Evolution of aromatic compounds during the second fermentation and aging of Brazilian sparkling wine

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Abstract. In this work, we evaluate the physic-chemical characteristics and volatile compounds during the second fermentation and aging of typical Brazilian sparkling wines. For this purpose, second fermentations were conducted by the traditional method using a base wine elaborated with Pinot Noir, Chardonnay and Riesling Italic, and fermented with *S. cerevisiae vr. bayanus* EC1118 strain. Samples were collected from 0 to 360 days, and evaluated with respect to the basic physic-chemical characteristics, yeast population, and the concentration of volatile compounds. The results showed that the second fermentation, other than an increment in the alcohol concentration leads to a small increase in volatile acidity, where total acidity decreased during fermentation, and increase again during aging. Yeast population declined rapidly after fermentation, but autolysis initiated just after 9 month of aging. Based on the concentration of volatile compounds, three profiles could be defined: (1) a fermentation profile defined by higher concentrations of acetates and lower concentrations of ethylates and fatty acids; (2) a post-fermentation profile with intermediary concentrations of acetates, and increased concentrations of fatty acids, and their ethyl esters; and (3) a mature profile with higher concentrations of diethyl succinate, fusel alcohols, volatile fatty acids, and their ethyl esters, and lower concentrations of acetates.

1. Introduction

The production of sparkling wines by the traditional method or "Champenoise" involves two fermentative and different steps. The first fermentation transforms the grape must into a base wine, in a process that is very similar to that used for high quality white wines. The second fermentation, "prise de mousse", occurs in the bottles after the addition of a "tirage solution" that includes yeast, sucrose, nutrients, and sometimes bentonite [1–3], and is followed by a biological aging in contact with lees in anaerobic conditions for 6–12 months [4–6].

The sensorial quality of sparkling wines is based on several organoleptic factors (visual, olfative, and gustative). The sparkling wine flavor are the result of a complex interactions between three factors: grape varietal aromas, yeast derived fermentative compounds, and enological methods adopted during the first and second fermentation, and aging processes [3,7,8].

Volatile compounds in wine have three origins: the grape (pre-fermentative aroma), the yeast during the first and second fermentation (fermentative aroma), and the aging during settling (post-fermentative aroma) [2,5,9]. Although several aromatic compounds are initially found in the grape, the dominant, and most important volatile compounds that contribute for the peculiar characteristics of sparkling wine (higher alcohols, fatty acids, acetates, ethyl esters, ketones, aldehydes, among others) are formed or transformed during fermentation and the post-fermentative process [3,10].

Representing just 1% of the alcohol mass (0.8 g L^{-1} to 1.2 g L^{-1}), the concentration of individual volatile compounds, as well as the equilibrium between them, have strong impact on wine bouquet [8]. The chemical and enzymatic reactions that occur during fermentation and aging are responsible for modifications on the concentration of volatile constituents, aromatic evolution, and can have important impact on the organoleptic characteristics of the final product.

Studies on sparkling wines aging on their lees showed that the wine develops a more complex aromatic profile. This behavior has been associated non-well defined chemical and enzymatic reactions, as well as the liberation of mannoproteins, peptides, amino acids, and other compounds by yeast autophagy and autolysis. Some authors consider the autophagic/autolytic process one of the most important factors affecting the sensorial quality of the aged sparkling wine [4,5,11]. However, the evaluation of volatile, olfative active compounds (OAV), along second fermentation and aging are scace.

In this context, the present research aimed to study physicochemical parameters, aromatic compounds, and yeast population, through the fermentation process and aging during a period of 360 days (one year).

2. Material and methods

2.1. Wine fermentation

The commercial base wine (Chardonnay −70%, Pinot Noir -20%, and Riesling Italic −10%) was elaborated at

Vinícola Cia. Piagentini (Caxias do Sul, Brazil). Briefly, grapes were destemmed, and pressed. The resulting musts were sulfited by the addition of 20 mg L^{-1} of potassium metabisulfite, and fermented at 18°C in steel less tanks with the *Saccharomyces cerevisiae* yeast strain Zymaflore X5®. After fermentation and assemblage, base wine was submitted to proteic stabilization for 8 days with 0.5 g L^{-1} of bentonite (Pentagel, Pedomini IOC), and tartaric stabilization for 10 days at −3°C. Base wine was filtered (0.4 μm) just before second fermentation.

For second fermentation, base wine was supplemented with 20 g L^{-1} of sucrose, and yeast assimilable nitrogen was corrected to 180 mg L^{-1} by the addition of ammonia phosphate. The commercial *S. cerevisiae* vr.*bayanus* yeast strain Lalvin EC 1118® was rehydrated in a 0.3 g L^{-1} Superstart (Laffort) activator solution for 4 hous at room temperature. Yeast inoculation was performed following traditional enological methods, with an inoculum of 5×10^6 cells mL^{-1} determined by microscopic counting. After inoculation, the wine was immediately bottled, and second fermentation and aging were conducted in a dark acclimatized chamber at 14 ± 1°C.

2.2. Sampling

Samples were collected at 0, 7, 15, 30, 60, 90, 135, 180, 270 and 360 days during second fermentation and aging. Three different bottles were used for each period tested. Bottles were opened, homogenized, and a small aliquot collected for the evaluation of yeast population. Wine were then centrifuged at 3200 × g at 4°C for 15 minutes, and the supernatants stocked in 100 ml aliquots at −80°C for chemical analysis.

2.3. Yeast population monitoring

The number of viable yeasts was determined by serial dilutions and plating on YEPD medium (2% glucose, 2% peptone, 1% yeast extract, 2% agar). Colonies were counted following an incubation period of 48 h at 28°C, and viable yeasts expressed as colony forming units per ml (UFC mL^{-1}).

2.4. Analytical methods

Total acidity (meq L^{-1}), volatile acidity (meq L^{-1}), free SO_2 (mg L^{-1}), dry extract (g L^{-1}), dry extract (g L^{-1}), residual sugar (g L^{-1}), alcohol (% v/v) and density (20/20) were determined according to the International Organization of Vine and Wine methods [12].

The analysis of volatile compounds was performed on a gas chromatograph HP 6890 Agilent Technologies, with a flame ionization detector. The compounds were identified by comparison to authentic standards purchased from Sigma–Aldrich.

Determination of higher alcohols, acetaldehyde, ethyl acetate, and methanol: 70 μL of 4-methyl-2-pentanol solution (5 g L^{-1}, internal standard) were added into 5 mL of a distilled sample. The injection (1.0 μL) was performed in split mode to 60 mL min^{-1} at 220°C. It was used a capillary column CPWax 57CB (60 m, 250 μm and 0.25 μm). The carrier gas was hydrogen 5.0 at 2.0 mL min^{-1}, and the nitrogen as auxiliary gas at 37 mL min^{-1}. The oven temperature was 40°C for 5 min, 40–90°C at 3°C min^{-1}, 90–200°C at 10°C min^{-1}, 200°C for 5 min. The combustion was maintained with synthetic air at 350 mL min^{-1} and hydrogen at 35 mL min^{-1}. The detector temperature was 230°C.

Determination of esters, acetates, alcohols, and volatile acids: It was added 2 mL of 3-octanol (40 mg L^{-1}) and 2 mL of heptanoic acid (50 mg L^{-1}), as internal standards, and 0.3 mL of phosphoric acid (1:3) to 50 mL of sparkling wine degassed in ultrasound. The sample was subjected to three liquid/liquid row extractions (4:2:2) with a mixture of diethyl ether/n-hexane (1:1). The injection (2.0 μL) was performed in splitless mode at 60 mL min^{-1} at 240°C. It was used a capillary column CP Inowax (30 m, 250 μm and 0.25 μm). The carrier gas was the hydrogen 5.0 at 2.0 mL min^{-1} and the nitrogen as auxiliary gas at 37 mL min^{-1}. The oven temperature was 40°C for 5 min; 40–230°C at 3°C min^{-1}; 230°C for 20 min. The combustion was maintained with synthetic air flow at 350 mL min^{-1}, and hydrogen at 35 mL min^{-1}. The detector temperature was 230°C.

2.5. Statistical analyses

All analyses were carried out in triplicate for each of the five bottles of sparkling wine sampled at each time period, and the results expressed as mean values ± standard deviation. The SPSS 20.0 software for Windows (Chicago, IL, USA) was used for the analysis of variance (ANOVA), Tukey test ($p < 0.05$), and principal component analysis (PCA).

3. Results and discussion

Figure 1 shows the kinetics of ethanol production and total sugar consumption (Fig. 1A), and the evolution of total and volatile acidity (Fig. 1B), during the second fermentation and aging of a typical Brazilian sparkling wine. The initial ethanol concentration of base wine 11.89% (v/v) increased to 12.82% (v/v) during second fermentation (60 days), where the total sugar concentration felt from 22.2 g L^{-1} to 5.0 g L^{-1}. As expected, after 60 days both ethanol and sugar concentrations remained constant. Ethanol final concentration was higher than the average reported in Brazilian sparkling wines [13,14].

The total acidity of base wine (100.2 meq L^{-1}) was higher than that Spanish cava base wines [3], but is typical of Brazilian sparkling wines [13,14], reflecting the peculiar edaphoclimatic characteristics of Brazilian highlands. As can be observed in Fig. 1B, total acidity felt down rapidly after 30 days, and increasing again to 92.2 meq L^{-1} (360 days). Conversely, volatile acidity increased linearly during second fermentation and the first month of aging, and stabilized at approximately 9.5 meq L^{-1} after 120 days. The final volatile acidity was within the range reported for Brazilian sparkling wines [13], and below acetic acid threshold (16 meq L^{-1}). Volatile acidity is associated with the production of acetic acid by yeasts during fermentation and the hydrolysis of acetate esters through the maturation of sparkling wine [15].

Yeast population increased from 6×10^6 CFU/ml to 7.8×10^7 CFU/ml during second fermentation (0 to

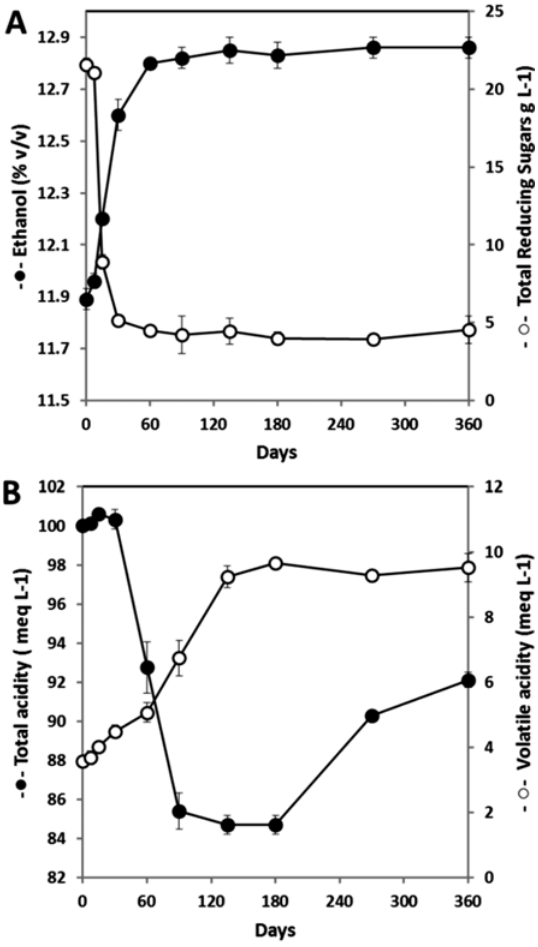

Figure 1. Fermentation parameters during second fermentation and aging of a typical Brazilian sparkling wine: (A) ethanol and total reducing sugars concentration, (B) total and volatile acidity. Data represent mean values of three replications.

30 days). After this period, viable yeast population rapidly decreased to approximately 1×10^6 UFC/ml (90 days), and showed a drastic reduction during aging, attaining less than 1×10^4 UFC/ml, 0.1% of the maximum population at 360 days. This reduction was accompanied by an increase of soluble peptides/proteins on wines after 240 days, indicating yeast autolysis.

Volatile profile analysis of Brazilian sparkling wine along second fermentation and aging led to the identification of twenty-seven compounds comprising aldehydes, alcohols, esters, and acids. Twenty compounds varied significantly along the evaluation period, but seven (2-methyl-1-butanol, hexanol, cis-3-hexen-1-ol, isobutyric acid, butyric acid, isovaleric acid, and 2-phenylethanol) did not show significant differences during the 360 days of observation. Among these compounds, butyric acid and 2-phenylethanol were above their olfative threshold values, and can contribute to the overall organoleptic characteristics of sparkling wine.

Acetaldehyde concentration increased during the first stages of second fermentation, and decreased right after, stabilizing in approximately 70 mg L^{-1} after 270 days. Acetaldehyde, an intermediary product of ethanol fermentation, decreased during wine maturation as it combines with polyphenols and other wine compounds [8]. At the observed concentration is considered a favorable aromatic compound [16].

The concentrations of higher alcohols in the sparkling wines assessed are within the inferior limits reported in wines [15], and consistent with those reported on Brazilian sparkling wines [13]. As expected, considering the low concentration of free amino acids in base wines [16], variation in higher alcohol content during second fermentation was relatively low, but in general, their concentration increased during second fermentation, and gradually declined during aging. The concentrations of 1-propanol and 2-methyl-1-propanol, produced as sub-products of the catabolism of threonine and valine, exhibited slight variation, and never surpassed the corresponding threshold values. Conversely, 3-methyl-1-butanol (isoamyl alcohol) derived from leucine catabolism, showed significant variation, and with OAV ranging between 3.75 (90 days) and 2.67 (360 days) may contribute with its typical marzipan aroma to the organoleptic characteristics of sparkling wine.

The isoamyl acetate, described as fruity aroma (banana and pear), showed a significant reduction throughout the 360 days period, falling from 88 to 17.6 OAV. Similarly, it was detected a significant reduction in the concentration of hexyl acetate (pear e fruits) and 2-phenylethyl acetate (roses, honey and tobacco). Together, these reductions can be responsible for the well document reduction of floral and fruity notes in aged sparkling wines [3,15].

The concentration of the seven volatile fatty acids identified in the samples were consistent with those previously reported [13,17], and most of them exceeded their respective olfative threshold values. Fatty acids are related to creamy, cheese and rancid notes, and at low concentrations give pleasant notes and contribute to the overall complexity of wines. In general, volatile fatty acids concentration increased during second fermentation, declined during the first months of aging, and increased again after nine months. Conversely, the concentration of ethyl esters of fatty acids which are described as fruity and pleasant aromatic compounds significantly decreased after nine month of aging, contributing to the overall loss of "fresh", "fruity" and "flowery" notes on aged wines [18]. This peculiar behavior of fatty acids and ethyl esters can be attributed to the adsorption by the lees [6], and the release during autolysis [1,19].

Diethyl succinate, the product of a spontaneous esterification reaction between succinic acid and ethanol, showed a constant and almost linear increase during the 360 days evaluation period. This volatile compound is post-fermentative being formed during the aging in contact with the lees, and has be considered an aging marker [5].

To obtain an overall vision of the evolution of volatile compounds during second fermentation and aging of a Brazilian sparkling wine, a principal component analysis (PCA) was carried out using a matrix that include the concentration of the 27 compounds analyzed. The plot of the two principal components which explained 76.37% of the total variance and the contribution of the variables are showed in Fig. 2 and Table 1, respectively.

As can be observed in Fig. 2, samples collected through second fermentation and aging were grouped in three clusters. The first group included base wine and the sparkling wine collected during second fermentation

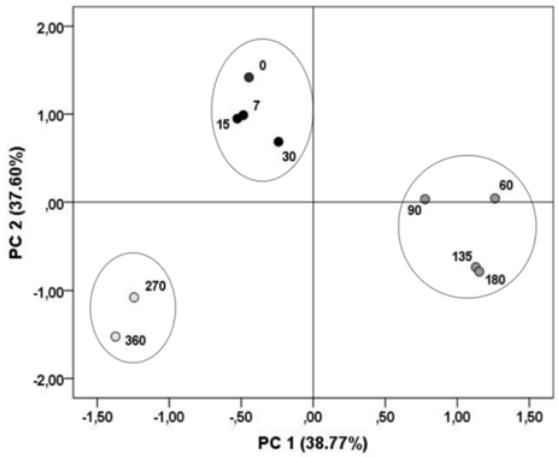

Figure 2. Principal components analysis based on the concentration of volatile compounds through the second fermentation and aging of Brazilian sparkling wines.

Table 1. Correlation between principal components and the concentration of volatile compounds.

	PC 1	PC2	PC 3
Variance Explained (%)	38.77	37.6	11.35
Acetaldehyde	−0.334	0.898	0.056
Methanol	−0.638	0.668	0.156
1-propanol	0.661	0.531	0.380
2-methyl-1-propanol	0.167	0.759	−0.109
2-methyl-1-butanol	0.823	0.487	0.139
3-methyl-1-butanol	0.875	0.411	−0.083
2-phenylethanol	−0.805	−0.009	0.349
Hexanol	−0.065	−0.963	0.042
Cis-3-hexen-1-ol	0.788	−0.529	−0.015
Trans-3-hexen-1-ol	0.802	−0.523	−0.174
Isobutyric acid	0.434	−0.695	0.306
Butyric acid	−0.392	−0.492	0.651
Hexanoic acid	−0.559	−0.049	0.819
Octanoic acid	−0.035	−0.279	0.924
Decanoic acid	0.808	0.270	0.416
Dodecanoic acid	0.946	0.168	0.017
Isovaleric acid	0.717	−0.574	0.312
Isoamyl acetate	0.286	0.923	0.202
Hexyl acetate	−0.520	0.804	0.196
2-phenylethyl acetate	−0.163	0.959	0.208
Ethyl butanoate	0.182	0.907	−0.012
Ethyl hexanoate	0.453	0.816	0.043
Ethyl octanoate	0.950	−0.222	0.045
Ethyl decanoate	0.616	−0.289	0.476
Ethyl dodecanoate	−0.990	−0.024	0.083
Ethyl acetate	0.613	0.237	0.141
Diethyl succinate	−0.393	−0.906	−0.089

(0 to 30 days). This group is clearly separated from the other groups by PC2, and was characterized by higher concentrations of methanol, 2-methyl-1-propanol, ethyl butyrate, isoamyl acetate, ethyl hexanoate, hexyl acetate, and 2-phenylethyl acetate, and lower concentrations of hexanol, isobutyric acid, isovaleric acid, and diethyl succinate. In general, these results confirm the graded loss of esters, particularly acetates, during the aging of the sparkling wines [15].

Groups 2 and 3, represented by the period between 60 to 180 days, and 270 to 360 days, respectively, showed a clear separation by the PC 1 (Figure 2). The compounds which determined the separation between these groups were mainly: 1-propanol (69.76 and 62.83 mg L^{-1}); 2-methyl-1-butanol (15.28 and 11.96 mg L^{-1}); 3-methyl-1-butanol (147.18 and 111.2 mg L^{-1}); ethyl octanoate (1.36 and 0.86 mg L^{-1}); diethyl succinate (1.14 and 3.08 mg L^{-1}); ethyl dodecanoate (0.01 and 0.14 mg L^{-1}); 2-phenylethyl acetate (12.23 and 13.77 mg L^{-1}); decanoic acid (1.74 and 0.85 mg L^{-1}), and dodecanoic acid (0.31 and 0.16 mg L^{-1}).

In summary, the results indicate that the composition of volatile compounds significantly change during second fermentation and aging. Three profiles defined by the concentration of specific volatile compounds can be detected: (1) a fermentation profile (0 to 30 days) defined by higher concentrations of acetates and lower concentrations of ethylates and fatty acids, (2) a post-fermentation profile with intermediary concentrations of acetates, and increased concentrations of fatty acids and their ethyl esters, and (3) a mature profile (>9 months) with higher concentrations of diethyl succinate, lower concentrations of acetates, fusel alcohols, and volatile fatty acids. This behavior allowed to produce different products: more fresh and fruity wines (just after second fermentation), and more "ripe", complex, and less fruity products after 9 month of aging.

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