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DOCTORAL THESIS

**CONTRIBUTIONS TO THE STUDY OF
VOLATILES COMPOUND OF GRAPES AND
WINES OBTAINED IN COTNARI
VINEYARD**

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SUMMARY

The doctoral thesis has as theme "Contributions to the study of volatile compounds from grapes and wines obtained in Cotnari vineyard".

The main objective of the thesis is the study of the volatile compounds from grapes and wines from Cotnari vineyard, as well as optimising the specific analysis methods, namely SPE liquid injection – „solid – phase extraction”, head-space injection (HS) and ITEX injection (in tube extraction).

The doctoral thesis is structured in six chapters, out of which three represents the general part and three the experimental part. The first part represents the viticultural ecosystem of Cotnari vineyard (chapter 1), the present state of research concerning volatile compounds of grapes and wine (chapter 2) as well as general information of gas-chromatography (chapter 3). The second part discusses aspects concerning the organisational frame (chapter 4), objectives of the research, material and research method (chapter 5) as well as results and discussions (chapter 6).

The natural environment of Cotnari vineyard shows that this famous vineyard has very favourable conditions for vine culture, some years being exceptional for grapes, fact that explains the specificity of typical Cotnari wines. Situated at the northern limit of vine culture, the vineyard has a valuable climate, where the local grape varieties Grasă de Cotnari, Tămâioasă românească, Frâncușă and Fetească albă, have optimal conditions for obtaining high quantitative and qualitative yields.

The complex analyses that have been done using gas-chromatographical techniques (GC), allowed the detection of many volatile compounds, whose presence can be associated to wine aroma.

The analysed wines are from 2005 and 2007 harvest for the Frâncușă grape variety, 1994, 2002 and 2003 for Fetească albă grape variety, 1979, 1984, 1996, 2000, 2002, 2003

and 2005 for *Grasă de Cotnari* variety and 1995, 2000, 2002, 2003 and 2005 for *Tămâioasă românească* grape sort. By analysing the main physical-chemical characteristics of the wines, one can say that the wines obtained from a 2000, 2002, 2003 and 2005 harvests were exceptional in order for their aging.

Beside the compounds identified in grapes, in wine appear a series of other compounds especially due to the fermentation process. In the case of the analysed wines, that were obtained in an industrial process, the identified volatile compounds depend very much on the used yeasts as well as the wine-making applied technology.

Analysing the compounds identified in grapes it was noted that the alcohols that are found in general are 1-dodecanol and 1-hexanol. Moreover, *Grasă de Cotnari* has in high quantity isoamyl alcohols. 1-Dodecanol (lauryl alcohol), a fatty alcohol, was found to be in the highest quantity in all grape varieties. This compound has a specific aroma of fat and wax.

Another identified fatty alcohol in grapes was 2-ethyl-hexan-1-ol. This compound appears in plants within the cycles of palmitates and oleates biosynthesis. The isomers 3-methyl-1-butanol (isoamyl alcohol) and 2-methyl-1-butanol (active amyl alcohol) could not have been separated in the present chromatographical conditions. Because of this they were reported as total amyl alcohol. These compounds offer a fruity character both to the grapes as well as to the wine. The presence of amyl alcohols in the analysed grape berries is surely due to a fermentation process started during their handling and storage.

Moreover, the following alcohols were identified: 1-butanol (appears due to a hydrogenation reaction of butyraldehyde and plays the role of enzymatic inhibitor in plants), 1-hexanol (it forms in plants from lipids oxidation's process under the action of lipoxidation), 2-methyl-1-propanol (present within the degradation cycle of valine in *Saccharomyces cerevisiae*), 1-octen-3-ol (considered an indicator of the health degree of grape harvests), 1-pentanol (appears in grape berries due to the transformation of norleucine through the Ehrlich way), trans-2-hexen-1-ol (appears within the biochemical way of β -13-lipoxidation and 13-hydroperoxidases).

In the grape berries, volatile acids were not identified, the only registered acid being the acetic acid. Its presence is based on the apparition of unwanted chemical processes that started from the harvest until the analysis process.

In what concerns the esters found in grapes, all the compounds that were identified

appeared during the samples' storage as a result of the degradation (fermentation) process.

Among the aldehydes, n-hexanal was identified in all the analysed grape varieties. Together with 2 hexenal, it appears as a secondary product within the biosynthesis of linoleic and α -linoleic acid, being found in highest percentual concentration among all identified compounds.

Concerning the ketones, acetophenone is registered in Fetească albă grape variety and is normally present in the degradation biochemical process of ethylbenzene (anaerobic). It is possible that its presence is due to the storage conditions of the grape samples. At the same time, 1-octen-3-one, aromatic compounds, with a metal and mushroomy sensation, was identified as well as 6-methyl-5-hepten-2-one that appears as product within the biosynthesis of cuticular wax in plants.

Following the ITEX analysis of the Grasă de Cotnari grape berries, no terpenic compounds were identified. However, contrary to the idea that Frâncușă grape variety is not an aromatic sort, a series of compounds was found (later, some of them were also identified in the wines as well), in total, representing 12,11 % of the total identified compounds. In Tămâioasă românească grape variety, the most terpenes were registered, among which α -terpineol, α -trienol, myrcene, β -ocimene.

In the grape berries, a high percentage was occupied by diethyl-phthalate (reported to the compounds identified in the chromatograms), that is largely used as a plasticizing agent in almost any polymeric material.

The analysed wines were from the wine collection (vinothèque) of S.C. Cotnari S.A. The oldest wine was that of Grasă from 1979, while the youngest was 2007 Frâncușă. The analysed wines were obtained by production technologies that were applied in the winery that year. The selective criteria for choosing the wines subject to this research were the years that had the best qualitative and quantitative harvests.

It can be observed that the following alcohols were identified in wines irrespective of the harvest year: amyl alcohols (3-methyl-butanol + 2-methyl-butanol), phenylethyl alcohol, 1-hexanol, isobutyl alcohol.

In the majority of analysed wine variants, 3-hexanol, also known as leaf alcohol, was found. This fact can lead to the conclusion, that, during processing, the grapes of Frâncușă and Fetească albă had a big amount of vegetal parts (leaves, shoot tips), while the grapes from Grasă de Cotnari and Tămâioasă românească varieties came in contact for a longer period of

time with the grape stalk during the premaceration process.

Among the used analyses methods, it was found out that for the ITEX method, the identified alcohols percentage is the highest.

Taking into consideration the Grasă de Cotnari grape variety, it was noticed that, through wine aging, due to the reductive processes that take place, a part of aldehydes, ketones or acids are transformed into the respective alcohols. This fact was demonstrated by all the three used analyses methods.

Only a few volatile acids that are found in wine are produced by yeast fermentation. High aliphatic acids (hexanoic, octanoic and decanoic) were identified in the studied wines, as product of yeast fermentation together with other esters.

Using ITEX analyses, formic acids was identified in all wines in a small percentage, its presence being justified by the possibility of a moldy grape harvest or the existence of a non-enzymatic oxidation of glyoxilic acid.

It is interesting to observe the presence of benzoic acid in small quantities, It is possible that it appears due to some natural biochemical processes in plants (benzoat I, II, III biosynthesis under the action of co-enzyme A).

In the analysed wines, a big percentage is represented by ethylic esters responsible for the specific fruity notes, that is stable in time. Their accumulation is influenced by the fermentation conditions: low temperatures, small quantities of nitrogen substances in the fermentation process.

It was shown that the head-space technique favours the extraction of higher quantities of esters. At the same time, the head-space technique identifies the ethyl acetate, one of the most important esters in wine. This is formed in small quantities under the action of yeasts, during the fermentation process, but it can also appear in high doses as a result of acetic bacteria intervention mostly during the barrel maturation phase, when the wine is still in contact with air.

Another identified ester was isoamyl acetate (3-methyl-1-butanol acetate) that appears during alcoholic fermentation and forms the fermentation aroma in young wines.

The wines that contain the lowest content of aldehydes are those from the Frâncușă grape variety.

The compound heptanal, identified in wines obtained from Fetească albă, Grasă de Cotnari and Tămâioasă românească, enriches them with a very strong fruity aroma.

Benzaldehyde is identified in the three wines mentioned above, and confers them a specific bitter almonds aroma.

Isovaleric aldehyde (3-methyl-butanal), identified in wines that are obtained from all the studied wines, has a strong apple-like odour.

Acetic aldehyde and formaldehyde could not be identified in the chromatograms because the specific signal was covered by that of ethyl alcohol (majority) in all of the studied cases.

The identified cetons were acetoin, acetol and diacetyl in the wines obtained from Tămăioasă românească. Acetol was also found in wines produced from Grasă de Cotnari and Fetească albă.

Acetone is present in all wines, except those made from Frâncușă. Diacetyl, in small concentrations, can influence positively wine's aroma, having a hazelnut hue. Acetoin enriches in the bouquet of the wine, in the studied wines the compound appeared due to the remanent sugar the wine had at sulphiting time.

The compound 3-methyl-4-heptanone, identified through SPE method in the 2007 Frâncușă, has not been reported by others in wine until now. Molecular mass is 128,21, boiling temperature is 150,47 °C, water solubility is 1371 mg/L, vapour pressure 525 Pa.

It is a known fact that terpenes are found in grapes and wine as free or as connected forms. The free forms have as glycosidic rest glucose, arabinose, rhamnose and apiose, these forms being non-volatile. In general, terpenes do not change during fermentation, although, some grape varieties, that contain β -glycosidases, can set terpenes free from their connected forms in normal wine-making conditions. The final composition of terpenes from the wines depends on the type and time of maceration.

Although the Frâncușă grape variety is not considered an aromatic grape sort, terpenic compounds were identified nonetheless in grape berries and the wines themselves. The grape berries contain myrcene, limonene and β -ocimene, while the wines register α -pinene and β -ocimene. At the same time, a big number of terpenes were identified in the Fetească albă and Grasă de Cotnari grapes, although not in significant quantities. In Fetească albă, a higher concentration of α -pinene and β -ocimene was registered. Grasă de Cotnari shows the highest quantity of α -terpineol. Terpenic compounds were not found in significant quantities. As expected, the grape berries and wines from Tămăioasă Românească had the highest number and biggest quantities of terpenic compounds.

Compared to the findings from the specific international literature concerning the grape varieties taken into study in the thesis, Tămâioasă românească grape variety registered the highest number of terpenes. It can also be noted that the SPE method, as well as the other two are efficient in extracting and identifying the terpenic compounds. This fact, corroborated with the following quantitative analyses can bring precious information on aroma characteristics of the Tămâioasă românească grape variety and wines and not only.

From what it was registered, the cartridge SPE extraction is the most useful in separating lactones. The main identified lactones were butirolactone, pantolactone, γ -caprolactone, δ -nonalactone.

Even if phthalates are not specific to the vine or fermentative processes, in the conditions where the processing of the grapes is done using modern methods, the appearance of this class of compounds is inevitable. In the case of wines, possible contamination sources are plastic bags used in pesticides transport, plastic buckets in which the grapes are harvested, plastic bags for grape harvest.

Diethylphthalate and dimethylphthalate were identified in all analysed samples. Dibutylphthalate and diisobutylphthalate were identified in all wines, except the ones obtained from Fetească albă. It was noticed that in order to identify this class of compounds only ITEX and head-space were useful. The initial extraction done by SPE was not useful as these compounds are not soluble in dichloromethane (used solvent in this case).

The research done within the doctoral thesis lead to the identification of the main volatile compounds from grapes and wines from Cotnari vineyard, obtaining therefore an exhaustive analysis per year of evaluating the volatile compounds from wines.

The degree of novelty brought by this thesis is the use of the three modern technologies of the wine samples, namely solid phase extraction (SPE), head-space (analysis of the vapors at the top of a liquid) and ITEX (extraction and tube concentration - "in tube extraction"). These can be applied in a complementary way in order to identify and quantitative determination of a higher number of compounds.