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IDENTIFICATION OF THE MAIN VOLATILE COMPOUNDS RESPONSIBLE FOR THE AROMA OF *SAUVIGNON BLANC* WINES

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Abstract

Our research aimed at identifying the main volatile compounds, including the volatile thiols, from 2 Sauvignon Blanc wines, obtained in Dragasani vineyard, 2014 harvest. Wines were obtained using 2 yeast strains of Saccharomyces cerevisiae, one commercial used in wine industry and the second being obtained by screening, in Dragasani vineyard. By GC/MS technique were identified and determined quantitatively three compounds of the mercaptans group, having an important contribution in the Sauvignon aroma, namely: 4-mercapto-4-metylpentan-2-one, 3-mercaptohexan-1-ol and 3-mercaptohexyl acetate. The results showed the positive influence of the yeast strain Saccharomyces cerevisiae selected from Dragasani area, over concentration in volatile thiols and consequently, on the flavor of Sauvignon wine, obtained by fermentation with this strain.

***Key words**: GC/MS method, 4-mercapto-4-metylpentan-2-one, Sauvignon flavor*

INTRODUCTION

Aromatic features are essential factors of the wines quality. Aroma and wines typology are due to a large number of volatile compounds that make up their aromatic expression [42]. The wines flavour is the result of three aromas categories, namely: varietal aromas, fermentation aromas and aging aromas (the bouquet of wines). Therefore, the flavor of wines depends on a multitude of factors. The study of these factors influence on the aromatic composition of grapes and wines, and therefore on the quality of wines, is complex and refers not only to the influence of each factor, but also to the interaction between them [16].

Varietal aromas, or aromatic potential of grapes, result from the complex interaction between: the vine variety and the rootstock used [16], "*terroir*" (climatic factors like microclimate, temperature, light, water reserve, and soil factors like soil nature) and cultural techniques applied to wines [13].

The initial aromatic potential of the grapes changes later in the course of alcoholic fermentation, as well as during the wines aging [22].

Thus, the *fermentation flavor* is due to the different physico-chemical and enzymatic processes, determined by yeast activity. During alcoholic fermentation, the *Saccharomyces cerevisiae* yeasts, the species most commonly used in winemaking, act on terpenic compounds, by hydrolyzing the glycosides and reducing the free terpene alcohols, leading to new flavor compounds. Fermentation flavors depend on the oenological methods and techniques applied in the bioprocess of wine obtaining. Fermentation monitoring and the strain of yeast used in the fermentation process are of great importance [42].

Subsequently, during wine maturation and aging, other compounds with an important role in the aroma, occur in a reducing environment. These compounds form *the aging aroma* (the tertiary aroma or the bouquet of wines) are depending on maturation and aging conditions. More than 1000 volatile compounds have been identified in wines, but only a relatively small percentage of 10-15% occurs in their flavor [43]. Although a small number of wine varieties belong to the group of aromatic wines (*Muscat*, *Tămâioasă*, *Traminer*), the grapes of all varieties contain volatile substances, from various chemical classes, that determine the variety specificity. These substances are mainly acids, alcohols, esters and aldehydes. In wine, volatile aromatic compounds result from the complex association of the substances named above, with phenols, proteins, ethyl alcohol, glycerol, polyglucides, organic acids etc [23]. Aromatic varieties have a higher content of flavors, especially terpenes.

Varietal aromas (biosynthetic compounds in grapes) are represented by two groups of compounds [23]: flavor precursors, which are non-volatile and non-aromatic compounds: fatty acids, glycosides, phenolic acids [8] and free flavors. Free flavors are represented in grapes by 3 classes of compounds:

-terpenic compounds and norisoprenoids, with floral and tropical fruits aroma (in aromatic varieties, namely *Muscat*);

-methoxypyrazines, with herbal vegetal flavors, in *Sauvignon* and *Cabernet Sauvignon* varieties [1, 9].

- rotundone (especially in the *Syrah* variety).

The *Sauvignon Blanc* is considered a half aromatic variety [21], the aromatic flavor being determined mainly by thiols (mercaptans). Thiols are found in grapes like flavor precursors, bound to an amino acid (cysteine, glutathione), in odorless form and during alcoholic fermentation are converted into aromatic thiols [18]. The potential odor occurs only in wine, during alcoholic fermentation, when flavor precursors are degraded by the yeast, under the action of enzymes in aromatic thiols [13, 35].

The main volatile thiols identified in wine are:

-3-mercaptohexanol (3MH), with a sensory perception threshold of 60 ng/L, with citrus, grapefruit and exotic fruits aroma [5];

-3-mercaptohexyl acetate (A3MH), with a sensory threshold of 4 ng/L, which prints a cranberry and tropical fruit aroma [37];

- 4-mercapto-4-methylpentan-2-one (4MMP), with a perceptual threshold of 0.8 ng/L, whose flavor reminiscent of cranberries;

-4 – mercapto – 4 – methylpentan – 2 - ol (4MMP).

In addition to these compounds, free aromas are found, such as monoterpenes, pyrazines, etc. According to some authors [14, 15, 19] the main volatile compounds involved in the *Sauvignon* flavor are mercaptans (especially 4-mercapto-4-methylpentan-2-one), other authors considering that methoxypyrazines are the decisive compounds in the variety flavor. After Tominaga (1998) thiol 3-mercaptohexanol and its derivative, 3-mercaptohexyl acetate is the "key" of the specific flavor of *Sauvignon Blanc* wine [40].

Sauvignon varietal aroma composition depends on several factors [28]. Thus, studies conducted on the *Sauvignon Blanc* cultivated in South Africa [31], showed the influence of area microclimate, temperature and light mainly, on the content of grapes in monoterpenes, C13 - norisoprenoids and pyrazines. The concentration in monoterpenes increases parallel to the accumulation of sugars. An optimal temperature and a better illumination of the grapes are leading to the growth of terpenes concentration [27].

Contrary, in the case of methoxypyrazines, strong light and high temperature have a negative influence on the concentration in these compounds, leading even to their degradation [30, 32]. Thus, the aroma profile of *Sauvignon* will depend on climatic conditions and the microclimate of the area, concerning the variable content of monoterpenes and methoxipirazines respectively [29].

In terms of thiols, microclimate conditions influence the content in various volatile thiols, grapes having an aromatic profile dominated either by tropical fruits and flowers (due to 3 – mercaptohexan – 1 - ol) or green pepper, asparagus, due to 4 – mercapto – 4 – methylpentan – 1 - ol [16].

Cultural techniques applied to vine also have a great influence on the aromatic potential of the *Sauvignon* variety. Some authors point out that under the conditions of a high temperature climate, the exposure of grapes to light (by defoliating the calves around the grapes) is contraindicated, because a temperature above 35°C at their level, disturbs the metabolism of flavor compounds. They recommend that

Sauvignon plantations have to be placed in cooler microclimates, or with a higher water regime, through irrigation of vineyard culture [10]. A cool-night microclimate is appropriate for preserving the aromatic potential of grapes. A water stress during vegetation on the *Sauvignon* variety is always correlated with a very low accumulation of cysteine precursors, namely precursors of volatile thiols [10]. A proper hydrological regime leads to a fresh, fruit flavor wine production.

The concentration of wines in volatile thiols is also influenced by the assimilable nitrogen content of the must, content that depends on soil fertility. Thus, in the case of a non-fertilized crop (nitrogen deficiency), the concentration of the main must thiols (4-mercapto-4-methylpentan-2-one, 4-mercapto-4-methylpentan-2-ol and 3-mercaptohexanol) is much lower [26].

Lacroux (2008) showed the importance of the grape must content in nitrogen and especially its nature (ammonium or amino acids), on yeasts and therefore thiols production [26]. Also, thiols are highly oxidative compounds in an environment without SO_2 protection, especially in a quinone rich must [24].

Thus, for wines where these compounds play an important role in flavor, such as *Sauvignon* wine, hyperoxigenation of must before antioxidant treatment is contraindicated [6].

In the course of alcoholic fermentation, besides sulfur compounds (mercaptans), numerous volatile compounds form, through secondary metabolism of yeasts, numerous volatile compounds, namely the class of fermentative aromas, also called secondary flavors. Fermentative aromas are classified into four groups: the group of higher alcohols, the fatty acid group, the ester and acetylene group, and the group of sulfur compounds [9].

The formation and concentration of volatile compounds in wine is influenced by numerous factors linked to the fermentation process: temperature, pH, composition of the grape must and especially, the yeast strains that make the alcoholic fermentation [2, 17, 25, 33].

The influence of yeasts on the flavor of wine has been studied by numerous researchers, especially concerning the production of sulfur compounds (volatile thiols). Conversion into volatile thiols (sulfur compounds) made during alcoholic fermentation is dependent on yeasts first, in the sense that different yeasts strains produce more thiols than others [4]. On the other hand, the composition of must in flavor precursors, as well as fermentation parameters, influence the production of wines thiols [20].

The production of higher alcohols during alcoholic fermentation also varies depending on yeasts strains, especially on the yeast capacity of using amino acids in the synthesis of higher alcohols [42].

Esters are particularly important for the aroma of young wines, where they participate with their fruity (red fruits, bananas, pineapple etc.) and floral odors. The formation of ethyl esters is positively influenced by a reduced fermentation rate, due to a low temperature and low wax turbidity, as well as a strict anaerobiosis. Acetate biosynthesis is influenced by both yew strain used and the content in higher alcohols [23].

MATERIALS AND METHODS

Analyzed wines. Two wines obtained from the *Sauvignon Blanc* grapes variety in Drăgăşani vineyard, 2014 harvest, were analyzed. The wines analyzed were obtained by controlled alcoholic fermentation, with 2 yeast strains of the species *Saccharomyces cerevisiae*: a commercial strain produced by Sodinal, France (*Sauvignon* S_1) and *Saccharomyces cerevisiae* strain obtained by screening from the Drăgăşani vineyard (*Sauvignon* S_2). The conditions for obtaining the wines were similar: 229 g/L sugar concentration of the must; similar fermentation parameters (temperature, pH etc).

Sauvignon wines were analyzed in terms of physico-chemical parameters: alcoholic strength (vol% alcohol), residual sugar content (g/L), total acidity (g/L sulfuric acid), total dry extract (g/L), volatile acidity (g/L acetic acid) and glycerol (g/L). All analyses were performed according to standard methods [39]: ebulliometer method for alcoholic strength; titrimetric method for total acidity; distillation method Saunier-Cazenave for volatile acidity;

Tabarié method for total dry extract and volumetric method for glycerol.

Specific Extraction of Volatile Compounds. 200 mL of wine, placed in a conical flask, were successively extracted (3x20 min) at 0°C with 3x25 mL of freshly distilled dichloromethane and then centrifuged for 15 min. at 3000 rot/min. The three organic extracts were pooled, dried with anhydrous sodium sulfate and concentrated to 5 mL in a Danish concentrator (45°C), then to 1 mL under a stream of nitrogen [3, 12].

Specific Extraction of Volatile Thiols. A volume of 500 mL of wine containing 4-methoxy-2-methyl-2-mercaptobutane, as an internal standard, was brought to pH 7.0 with sodium hydroxide solution and was extracted successively twice, with 100 mL dichloromethane, with magnetic stirring for 5 min; the organic phases were centrifuged for 5 min., at 3000 rot/min, to break the emulsion and were separated in a funnel; the obtained organic phase was then extracted successively twice, with 20 mL p-hydroxymercurbenzoate solution, for 5 min.

The two aqueous phases, from the extractions, were pooled and brought to pH 7.0 by addition of a 5% hydrochloric acid solution; the obtained solution was loaded into a strongly basic anion exchange column; the volatile thiols were released from the complex thiol-p-hydroxymercurbenzoate, fixed on the column, by percolating for 40 min with a cysteine solution adjusted to pH 7.0. The organic phases were collected, dried on anhydrous sodium sulfate and concentrated under nitrogen flow [40].

GC/MS. Determination of volatile aromatic compounds in wine was performed using a Hewlett Packard 5890 gas chromatograph series II coupled to a mass spectrometer Hewlett Packard 5972 series II.

Quantitative analysis of volatile compounds identified in Sauvignon wines by GC/MS. 1μL from each extract was injected into an HP 5-MS capillary column with dimensions: 30 m x 0.25 mm x 0.25 mm (film thickness). Column temperature: 30°C for 10 min., followed by temperature gradient 10° min^{-1} up to 80° C, then gradient of 25°C/min. up to 250°C where stationed 10 minutes. Detector and injector temperatures are: 280° C and 250° C resp. Carrier gas is He, flow-0.5 ml min^{-1}. MSD conditions are: temperature 180° C ion source, ionization energy 70 eV, mass limit of 20-400 amu, electronic multiplier voltage 1700V, scan rate 1.60 s^{-1}. Injection mode: split, opening after 60 sec. and the split flow 20 mL min^{-1}. Quantitative determination and identification of volatile compounds based on the comparison of retention indices (RI), mass spectra and the estate of odors. Identification is based on the standard MS library Wiley [7, 38].

Quantitative analysis of volatile thiols identified in Sauvignon wines by GC/MS [40, 41]. 2 μL from extract was injected into an HP 5-MS capillary column. The three volatile thiols were detected: 4-mercapto-4-metylpentan-2-one, 3-mercaptohexan-1-ol and 3-mercaptohexyl acetate.

RESULTS AND DISCUSSIONS

Chemical and sensorial analyses of wines. The two *Sauvignon Blanc* wines from the Romanian wine region Drăgăşani, harvest 2014: *Sauvignon* S_1 and *Sauvignon* S_2 were analyzed chemical and sensorial.

Results are presented in Table 1.

The *Sauvignon Blanc* variety is grown in Romania in 41 vineyards, but in the Drăgăşani vineyard it seems that it finds the best conditions, the wine produced here being special [11]. In sensorial analysis, the two *Sauvignon* were characterized as dry wines, with a bright yellow color with light green reflections, medium-sized, well-structured. Both wines showed a gentle acidity, perfectly balanced with alcohol.

In terms of flavor, the general impression shows that the *Sauvignon 1* sample had a more intense flavor, elder, jasmine and melon.

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Table 1. The main physical-chemical parameters of wines

Wine sample	Alcoholic strength (vol% alcohol)	Residual sugar content (g/L sugar)	Total acidity (g/L sulfuric acid)	Glycerol (g/L)	Total dry extract (g/L)	Volatile acidity (g/L acetic acid)
Sauvignon S_1	13.35	1.8	5.8	8.9	22	0.35
Sauvignon S_2	13.18	3.8	5.45	9.4	21	0.56

The *Sauvignon 2* sample was characterized at tasting, with a delicate but expressive, complex flavor; the taste was fruity, fresh, with exotic fruit aromas, vine flowers and acacia flowers. In addition, the *Sauvignon 2* sample featured typical mineral notes, and the wine was finally characterized with more variety and region typology.

The alcoholic strength of the two wines varied very little in favor of the *Sauvignon 1*sample, due to the fact that the commercial yeast strain showed a very good ability to metabolize the sugars. The sample fermented with the local yeast strain showed a residual sugar content of 3.8 g/L.

From the point of view of total acidity, although its value is slightly different, both wines showed a pleasant acidity, in balance with the alcoholic strength.

Regarding glycerol, a compound that is positively involved in the quality of wines, the local strain led to a higher concentration of glycerol, although the sample obtained with the commercial yeast strain showed a higher alcoholic concentration.

Volatile acidity, a very important parameter in the quality and health of wines, showed normal values for both samples. However, it was noted that the local strain led to higher production of volatile acids (0.56 g/L acetic acid).

GC/MS wines analysis. Identification of volatile compounds.

Fermentation flavors are very important for the typical wines, which are responsible for their wineiness and their specific character. Fermentative aromas are classified into four groups: the group of higher alcohols, the fatty acid group, the ester and acetylene group, and the sulfur compound group [4].

In the analyzed wines, volatile compounds from the group of esters, higher alcohols, lactones and terpenes, as well as from the group of sulfur compounds (thiols or mercaptans) were identified.

Esters are especially important for the aroma of young wines, to which they participate with their fruity and floral odors. Their contribution is however, much more complex, because it involves the aromatic potential of the variety, which is in a complex relationship with other factors (ex. yeast strain).

During alcoholic fermentation, ethyl esters and acetals of higher alcohols are formed. Among the ethyl esters, especially ethyl butanoate, ethyl hexanoate, ethyl octanoate and ethyl decanoate, are involved in the aroma of young wines, imparting odors of flowers, apples, strawberries, pineapples, etc. [34]. Of the branched esters, an important role in the aroma of the wines seems to have ethyl 2-methylpropanoate and ethyl 2- and 3-methylbutanoate

When analyzing the two samples of wine, it was observed that the local strain led to the production of a larger quantity of ethyl esters (Fig. 1).

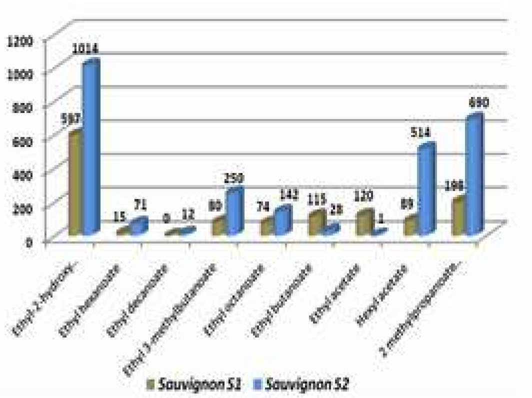

Fig. 1. The main esters of a *Sauvignon* wines (mg/L)

Superior Alcohols. With the exception of 2-phenylethanol containing floral, rose flavors, the aroma of the other higher alcohols is less favorable to the aroma of wines [36].

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Many authors believe that a content of less than 300 mg /L of higher alcohols has a positive effect on wine aroma; the presence of higher alcohols above the level of 1 g/L leads to olfactory defects of wine.

From Fig. 2 it can be seen that the sample obtained by fermentation with local yeast had a very low content of superior alcohols, in the form of traces, except 2-methyl-1-propanol (alcoholic, malt, vinified).

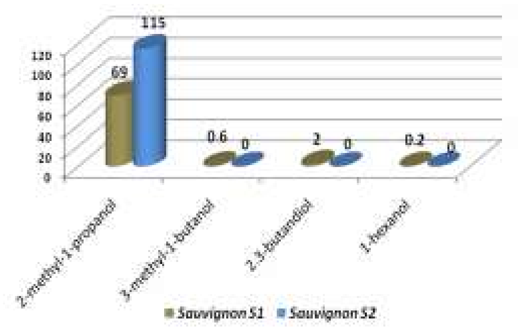

Fig.2. Concentration of aliphatic alcohols in *Sauvignon* wines (mg/L)

Terpenes. Monoterpenols are specific to aromatic varieties present in the grapes of *Muscat*, *Tamâioasă*, etc., but also in the aroma of wines from other varieties. In *Sauvignon*, in both samples, only linalone (monoterpenol which imparts aroma of rosewood, coriander) was identified (Fig. 3).

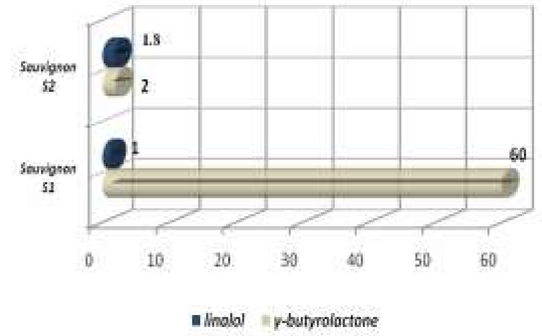

Fig.3. Concentration of terpenes and lactones in *Sauvignon* wines (mg/L)

GC/MS wines analysis. Identification of volatile thiols.

Volatile thiols, aromatic compounds that impress citrus aromas, exotic fruits, cranberries etc., have been identified in wines of the variety like *Colombard, Merlot, Cabernet, Grenache, Cinsaut, Malbec* etc.

After Tominaga (1998) 3-mercapto-hexanol thiol and its derivative, 3-mercaptohexyl acetate is the "key" to the specific flavor of *Sauvignon Blanc* wine [40]. The conversion of precursors into thiols is dependent on yew, first of all, in the sense that different strains of yeasts produce more thiols than others.

In the analyzed wines 3 volatile thiols were identified: 3-mercaptohexanol (3MH); 3-mercaptohexyl acetate (3MHA) and 4-mercapto-4-methylpentan-2-one (4MMP).

GC/MS analysis showed that the local strain led to the production of a larger amount of 3-mercaptohexanol and 3-mercaptohexyl acetate (Fig. 4 and Fig. 5).

Fig.4. The concentration in *3-mercaptohexan-1-ol* from the *Sauvignon* wines

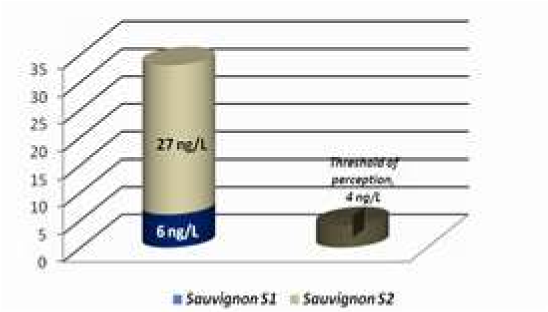

Fig.5. The concentration in *3-mercaptohexyl acetate* from the *Sauvignon* wines

Some authors state that the specific flavor of *Sauvignon* wine is given by the 4-mercapto-4-methylpentan-2-one thiol found in some French wines, in concentrations up to 50ng/L. A higher concentration of 4-mercapto – 4 – methylpentan – 2 - one was identified in *Sauvignon* 2, a wine produced by fermentation with the commercial strain (Fig. 6).

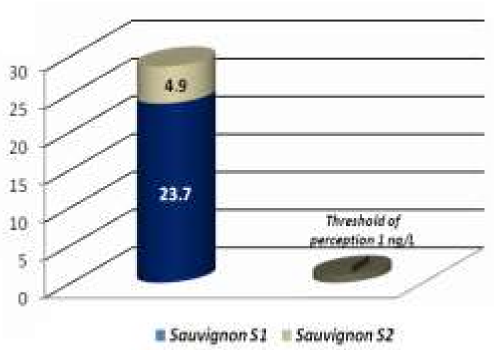

Fig. 6. The concentration in *4-mercapto-4-metylpentan-2-one* from the *Sauvignon* wines

CONCLUSIONS

In sensorial analysis, the two *Sauvignon* were characterized as dry wines, with a bright yellow color with light green reflections, medium-sized, well-structured.

Both wines showed a gentle acidity, perfectly balanced with alcohol.

Sauvignon 1 sample had a more intense flavor, elder, jasmine and melon. The *Sauvignon 2* sample was characterized, at tasting, with a delicate but expressive, complex flavor; the taste is fruity, fresh, with exotic fruit aromas, vine flowers and acacia flowers. The *Sauvignon 2* sample featured typical mineral notes, and the wine was finally characterized with more variety and region typology.

The alcoholic strength of the two wines varied very little in favor of the *Sauvignon 1* sample, due to the fact that the commercial yeast strain showed a very good ability to metabolize the sugars. The sample fermented with the local yeast strain showed a residual sugar content of 3.8 g/L.

The local strain led to a higher concentration of glycerol, although the sample obtained with the commercial yeast strain showed a higher alcoholic concentration.

Volatile acidity showed normal values for both samples. The local strain led to higher production of volatile acids (0.56 g/L acetic acid).

In the analyzed wines, volatile compounds from the group of esters, higher alcohols, lactones and terpenes, as well as from the group of sulfur compounds (thiols or mercaptans) were identified.

The local strain led to the production of a larger quantity of ethyl esters. The sample obtained by fermentation with local yeast had a very low content of superior alcohols, in the form of traces, except 2-methyl-1-propanol.

In *Sauvignon*, in both samples, only linalone (monoterpenol which imparts aroma of rosewood, coriander, etc.) was identified.

In the analyzed wines, 3 volatile thiols were identified: 3 - mercaptohexanol (3MH); 3 - mercaptohexyl acetate (3MHA) and 4-mercapto-4-methylpentan-2-one (4MMP).

GC/MS analysis showed that the local strain led to the production of a larger amount of 3-mercaptohexanol and 3-mercaptohexyl acetate.

A higher concentration of 4-mercapto-4-methylpentan-2-one was identified in *Sauvignon* 2, a wine produced by fermentation with the commercial strain.

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