STUDIES REGARDING OPTIMISATION OF PRODUCTION TECHNOLOGIES OF FLAVOURED WINES IN IASI VINEYARD

STUDII PRIVIND OPTIMIZAREA TEHNOLOGIEI DE OBTINERE A VINURILOR AROMATE ÎN PODGORIA IAŞI

COLIBABA CINTIA, COTEA V. V., NECHITA B., TUDOSE SANDU-VILLE ST., ZAMFIR C.
University of Agricultural Sciences and Veterinary Medicine Iaşi

Abstract. *This study has the following objectives: testing new production technologies and analyses and control techniques, determining flavour compounds in Romanian wines, minor technological corrections and their impact, online analysis and its influence on aroma evolution, identifying flavour substances in Muscat Ottonel wines and monitoring aroma evolution in wines. For this, three specific yeast types for flavoured wines were used, whereas the witness sample was spiked with yeast specific to non-aromatic wines. In the same way described, two types of enzymes were used. 7 samples were obtained. After a two week fermentation, at maximum 15°C, the wine samples were filtered and bottled. Gas-chromatography mass-spectrometry was used to determine individual terpenic compounds (linalool, geraniol, nerol, citronellol etc).*

Rezumat. *Studiul propus se refera la testarea unor noi tehnologii de productie si tehnici de analiza si control, determinarea unor compusi de aroma din vinurile romanesti, corectii tehnologice minore si impactul lor, analiza online si influenta asupra evolutiei aromei, identificarea substantelor aromate din vinurile de Muscat Ottonel si monitorizarea evolutiei aromelor din vin. Astfel, 100 litrii mustuială Muscat Ottonel au fost însămânțați cu 3 tipuri de levuri specifice vinurilor aromate iar proba martor a fost însămânțată cu levuri specifice vinurilor nearomate. În acelaşi fel, s-au folosit 2 tipuri de enzime specifice obtinerii vinurilor aromate. Astfel, au fost obținute 7 probe care sa sintetizeze tehnologia de macerație clasică. Probele au fermentat pe parcursul a doua săptămâni, la temperaturi scăzute, de maxim 15°C. După ce fermentația s-a încheiat, probele au fost filtrate prin hârtie de filtru şi îmbuteliate. Se utilizează cromatografia în fază gazoasă cuplată cu spectrometria de masa, prin care se determină aromele terpenice individuale (linalool, geraniol, nerol, citronelol etc) din vin.*

Wine's aromatic profile depends on the grape sort, maturity degree at harvest, yeast activity, prefermentative procedures and aging (Ebeler, 2001; Schreirer, 1979). In literature, about 800 aroma compounds in wine are found: alcohols, aldehides, cetones, esters, acids and monoterpenic compounds (Aznar, Lopez, Cacho şi Ferreira, 2001). The aroma compounds responsible for the special flavour of Muscat (Marais, 1983) and Tămâioasa come from the grape's skin, being monoterpenic substances.

MATERIAL AND METHOD

Romanian varieties of Muscat Ottonel from Copou vineyard have been used. Muscat Ottonel, a wine full of flavour, represents one of the attractions of Iasi wine center. Well known as a dry wine, or as demi dry-demi sweet even sweet in exceptional years, Muscat

Ottonel is fine, generous with a persistent remanence and incredibly agreeable. The experimental part of my paper is based on the study and comparison of different maceration techniques of Muscat grapes. The obtained wines will be analysed by gas-chromatography and the differentiated identification of aroma compounds, according to the maceration method applied, will be developed. 100 litres of Muscat Ottonel pomace was spiked with three specific yeast types for flavoured wines (Fermol Aromatic, Fermactive Muscat and Fermol Grand Rouge Nature) whereas the witness sample was spiked with yeast specific to non-aromatic wines (Fermactive AP). In the same way described, two types of enzymes, Zymarome G and Zymoclaire Muscat were used. 7 samples were obtained. The fermentation lasted for 2 weeks, at low temperatures of 15°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 adding SO_2 protection 2-3 mL /bottle.

1) Headspace gas-chromatography method:

Gas chromatography coupled with mass-spectrometry is used to determine aroma compounds in wines (terpen compounds, alcohols, acids, esters, aldehides etc).

The wine sample is introduced in the specific vial; it is thermostated at constant temperature, until equilibrium between the two phases is reached. Part of the gas phase (headspace_ is injected into the column to be analysed. The transfer of the sample can be done manually or automatically. 1000 µL headspace gas is injected, splitless mode. Carrier gas flow (He) 1mL/min; temperature rising from 35°C to 250°C, 5°C/min, 250°C for 2 minutes. Injector temperature 220°C, detector temperature 250°C. Scan detection was done between 30 m/z – 200 m/z (detector sensibility 1,0 kv) and50m/z - 200m/z (detector sensibility 1,1 kV). Headspace working conditions: the sample vial is transferred to the heating compartment, temperature reaches 88°C in 20 minutes. Meanwhile, the two phases (gas and liquid) reach equilibrium. A part of the headspace is transferred by means of an hermetic syringe, at 90°C and injected into the GC. Syringe temperature is 130°C, rotation speed of the container is 500 rpm. Injection speed 1000 µL/s.

2) 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°C to 250°C, 5°C/min, 250°C for 27 minutes. Injector temperature 220°C, detector temperature 250°C. Scan detection was done between 30 m/z – 200 m/z (detector sensibility 1,0 kv) and50m/z - 200m/z (detector sensibility 1,1 kV)

RESULTS AND DISCUSSIONS

A Shimadzu GC-2010 gas chromatograph, coupled with a GCMS – QP 2010 Plus mass-spectrometer was used.

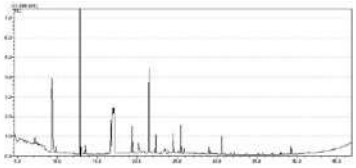

Fig.1. Chromatogram obtained by applying the headspace method

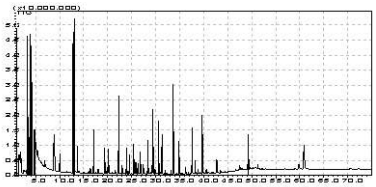

Fig.2. Chromatogram obtained by applying the SPE method

The aroma compounds were determined with the NIST05 spectrum library. An acceptable percentage of probability was considered higher than 70%.

Table 1.

Gas chromatographic analysis – Headspace extraction method

Sample	Mass range	Identified compound	Percentage	Retention time (min.)
M.O., e. G, l. Ro	50mz -200 mz	camfen	97%	7,7
		β-mircen	92%	11,2
		limonen	92%	12,0
		α-pinen	90%	14,03
		cis-2-pinanol	85%	16,10
		linalool	95%	22,34
		hotrienol	87%	23,8
M.O., e. M, l. Ro	50 mz -200 mz	camfen	92%	7,6
		β-mircen	86%	11.16
		limonen	80%	12,03
		α-pinen	85%	14,01
		4-caren	86%	14,80
		nerol	81%	21,35
		linalool	95%	22,35
		borneol	73%	25,16
M.O., e. M, l. Mo	50 mz -200 mz	camfen	85%	7,61
		β-mircen	85%	11,25
		limonen	91%	12,13
		α-pinen	89%	14,07
		β-cis-ocimen	94%	14,15
		cis-2- pinanol	85%	16,21
		nerol	76%	21,35
		linalool	97%	22,35
		isoborneol	73%	25,17

M.O,. e.G, l. Mo	50 mz -200 mz	camfen	98%	7,60
		β-mircen	87%	11,14
		limonen	91%	12,06
		α-pinen	79%	13,52
		cis-2- pinanol	84%	16,19
		linalool	96%	22,34
M.O,. e.M, l. Ar	50 mz -200 mz	camfen	92%	7,6
		β-mircen	78%	11,20
		limonen	88%	12,00
		β-cis-ocimen	91%	14,05
		ocimen	87%	14,85
		cis-Linalool Oxid	73%	19,63
		nerol	70%	21,35
		linalool	95%	22,35
		Bergamoten	72%	29,35
		pivalat limonen-6-ol	73%	34,4
M.O,. e.G, l. Ar	50 mz -200 mz	camfen	97%	7,63
		β-mircen	86%	11,07
		limonen	93%	11,98
		α-pinen	94%	13,98
		cis-2- pinanol	86%	16,17
		α-terpineol	74%	19,50
		nerol	83%	21,30
		linalool	97%	22,33
M.O,. e.M, l. Ap (Witness sample for enzymatic maceration)	50 mz -200 mz	camfen	94%	7,58
		β-mircen	70%	11,19
		limonen	81%	12,10
		α-pinen	84%	14,06
		4-caren	85%	14,85
		cis-2- pinanol	79%	16,21
		nerol	74%	21,36
		linalol	96%	22,33
		borneol	71%	25,16
M.O. Witness (spontaneous maceration, without maceration enzymes)	50-200	camfen	97%	7,62
		limonen	90%	12,12
		3-caren	93%	14,80
		linalol	96%	22,33

Table 2.

Gas chromatographic analysis – SPE extraction method

Sample	Mass range	Identified compound	Percentage	Retention time (min.)
M.O,. e.G, l. Ar	30 mz -200 mz	β-mircen	95%	11,45
		D-limonen	90%	12,35
		Ocimene	87%	14,20
		linalol	95%	21,95
		hotrienol	96%	23,9
		beta-Citronellol	95%	97,5
		Nerol	94%	28,23
M.O,. e.G, l. Ar	50 mz -200 mz	β-mircen	94%	11,43
		limonen	92%	12,42
		β-cis-ocimen	90%	14,18
		linalol	95%	21,93
		hotrienol	96%	23,90
		α - terpineol	94%	25,86
		β-Citronellol	94%	26,47
		nerol	92%	28,20
		Lemonol	95%	29,25

Alcohols: 2-amino-1,3-propandiol, 2-propil-1-heptanol,4-etil-1-octin-3-ol, β-fenoxiethyl alcohol, methanol, 1-butanol, 1-hexanol, 1-pentanol, 1-octanol, 1-octen-3-ol, 2-etil-1-hexanol, 2-etil-1-butanol, 3,7-dimetil-1,6-octadien-3-ol, 3-pentanol, 1-nonanol, 3,7-dimetil-2,6-octadien-1-ol, alcohol benzilic, phenyl ethyl alcohol, indol-3-ethanol, 3-methyl-1-pentanol, 3-hexen-1-ol, 2-Octen-1-ol, benzilic alcohol, 1-dodecanol, isohexil alcohol, 2,3-Butandiol, 1-metoxi-2-butanol.

Acids: nonanoic acid, enantic acid, acetic acid, formic acid, n-decanoic acid, neric acid, p-hidroximandelic acid, pentadecanoic acid, acetic acid, izobutiric acid, hexanoic acid, octanoic acid, butanoic acid, octadecanoic acid

Aldehides: acetaldehide, isovaleric aldehide, phenilglioxal, hidroximetilfurfural, furfural, 2- methyl propanal, 3 methyl butanal, 5-hidroxilmetil-2-furancarboxaldehide, 4-Hidroxi-2-metoxicinamaldehide

Alcans: hexadecane, nonadecane, 2,6,11-trimetildodecane,3-methil pentane

Esters: ester of the phtalic acid, methyl acetate, ethyl acetate, dipropil sulphite, ethyl butirate, acetate 3-methyl 1-butanol (banana flavour), butanoate ethyl, caproate ethyl, caprilate ethyl, isovalerate ethyl, decanoate ethyl, propanoat ethyl, ethyl isobutirate, octanoate ethyl, hexanoate ethyl, propaonate hexil, ethyl hexil benzoate, ethyl isovalerate, isoamil caproate

Cetones: butilacetone, isopropyl phenyl cetone, phenyl methyl cetone

CONCLUSIONS

The following conclusions have been drawn from the analysis of the obtained spectrums:

- Muscat Ottonel has intense aromatic properties, mainly given by terpenic compounds and esters, out of which, the most important is linalool
- Best mass range for identifying aroma compounds is 50mz -200mz
- The compounds identified in my study are confirmed by research done in the field
- Headspace method leads to identification of fewer compounds , with a lower probability percentage
- SPE method identifies more terpenic compounds than the headspace one: butirolactone, citronelol, lemonol
- The headspace method is very much influenced by the matrix, as well as repartition coefficients of the compounds during the liquid and gas phases.
- Zymoclaire Muscat enzyme, during the headspace method, accentuates the terpenic compounds borneol, nerol, bergamoten, pivalat limonen and cis linalol oxide.

REFERENCES

1. Cotea V.D., 1985 - *Tratat de oenologie*, vol. 1. Ed. Ceres, Bucureşti.

2. Pomohaci N., Stoian V., Gheorghita M., Sirghi C., Cotea V.V., Namolosanu I., 2000 - *Oenologie. Volumul 1: Prelucrarea strugurilor şi producerea vinurilor.* Editura Ceres.

3. McNair H., Miller J., 1998 - *Basic Gas Cromatography*. Ed. John Wiley and Sons.

4. Teisseire P. J., 1991 - *Chemie des substances odorants*. Ed. Lavoisier.

5. Ţârdea C., 2007 - *Chimia şi analiza vinului*. Ed Ion Ionescu de la Brad.

6. 2005, *** *Recueil des méthodes internationales d'analyse des vins et de moûts*. Office International de la Vigne et du Vin, Édition Officielle, Paris.