	Groups	Content of Aqueous Materials (%)	Anthocyanins (mg/L)	Total Sugar (g/L)	Alcohol Percentage (%)	Total Acid (g/L)	pH	Total Phenols (mg/L)
0	-	20.50 ± 0.00	25.83 ± 2.01	155.86 ± 0.26	0.00 ± 0.00	9.54 ± 0.30	3.55 ± 0.01	306.97 ± 1.01
1	CTF	18.15 ± 0.08^a	8.63 ± 0.92^a	124.22 ± 2.50^a	1.50 ± 0.50^b	12.59 ± 0.22^b	3.33 ± 0.01^a	453.10 ± 2.10^b
	NTF	20.16 ± 0.13^b	23.04 ± 1.17^b	151.44 ± 6.09^b	0.00 ± 0.00^a	9.52 ± 0.28^a	3.56 ± 0.01^b	349.73 ± 4.55^a
2	CTF	14.57 ± 0.14^a	4.06 ± 0.34^a	75.75 ± 2.27^a	3.00 ± 0.00^b	15.46 ± 0.29^b	3.31 ± 0.01^a	491.14 ± 6.17^b
	NTF	20.14 ± 0.12^b	17.36 ± 0.29^b	149.54 ± 0.44^b	0.00 ± 0.00^a	9.52 ± 0.28^a	3.55 ± 0.00^b	318.08 ± 3.64^a
3	CTF	11.36 ± 0.09^a	5.29 ± 0.67^a	56.87 ± 2.18^a	4.67 ± 0.58^b	16.10 ± 0.50^b	3.29 ± 0.01^a	498.89 ± 6.62^b
	NTF	19.01 ± 0.01^b	7.24 ± 0.10^b	134.82 ± 1.87^b	0.83 ± 0.29^a	10.69 ± 0.19^a	3.34 ± 0.02^b	450.07 ± 5.56^a
4	CTF	9.34 ± 0.10^a	4.95 ± 0.35^a	40.60 ± 0.83^a	6.67 ± 0.29^b	16.08 ± 0.00^b	3.31 ± 0.02^b	508.32 ± 7.30^b
	NTF	17.35 ± 0.09^b	3.45 ± 1.75^a	113.97 ± 0.23^b	1.83 ± 0.29^a	12.16 ± 0.37^a	3.23 ± 0.01^a	476.67 ± 13.66^a
5	CTF	7.96 ± 0.14^a	5.51 ± 0.50^b	16.81 ± 0.15^a	7.17 ± 0.29^b	16.40 ± 0.55^b	3.31 ± 0.00^b	450.74 ± 9.81^a
	NTF	15.29 ± 0.15^b	1.72 ± 0.48^a	81.44 ± 1.34^b	2.91 ± 0.08^a	14.40 ± 0.24^a	3.20 ± 0.01^a	501.92 ± 5.34^b
6	CTF	7.09 ± 0.16^a	6.62 ± 0.39^b	13.79 ± 0.09^a	8.33 ± 0.29^b	16.96 ± 0.37^b	3.30 ± 0.01^b	430.54 ± 6.49^a
	NTF	13.20 ± 0.20^b	1.89 ± 0.26^a	69.83 ± 1.43^b	4.50 ± 0.14^a	15.34 ± 0.24^a	3.21 ± 0.01^a	454.44 ± 2.67^b
7	CTF	6.36 ± 0.06^a	6.18 ± 0.33^b	3.91 ± 0.13^a	10.17 ± 0.29^b	17.52 ± 0.48^a	3.31 ± 0.01^b	428.52 ± 2.10^a
	NTF	8.55 ± 0.08^b	1.78 ± 0.39^a	40.17 ± 1.50^b	6.16 ± 0.29^a	16.90 ± 0.25^a	3.21 ± 0.01^a	453.77 ± 2.10^b

Note: a ($p < 0.05$) and b ($p < 0.01$).

4.1. Fungal community microbial diversity analysis

The diversity of the fungal community at the genus level in NTF and CTF samples contained 135 different genera. The most abundant genera were Geotrichum, Pichia, Aspergillus, and Saccharomyces. Geotrichum is known for its high biotransformation activity, producing a variety of volatile flavor compounds related to the typical flavors of fruits, cheese, caramel, and butter, thereby enhancing the sensory properties of fermented products. In addition, Pichia and Aspergillus produce ethyl acetate that contributes to more odoriferous attractiveness besides its complexity in the wine flavor while ensuring that red wine color does not fade. Besides these, Saccharomyces is also an important yeast in winemaking, since it can secret glycosidases that cleave the glycosidic bond, thus liberating constituents that have flavor and aroma from their precursors. Though abundance during fermentation is the crucial phase for wine aroma expression, 389 species were reported from CTF, the most common of which being Geotrichum, Pichia, Aspergillus, and Saccharomyces again. Notably, the commercially available Saccharomyces cerevisiae soon surpassed the formerly predominant genera. The latter are particularly adapted to high alcohol conditions that prevail in CTFs. Saccharomyces cerevisiae is also very important in fermentation since it can easily convert carbohydrates into alcohol which determines a considerable influence on yield, flavor, and aroma of the final liquor. Initially, non-Saccharomyces yeasts were dominant in CTF; however, as alcohol concentration increased, Saccharomyces cerevisiae gradually took over, showing its competitive advantage in such conditions. The diverse fungal communities and their interactions during fermentation are pivotal in influencing the synthesis of flavor compounds, ultimately imparting the passion fruit wine with its distinct fruity characteristics. **Figure 7** shows a microbial diversity analysis of the fungal community at the genus level, which indicates a dynamic change in community structure during fermentation.

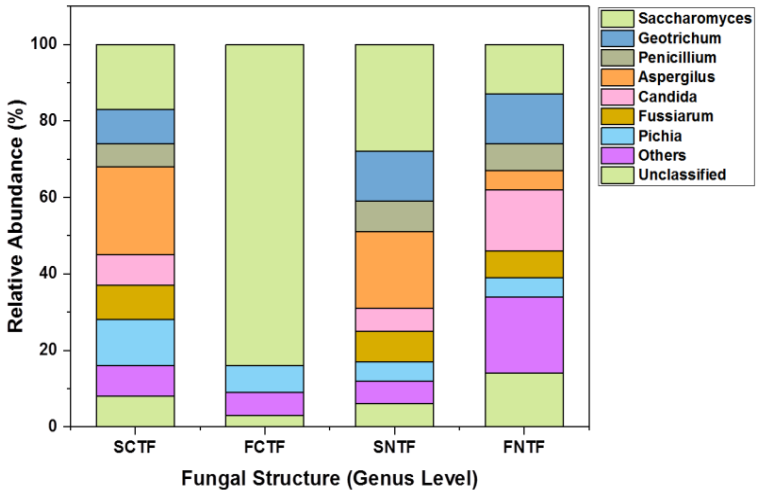

Figure 7. Fungal community genus diversity.

4.2. Bacterial community microbial diversity analysis

The bacterial genera found in passion fruit wine's CTF and NTF. In NTF, the most frequently observed bacterial genera are Paucibacter, Pantoea, Escherichia

schidelliformis, Achromobacter, Lactobacillus, and Bifidobacterium. The Lactobacillus and Bifidobacterium species are aerobic and acid-tolerant and thus can survive the fermentation environment. As fermentation continues, these genera dominate NTF in contributing to the fermentation process and Paucibacter and Pantoea decline as alcohol levels increase. The shift depicts how certain populations of bacteria can adapt to and thrive under specific conditions of fermentation. In CTF, the dominant genera of bacteria are Streptococcus, Bifidobacterium, Pantoea, and Lactobacillus. It grows initially but then its population is significantly decreased during fermentation because of high alcohol concentration that does not favor the survival of Paucibacter. Lactobacillus is important, as this strain contributes towards desirable flavor formation but also to acidity resistance, therefore a prerequisite for the wine sensory attribute development. On the other hand, the commercial strains used in CTF may repress the growth of less-dominant bacteria, which may result in a smaller range of dominant genera than in NTF. This illustrates how the selection of fermentation strains can play a key role in influencing the general microbial composition and, hence, the traits of the wine. **Figure 8**, visually presents these differences in bacterial genera between NTF and CTF, emphasizing the dynamics of bacterial interactions during fermentation.

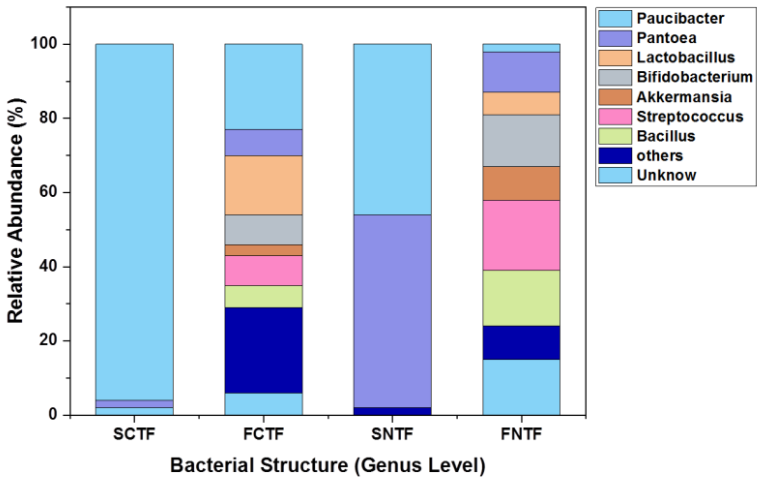

Figure 8. Bacterial community genus diversity.

During the fermenting process, researchers found 176 permanent compounds in passion fruit wine. The chemical classes within them comprise twelve amines, ten nucleotides, fifty organic acids, twenty-one fatty acids, twenty-one alcohols, seven ketones, ten phenols, seven esters, eleven amines, and thirty-six others, which result in a wine with great complexity in flavor and aroma. In the CTF, acidic compounds increase initially and reflect the active fermentation process as microorganisms metabolize sugars into acids, which can enhance the overall taste profile of the wine. However, these acidic compounds later declined as fermentation progressed, possibly due to conversion into other metabolites or because they became less prominent relative to alcohol production. On the other hand, sugar levels declined gradually throughout the fermentation process, which is expected since the yeasts, will break down the sugars into alcohol and carbon dioxide. Conversely, the NTF subgroup

showed more organic acids than the CTF group after fermentation. This could be because wild micro-flora existing in the NTF may produce other organic acids which contribute to a more complicated flavor profile. **Figure 9** among the non-volatile compounds of passion fruit wine, there is a wide range, which will be discussed below: Hence, it is very crucial to understand these compounds and their interplays at fermentation time to serve towards optimal flavoring and optimal quality wines.

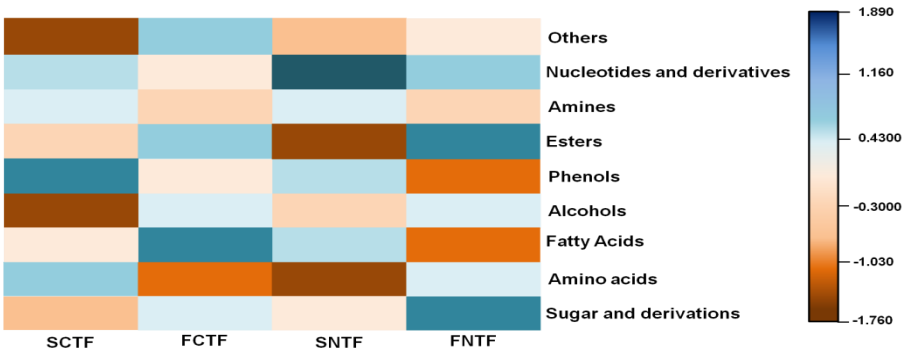

Figure 9. Metabolites types analysis.

4.3. Volatile aromatic chemicals analysis

During the maturation process of passion fruit wine, scientists identified 128 volatile taste compounds. These compounds play a vital role in the definition of the sensory profile of wine. The chemical classes identified include ester compounds, alcohols, ketones, organic acids, alkenes, benzene rings, aromatic compounds, amines, and nitrogen-containing compounds such as furan, pyrazine, and pyridine. All these compounds contribute uniquely to the wine's aroma and flavor complexity. The most significant group of volatile compounds for esters is that they contribute to the fruity fragrance. At first, it has a fruity smell because of active yeast metabolism during fermentation. Later on, it will decrease with time because that indicates that the producing mechanism and degradation mechanism are balancing each other. Ethyl esters contribute to floral and strawberry notes. One particular ester is the carboxylate of 5-3-methyl-4-hydroxy-3-5-pyrazole; when it is methylated, it possesses a specific aroma that enhances the overall sensory perception of wine. As fermentation progresses, the metabolism of amino acids and glycolysis increase the formation of such alcohols as benzyl alcohol, 1-butanol, and phenylethyl alcohol with their respective aromas of rose, apple, and almond. Levels of ketones like 3-hexanone will decline, yet some of these give flavors reminiscent of grapes. Volatile acids have an extreme impact on aroma while also adding a level of complexity to the wine. Levels of alkanes and alkenes continue to build and contribute more flavor. The concentration of the benzene ring compounds also varies, for instance, volatile phenols like 2,4-di-tert-butylphenol add complexity but overpower fruity aromas if present in excess concentrations. Data summarizing the results are presented as mean ± SD (n = 3) in **Table 2**, where the letters a (p< 0.01) and b (p< 0.05) indicate significant differences in compound concentrations. This analysis will depict the dynamic interplay of volatile compounds during the maturation of passion fruit wine as well as the contribution that such volatile compounds might offer toward its rich, harmonious flavor profile.

Table 2. Volatile aromatic chemicals recognizing.

Samples	Compounds	SCTF	FCTF	SNTF	FNTF
S1	$C_{12}H_{24}O_2$	0.02 ± 0.01^a	1.29 ± 0.14^b	nr	0.62 ± 0.03^a
S2	$C_{13}H_{28}O_2$	nr	0.02 ± 0.00^a	nr	0.02 ± 0.00^a
S3	$C_{12}H_{18}O_2$	0.06 ± 0.01^a	0.06 ± 0.01^a	0.07 ± 0.00 a	0.08 ± 0.01^b
S4	$C_{12}H_{24}O_2$	0.04 ± 0.01^a	0.24 ± 0.04^a	nr	0.26 ± 0.04^a
S5	$C_{18}H_{39}O_4P$	0.009 ± 0.001^b	0.009 ± 0.000^b	0.02 ± 0.001^b	0.03 ± 0.01^b
S6	$C_{14}H_{28}O_2$	0.003 ± 0.000^b	0.39 ± 0.006^b	nr	0.13 ± 0.001^a
S7	$C_{14}H_{19}N_3O_4$	nr	0.06 ± 0.02^b	nr	0.02 ± 0.00^a
S8	$C_{11}H_{13}N_3O_2$	0.23 ± 0.01^a	0.09 ± 0.00^a	0.24 ± 0.01^b	0.12 ± 0.01^b
S9	$C_{13}H_{14}O_3$	0.09 ± 0.00^a	0.03 ± 0.00^b	0.09 ± 0.01^a	0.02 ± 0.00^a
S10	$C_{14}H_{26}O_2$	0.009 ± 0.000^b	0.002 ± 0.000^b	0.01 ± 0.000^b	0.002 ± 0.001^b
S11	$C_{18}H_{23}O_4P$	0.01 ± 0.001^b	0.15 ± 0.001^b	0.01 ± 0.000^b	0.13 ± 0.000^b
S12	$C_{13}H_{18}O_4$	0.02 ± 0.00^a	0.03 ± 0.00^a	0.02 ± 0.00^a	0.13 ± 0.01^b
S13	$C_{11}H_{13}NO_2$	nr	0.01 ± 0.00^a	0.01 ± 0.00 a	0.01 ± 0.00^a
S14	$C_8H_{18}O_3$	0.09 ± 0.00^a	nr	0.09 ± 0.01^a	0.02 ± 0.00^b
S15	$C_4H_8O_4$	nr	0.07 ± 0.01^b	nr	0.04 ± 0.00^a
S16	$C_7H_{14}O_4$	0.01 ± 0.00^a	0.01 ± 0.00^a	0.01 ± 0.00^a	0.02 ± 0.00^b
S17	$C_7H_{14}O_2$	nr	0.09 ± 0.02^a	nr	0.12 ± 0.01^b
S18	$C_{11}H_{12}O_2$	0.019 ± 0.001^b	0.028 ± 0.001^b	0.02 ± 0.001^b	0.029 ± 0.000^b
S19	$C_8H_{18}O_2$	0.01 ± 0.00^b	0.04 ± 0.01^b	nr	0.03 ± 0.00^a
S20	$C_{13}H_{11}N_2O_2S$	0.02 ± 0.00^a	0.02 ± 0.01^a	0.02 ± 0.00^a	0.02 ± 0.00^a

Note:$C_{12}H_{24}O_2$ depicts the Decanoic acid, ethyl ester, $C_{13}H_{28}O_2$ represents the Octanoic acid, 3-methylbutyl ester, $C_{12}H_{18}O_2$ corresponds to the Terpinyl formate, $C_{12}H_{24}O_2$ indicates the Octanoic acid, ethyl ester, $C_{18}H_{39}O_4P$ refers to the Butylphosphonic acid, dodecyl isohexyl ester, $C_{14}H_{28}O_2$ signifies the Dodecanoic acid, ethyl ester, $C_{14}H_{19}N_3O_4$denotes the O-Methyl-DL-serine, N-dimethylaminomethylene ester, $C_{11}H_{13}N_3O_2$represents the Ethyl 6-methylpyridine-2-carboxylate, $C_{13}H_{14}O_3$ depicts the 2-(4-Methoxyphenyl)ethyl acetate,$C_{14}H_{26}O_2$ corresponds to the Vinyl caprylate, $C_{18}H_{23}O_4P$ signifies the phosphonic butyl acid, di(2-phenylethyl) ester, $C_{13}H_{18}O_4$ indicates the ethylene 3-pentyl sucrose ester, $C_{11}H_{13}NO_2$ refers to the 2-propionic alcohol Ester of methyl, 4-cyanophenyl derivative, $C_8H_{18}O_3$ denotes the 3-hydroxy-hexanoic ethanol ester of ethyl, $C_4H_8O_4$ represents the Ethylene glycol acetate formate, $C_7H_{14}O_4$ indicates the Propanoic acid, 2-hydroxy-, ethyl ester, $C_7H_{14}O_2$ signifies the 2-Pentanol, acetate,$C_{11}H_{12}O_2$ denotes the esters of ethyl of 4-ethenyl-benzeneacetic acidity, $C_8H_{18}O_2$ depicts the Hexanoic acid, ethyl ester and $C_{13}H_{11}N_2O_2S$ refers to the Benzoic acids acetic [1,2,5]Acetate of thiadiazol-5-yl.a ($p < 0.01$) and b ($p < 0.05$)

5. Conclusion

The study demonstrates that during fruit wine fermentation, sterilizing treatments have a major impact on the synthesis and fluctuations of the microbiological population of important biomolecules. It highlights the significance of microbial diversity in affecting fruit wine's aroma quality by revealing that certain fungal and bacterial species positively connect with aroma compound synthesis. This research can improve the aromatic profile of fruit wines and optimize fermentation procedures. Using two distinct fermentation techniques, controlled fermentation CTF and NTF, high-throughput sequencing technology was utilized in this work to investigate the wide range of populations of microbes throughout passion fruit wine manufacturing.

The findings showed a considerable variation in the two approaches' microbial diversity. The gram-positive *Paucibacter, Antarctica, the microorganism, Lactobacillus, and the Bifidobacterium* were the most prevalent bacterial genera in NTF. The most common bacterial species in CTF were *Streptococcus, Bifidobacterium, Pantoea, and Lactobacillus*, whereas the most common fungus genera were Geotrichum, Pichia, Aspergillus, and Saccharomyces. As the fermentation process becomes more sophisticated, the study found that the colony architectures in NTF and CTF differed greatly.

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