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DOCTORAL THESIS ABSTRACT

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Sparkling wines are characterized by high concentrations of carbon dioxide, being considered *special wines*. In accordance with the traditional method of obtaining sparkling wines, they are subjected to a double fermentation process, thus after the first alcoholic fermentation and following the addition of the *tiraje liqueur*, the wines are subjected to the second fermentation (Coehlo et al., 2009). There are many factors involved in the chemical composition of sparkling wines, such as variety, cultivation technology, quality of the base wine, type of inoculated yeasts for secondary fermentation, etc. However, the second fermentation and the aging stage on yeast sediment are key factors on the final quality of sparkling wines (Pozo-Bayón et al., 2009). Correlated with the new generation's desire for different organoleptic sensations, the doctoral thesis entitled *„Studies on the influence of different yeast strains on the quality of sparkling wines produced by traditional method”* aims to study the influence of yeasts (Saccharomyces spp.) (used in the second fermentation) on the quality of white sparkling wines. The present research focuses on the evaluation of some chemical constituents (organic acids, metals, volatile compounds), but also on the evaluation of the sensory profile of the obtained sparkling wines. To fulfill the general purpose, the experimental protocol assumed the establishment of the following specific objectives: studying the specialized literature and selecting a relevant database; establishing the experimental protocol and activities; realization of experimental variants; analysis of the general physicochemical properties of the obtained samples; evaluating the level of metals in sparkling wines; analysing the content of organic acids; determining of the main volatile compounds in the final samples; studying the sensory characteristics of the analysed variants; statistical interpretation of the data and their comparison with the results from the specialized literature.

For obtaining the experimental sparkling wines, Muscat Ottonel grapes, cultivated in the Iasi-Copou Vineyard were used. The experimental samples and laboratory analyses were carried out in the experimental winery and the Oenology Laboratory of the Faculty of Horticulture, "Ion Ionescu de a Brad" Iasi University of Life Sciences. Thus, five experimental variants were made. The grapes were manually harvested at full maturity in the autumn of 2015. In order to carry out the alcoholic fermentation, various yeasts belonging to the *Saccharomyces* species were inoculated. The fermentation process took place under temperature-controlled conditions (20 °C). The fermentation process took place under temperature-controlled conditions (20 °C). After the completion of the primary fermentation, the initially wine (V0) showed an alcoholic strength of 12.5 % alc vol. To reduce this level and obtain a predetermined alcoholic concentration (10.5 % vol.), reverse osmosis was applied (resulting in V0'). Secondary fermentation took place in bottles, being inoculated with different commercial yeasts, as follows: IOC FIZZ™ - V1; IOC DIVINE™ - V2; LEVULIA CRISTAL™ - V3; IOC 18-2007™ - V4. The results of the physicochemical analyses of the analysed sparkling wines fall within the limits allowed by the norms of the International Organization of Vine and Wine (OIV, 2020). Given that the same base wine was used to obtain them, the possible differences are attributed to the inoculation of different types of yeasts in order to carry out the secondary fermentation. Minor changes were observed in the main physicochemical parameters analyzed at 11 months after the production of the samples, compared to the results recorded at 6 months. Depending on the level of residual sugars, the experimental samples can be classified as brut sparkling wines. The alcoholic concentration of the analysed sparkling wines varied from 11.3 % vol (V2 and V4) to 11.6 % volume (V1 and V3). Parameters such as density, total acidity, residual sugars and non-reducing extract have dependent values with important differences between samples. Analysis of variance indicated significant differences between the physicochemical characteristics of the control sample and those containing yeasts ($p < 0.001$ for most parameters). The values were also influenced by the type of inoculated yeasts ($p < 0.001$ for most pairs of media, $p < 0.05$ for volatile acidity in both periods of analysis, non-significant differences for density). The Tukey HSD post-hoc test highlights the pairs of variants that significantly differed for each individual physicochemical indicator. Thus, in the case of density, the differences concerned the control sample (V0) compared to the variants V1, V2, V3 and V4, which contained yeasts. Also, a minor influence on the physicochemical quality is observed in the samples in which yeasts were inoculated.

The metal ion profile of sparkling wines plays an important role in modulating their sensory attributes. The metal composition of wine during fermentation, maturation and storage is not stable. The amount of potassium and calcium increases during storage (from 6 to 11 months), while the level of magnesium and sodium ions decreases considerably during the storage period. During fermentation, yeasts consume significant amounts of metal ions such as calcium, cooper, iron, potassium and magnesium (Cotea, 2009). Also, following the sedimentation and yeast removal processes, important amounts of the analysed metal ions are removed. However, higher concentrations of some compounds were identified in the samples in which yeasts were inoculated for the second fermentation. In addition, comparing the concentrations of iron, cooper, nickel and manganese in samples V1-V4, an upward dynamic is observed during the storage-ripening period. This phenomenon could suggest the susceptibility of the wine samples to the occurrence of turbidity or sedimentation, as well as the deterioration of the sensory quality, correlated with the catalytic and oxidative action of the studied cations (especially iron and cooper). According to Galani-Nikolakaki et al. (2002), the main source of iron contamination is soil, as well as utensils and machinery used during production and processing. The analysis of variance reveals a significant influence of the metal content according to the type of inoculated yeasts. Also, the post-hoc test indicates the pairs of means between which there are significant differences (generally, $p < 0.01$, at least two means being significantly different at the 99 % significance level, regardless of the analysing moment).

The organic acids in the wine come mainly from the raw material, from the administered treatments or they can result from the metabolic processes of the microorganisms. An optimal level of organic acids ensures the balanced, fresh and refreshing character of the wines, while high levels will impress intensely sour taste. The results highlight a higher content in the analysed organic acids in the case of the base wine, compared to the sparkling wines. Both malic acid and tartaric acid represent approximately 90 % of the total organic acids in wine (Robles et al., 2019). The analysed samples showed values between 6.33 g/L (V1) and 6.46 g/L (V2) malic acid after 6 months of storage and between 5.61 g/L (V3') and 6, 63 g/L (V2') after 12 months. An increasing trend of malic acid concentration was noted between 6 and 12 months of storage of most varieties, except for sample V3, where the content of this acid registered important decreases over time. After malic acid, the most abundant acid was tartaric acid, with an average concentration ranging from 0.99 to 5.97 g/L. Tartaric acid values remain almost constant between the two moments of analysis (6 months and 11 months), registering a slight decrease in the case of the V3 variant and an increase in the case of the V4 variant. Along with the first two acids presented, citric acid contributes significantly to defining the colour and stability of sparkling wines. A higher amount of citric acid allows the detection of counterfeiting (Ivanova-Petropulos et al., 2020). Values ranged from 0.58 g/L (V1 and V3) – 0.61 g/L (V4) at 6 months and 0.51 g/L (V3 and V4) – 0.59 g/L (V1) at 11 months. Lactic acid concentrations varied with yeast activity, usually being formed from the breakdown of malic acid. The highest values of this compound were determined in samples V1 (0.42 g/L) and V4 (0.40 g/L) after 6 months of storage, going on to register important accumulations up to 12 months. Succinic acid is formed early in fermentation depending on the inoculated yeast strain and varies with nitrogen composition

(Waterhouse et al., 2016). Regarding the samples analysed in the first stage, the highest succinic acid content was identified in V3 and V4 samples (0.31 g/L), followed by V2 (0.20 g/L) and V1 (with 0.18 g/L), in the base wine finding values 8 - 10 times higher. The succinic acid content increased during storage in all variants. In a first stage, the concentration of organic acids showed minor variations in relation to the type of yeasts inoculated for the second fermentation ($p > 0.05$). Significant changes were obtained between the two analysis points (6 and 11 months of storage). For example, in the case of malic acid, post-hoc analysis revealed significant differences ($p < 0.05$) between sample means V1-V4, V1-V5, and V2-V3 for determinations made 6 months after storage. Further, analyzes at 12 months showed significant differences ($p < 0.01$) between the V1-V2, V1-V3, V2-V3 and V3-V4 groups.

The aroma profile is a major factor that determines the character and quality of the sparkling wine, but also its acceptability and competitiveness in the market. Complexity of the aroma profile during fermentation increases significantly due to the synthesis of volatile compounds by *Saccharomyces cerevisiae* yeasts. Yeasts play an important role in defining the characteristics of sparkling wine, including alcohol concentration, carbon dioxide overpressure, the presence of mannoproteins and precursors to various aroma compounds. Quantified volatile compounds (represented by esters, acids, alcohols and terpenes) were separated into their chemical classes. Esters contribute to the sensory characteristics of wines, being mainly responsible for floral and fruity notes. Of these, compounds such as ethyl octanoate, ethyl decanoate, ethyl laureate, isopropyl myristate, ethyl palmitate and ethyl oleate were identified by gas chromatography analysis. According to Muñoz-Redondo et al. (2020), several esters are considered markers of the second fermentation. Isoamyl acetate is usually derived from yeast metabolism during alcoholic fermentation. This compound contributes to the fruity aroma and gives complexity to white wines (Vigentini et al., 2017). Its concentrations ranged from 11.71 µg/L in V2 sample to 22.78 µg/L in V4 sample. 2-Phenethyl acetate is generally produced by phenolic precursor yeasts during the ripening stage and is characterized by sweet notes of honey and flowers. The highest level of this compound was identified in samples V1 and V3, while the lowest concentration was recorded in the case of variant V2. According to Genovese et al. (2013), ethyl decanoate (floral odor) and 2-phenylethyl acetate (rose aroma) may exhibit synergistic effect, even at low levels. The values of 2-phenylethyl acetate reported by Torchio et al. (2012) were comparable to the results obtained in the present experiment (22.33 to 47.72 µg/L). Diethyl succinate is usually formed during alcoholic fermentation. Sample V2 displayed the highest level of this compound (62.58 µg/L). According to Torrens et al. (2008) and Riu-Aumatell et al. (2006), diethyl succinate represents one of the esters specific to the maturation period. Their proportions can indicate significant increases during the second fermentation, as a result of contact with yeast cells. Acids can come from the raw material grapes, but they can also result from chemical reactions carried out during alcoholic fermentation. The high concentrations of decanoic acid, in conjunction with the high values of octanoic acid, determine a lasting inhibitory effect on the growth and development of yeasts (Riu-Aumatell et al., 2006; Delfini et al., 2001). In the analysed samples, the content of octanoic acid varied from 580.64 µg/L in the V1 variant to 258.79 µg/L in the V2 sample. Decanoic acid reached a maximum concentration in V1 sample (145.25 µg/L) and a minimum value in sample V2 (11.36 µg/L). Alcohols represent secondary aromatic components derived from the transformation of sugars and amino acids during the fermentation process, with a significant influence on the sensory profile of the wine (Nascimento et al., 2018; Caliari et al., 2015). Regarding the level of alcohols, isoamyl alcohol, 4-octanol, 1-heptanol and 2-phenylethyl were representative in the analysed samples. Isoamyl alcohol generally represents more than 50 % of the alcohols responsible for the fusel note (Ribéreau-Gayon et al., 2004). The experimental samples presented amounts from 1019.50 µg/L of isoamyl alcohol in the V3 sample, to 485.91 µg/L in the case of the V2 variant. 1-heptanol was identified in large quantities in the V2 variant, providing a pleasant vegetal odour and fruity notes (apples and bananas). Phenylethyl alcohol, a volatile compound with a pleasant sweet, floral and honey-like odour, was detected in all analysed samples. Higher amounts of this compound were identified in samples V1 (1150.12 μg/L) and V4 (683.46 μg/L).

Terpenes are secondary metabolites that come from grapes. Of these, L-linalool gives a fresh, spicy, floral aroma in wines, with hints of lemon. V1 presented the highest level of L-linalool (138.86 μg/L), followed by V3 (120.43 μg/L, V4 (44.31 μg/L) and V2 (16.65 μg/L). This compound is transformed by the action of acids into geraniol, nerol and terpineol, respectively (Lengyel, 2012). Of these, terpineol contributes to the definition of the fruity (melon) and floral (lilac) odour. The compound consists of monoterpene-glycosides in an acidic environment (Strauss, 1986). Following analysis by gas chromatography, the highest concentration was identified in V1 (42.79 μg/L), followed by variants V3 (41.40 μg/L), V2 (28.19 μg/L) and V4 (24.19 μg/L). This compound may originate from grapes (in low concentrations) and exhibits high olfactory perception (Lengyel, 2012). The data revealed a significant positive impact on the volatile profile following yeast supplementation. It is noted that the yeasts inoculated in samples V1 and V3 showed the greatest influence on most of the studied volatile compounds ($p < 0.05$). The null hypothesis that yeasts would not affect the volatile profile of sparkling wines is thus rejected. Regarding the results of the Tukey HSD (honest significant differences) test for differences between the various pairs of samples, significant results can be observed between V1 and V3 in the case of 1-heptanol and terpineol. Significant differences between V2 and V4 samples were obtained, regarding butyric acid, linalool and terpineol. In the case of diethyl succinate and decanoic acid, significant difference was obtained between variants V3 and V4 ($p < 0.05$). The principal component analysis describes the variations in the composition of volatile compounds of sparkling wines produced under the influence of different strains of commercial yeasts. The first factor described 59.82 % of the data variability and was closely correlated with most of the volatile compounds identified (ethyl octanoate and decanoate, 2-phenethyl acetate, ethyl laurate, hexanoic, octanoic, decanoic and 9-decenoic acid, alcohol isoamyl, 4-octanol, phenylethyl alcohol, linalool L and α-terpineol). Therefore, these components show high correlation with most of the volatile compounds identified in the analyzed samples. The first two principal components accounted for approximately 85% of the total variability in the data. The first principal component that explained most of the total variability in the data (59.82 %) was highly correlated with isoamyl acetate, ethyl decanoate, ethyl laurate, isoamyl alcohol and linaol (in all cases showed higher factor loadings greater than 0.90). For the second main component, diethyl succinate and isopropyl myristate showed high and positive values. The principal component analysis plots show a positive correlation of 1-heptanol with butyric acid (r close to +1) but a negative correlation with isopropyl myristate (r close to −1). In addition, linalool and ethyl decanoate are positively correlated, while linalool and butyric acid show a negative correlation. Ethyl octanoate, ethyl decanoate and isoamyl alcohol, the most widespread volatile substances in the analyzed samples, are also positively correlated. On the other hand, they are negatively correlated with butyric acid and 1-heptanol (positioned in the opposite direction). The variables related to the factor 1 allow the differentiation of the samples according to the volatile fraction. Since one of the aims of the experiment was to evaluate the influence of the inoculated yeasts on the volatile fraction of the experimental sparkling wines, the data confirm that different preparations can generate different concentrations of volatile compounds.

From a sensory point of view, the analysed sparkling wines are defined by intensely fruity (bananas, apples) and floral (elder flowers) notes, associated with high levels of esters (e.g. ethyl octanoate and ethyl decanoate). From the point of view of the concentration in which they are found, compounds such as isoamyl acetate, ethyl palmitate, 4-octanol or 1-heptanol did not show a significant contribution to the highlighted sensory profile. According to the sensory analysis, all the sparkling wines obtained were characterized as balanced from a taste point of view, with increased persistence, high acidity (which impressed freshness) and good texture (especially sample V4). The V1 variant was noted for its floral notes (elder flowers), while the fruity character was predominant in the V2 (especially green bananas). The samples also differed in terms of effervescence. The ability of sparkling wines to produce foam, as well as their degree of effervescence, depends on its composition and the applied production technology. Sample V1 received the highest score for this descriptor, while V4 presented the lowest effervescence. The intensity of the sweet taste is lower the higher the degree of effervescence was perceived. Regarding the correlation between volatile compounds and sensory perception, the fruity notes (apples, green bananas, peaches) of the experimental samples can be explained by the presence of large amounts of ethyl octanoate, ethyl decanoate or diethyl succinate. The floral smell is mainly due to high concentrations of phenylethyl alcohol. The samples were perceived to be balanced from a taste point of view. A significant influence of the yeasts on the final sensory profile was noted, with the control sample showing the lowest scores for most of the descriptors followed. V4 variant stood out with high acidity and effervescence, good texture (highest note) and increased persistence. The sample rated V2 was appreciated for intensely fruity notes (apples, melon), while the V3 variant stood out for its vegetal, yeasty, but also apple character.

The results showed a minor influence of yeasts on the physicochemical properties of sparkling wines, but an essential impact on the content of volatile compounds and their sensory characteristics. Also, the profile of organic acids and metals was greatly influenced by the type of inoculated yeasts. The data contribute to the completion, improvement and enrichment of the specialized literature in the field of the topic addressed, as well as to the optimization of existing winemaking technologies.