

Influence of different commercial yeasts on volatile profile of sparkling wines

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Abstract: Sparkling wine producing and consumption are in constant increase in the last decade and show no sign of slowing. Prior consumption, origin, grape variety, occasion, price and sensorial perception are factors that typically influence wine consumers' purchasing and consumption behavior. The presence of volatile compounds in sparkling wines, with direct impact on their organoleptic characteristics, is influenced by several factors, such as the base-wine characteristics, grapes cultivar conditions, the used yeasts, the aging stage or wine-making practices. For this study, five sparkling wines variants from Muscat Ottonel grape variety were obtained. For the experimental samples, the grapes were processed by applying secondary fermentation in bottles. The base-wine was obtained through reverse osmosis and had a predetermined alcoholic concentration (10.5 % vol.). This study aimed to analyze the influence of different commercial yeasts (IOC FIZZ™, IOC DIVINE™, LEVULIA CRISTAL™, IOC 18-2007™) on the volatile composition of experimental sparkling wines. The obtained sparkling wines were characterized by the physical-chemical parameters (according to OIV methods of analysis) and volatile fraction (using gas-chromatography). Data showed an important impact on the concentration of the volatile compounds ($p < 0.05$), depending on the type of inoculated yeast for the second fermentation and only a minor influence on the physical-chemical parameters was registered.

Keywords: reverse-osmosis, terpenes, yeasts, ethanol reduction, secondary bottle fermentation

1. Introduction

Wine quality, stability and sensorial characteristics depend on the chemical composition of raw materials, environmental conditions and viticulture management. In the last years, the alcohol concentration in wines has increased, because of various agents, especially climate change [1,2]. At the same time, many consumers require lower alcohol beverages (9 % - 13 % v/v) as a result of health and social concerns (i.e., traffic penalties) [3,4].

Several techniques can be applied to produce low alcohol wines, mainly by using grape juice with low sugar content, selected yeasts, or an earlier interruption of alcoholic fermentation [5]. To obtain predetermined alcohol strength in wine, heat, or membrane-based processes can be employed. However, using heat-based processes leads to the loss of important volatiles [6,7]. Several membrane-based procedures could be used to reduce the wine alcohol content [8], aiding in preserving the sensory characteristic of the initial wine [5]. These procedures (i.e. nanofiltration, reverse osmosis) have an important benefit revealing a low energy consumption when working at low to moderate temperatures. Reverse osmosis represents a successfully-employed procedure for reducing the alcohol strength (it can be used to reduce only 1 % or 2 % (v/v) of the alcohol) and has the benefit of generating a minimal negative

sensory features) since it is performed at low temperatures [9].

Sparkling wine producing and consumption have constantly increased in the last decade and show no sign of slowing down. Consumption of this beverage shows a change from mainly festive to more regular occasions and less specific manner [10]. Generally, consumers tend to rely on extrinsic cues, such as price, packaging, labeling and brand to evaluate quality and mitigate risk. Prior consumption, style, country of origin, grape variety, occasion, price and sensorial perception are factors that typically influence wine consumers' purchasing and consumption behavior [11-13]. According to the traditional method, these products suffer a double fermentation process, so after the first alcoholic fermentation, the wines are subjected to a second one by adding tirage liquor [14-15]. Aroma profile constitutes a major factor determining the sparkling wine's typicality and quality but also its acceptability and competitiveness on the market. The traditional method usually generates a rich sensory profile [5] with over 800 different compounds that represent the volatile fraction of wine but only few of them are odor-active [17-21]. The volatile fraction comprises several classes of organic compounds in sparkling wines, such as esters, alcohols, organic acids, ketones, aldehydes, and terpenes [22]. Their concentration depends on the grape variety, climatic or biological factors and wine-making practices [23-25]. The activity of yeasts strains during the alcoholic fermentation is regarded as a significant agent contributing to the volatile fraction and organoleptic feature of sparkling wines [26]. The aroma profile increases its complexity during alcoholic fermentation due to the synthesis of significant volatile compounds by the *Saccharomyces cerevisiae* yeast strains and the release of varietal aroma precursors. The type and quantity of the synthesized volatile compounds are dependent on multiple factors, such as nitrogen content of the must, fermentation temperature and yeast strain [27].

Ethanol is the most abundant volatile compound in wine and it can change the sensory perception of aroma compounds. Volatile compounds can have different origins (from the raw material, the first or second fermentation or by the aging stage) and have distinct physical-chemical properties, such as polarity, volatility and odor impact as a result of the functional groups that exist in the molecule [28-29].

Yeasts present an important role defining sparkling wine's features, including ethanol content, carbon dioxide overpressure, mannoproteins and precursors of aroma compounds levels. Most of the revealed compounds contribute positively to the sensorial properties and foaming characteristics of the final product [30,15]. According to di Gianvito *et al.* [29], distinct flocculent *Saccharomyces cerevisiae* wine strains with diverse flocculation degrees can generate a substantial diversification of aroma molecules in terms of quantitative and qualitative views. Because the in-bottle fermentation of sparkling wines is usually triggered by few commercialized *Saccharomyces cerevisiae* strains [31], suggest the exploitation of the natural multiplication of yeast population that aimed to introduce variability in sparkling wines throughout the refermentation step. Each yeast species can contribute to the formation of aromatic compounds through specific metabolic pathways and differences in flavor production can be observed at the strain level. The results obtained at the end of the aging time (18th month) prove that important differences among the strains are obtained for the alcohol content, the achieved carbon dioxide pressure and the sensorial traits of final samples.

Numerous studies have focused on the volatile fraction of sparkling wines and its sensorial implication [10,14,15,25,32]. Englezos *et al.* [32] studied the effect of mixed fermentations of *Starmerella bacillaris* with different *Saccharomyces cerevisiae* strains on the volatile profile and physicochemical composition of Barbera wines. The wines produced from mixed cultures displayed lower levels of ethanol, acetic acid and ethyl acetate, higher amounts of higher alcohols and pleasant smell esters compared to the wine fermented with *Saccharomyces cerevisiae* alone. Lencioni *et al.* [35] compared mixed fermentation of selected strain

Saccharomyces cerevisiae and showed that the mixed fermentation produced a significant concentration of 2-phenylethanol and a reduction of volatile acidity.

Following the consumer's tendency to prefer lower alcoholic beverages corroborated with the new generations' wish for different organoleptic sensations, this study aimed to observe the influence of different specific commercial yeasts on the volatile composition of experimental sparkling wines.

2. Materials and Methods

2.1. Grapes and wine-making procedure

Five sparkling wine variants from Muscat Ottonel grape variety were obtained. The grapes were manually harvested in autumn of 2018 at full maturity from Iași vineyard, Romania. The experimental wine (V0) had 12.5 % vol. and the reverse osmosis procedure was used for obtaining the base-wine (V0'), with a predetermined alcoholic concentration (10.5 % vol.).

The alcoholic fermentation was started by yeast strains inoculation (*Saccharomyces* spp.) at controlled temperature (18 °C). After the first fermentation, the tirage liquor (a mixture of selected yeast strains, 24 g L⁻¹ sugar and wine) was added and after that, the experimental mixture was bottled. The sugar concentration determines the sweetness degree of the wine and its pressure in the bottle.

For the second fermentation, four commercial yeasts (IOC FIZZ™, IOC DIVINE™, LEVULIA CRISTAL™, IOC 18-2007™) were compared (resulting V1, V2, V3 and V4 variants). The analyzed products are commonly used for sparkling wine production and recommended by the Institut Œnologique de Champagne. Each commercial product was inoculated according to the producer's specification and legislation in force (20 g/hL).

The second fermentation (that took place in the bottle) and aging in contact with lees (at 12 °C) lasted 15 months. After aging phase, gravity drives the sediment lees to the bottle's neck. This process, known as remuage, has been performed by manually turning the bottle around 1/8 of a turn for about 15 days. Bottle inclination is progressively increased until they are perpendicular on the rack. Disgorging was applied by freezing at -25 °C the upper part of the bottle, making it easier for the pressure built in the bottle to eliminate the lees. As the majority of the yeasts have either been expelled at the disgorgement stage, the sparkling wine in the bottle was then clear. After the disgorging phase, the expedition liquor was added, also known as "dosage" operation. In the end, the corks, labels and muselets/wire cages were added.

Samples were stored under controlled conditions (70 % humidity, 8 °C temperature and no light exposure) and analyzed after 6 months. All the analyses were conducted in triplicate.

2.2. Chemicals

A C7-C40 hydrocarbon mixture in hexane (Sigma–Aldrich, Cat. Number U-49451) was used for the determination of LRI in the HP-5MS, GC capillary column. All reagents and standards used were of analytical grade and supplied by Sigma–Aldrich or Merck.

2.3. Methods of analysis

Physical-chemical parameters were performed according to the International Organization of Vine and Wine Compendium methods of analysis (2019): total (g L⁻¹ tartaric acid) and volatile acidity (g L⁻¹ acetic acid), alcoholic strength (% vol.), pH, density, reductive sugars (g L⁻¹), free and total sulphur dioxide (mg L⁻¹) and non-reductive extract (g L⁻¹).

Volatile compounds were quantified using a GC-7890A chromatograph, an MSD 5975 instrument from Agilent Technologies and a Multi-Purpose-Sampler from Gerstel (Mülheim an der Ruhr, Germany), all governed via the software Chemstation (Agilent Technologies) and Maestro (Gerstel).

with a 10 mm long, 0.5 mm thick piece of polydimethylsiloxane (PDMS) film. According to Vararu *et al.* [36], a 10 mL vial was filled with 0.5 mL of sample and 0.1 mL of an internal standard solution consisting of 0.4464 mg L⁻¹ ethyl nonanoate in pure ethanol plus a solution of 12 % (v/v) ethanol adjusted to pH 3.5 with 2.6 g L⁻¹ tartaric acid and 2.2 g L⁻¹ potassium bitartrate to a final volume of 10 mL. The Twister was then placed in the vial and stirred at 1200 rpm at 20 °C for 100 min on a Variomag Multipoint 15 magnetic stirrer from Thermo Fisher Scientific, Inc. (Waltham, MA, USA). Finally, the Twister was removed, rinsed with water, dried with a cellulose tissue and transferred to a desorption tube that was placed in a Thermal Desorption Unit from Gerstel.

Compounds were thermally desorbed at an initial temperature of 35 °C for 0.1 s, using a 120 °C/min ramp to 280 °C for 10 min and a helium stream at 16 mL/min in the splitless mode into a Cooled Injection System (CIS-4) from Agilent Technologies furnished with an inlet liner packed with Tenax (3 x 2 mm). The CIS-4 was programmed as follows: 25 °C (for 0.05 s), 12 °C/s ramp to 280 °C (for 7 min); helium inlet flow, 16 mL/min. The 7890A GC instrument was equipped with an HP-5MS fused silica capillary column (30 m x 0.25 mm i.d., 0.25 µm film thickness) from Agilent Technologies (Wilmington, DE, USA). The oven initial temperature was set at 50 °C for 2 min and then increased with 4 °C/min to a final temperature of 190 °C that was maintained for 10 min. The MSD was used at 70 eV in the electron impact mode (EI), using the mass range from 35 to 550 Da at 150 °C.

Each sample was measured in triplicate. Peak identification of the aroma components was achieved by comparison of mass spectra with the mass spectral data collection from Wiley7N and NIST08 libraries.

Regarding the sensory perception, a professional panel of 20 tasters (represented by winemakers, laboratory personal and researchers) evaluated the obtained experimental samples. The wine quality was estimated by defining aroma descriptors that were chosen by the taster panels. The odor intensity of the analyzed parameters was evaluated by means of a hedonistic scale from 0 (absence) to 5 (maximum).

Statistical analysis was performed using XLSTAT software in Office Excel Package. The analysis of variance on volatile compounds was developed using the Anova One Way test. Since the Anova does not reveal which means are different from which, the Tukey's honestly significant difference test (HSD) was used to identify differences significant at $p = 0.05$ on 95 % confidence intervals. Also, PCA test (Principal Component Analysis) describes the changes in the composition of volatile compounds of samples treated with different yeasts. All results were expressed as mean \pm standard deviation.

3. Results and discussion

3.1. Physical-chemical parameters

The physical-chemical parameters of the analyzed sparkling wine samples are illustrated in Table 1. The type of inoculated yeasts showed only a minor impact on the physical-chemical parameters whose levels are within the limits allowed by OIV legislations [37]. Regarding the first two parameters (density and total acidity) significant differences can be observed between sparkling wine variants and the base wine used for its production.

The experimental samples showed the highest total acidity in V2 and V3 (6.9 g L⁻¹ tartaric acid) while the lowest value was registered in V4 variant (6.6 g L⁻¹ tartaric acid). The pH presented similar results in all samples (3 – 3.1).

There was no important difference observed among the sparkling wines in terms of pH values.

The lowest production of SO₂ was registered in V2 and V3 variants. Regarding the volatile acidity, V2 sample displayed the highest value. These results showed a lower performance of fermentation conducted with the inoculated yeast cultures.

Samples	ρ	T. A. (g tartaric acid/L)	V. A. (g acetic acid/L)	A. S. (% vol.)	Free SO ₂ (mg/L)	Total SO ₂ (mg/L)	R. S. (g/L)	N. E. (g/L)	pH
V0	0.9932	6.3	0.30	12.5	18	72	3.4	17.0	2.9
	±0.0001	±0.07	±0.02	±0.03	±0.47	±0.47	±0.13	±0.14	±0.02
V0'	0.9921	6.2	0.30	10.5	17	71	3.2	15.2	2.8
	±0.0001	±0.03	±0.01	±0.05	±0.47	±0.00	±0.05	±0.13	±0.05
V1	0.9905	6.7	0.30	11.6	5	56	0.7	14.5	3.1
	±0.0003	±0.02	±0.01	±0.07	±0.00	±0.47	±0.02	±0.04	±0.01
V2	0.9908	6.9	0.35	11.3	5	49	1.9	13.3	3.0
	±0.0001	±0.01	±0.01	±0.00	±0.00	±0.47	±0.01	±0.05	±0.01
V3	0.9906	6.9	0.30	11.6	5	51	1.9	13.5	3.0
	±0.0001	±0.03	±0.05	±0.00	±0.47	±0.47	±0.01	±0.01	±0.01
V4	0.9907	6.6	0.30	11.3	8	64	0.7	14.3	3.0
	±0.0002	±0.01	±0.09	±0.07	±0.47	±0.00	±0.02	±0.01	±0.01
<i>p</i> -value	0.0001	0.0001	0.0772	0.0772	0.0772	0.0772	0.0772	0.0772	0.0772

ρ – density; T. A. – total acidity; V. A. – volatile acidity; A. S. – alcohol strength; R. S. – residual sugar; N. E. – non-reductive extract. The results are expressed as mean \pm standard deviation of three sparkling wine bottles (triplicate). Analysis of variance was carried out by comparing each wine variants with base wine used for its production.

The amount of total sugars (0.7 – 3.4 g L⁻¹) in V2 and V3 variants is more than double compared to V1 and V4. The alcoholic strength of sparkling wines varied from 11.3 % vol. (V2 and V4) to 11.6 % vol. (V1 and V3).

Overall, all yeasts were able to complete fermentation (residual sugar content < 2 g L⁻¹).

Parameters such as density, total acidity, residual sugar and non-reductive extract have dependent values, with important differences between samples.

Comparable results were presented by Benucci and Esti [38].

3.2. Volatile fraction

Twenty volatile compounds (represented by esters, acids, alcohols, and terpenes) were quantified and separated into their chemical classes. The volatile compounds identified in the analyzed samples and their correlated odor descriptors are presented in Table 2.

Esters contribute to sensory features of wines, being responsible for their floral and fruity notes. Their concentrations are dependent on various factors, including yeast species, temperature and aeration degree during alcoholic fermentation and sugar content [24,39,40]. The majority of esters represent by-products of yeast metabolic action, with higher content in wine after cell division has slowed or essentially ceased. Straight-chain forms are synthesized from esterification of the corresponding acids which have been activated by acyl-S-CoA [41].

Esters like ethyl octanoate, ethyl decanoate, ethyl laureate, isopropyl myristate, ethyl palmitate and ethyl oleate were identified in analyzed samples. Their levels varied depending on the inoculated yeasts. According to Muñoz-Redondo *et al.* [10], some ester compounds are pointed out as markers of the second fermentation.

Isoamyl acetate is usually derived from yeast metabolism during the alcoholic fermentation. This compound contributes to a banana-like note and gives complexity to white wines [25,40]. Its concentrations varied from 11.71 μ g L⁻¹ in V2 sample to 22.78 μ g L⁻¹ in V4 sample.

2-Phenethyl acetate is generally produced by yeasts from phenolic precursors during the maturation stage and is characterized by sweet honey notes and flowers. The highest level of this compound was identified in V1 and V3 samples, while the lowest concentration was registered in V2 variant.

According to Genovese *et al.* [42], ethyl decanoate (floral) and 2-phenylethyl acetate (scent of rose) can show synergistic effect even at low concentrations. The values of 2-phenylethyl acetate published by Torchio *et al.* [43], were comparable with our data (22.33 to 47.72 $\mu\text{g L}^{-1}$).

Diethyl succinate is usually formed during the alcoholic fermentation. V2 sample displayed the highest level of this compound (62.58 $\mu\text{g L}^{-1}$). According to de Souza Nascimento *et al.* [25], this compound was on the most relevant esters in the volatile profile of Chenin Blanc sparkling wines. According to Torrens *et al.* [44] and Riu-Aumatell *et al.* [45], diethyl succinate represents one of the “ageing esters” which concentrations can increase in contact with yeast cells through the second fermentation.

Acids can originate from the plants, but also from the alcoholic fermentation, resulting in some fatty acids that have sensorial properties but also can supplement other roles. A combination of decanoic and octanoic acids is necessary to get a lasting inhibitory effect on yeast growth [46].

In the analyzed samples, the octanoic acid content varied from 580.64 $\mu\text{g L}^{-1}$ in V1 variant to 258.79 $\mu\text{g L}^{-1}$ in V2 sample. Decanoic acid reached a maximum concentration in the V1 sample (145.25 $\mu\text{g L}^{-1}$) and a minimum in the V2 sample (11.36 $\mu\text{g L}^{-1}$).

Alcohols represent secondary aromatic components derived from sugars and amino-acids transformation during the fermentation process, with a significant influence on wine's sensorial profile [25,40].

Concerning the alcohols level, isoamyl alcohol, 4-octanol, 1-heptanol and 2-phenylethyl were the most representative in resulting samples. Isoamyl alcohol generally accounts for more than 50 % of all fusel alcohols fractions [47]. The experimental samples presented a range from 1019.50 $\mu\text{g L}^{-1}$ on V3 sample to 485.91 $\mu\text{g L}^{-1}$ on V2 sample. These compounds were also identified in high proportion in Muscat Ottonel wines by Călugăr *et al.* [48].

A small part of the fusel alcohols may originate from grape-derived aldehydes by the reductive denitrification of amino-acids or throughout the synthesis of sugars [49,50]. The formation of higher alcohols during the fermentation stage is usually influenced by the wine-making techniques, inoculated yeasts, low amino-acids levels, low temperature and reduced pH degree [50,51]. The amount of higher alcohols produced during fermentation of the grape juice was found to vary considerably according to the yeast used.

1-heptanol was identified in large quantities on the V2 variant, assuring a pleasant vegetal odor and fruity notes (apples and banana). Phenylethyl alcohol, a volatile compound with pleasantly sweet, floral and honey odors was detected in all analyzed samples. Larger quantities of this compound were identified in V1 (1150.12 $\mu\text{g L}^{-1}$) and V4 (683.46 $\mu\text{g L}^{-1}$) samples. Its presence in wine is probably due to the degradation of branched-chain amino-acids following the Ehrlich pathway. The production of phenylethyl alcohol depends on the temperature level and inoculated yeast strains [52]. Data published by Torrens *et al.* [18] and Jaganatić Korenica *et al.* [53] also showed that high proportions of phenylethyl alcohol.

Terpenes represent secondary metabolites that originate from the grapes. However, the biosynthesis of monoterpenes by *Saccharomyces cerevisiae* in the absence of grape derived precursors was indicated to be a possible origin for aroma compounds in wine [54]. Terpenes play an important role in defining floral odour of wines that reminds of roses and are usually specific for the Muscat de Alexandria and white Frontignac grapes [55]. L-Linalool gives fresh-floral aroma to wines, reminiscent of spices and lemon notes. The highest concentration of linalool is usually registered in aromatic and semi-aromatic varieties, such as Muscat Ottonel, Tămâioasă Românească, Sauvignon blanc and Fetească albă [56]. V1 variant showed the highest level in L-linalool (138.86 $\mu\text{g L}^{-1}$), followed by V3 (120.43 $\mu\text{g L}^{-1}$), V4 (44.31 $\mu\text{g L}^{-1}$) and V2 (16.65 $\mu\text{g L}^{-1}$). This com-

α -terpineol, respectively [55].

α -terpineol usually gives wine a melon, sweet-floral, lilac-like perfume and it's formed out of monoterpene-glycosides in an acid environment [57]. The highest concentration was identified in the V1 sample ($42.79 \mu\text{g L}^{-1}$), followed by V3 ($41.40 \mu\text{g L}^{-1}$), V2 ($28.19 \mu\text{g L}^{-1}$) and V4 variant ($24.19 \mu\text{g L}^{-1}$). This compound can originate from the grape (in low concentrations) and have a high olfactory perception value [55]. High levels of linalool and α -terpineol were also identified in Moscato Giallo wines by Marcon *et al.* [58]. Comparable concentrations of linalool were identified in Muscat de Alexandria wines by Lanaridis *et al.* [59].

A significant impact of supplemented yeasts on the volatile profile was observed. V1 and V3 variants have been remarked to have the highest influence on the majority of aroma compounds. The null hypothesis that the type of yeast didn't affect the concentrations of the volatile compounds was rejected, the alternative one that in fact the yeast did affect the volatile content of the analyzed sparkling wines was confirmed ($p < 0.05$).

Regarding the results of Tukey's HSD test (Table 3), a significant difference between V1 – V3 in the case of 1-heptanol and α -terpineol variances can be observed. Also, statistically significant differences between V2 and V4 samples on the butyric acid, linalool and α -terpineol concentrations were registered. In the case of diethyl succinate and decanoic acid, the significant difference was represented by the V3 and V4 groups ($p < 0.05$).

Table 2: Volatile fraction of resulted sparkling wine

No	V. C. ($\mu\text{g L}^{-1}$)	V1	V2	V3	V4	ODOUR DESCRIPTORS	REF.
ESTERS							
1	Isoamyl acetate	17.83±0.06*	11.71±0.15*	17.89±0.23*	22.78±0.11*	fruity, banana	[28,60]
2	Ethyl octanoate	7998.72±0.15*	5285.90±0.08*	7162.47±0.21*	6789.59±0.31*	fruity, banana, apple, pineapple, pears, floral, sweet, soap	[7,61,62]
3	Ethyl decanoate	2177.35±0.35*	985.37±0.20*	2126.20±0.11*	1593.61±0.30*	fruity, apple, waxy, oily	[62]
4	Diethyl succinate	53.58±0.90*	62.58±0.01*	49.52±0.11*	54.40±0.57*	fruity, floral, waxy, dusty	[7]
5	2-Phenethyl acetate	34.87±1.57*	22.33±0.81*	47.72±0.44*	28.18±0.16*	floral, sweet, fruity, honey	[44,63]
6	Ethyl laurate	162.34±0.51*	56.71±0.01*	136.25±0.45*	110.49±0.82*	floral, fruity, grassy, woody	[44,64]
7	Isopropyl myristate	14.87±0.17*	16.17±0.50*	15.99±0.11*	13.98±0.76*	faint, oily, fatty	[64,65]
8	Ethyl palmitate	15.64±0.98*	7.89±0.15*	15.98±0.15*	8.51±0.22*	waxy, fruity, creamy and milky with a vanilla balsamic nuance	[44]
9	Ethyl oleate	159.21±0.08*	132.12±0.16*	198.97±0.19*	108.42±0.23*	fatty, oily, dairy, milky, waxy, tallow	[44]
ACIDS							
10	Butyric acid	nd	9.81±0.57*	nd	6.32±0.11*	cheese, rancid, sweet, animal	[7,62]
11	Hexanoic acid	326.09±0.25*	189.98±0.11*	227.50±0.70*	230.34±0.45*	fatty	[44]
12	Octanoic acid	580.64±3.22*	258.79±2.23*	367.50±0.40*	nd	cheese	[40]
13	Decanoic acid	145.25±0.59*	11.36±0.06	13.01±0.06*	16.91±0.14*	rancid, sour, oily, unpleasant, woody	[62,67]
14	9-Decenoic acid	6.90±2.25	6.75±0.98*	4.37±1.30	4.00±2.20	waxy orange, reminiscent of kiwi-fruit, fruity and milky, drying down to a clean cucumber / melon note	[64,65]
ALCOHOLS							
15	Isoamyl alcohol	1001.47±0.23*	485.91±0.16*	1019.50±0.02*	693.63±0.50*	alcohol, nail polish, bananas	[23,62]
16	4-Octanol	5.62±0.59	5.53±0.75	6.13±0.40	5.14±0.56	-	-
17	1-Heptanol	5.89±0.54*	28.70±0.04*	10.06±0.40*	18.76±0.45*	green, musty, leafy, violet, herbal, green, sweet, woody, peony	[62,68]
18	Phenylethyl alcohol	1150.12±0.23*	884.56±0.14*	973.18±0.03*	683.46±0.01*	floral, rose, dried rose, rose water	[68]
TERPENIC COMPOUNDS							
19	Linalool L	138.86±0.06*	16.65±0.55*	120.43±0.01*	44.31±2.22*	citrus, floral, sweet, bois de rose, woody, green blueberry	[62,68]
20	α -terpineol	42.79±0.40*	28.19±0.14*	41.40±0.02*	24.19±0.85*	pine like, terpene, lilac, citrus, woody, floral	[65,68]

The results are expressed as mean \pm standard deviation of three sparkling wine bottles; V.C.– Volatile compounds; nd – not detected or below detection limit; *statistically significant.

Table 3. Significant results of Tukey HSD post-hoc test.

Variables	Groups	Diff	<i>p</i>	95 % confidence interval for mean	
				Lower bond	Upper bond
Diethyl succinate	V3-V4	4.8800	0.0000	3.7558	6.0042
Butyric acid	V2 -V4	-3.4900	0.0000	-4.0993	-2.8807
	V2-V3	1.6500	0.0000	1.0073	2.2927
Decanoic acid	V3-V4	3.9000	0.0021	3.2573	4.5427
	V1-V3	4.1700	0.0000	3.3200	5.0200
Linalool L	V2-V3	-4.220	0.0011	-6.6216	-1.8184
α -terpineol	V1-V3	-1.3900	0.0065	-2.3872	-0.3928
	V2-V4	-4.0000	0.0000	-4.9972	-3.0028

Table 4. Factor loadings of the experimental samples.

	Factor 1	Factor 2	Factor 3
Eigenvalue	11.964	5.081	2.955
Variability (%)	59.820	25.404	14.775
Cumulative %	59.820	85.225	100.000
Isoamyl acetate	0.264	-0.915	-0.305
Ethyl octanoate	0.924	-0.317	-0.211
Ethyl decanoate	0.944	-0.319	0.089
Diethyl succinate	-0.219	0.941	0.257
2-Phenethyl acetate	0.727	-0.308	0.613
Ethyl laurate	0.951	-0.287	-0.118
Isopropyl myristate	0.234	0.844	0.483
Ethyl palmitate	-0.261	-0.413	0.873
Ethyl oleate	0.664	0.231	0.711
Butyric acid	-0.964	0.186	-0.192
Hexanoic acid	0.857	0.057	-0.512
Octanoic acid	0.788	0.611	0.075
Decanoic acid	0.753	0.326	-0.571
9-Decenoic acid	0.687	0.651	-0.321
Isoamyl alcohol	0.952	-0.232	0.202
4-Octanol	0.832	-0.516	0.203
1-Heptanol	-0.973	0.228	0.012
Phenylethyl alcohol	0.796	0.603	0.060
Linalool L	0.992	-0.044	0.117
α -terpineol	0.786	0.602	-0.139

The bold numbers indicate the higher weight of each compound in each factor.

PCA test describes the changes in the composition of volatile compounds of sparkling wines produced by different yeast strains. The factorial analysis selected three factors with an eigenvalue greater than 1, which explained the 100 % of the total variance. However, the variables associated with two factors were enough to explain more than 85 % of total variability. Table 4 shows the loadings for each variable on the selected factor, as well as the eigenvalue and the cumulative variance. The variables with higher loading values contribute most significantly to the explanatory meaning of the factors (marked in bold). The first factor explained the 59.82 % of the data variability and was strongly correlated with most of the volatile compounds (ethyl octanoate, ethyl decanoate, 2-phenethyl acetate, ethyl laurate, ethyl laurate, hexanoic acid, octanoic acid, decanoic acid, 9-decenoic acid, isoamyl alcohol, 4-octanol, phenylethyl alcohol, linalool L, α -terpineol). This fact indicates that these volatile compounds are highly correlated with most of the volatile compounds present in analyzed samples.

Through the PCA (Figure 2) it was possible to observe that the two principal components explain around 85 % of the total data variability. The first principal component that explained most of the total variability of the data (59.82 %) was strongly correlated with isoamyl acetate, ethyl decanoate, ethyl laurate, isoamyl alcohol and linalool showed in all cases factor loadings greater than 0.90. For the second principal component, diethyl succinate and isopropyl myristate showed high and positive values.

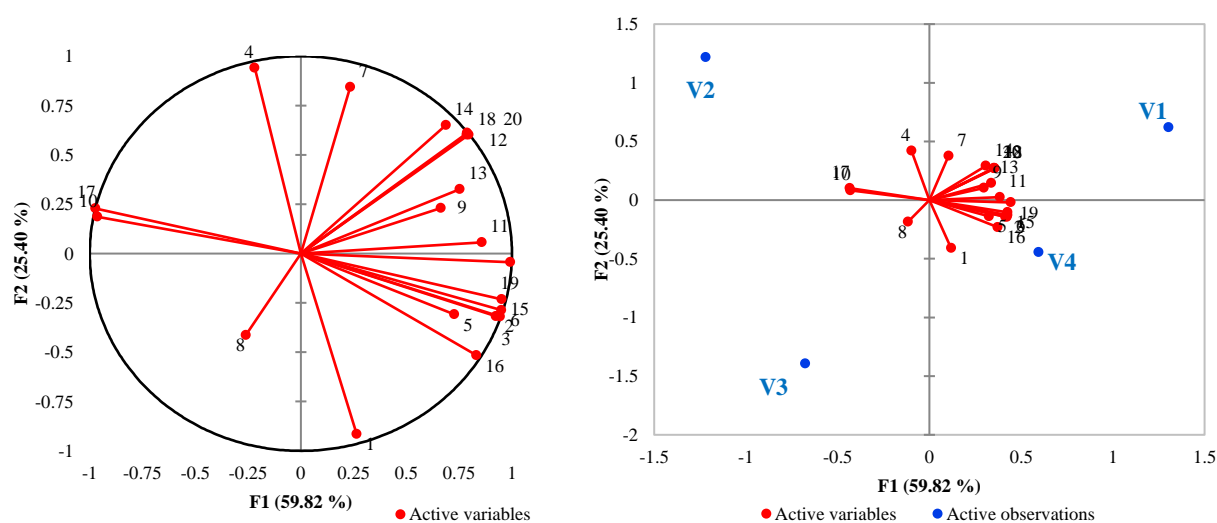


Figure 1: Principal component analysis (PCA) based on the results for volatile compounds: 1-Isoamyl acetate; 2-Ethyl octanoate; 3-Ethyl decanoate; 4-Diethyl succinate; 5 - 2-Phenethyl acetate; 6-Ethyl laurate; 7-Isopropyl myristate; 8-Ethyl palmitate; 9-Ethyl oleate; 10-Butyric acid; 11- Hexanoic acid; 12 – Octanoic acid; 13-Decanoic acid; 14-9-Decenoic acid; 15-Isoamyl alcohol; 16- 4-Octanol; 17-1-Heptanol; 18- Phenylethyl alcohol; 19-Linalool L; 20- α -terpineol; V1 - FIZZ™, V2 - IOC DIVINE™, V3 - LEVULIA CRISTAL™, V4 - IOC 18-2007™

The correlation circles (Figure 1) show a projection of the initial variables in the factors space. It can be observed that 1-heptanol is positively correlated with butyric acid (r close to +1), but negatively correlated with isopropyl myristate (r close to -1). Also, linalool and ethyl decanoate are positively correlated, while linalool and butyric acid

show a negative correlation. The biplot chart enables observations and variables made on a two-dimensional map and to identify the trends. As can be seen in the mentioned figure, the variables associated with factor 1 permit to differentiate the sparkling wines by volatile fraction.

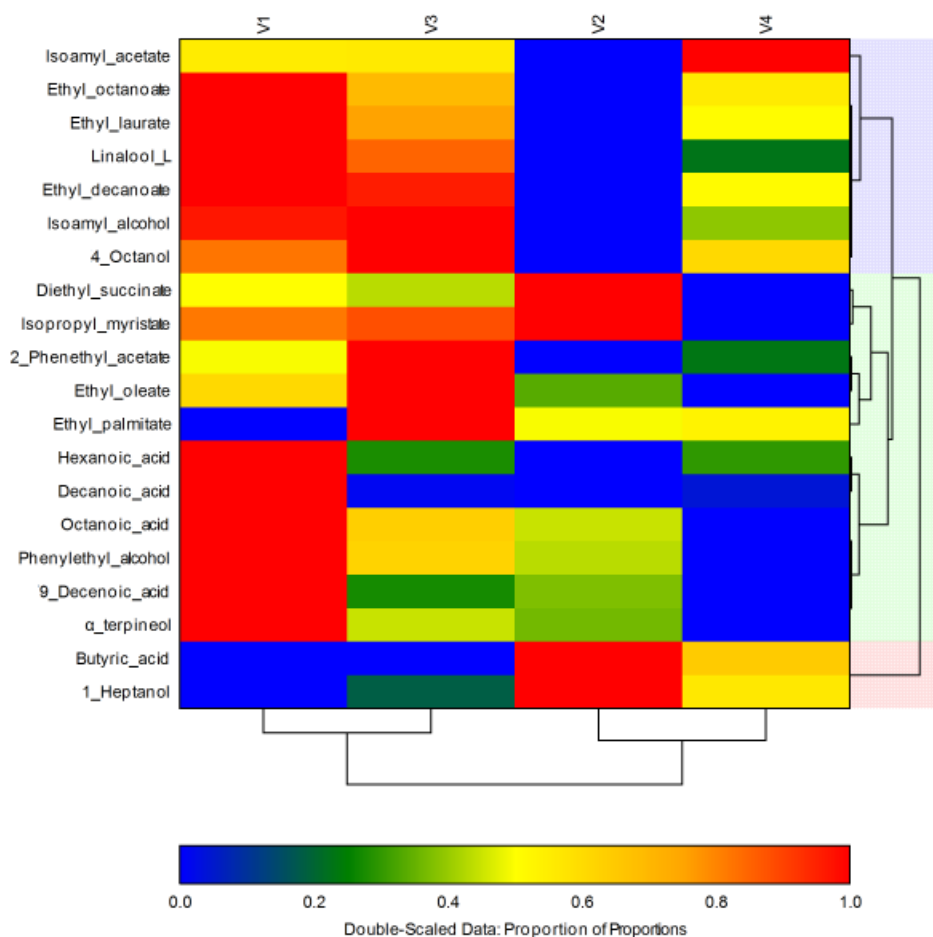


Figure 2: Heat map obtained using the concentration of each volatile compound in resulted experimental sparkling wines. Clusters related to the grouping of volatiles and samples were designed. Samples represented with blue color showed the lowest concentrations of the separated compound while the highest levels are represented by red color.

Since this article aimed to evaluate the influence of inoculated yeasts on the volatile fraction of experimental sparkling wines, the data confirms that different yeast can generate different levels of volatile compounds.

The heat map (Figure 2) was obtained using the identified concentration of each volatile compound according to Table 2. Data is displayed in a grid where each row represents a quantified volatile compound and each column represents a sample. The color and intensity of the boxes is used to represent changes on each compound concentration. In the figure, it can be observed that red color indicates the highest concentrations of each substance and blue represents the lowest. The order of the rows is determined by performing hierarchical cluster analyses of the rows.

Several works studying similar products [25,29,69,70,71] have reported significant influence in sparkling wine's aroma compounds and only a minor influence on the physical-chemical parameters.

Many studies that refer to the volatile compounds evolution during the ageing of sparkling wines are contradictory. Some of the reasons for the lack of agreement amongst the different papers could have been due to differences in the experimental conditions, but also because of the simultaneous degradation and synthesis of volatile fraction that occurs through the ageing stage of wine with yeast, resulting that at any given time either of these processes can predominate. According to Torrens *et al.* [44], different commercial yeast strain presents significant impact on the chemical and volatile composition of the sparkling wines, with major repercussion on their sensory profile.

3. Sensory analysis

The sensory perception of sparkling wines is given by the interaction of different volatile constituents. The character of sparkling wine is usually influenced by its effervescence, sweetness, acidity or bitterness and is generated by non-volatile compounds soluble in water or alcohol mixture [60].



Figure 3: Sensory perception of experimental sparkling wines. The odor intensity of the analyzed parameters was evaluated by means of a hedonistic scale from 0 (absence) to 5 (maximum).

Regarding the odor descriptors (Table 2), the resulting sparkling wines are defined by their fruity (especially banana-like and apple) and floral notes (elderflower), due to their high levels of esters (e.g. ethyl octanoate and ethyl decanoate). Isoamyl acetate,

ethyl palmitate, 4-octanol or 1-heptanol did not significantly contribute to the final aroma profile of experimental samples.

According to the sensory analysis (Figure 3), major differences can be observed due to the type of inoculated yeasts. All sparkling wines were characterized as balanced, with great persistence, acidity (that imprinted freshness) and good texture (especially V4 sample). V1 variant was remarked for its floral odor (elderflowers) while fruity notes were dominant in V2 sample (especially green banana).

Regarding the correlation between the volatile compounds and sensorial perception, the fruity notes (apple, green banana, peach) of experimental samples can be explained by the presence of ethyl octanoate, ethyl decanoate or diethyl succinate. The floral odor is mainly due to the high concentrations of phenylethyl alcohol.

5. Conclusions

According to the results, yeasts can influence the final quality of wines in varying degrees, for example V3 and V4 showed the lowest production of volatile acidity and total sulphur dioxide. The type of inoculated yeasts for the second fermentation showed a statistical significance on the majority of volatile compounds concentrations ($p < 0.05$). The obtained results confirm that yeasts significantly contribute to the enrichment of the volatile profile of wines. V1 variant shows the highest concentrations of the majority of aroma compounds while V2 and V3 presented the lowest levels. Following the sensory analysis, key differences can be observed due to the type of inoculated yeast. Ethyl octanoate and ethyl decanoate were representatives for all variants, defining their fruity (especially banana, apple) and floral notes (elderflower).

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