STUDIES ON THE INFLUENCE OF MACERATION TECHNIQUES ON TĂMÂIOASĂ ROMÂNEASCĂ WINE'S FREE TERPENIC COMPOUNDS

STUDII ASUPRA COMPUŞILOR TERPENICI ÎN VINURILE OBŢINUTE DIN SOIUL TĂMÂIOASĂ ROMÂNEASCĂ PRIN DIFERITE PROCEDEE DE MACERARE

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Abstract: *The volatile compounds in local origin Romanian wines represent an insufficiently researched domain. This study wants to identify aroma substances in Tămâioasă românească wines from Cotnari vineyard, in the north-east of Romania. During 2008 harvest, different maceration technologies were applied to Tamâioasă românească grapes like: classical maceration, cryo-maceration, ultrasound maceration and microwave maceration. A SPE extraction prior to gas-chromatography mass-spectrometry was used to determine individual aroma compounds. As expected, Tamâioasă românească has terpenic compounds that vary in number in quantity according to the used maceration method.*

Key words: terpenes, *Tamâioasă românească, gaschromatography, SPE extraction*

Rezumat: *Studiul propus se referă la testarea unor noi tehnologii de producţie şi impactul lor asupra unor compuşi de aroma din vinuri. Au fost folosite vinuri obţinute din Tămâioasă românească din podgoria Cotnari. Strugurii s-au recoltat la maturare tehnologică, în 2008. Strugurii au fost procesaţi urmând fluxul tehnologic general pentru obţinerea de vinuri aromate, dar accentul a fost pus pe faza de macerare, efectuată atat pe cale clasică, prin macerare pe boştină cât şi aplicând metodele moderne, gen macerare enzimatica, macerare cu microunde, macerare cu ultrasunete, criomacerare. Extractul obţinut prin separare SPE a fost gaz-cromatografiat şi analizat prin spectrometrie de masă. In vinurile de Tămâioasă românească au fost identificate terpene, ce variaza in numar si cantitate in functie de metoda de macerare folosita.*

Cuvinte cheie: terpene, *Tămâioasă românească, gazcromatografie, extractie SPE*

INTRODUCTION

Wine aroma is one of the most important parameters that determine wine character and quality, and it depends on the variety, grape maturity, prefermentative and vinification procedures, yeast activity, and wine aging.

Several authors (Pomohaci et al., 2000, Nechita et al, 2007, Ribérau-Gayon et al., 2000) underline that terpenic compounds play a significant role in varietal wine aroma because of their characteristic fruity-flowery odour. The main

representatives of terpenes in grapes and wine are monoterpene alcohols linalool, geraniol, nerol, citronellol and α-terpineol (Ebeler S.E, 2001**)**. Since the majority of monoterpenes is contained in berry skin (Schreier P., 1979), type and duration of maceration can significantly influence their concentration in must and wine (Radeka S., 2008). In winemaking practice, the most frequently applied are maceration treatments at temperatures from 20 to 25°C, and cold soak maceration with skin contact at 5–8°C. Skin contact at temperatures from 20 to 25°C leads to increased extraction of the phenolic compounds that increase wine astringency and bitterness, and with time oxidize, creating undesirable odours that suppress varietal aroma, as well as browning of must and wine (Cotea V.D., 1985).

On the contrary, a cold soak maceration leads to an increased extraction of aromatic compounds (Radeka S., 2005) from berry skin cells, while the additional undesirable extraction of phenolic fraction is reduced to the highest possible degree. Namely, cold soak low temperatures favour must and wine enrichment with terpenic compounds (Marais J., 1983).

This study wants to identify the terpenic compounds found in Tămâioasă românească wines from Cotnari vineyard, in the north-east of Romania and to underline the effect the type of applied maceration has on the extraction of these volatile compounds.

Three types of categories of monoterpenes exist in grapes with some interrelationships between the categories (Rapp A., 1986).

On the top of the complex are the free aroma compounds, commonly dominated by linalool, geraniol, and nerol, together with the pyran and furan forms of the linalool oxides. However, depending on how the juice has been treated and on factors, which may include climate, many additional monoterpenes can be found in this group, i.e. citronellol, α-terpineol, hotrienol, nerol oxide, myrcenol, the ocimenols plus several other oxides, aldehydes and hydrocarbons. In wines, several monoterpene ethyl ethers and acetate esters have also been found among the free aroma compounds.

Second, there are the polyhydroxylated forms of the monoterpenes, or free odourless polyols. A most significant feature of the polyols is that, although these compounds make no direct contribution to the aroma, some of them are reactive and can break down with great ease to give pleasant and potent volatiles, i.e. diendiol (3,7-dimethylocta-1,5-diene-3,7-diol) can give hotrienol and nerol oxide.

Third, there are the glycosidically conjugated forms of the monoterpenes which also make no direct contribution to the aroma of the grape. Glycosides are, in most cases, more abundant than the unglycosilated forms of individual monoterpenes and polyols (Teisseire P. J., 1991).

MATERIAL AND METHOD

The experimental part of my paper is based on the study and comparison of different maceration techniques of Tămâioasă românească grapes. The obtained wines will be analysed by gas-chromatography and the differentiated identification of terpenic compounds, according to the maceration method applied, will be developed.

Romanian variety Tămâioasă românească from Cotnari vineyard has been used, harvest of 2008. The experimental 100 litres of Tămâioasă românească marc was divided into equal parts to which several maceration techniques were applied: ultrasound maceration, microwave maceration, cryo-maceration and simple skin maceration.

For the ultrasound maceration, marc was ultrasoned for 15 minutes, at 35kHz (2000W, 30 L volume).

Microwave maceration was done at 650 W for 10 minutes. It was observed that the color compounds migrated into the pulp, therefore the grape berry changed color and became less translucid, even opaque. The skin of the grape berry tore, as the internal pressure was too high. Water evaporated and the volume reduced. Microwave maceration was applied to whole grape berries and grape marc.

For cryo-maceration, the grapes were first frozen at -20 ^{o}C, then destemmed, crushed and pressed while still frozen.

Classical maceration was done by allowing must to remain in contact with the berry skins for 12 hours, adding no enyzmes.

The control sample was obtained by pressing the Tămâioasă românească grapes immediately.

Saccharomyces cerevisiae yeast strains were used in order to induce fermentation, which lasted for 2 weeks, at low temperatures of 15 ^{o}C. Enovit, a fermentation activator, was added in the beginning of the second week, to make sure that there was not sugar remaining in the wine, which would lead to a second fermentation. The wine samples were filtered and bottled, not before sulphiting.

SPE extraction gas chromatography method:

50 mL wine were passed through a LiChrolut RP-18 (40-63 µm) 200 mg cartridge and LiChrolut EN (40-120 µm) 100 mg, 6 mL cartridge. The bed was first conditioned by washing it with 10 mL dichloromethane, 10 mL methanol and 10 mL ethanol 13 % v/V solution. After being force dried for 20 minutes, the sample was passed through. The aroma compounds were recuperated by washing the bed with 1,5 mL dichloromethane. 1000 µL extract, splitless mode are injected into the GC. Carrier gas flow (He) 1mL/min; temperature rising from 35^{o}C to 250^{o}C, 5^{o}C/min, 250^{o}C for 27 minutes. Injector temperature 220^{o}C, detector temperature 250^{o}C. Terpenic compounds were identified by comparison of their fragmentation patterns with those of reference compounds in spectrum libraries NIST08, Wiley08 and SZTERP.

RESULTS AND DISCUSSIONS

A Shimadzu GC-2010 gas chromatograph, coupled with a GCMS – QP 2010 Plus mass-spectrometer was used. The identified terpenes and their peak areas are registered in table 1.

Table 1

Terpenic compounds and their peak areas identified in Tămâioasă românească wines obtained through different maceration technologies

TR 2008 control	
Area	Terpenic compound
1729324	Limonene
4027946	Linalool
1136176	Hotrienol
1208369	Linalyl propionate

1635130	3,7-Octadiene-2,6-diol, 2,6-dimethyl-
564206	7-Octene-2,6-diol, 2,6-dimethyl-
1048242	2,7-Octadiene-1,6-diol, 2,6-dimethyl-
TR microwave marc maceration 650 W 2008	
Area	Terpenic compound
3133287	Linalool
767731	Hotrienol
588680	Nerol
1971865	3,7-dimethyl-1,5-octadien-3,7-diol
798632	7-Octene-2,6-diol, 2,6-dimethyl-
TR microwave grape berries maceration 650 W 2008	
Area	Terpenic compound
613285	Linalool oxide
3967532	Linalool
2083529	Hotrienol
639931	trans-Geraniol
814692	3,7-Octadiene-2,6-diol, 2,6-dimethyl-
885509	3,7-Dimethyloct-1-en-3,7-diol
TR simple maceration 12 h 2008	
Area	Terpenic compound
695191	Linalool oxide trans
6260691	Linalool
1246652	Hotrienol
1939399	Linalyl propionate
850190	Citronellol
259104	Trans-Geraniol
1138281	Nerol
3490192	3,7-Octadiene-2,6-diol, 2,6-dimethyl-
1707419	3,7-Dimethyloct-1-en-3,7-diol
651282	Dihydromyrcenol
3087955	2,7-Octadiene-1,6-diol, 2,6-dimethyl-
1132496	Geranic acid
TR cryomaceration 2008	
Area	Terpenic compound
613285	Linalool oxide

3967532	Linalool
2083529	Hotrienol
639931	trans-Geraniol
814692	3,7-Octadiene-2,6-diol, 2,6-dimethyl-
885509	3,7-Dimethyloct-1-en-3,7-diol
TR ultrasounds 15 minutes 2008	
Area	Terpenic compound
3254403	Linalool
821104	Hotrienol
567746	Nerol
2455109	3,7-Octadiene-2,6-diol-2,6-dimethyl-
1175077	3,7-Dimethyloct-1-en-3,7-diol
1429564	2,7-Octadiene-1,6-diol, 2,6-dimethyl-

Tămâioasă românească's analysis confirms its profound aromatic character, by identifying compounds with high aromatic qualities: linalool, linalool oxide cis and trans, limonene, hotrienol, nerol, trans-geraniol, citronellol, dihydromyrcenol, linalool derivates, all terpenic compounds that are specific to aromatic grape varieties, like Tamâioasă românească.

As it can be observed from the above tables, simple maceration for 12 hours extracted the most aroma compounds, even if α – terpineol was not identified in this wine sample. α – terpineol was found in the wine samples obtained by cryomaceration, ultrasounds maceration and grape berries microwave maceration.

Linalool, compound specific for Muscat obtained wines, was, on the other hand, found in all of the six wine samples, as was hotrienol, although in smaller quantities. It was best extracted by simple maceration for 12 hours. The second highest levels were found in the control sample, cryomaceration and grape marc microwave maceration. The lowest extraction levels are in the wine saples obtained by ultrasound maceration and marc microwave maceration.

Hotrienol, another aromatic compound, was also found in all six wine samples, in smaller quantities that linalool. Hotrienol was best extracted by cryomaceration and grape berries microwave maceration, on the second place being found in the wine obtained by simple maceration for 12 hours. Third and fourth place are occupied by the control sample and the wine made through ultrasound maceration and marc microwave maceration.

Other monoterpenes that contribute to the wine's aroma were found in the wine sample obtained by simple maceration for 12 hours: citronellol, dyhidromyrcenol, geranic acid. Limonene appeared in the control wine sample, demonstrating thus that this compound appears in the pulp of the grape as well.

Trans-geraniol, an isomer of nerol, appears in the wines obtained by grape berry microwave maceration, cryomaceration and simple maceration for 12 hours,

having the highest level in the first two samples. Nerol, on the other hand, has been found in the wines made by ultrasounds maceration, simple maceration and marc microwave maceration.

CONCLUSIONS

SPE extraction and GC MS analysis of wines proves to be satisfactory concerning identification of volatile compounds, as known from literature (Ţârdea, 2007). In ulterior studies, the reasearch will comprise quantitative results obtained by using internal standard.

Terpenic compounds proved to be better influenced by simple maceration extraction, for short periods of time.

Although, in literature, Tămâioasă românească's specific terpenic compound is shown to be α – terpineol, this study proved that linalool is found in all of the obtained wine samples.

Considering the fact that the used maceration techniques (ultrasounds, cryomaceration) are new in the wine-making process they do have a certain efficiency in the process of aromatic compounds extraction.

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