



Improving the aging aroma profiles of Italian Riesling and Petit Verdot Wines: Impact of spontaneous and inoculated fermentation processes

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ABSTRACT

The study employed gas chromatography-ion mobility spectrometry to differentiate between wines undergoing spontaneous fermentation and inoculated fermentation, with aging periods of 3, 9, and 15 months. The results indicate that throughout the three aging periods, there was a notable increase in the levels of ethyl hexanoate (Monomer, M), 2-methyl butanal, ethyl octanoate (M), ethyl octanoate (Dimer, D), propyl acetate, and 3-methylbutanal in the spontaneous Italian Riesling wine (RS). Furthermore, the compounds isoamyl acetate (M), ethyl formate (D), 4-methyl-2-pentanone (M), and ethyl formate (M) demonstrated the highest concentrations at 15 months in RS, accordingly, these compounds displayed a consistent upward trend throughout the aging period. A total of 14 volatile compounds exhibited an upward trend from 3 to 15 months in the spontaneous fermentation of Petit Verdot Wine (VS). Subsequently, these compounds attained their maximum levels. Spontaneous fermentation effectively enhances the aromatic characteristics of wines, consequently improving their capacity for aging.

1. Introduction

The presence of a unified wine style poses a significant obstacle to the attainment of long-term, sustainable growth within the wine industry (Wei et al., 2022). Meanwhile, it negates the influence of the terroir on the characteristics of wine. The aroma profile is a crucial aspect of winemaking, representing one of the significant terroir expressions that holds immense value for winemakers globally (Pinu, 2018). The aroma characteristics of wine can be classified into three categories, fruit aromas, fermentation aromas, and aging aromas (Cao et al., 2022). Wine aroma is commonly associated with the existence of volatile compounds that include alcohols, aldehydes, acids, esters, ketones, and terpenes, which are related to the variety, environmental conditions, vineyard practices, the winemaking process, and storage methods (Ubeda, Pena-Neira, & Gil, 2022; Wu et al., 2022; Zhu et al., 2022). The prevalence of commercial yeast in the winemaking process contributes to the homogeneity of wine flavors (Puertas et al., 2018; Xi et al., 2021). In contemporary times, the utilization of commercial yeasts is prevalent owing to their prompt initiation of fermentation and

efficient conversion of sugar into a substantial quantity of alcohol, which rendering them highly manageable within an industrial context (Sen, 2021). The biosynthesis of various compounds that contribute to the aroma of wine is influenced by the metabolic activity of yeast during the process of alcoholic fermentation (Carpena et al., 2021; Naselli et al., 2023). Moreover, these compounds give rise to secondary aromas that have been shown to be impacted by multiple factors. Among these factors, the impact of microorganisms is considered the most significant in shaping the final aroma profile of wine (Carpena et al., 2021). However, the significance of the microorganism in determining the impact of terroir on the final aroma profile of wine, has frequently been disregarded by researchers. In this regard, the utilization of indigenous yeast derived from grape surfaces exemplifies an alternative approach to wine production, aiming to create wines that accurately reflect the unique characteristics of the local terroir (Wei et al., 2022).

The microbial ecosystem present in grapes and wine, which includes *Saccharomyces* and non-*Saccharomyces* yeasts, as well as lactic acid bacteria, is considered a crucial element in determining the aroma of wine and influencing consumer preferences, as recognized by

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winemakers and oenologists (Belda et al., 2017). Indigenous yeasts are frequently present in grapes, vineyards, and natural surroundings, with those originating from the same region as the grape variety exhibiting a greater propensity for maintaining the inherent characteristics of the grapes (Wang et al., 2022; Xi et al., 2021). Furthermore, it is worth noting that non-*Saccharomyces* yeasts have a substantial impact on the initiation of spontaneous fermentation, given their higher prevalence in the vineyard compared to *Saccharomyces cerevisiae* (Borren & Tian, 2021). In relation to this matter, Wang et al. (2022), conducted a study which demonstrated that the levels of lactic acid in spontaneous fermentation processes, utilizing H30 and YT13 (indigenous yeast strains obtained from vineyards in Yantai, China), increased when compared to the control group. In contrast, the concentration of ferulic acid content was 1.4 times that of the inoculated fermentation group (Wang et al., 2022). Moreover, research conducted by Xi et al. (2021) also demonstrated that the microorganisms employed in the production of spontaneous wines exhibit a remarkable aptitude for fermentation and yield higher quantities of glycerol, thereby enhancing the sweetness and smoothness of the final flavor profile. The selection of the starter yeast not only impacts the development of secondary aromas during the fermentation process, but also influences the transformation of aromatic compounds as the wine ages, thereby contributing to its aging potential (Caridi et al., 2021). Correspondingly, there has been a growing trend in utilizing non-*Saccharomyces* yeasts, primarily attributed to their capacity for enhancing aroma characteristics (Carpena et al., 2021). These studies provide additional confirmation regarding the involvement of spontaneous fermentation microorganisms in the development of wine's aroma and aging process.

At present, oak chips have been proven effective in augmenting the aroma/flavor of wine, as well as enhancing its mouthfeel in traditional winemaking (Liang et al., 2021). However, the incorporation of non-grape materials may introduce supplementary compounds, thereby resulting in an unstable microbial environment and a homogenized wine style. Furthermore, the utilization of sulfur dioxide (SO_2) is commonly employed to enhance the stability of the microbial fermentation environment through the elimination of free radicals produced by oxidation reactions and the suppression of indigenous microorganisms (Marchante et al., 2020). Therefore, the utilization of indigenous microorganisms has the potential to significantly decrease the addition of SO_2 , primarily through antagonistic mechanisms such as the production of antimicrobial peptides by yeasts (Comuzzo & Zironi, 2013; Wei et al., 2022). The rising consumer consciousness regarding sustainable development matters and the growing demand for terroir wines, have created potential avenues for the future advancement of the wine industry, wherein spontaneous fermentation wines play a significant role (Wei et al., 2022). Our study hypothesizes that the identification of volatile aroma compounds, enhancement of sensory quality, and influence during bottle-aging of spontaneous fermentation in white and red wine are significant factors that influence the overall vinification process. Accordingly, this study, adopting Italian Riesling and Petit Verdot grape varieties in the Yantai Penglai wine region. To ensure accurate comparisons, we maintained consistent experimental conditions for the comparison between inoculated fermentation method with utilization of commercial yeast and the spontaneous fermentation method relying solely on natural microorganism without any introduction of foreign yeasts. The examination of aroma changes and sensory quality in spontaneous and inoculated fermented wines throughout the aging process can provide insights into the indigenous characteristics of spontaneous fermentation in terms of aroma, as well as determine which fermentation style approach is more effective in enhancing the sensory quality of wines during aging. This study offers additional scholarly references and a robust theoretical framework to support the utilization of spontaneous fermentation in industrial manufacturing process.

2. Material and method

2.1. Harvesting grapes and winemaking

Italian Riesling and Petit Verdot grape varieties, sourced from Junding vineyards (E120°50'37", N37°45'22") were subjected to sustainable viticultural practices. The practice included the cultivation of grass between the rows, and covering the branches within the rows, and the exclusion of chemical fertilizers and pesticides (Li, 2023). Subsequently, they were collected and screened. The horticultural maturity of Italian Riesling was determined to be 162.53 ± 3.9 (g/L) for reducing sugar content, 6.21 ± 0.1 (g/L, as tartaric acid) for titratable acid, and 3.12 ± 0.01 for pH, respectively. On the other hand, the horticultural maturity of Petit Verdot was determined to be 152.15 ± 0.12 (g/L) for reducing sugar content, 7.98 ± 0.36 (g/L, as tartaric acid) for titratable acid, and 3.2 ± 0.01 for pH, respectively. Ultraviolet (UV) irradiation and ozone were used to sanitize all brewing implements. The hand-pressed grape mash was divided into three parallel groups, each consisting of two treatments that were conducted simultaneously. The fermentation process was carried out in triplicate. After cooling to 15 °C and undergoing 12 h of clarification at this temperature, the inoculated fermentation group was inoculated with commercial yeast strains, namely ZYMAFLORE® X16 from LAFFORT® (Italian Riesling) and Lalvin D80® from Lallemmand® (Petit Verdot). In contrast, the spontaneous fermentation group was left uninoculated. The details of inoculated commercial strains were supplied in [supplementary data](#). All wines were bottled after the fermentation process. The wine samples were obtained at the end of 3, 9, and 15 months during the aging period. The spontaneous fermentation of Italian Riesling Wine was designated RS, while the inoculated fermentation was designated RI. Furthermore, the spontaneous fermentation of Petit Verdot Wine was designated VS, whereas the inoculated fermentation of Petit Verdot Wine was designated VI. [Fig. S1](#) illustrates the flowchart.

2.2. Determination of volatile compounds by headspace gas chromatography-ion mobility spectrometry (HS-GC-IMS)

A 3 mL sample of wine was incubated at 65 °C for 10 min in a 20 mL headspace vial prior to injection. The first passed through the primary separation of the gas chromatography column, then entered the ion migration tube, where the molecules to be measured were ionized in the ionization zone and migrated under the influence of the electric field and the reverse drift gas to the Faraday disk for detection and secondary separation. [Table S1](#) depicts the condition analyzed. The analysis software that came with the instrument consisted of VOCal and three plugins, each of which can be used to analyze samples from a unique perspective. The retention index (RI) of all samples was calculated using the C4-C9 ketone as external references. The volatile compounds were qualitatively characterized by comparing the RI and drift time (DT) with the NIST (National Institute of Standards and Technology, an official website of the United States government) and IMS (ion mobility spectroscopy, G.M.S., Dortmund, Germany) databases (Song et al., 2023; Rong et al., 2023; Yin et al., 2023; Wang et al., 2021). Detailed information (RI, RT, and DT) of volatile compounds is shown in the [Table S2](#). All samples were analyzed in quadruplicate and peak area is shown in the [Table S3 and S4](#).

2.3. Sensory evaluation

The sensory evaluation was conducted in accordance with the method reported by Lan et al. (2021). The trained sensory panel was comprised of twelve assessors (7 females and 5 males) from the College of Enology, Northwest A&F University, China, who had all successfully completed the College's wine tasting course. In the first trial, the sensory panel evaluated the appearance (clarity and hue), aroma (purity, strength, and quality), taste (purity, sweetness, acidity, bitterness,

alcohol, balance, strength, persistence, quality and astringency for Petit Verdot), and overall rating of each sample. Table S5 displays the specifics of the sensory evaluation scoring sheet. In order to complete the online sensory evaluation of wine samples, the Easy Sensory Analysis System (v2.0), a software developed by the China National Institute of Standardization (CNIS) was utilized. In the second experiment, the panel sniffed samples to identify aromatic descriptors. After brief training of sensory panelists with reference flavors, each wine sample was evaluated. In the online system, sensory panelists were asked to describe the aroma of each wine sample, write a minimum of three and a maximum of five descriptors and rate the intensity of each descriptor from 1 (weakest) to 5 (strongest). All tasters were instructed to independently complete all tasting texts and were prohibited from communicating with one another during the experiments.

2.4. Statistical analysis

Principal component analysis (PCA) was performed using the Dynamic PCA plug-in (G.A.S., Dortmund, Germany). Details of the analytical software used in the current study, as well as their functions are provided below: a) VOCal: Each graph point represents a volatile organic compound (VOC). b) Reporter plug-in: This included direct comparison of spectral differences between samples using the Reporter plug-in (3D spectra, 2D top view, and difference spectra). c) Gallery Plot plug-in: A fingerprint comparison was conducted to visually and quantitatively compare the differences in VOCs between samples. d) Dynamic

PCA plug-in: Here, the dynamic principal component analysis for clustering samples and rapidly identifying unidentified sample species was implemented.

3. Results and discussion

3.1. The spontaneous fermentation promoted volatile aroma compounds at bottle-aging periods for Italian Riesling

PCA was conducted in order to enhance the visual representation of the distinctions between RS and RI wine samples throughout the aging period (Fig. 1). At time points of 3 months (Fig. 1A), 9 months (Fig. 1B), and 15 months (Fig. 1C), significant variations in volatile aroma compounds were observed between the RS and RI groups. The initial two components acquired accounted for a total of 79 % of the overall variation, with Principal Component 1 (PC1) explaining 68 % and Principal Component 2 (PC2) explaining 11 % of the variation observed at 3 months. At 9 months, the initial two acquired components accounted for 80 % of the total variation, with 58 % attributed to PC1 and 22 % attributed to PC2. Furthermore, the initial two acquired components accounted for 84 % of the total variance, with PC1 explaining 66 % and PC2 accounting for 18 % at 15 months. At 3 months, the RS treatment resulted in an elevation in the concentration of 18 volatile aroma compounds, including ethyl octanoate (D), propyl acetate, and propanal (D). Conversely, the RI treatment led to a reduction in the concentration of 10 volatile aroma compounds, such as 3-octanol, 1-hexanol (M), and

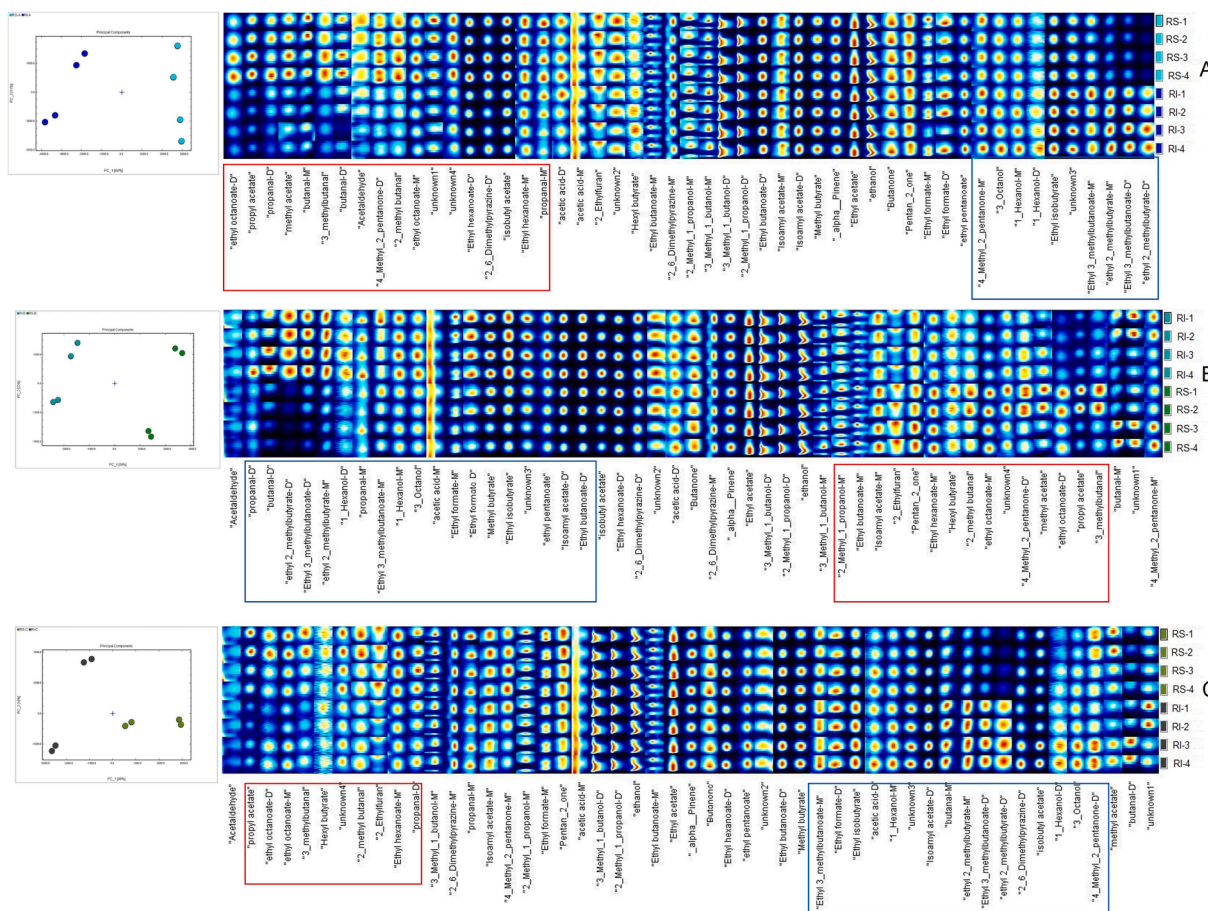


Fig. 1. The aroma volatile compounds detected by GC-IMS after 3-month, 9-month, and 15-month aging. Principle components analysis (PCA) and fingerprint of spontaneous fermented Italian Riesling wine (RS) and inoculated fermented Italian Riesling wine (RI) at 3-month aging (A). Principle components analysis (PCA) and fingerprint of spontaneous fermented Italian Riesling wine (RS) and inoculated fermented Italian Riesling wine (RI) at 9-month aging (B). Principle components analysis (PCA) and fingerprint of spontaneous fermented Italian Riesling wine (RS) and inoculated fermented Italian Riesling wine (RI) at 15-month (C). The red box means that the aroma compounds were higher in RS than RI. The blue box means that the aroma compounds were higher in RI than RS. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

1-hexanol (D). At 9 months, the treatment with RS increased the concentration of 15 volatile aroma compounds, including ethyl octanoate (M), propyl acetate, and ethyl octanoate (D), and decreased the concentration of 19 volatile aroma compounds, including 3-octanol, 1-hexanol (M), and 1-hexanol (D). At 15 months, the treatment of RS increased the concentration of 10 volatile aroma compounds, including ethyl octanoate (D), ethyl octanoate (M), propyl acetate, and propanal (D), whereas the treatment of RI decreased the concentration of 16 volatile aroma compounds, such as 3-octanol, 1-hexanol (M), and 1-hexanol (D). Three times, RS exhibited higher concentrations of ethyl hexanoate (M), 2-methyl butanal, ethyl octanoate (M), ethyl octanoate (D), propyl acetate, and 3-methylbutanal compared to RI. The utilization of commercial yeast for inoculation has been reported to result in the eradication or suppression of a significant portion of the microbiota, such as non-*Saccharomyces* yeast (van Wyk et al., 2019). Therefore, the utilization of a solitary strain of yeast is expected to exert an influence on the attributes of the wine's aroma (King et al., 2008). In this study, the olfactory characteristics of ethyl hexanoate have been elucidated, revealing a sweet fruity aroma profile with distinct notes of pineapple and green apple, along with a low odor threshold (=5) (Naselli et al., 2023; Pino & Queris, 2010). In addition, the olfactory perception of ethyl octanoate has been characterized as fruity and reminiscent of pear, exhibiting a relatively low odor threshold (=2) (Naselli et al., 2023; Pino & Queris, 2010). The flavor characteristics of propyl acetate were

characterized as having fruity notes reminiscent of celery, raspberry, and pear (Feng et al., 2022). In addition, the aroma of 3-methylbutanal was characterized as apple scent (Cao et al., 2022). Therefore, when compared to RI, the aforementioned aroma compounds exhibited an augmentation of the fruity aroma in RS. Moreover, the family of poly-functional thiols with fruity characteristics holds significant influence in the realm of yeast-modified compounds, particularly in the context of white wines (Cordente et al., 2022). As per the findings, it was consistently observed that ethyl isobutyrate, ethyl 2-methylbutyrate (M), ethyl 2-methylbutyrate (D), ethyl 3-methylbutanoate (M), ethyl 3-methylbutanoate (D), 1-hexanol (D), 1-hexanol (M), and 3-octanol exhibited a consistently lower concentration in RS compared to RI. According to Puertas et al., (2018), ethyl isobutyrate serves as a volatile indicator for the occurrence of the second fermentation process in bottles, facilitated by yeast. Hence, the viability of the commercial yeast persisted until the bottle underwent aging thereby exhibiting a prolonged duration of activity. The aroma of ethyl 3-methylbutanoate-M and -D was characterized as aromatic and fruity, reminiscent of the aroma found in wine (Cao et al., 2022). Furthermore, the aroma of 1-hexanol-D and -M was described as having grassy notes (Cao et al., 2022). In addition, the olfactory perception of 3-octanol was characterized as possessing an earthy and mushroom-like aroma. Hence, it was determined that RS exhibited a more pronounced reduction in pungent odors compared to RI.

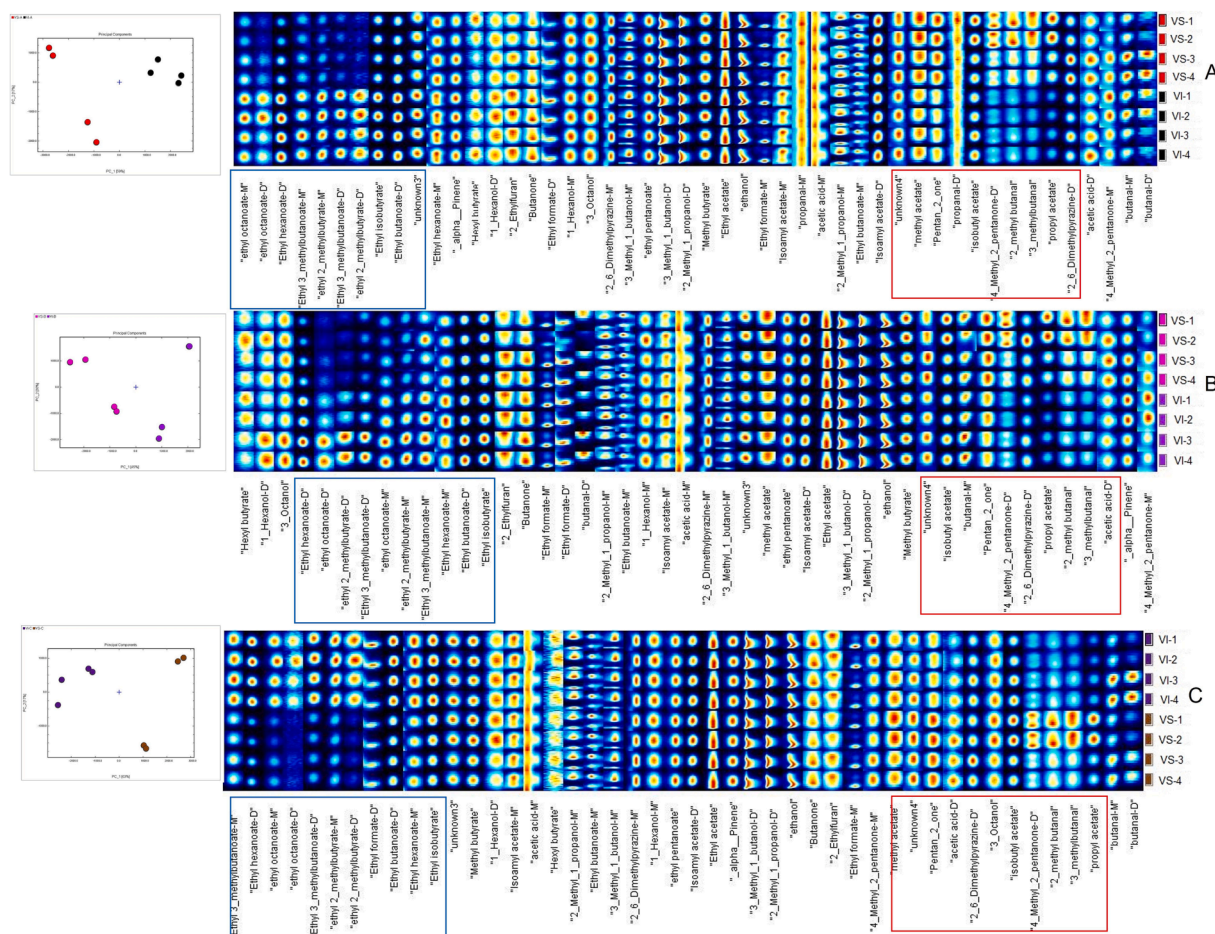


Fig. 2. The aroma volatile compounds detected by GC-IMS after 3-month, 9-month, and 15-month aging. Principle components analysis (PCA) and fingerprint of spontaneous fermented Petit Verdot wine (VS) and inoculated fermented Petit Verdot wine (VI) at 3-month aging (A). Principle components analysis (PCA) and fingerprint of spontaneous fermented Petit Verdot wine (VS) and inoculated fermented Petit Verdot wine (VI) at 9-month aging (B). Principle components analysis (PCA) and fingerprint of spontaneous fermented Petit Verdot wine (VS) and inoculated fermented Petit Verdot wine (VI) at 15-month aging (C). The red box means that the aroma compounds were higher in VS than VI. The blue box means that the aroma compounds were higher in VI than VS. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. The spontaneous fermentation promoted volatile aroma compounds at bottle-aging periods for Petit Verdot

PCA was also performed (Fig. 2) to enhance the visualization of the variations in wine samples between VS and VI over the course of the aging period. Accordingly, a notable difference in the volatile aroma compounds between VS and VI at 3 months (Fig. 2A), 9 months (Fig. 2B) and 15 months (Fig. 2C) was observed. At 3 months, the initial two acquired components accounted for 76 % of the total variation, with PC1 contributing 59 % and PC2 contributing 17 %. In addition, the first two components acquired accounted for 75 % of the total variation, with 45 % attributed to PC1 and 30 % to PC2 at 9 months. Moreover, the initial two components acquired accounted for 80 % of the overall variation, with 63 % attributed to PC1 and 17 % to PC2 at 15 months. The treatment of VS increased the concentration of 10 volatile aroma compounds, including methyl acetate, isobutyl acetate, and propanal (D), decreased the concentration of 10 volatile aroma compounds, including ethyl octanoate (M), ethyl octanoate (D), and ethyl hexanoate (D), in comparison to the treatment of VI at 3 months. The treatment of VS increased the concentration of 10 volatile aroma compounds, such as isobutyl acetate, propyl acetate, and acetic acid (D) while decreased the concentration of 10 volatile aroma compounds, such as ethyl hexanoate (D), ethyl octanoate (D), and ethyl isobutyrate when compared to the treatment of VI at 9 months. The treatment of VS increased the concentration of 11 volatile aroma compounds, such as methyl acetate, isobutyl acetate, propyl acetate, and acetic acid (D) while the treatment of VI decreased the concentration of 11 volatile aroma compounds, such as ethyl hexanoate (D), ethyl octanoate (M), and ethyl octanoate (D). Isobutyl acetate, pentan-2-one, 4-methyl-2-pentanone (D), 2,6-dimethylpyrazine (D), 2-methyl butanal, 3-methyl butanal, and propyl acetate exhibited higher levels of abundance in VS compared to VI across all three time. According to Cao et al., (2022) the aroma description of isobutyl acetate was ripe fruit. In addition, Cao et al., (2022) described the aroma of 4-methyl-2-pentanone as pleasant keto-like odor. The concentrations of RS and VS were found to be higher in propyl acetate, 2-methyl butanal, and 3-methyl butanal compared to RI and VI,

respectively. Ethyl isobutyrate, ethyl 2-methylbutyrate (M), ethyl 2-methylbutyrate (D), ethyl 3-methylbutanoate (M), ethyl 3-methylbutanoate (D), ethyl butanoate (D), ethyl hexanoate (D), ethyl octanoate (D), ethyl octanoate (M) were found lower in VS than VI across all three time. The compounds ethyl 2-methylbutyrate (M), ethyl 2-methylbutyrate (D), ethyl 3-methylbutanoate (M), and ethyl 3-methylbutanoate (D) exhibited higher RI compared to RS. The aroma description of ethyl butanoate (D) is characterized by the presence of strawberry flavors, as well as floral and fruity aromas (Cao et al., 2022). According to Cao et al., (2022), the aroma description of ethyl hexanoate (D) was akin to currants and pineapples. In addition, the aromas of ethyl octanoate-D and -M were fruity with pineapple, apple and brandy wine notes (Cao et al., 2022). The behavior of fermentative branched fatty acid ethyl esters, which are associated with yeast nitrogen metabolism, exhibits distinct differences from their straight-chain counterparts during the process of wine aging, which are linked to yeast lipid metabolism (Kong et al., 2022). Esters, such as ethyl butanoate, ethyl hexanoate, and ethyl octanoate, play a significant role in enhancing the aromatic characteristics of wine, thereby contributing to its overall quality (Zhao et al., 2021). As observed, non-*Saccharomyces* yeasts exhibit a wider range of volatile aroma compounds in comparison to *S. cerevisiae* (Borren & Tian, 2021).

3.3. RS's and RI's volatile aroma compounds changed during bottle-aging

PCA was performed (Fig. 3) in order to better visualize the dynamic change in wine samples from 3 to 15 months during the aging period. Significant differences in the volatile aroma compounds were observed among the RS samples collected at 3 months, 9 months, and 15 months (Fig. 3a). The first two components acquired collectively accounted for 73 % of the overall variance, with PC1 accounting for 51 % of the variance and PC2 accounting for 22 %. The concentrations of isoamyl acetate (M), ethyl formate (D), 4-methyl-2-pentanone (M), and ethyl formate (M) exhibited their highest levels at 15 months. Consequently, these compounds exhibited an aging-related increase in RS. Methyl acetate, propyl acetate, isoamyl acetate (D), isobutyl acetate, ethyl

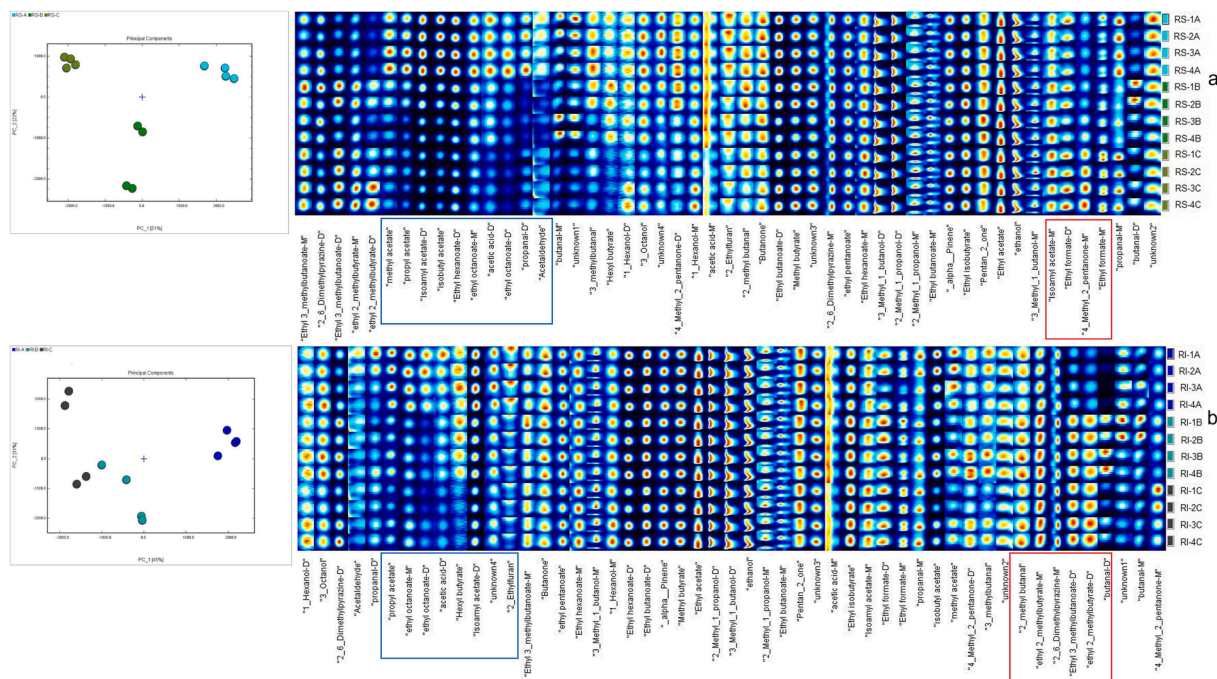


Fig. 3. The dynamic change of spontaneously fermented Italian Riesling wine (RS) from 3-month (RS-A) to 9-month (RS-B) to 15-month (RS-C) and PCA analysis among RS-A, RS-B, and RS-C (a). The dynamic change of inoculated fermented Italian Riesling wine (RI) from 3-month (RS-A) to 9-month (RS-B) to 15-month (RS-C) and PCA analysis among RS-A, RS-B, and RS-C (b). The red box means that the aroma compounds were highest at 3-month. The blue box means that the aroma compounds were highest at 15-month. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

hexanoate (D), ethyl octanoate (M), acetic acid (D), ethyl octanoate (D), propanal (D), and acetaldehyde had the highest concentrations at 3 months. As a result, these compounds exhibited a decline in RS as age increased. Significant variations in the levels of volatile aroma compounds were observed among the 3 months, 9 months, and 15 months in the RI (Fig. 3b). The first two components obtained accounted for 76 % of the overall variation, with PC1 accounting for 45 % and PC2 for 31 %. The highest concentrations of propyl acetate, ethyl octanoate (M), ethyl octanoate (D), acetic acid (D), hexyl butyrate, isoamyl acetate (D), and 2-ethylfuran were observed at 3-month. Conversely, the lowest concentrations of 2-methyl-butanal, ethyl 2-methylbutyrate (M), butanal (D), 2,6-dimethylpyrazine (M), ethyl 3-methylbutanoate (D), and ethyl 2-methylbutyrate (D) were observed at 3 months. The data from various sources support the notion that the selection of wine yeast plays a crucial role in enhancing the antioxidant levels found in red wine (Caridi et al., 2015). The volatile compound profiles of the wine samples underwent changes both during the process of maturation and subsequent to the period of bottle aging (Ubeda et al., 2022). Hence, the variations in microorganisms across different treatments exert an influence on the temporal evolution of volatile aroma compounds throughout the aging process.

3.4. VS's and VI's volatile aroma compounds changed during bottle-aging

PCA was performed (Fig. 4) in order to better visualize of the dynamic change of wine samples from 3 months to 15 months of aging. Significant variations in volatile aroma compounds were observed among the VS samples collected at 3 months, 9 months, and 15 months (Fig. 4a). The first two components obtained accounted for 77 % of the variance, with PC1 accounting for 55 % and PC2 for 22 %. A total of 14 volatile aroma compounds exhibited a progressive increase over a span of 3 to 15 months. These compounds include α -pinene, isoamyl acetate (M), ethyl formate (D), 4-methyl-2-pentanone (M), ethyl formate (M),

ethyl 3-methylbutanoate (D), ethyl 3-methylbutanoate (M). At 15 months, all individuals had attained their maximum value. One of the aroma descriptions attributed to α -pinene was that of a fresh camphor scent with a hint of sweetness (Sun et al., 2022). From 3 months to 15 months, a total of 10 compounds, such as methyl butyrate, propyl acetate, isoamyl acetate (D), isobutyl acetate, ethyl hexanoate (D), 3-octanol, hexyl butyrate, etc., gradually decreased. They all achieved the highest value at 3 months. Significant variations in volatile aroma compounds were observed among the VI samples collected at 3 months, 9 months, and 15 months VI samples in terms of volatile aroma compounds (Fig. 4b). The initial two components acquired accounted for 80 % of the overall variation, with 62 % attributed to PC1 and 18 % attributed to PC2. The compounds butanone, 1-hexanol (D), 3-octanol, 4-methyl-2-pentanone (D), 2,6-dimethylpyrazine (D), 2-methyl butanal, 3-methylbutanal, and 2-ethylfuran exhibited a declining pattern over the course of 3 months to 15 months. The compounds ethyl 2-methylbutyrate (M), ethyl 2-methylbutyrate (D), 4-methyl-2-pentanone (M), ethyl 3-methylbutanoate (D), ethyl 3-methylbutanoate (M), ethyl isobutyrate, ethyl formate (D), ethyl hexanoate (M), and isoamyl acetate (M) exhibited the lowest levels at 3 months. Subsequently, these compounds demonstrated an upward trend from 3 months to 15 months. The initial young wine exhibited a notable disparity between acid levels and ester levels, with the former being considerably higher than the latter. As the wine aged, a substantial deviation from the acid-ester equilibrium was observed, characterized by a significant increase in esterification ratios (Kong et al., 2022). In contrast VI, the process of aging exhibited a more pronounced augmentation in aroma volatile compounds and a heightened capacity for further maturation.

3.5. Changes in sensory characteristics

The sensory quality data are depicted in the form of radargrams (Fig. 5). Additionally, the corresponding error was shown in Table S6. As

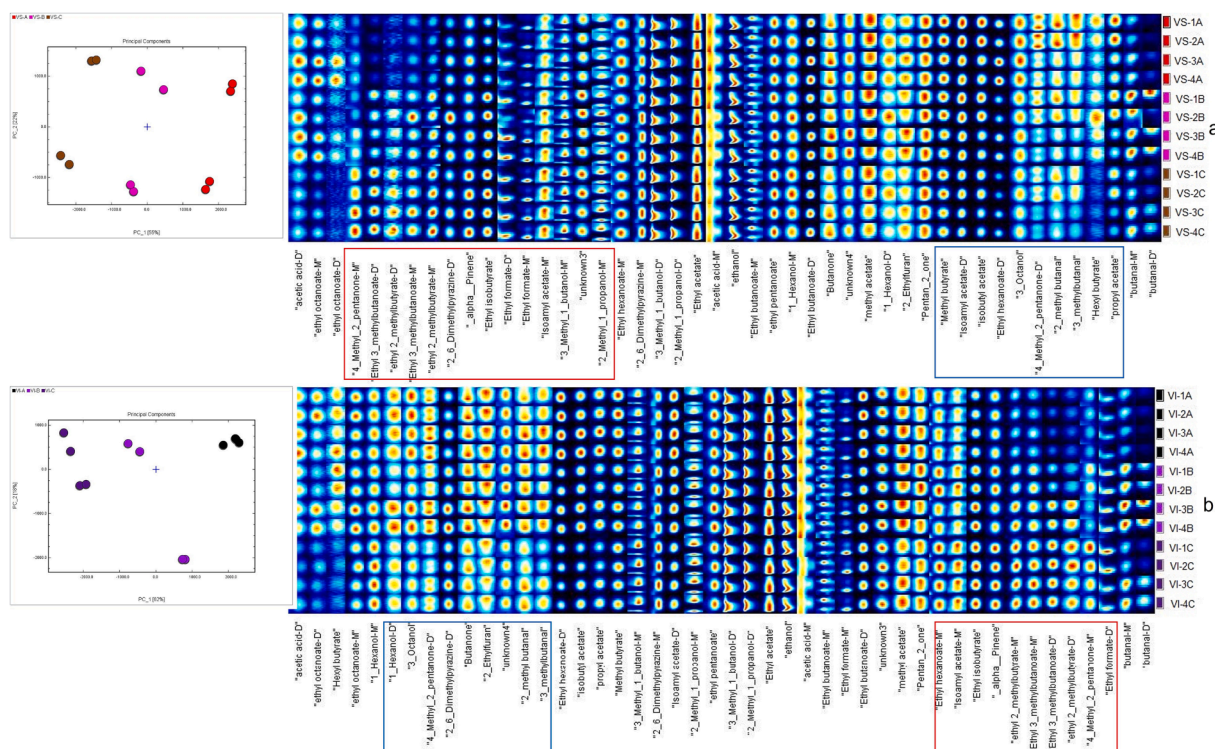


Fig. 4. The dynamic change of spontaneous fermented Petit Verdot wine (VS) from 3-month (VS-A) to 9-month (VS-B) to 15-month (VS-C) and PCA analysis among VS-A, VS-B, and VS-C (a). The dynamic change of inoculated fermented Petit Verdot wine (VI) from 3-month (VI-A) to 9-month (VI-B) to 15-month (VI-C) and PCA analysis among VI-A, VI-B, and VI-C (b). The red box means that the aroma compounds were highest at 15-month. The blue box means that the aroma compounds were highest at 3-month. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

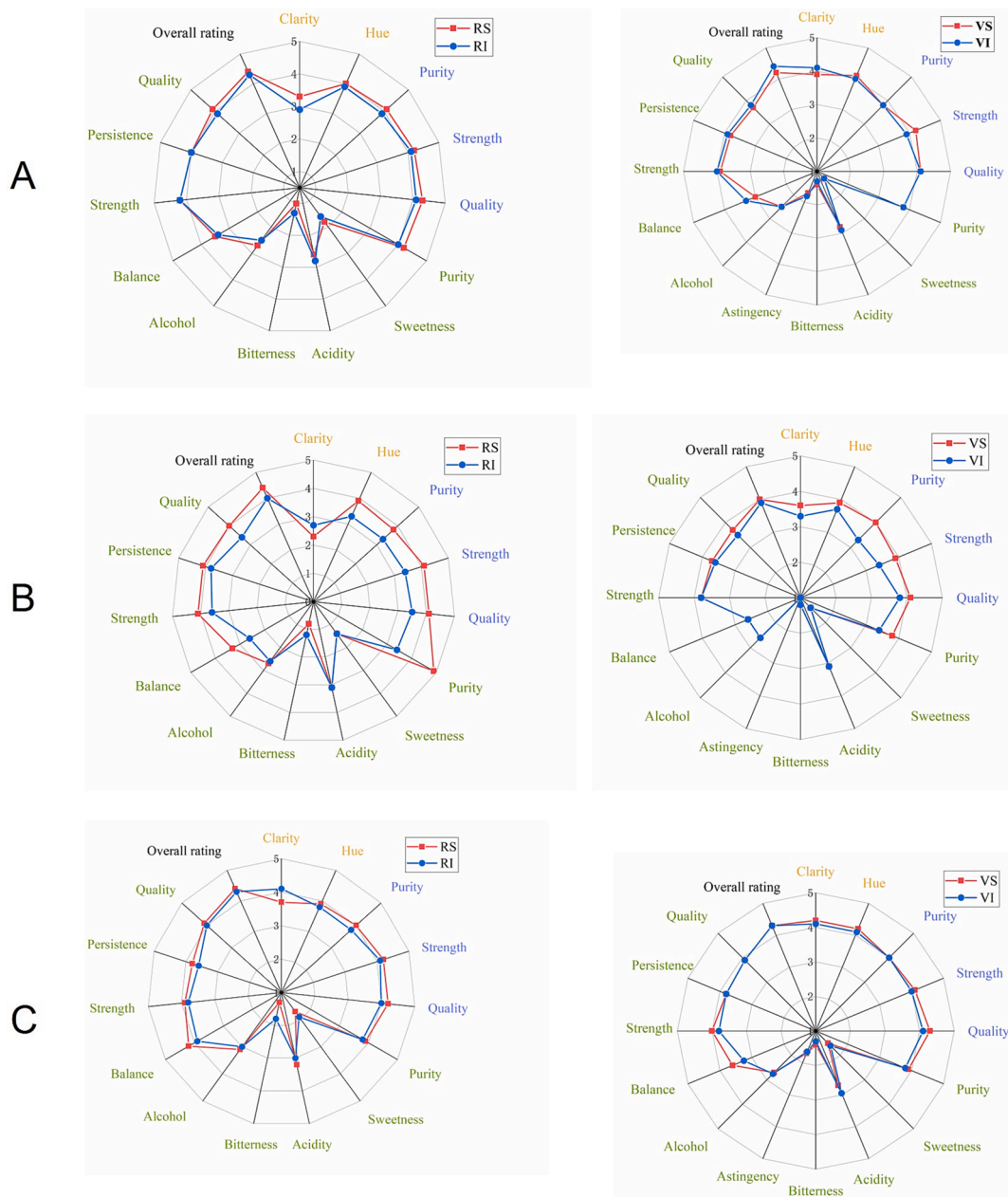


Fig. 5. Sensory evaluation by tasting panel. Spontaneous fermented Italian Riesling wine (RS) compared with inoculated fermented Italian Riesling wine (RI) and spontaneous fermented Petit Verdot wine (VS) compared with inoculated fermented Petit Verdot wine (VI) at 3-month aging (A). Spontaneous fermented Italian Riesling wine (RS) compared with inoculated fermented Italian Riesling wine (RI) and spontaneous fermented Petit Verdot wine (VS) compared with inoculated fermented Petit Verdot wine (VI) at 9-month aging (B). Spontaneous fermented Italian Riesling wine (RS) compared with inoculated fermented Italian Riesling wine (RI) and spontaneous fermented Petit Verdot wine (VS) compared with inoculated fermented Petit Verdot wine (VI) at 15-month aging (C).

illustrated in Fig. 5, the wines were evaluated an assigned a rating of “good” (4 to 5 points) with the exception of VI at 9 months. Notably, discernible differences were observed between the wines that underwent inoculation and those that underwent spontaneous fermentation. The aromatic descriptors of purity, strength, and quality (indicated by blue words) exhibited higher scores in the spontaneous treatment compared to the inoculation treatment for Italian Riesling and Petit Verdot wines, spanning a period of 3 to 15 months (Fig. 5). The taste attributes of alcohol, balance, strength, persistence, and quality were found to have higher ratings in Italian Riesling wines aged between 3 and 15 months when subjected to spontaneous fermentation compared to those subjected to inoculation (Fig. 5). The Petit Verdot wine displayed a lack of consistent trends. During the entire aging process, it was observed that the aroma score of RS wine was consistently exceeded

than that of RI wine. Similarly, the aroma score pattern of Petit Verdot wine samples followed the same trend (Fig. 5). In order to acquire further understanding regarding the impact of yeast on the sensory characteristics of wine, an analysis was conducted on the frequency and rating of the aromatic descriptors of the wine over a period of 3 to 15 months (Fig. 6). At the 15 months aging period, interval, there was a correspondence observed between the change in aroma score and the aromatic description (Fig. 6C). Similarly, the fragrance of apple, peach, and grapefruit in RS wine exhibited higher ratings compared to those found in RI wine after 15 months aged (Fig. 6C). This finding aligns with the outcomes presented in section 3.1, which indicated that RS wine contained greater concentrations of compounds associated with apple and grapefruit such as ethyl octanoate (D), ethyl octanoate (M), and propyl acetate. Hence, it is apparent that the sensory characteristics of

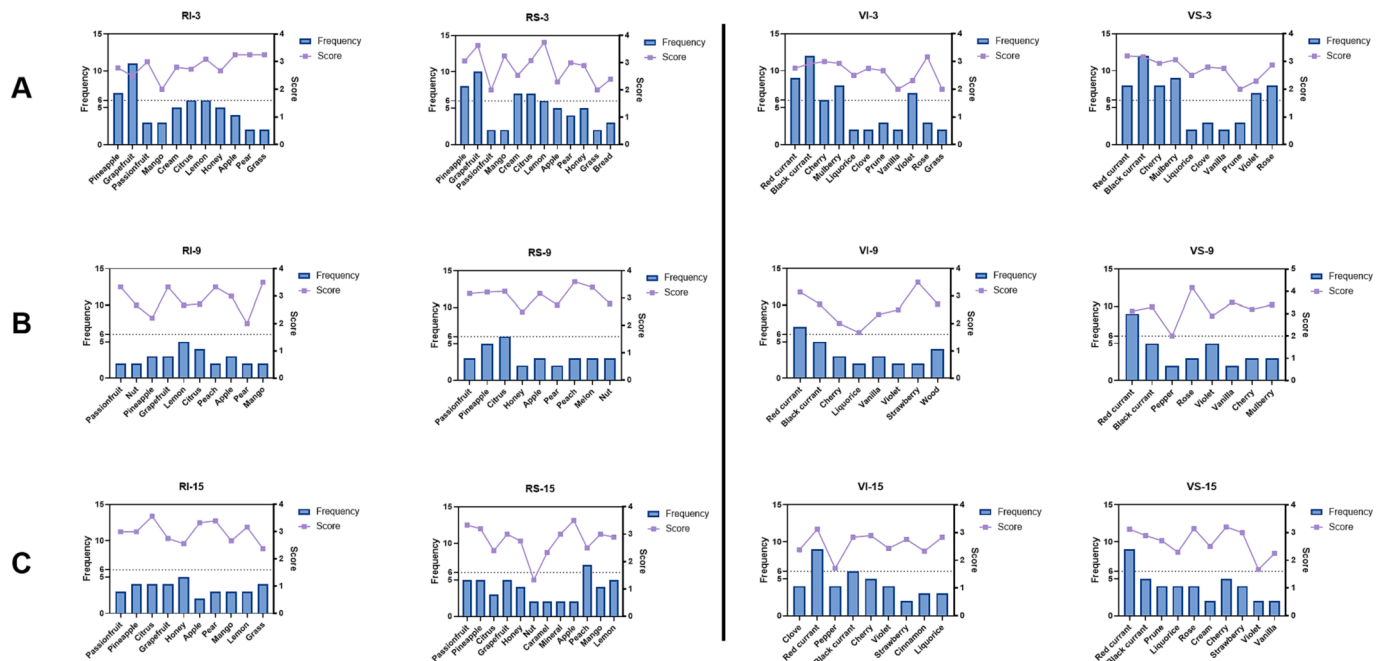


Fig. 6. The frequency and score of aroma description words by tasting panel. (A) 3 month (B) 9 month (C) 15 month.

inoculated wine may exhibit a lower compared to spontaneous wine, as indicated by the findings presented in section 3.1. These findings reveal that RS exhibited higher levels of ethyl hexanoate (M), 2-methyl butanal, ethyl octanoate (M), ethyl octanoate (D), propyl acetate, and 3-methylbutanal in comparison to RI. In the context of Italian Riesling wine samples aged for 3 months, it was observed that the intensity of pineapple, grapefruit, and lemon aromas increased, while the aromas of passionfruit, apple, pear, and grass decreased in the RS-3 samples compared to the RI-3 samples (Fig. 6A). This finding aligns with the results presented in section 3.1, which indicated that the RS samples contained higher concentrations of compounds associated with pineapple and grapefruit aromas, such as ethyl hexanoate (M), ethyl octanoate (M), and ethyl octanoate (D). Based on the studies conducted by Saltman et al. (2017), enhancing the citrus aroma and red and dark fruit aromas while reducing green notes has been shown to enhance the sensory attributes of wine and potentially increase its acceptability among certain consumer groups. The process of mixed fermentation contributes to the development of tropical fruit flavors and heightened aromatic intensity in white wines, whereas red wines tend to showcase more prominent fruity and spicy characteristics (Padilla, Gil, & Manzanarez, 2016). Furthermore, it should be noted that the hybrid yeast strains mentioned above have the capability to generate wines with unique aromatic profiles, which differ from the wines produced using commercial wine yeast (Bellon et al., 2011). Sensory analysis by a trained panel of wines produced by different fermentation styles revealed significant differences in fruity aromas, specifically pertaining to red and dark fruit aromas (Takush & Osborne, 2012). The results of the sensory evaluation indicate that the sensory attributes of wine can be enhanced through spontaneous fermentation, particularly in the context of white wines, as observed during the aging process. Furthermore, the findings pertaining to aromatic descriptors indicate that the process of spontaneous fermentation has a substantial positive impact on the overall aroma characteristics and consumer acceptance of wines.

4. Conclusion

This research examines and contrasts the aromatic and sensory attributes of wines produced through traditional fermentation methods and spontaneous fermentation methods, focusing specifically on the

changes that occur during the aging process in bottles. The study provides initial observations regarding the influence of indigenous microorganisms on the unique characteristics of the vineyard site, commonly referred to as terroir. The presence of diverse microorganisms during two distinct fermentation processes resulted in notable variations in aroma-active compounds, which subsequently manifested in the sensory attributes, aroma profiles, and overall aromas of the wine. Based on the available data, it can be observed that the process of spontaneous fermentation has a positive impact on the sensory attributes of wines, particularly in terms of enhancing their aromatic characteristics as they undergo aging. Therefore, it is imperative to investigate in future research whether spontaneous wine possesses stronger antioxidant properties. Furthermore, the interrelationship between indigenous flora and each flavor substance should be investigated in further research to provide a stronger theoretical foundation for the application of spontaneous fermentation in industrial production.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fochx.2023.100978>.

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