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GC/MS characterization of the volatiles isolated from the wines obtained from the indigenous cultivar *Feteasca Regalã*

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Abstract. To improve the quality of the wine obtained from the indigenous cultivar *Feteasca regalã*, six selected yeast strains were used for wine making at a pilot scale. Their quality was established after routine chemical analysis, organoleptic control and gas chromatographic studies concerning volatile compounds. 52 compounds were identified using gas chromatography/mass spectrometry. Two strains from yeast species *S. cerevisiae var. ellipsoideus* and *S. carlsbergensis* were more valuable than commercial yeast usually used in this region.

Key words. wine – volatiles – gas chromatography/mass spectrometry – yeast – *Feteasca regalã* cultivar.

Introduction

At present there is a tendency in the countries with a developed wine making for selecting yeast strains appropriate for securing the authenticity in the specific climatic and edaphic conditions of the zone [17].

Romania has an old tradition for selection of some valuable yeasts originating from the most famous viticultural regions: Cotnari (for the indigenous cultivars: *Grasã, Fetească albã, Frâncusã*), Iasi (*Fetească neagrã*), Pietroasele (*Grasã, Tãmâioasã românească*), Drãgãsani (*Tãmâioasã românească, Crâmposie, Negru vârtos*), Odobesti (*Galbenã, Tãmâioasã românească*), Dealu Mare (*Pietroasa*), Blaj (*Fetească albã*) [12].

Starting from the necessity of selecting some valuable strains for improving the quality of young wines, the present research has been initiated, beginning with a region where such an investigation has not been performed up to now - the viticultural region Panciu. The aim was to improve the quality of the wine obtained from the indigenous cultivar *Feteasca regalã*.

The assessment of the most valuable strain has been performed by using some routine chemical methods, by gas chromatography/mass spectrometry and by organoleptic analysis. Their combination is absolutely necessary both for establishing the quality and for the identification of the compounds which confer aroma to the wine. The majority of these are found in relatively low concentrations (μg/L) [14] and for this, some techniques for volatile concentration has been used: wine distillation, the solvent extraction followed by extract concentration [9], volatiles concentration by sorbent extraction [8] or the analysis with consacrated systems for volatiles like headspace [10,18,19], purge and trap [7,13,21] or solid phase microextraction [4]. Recently it was also proposed a method of injecting large sample volumes (up to 100 μL) of solvent extracts, without the concentration of the sample [15]. Many of this extraction techniques had already mentioned in some reviews dealing with volatiles constituents in wines [5,6,22].

In our studies we used the solvent extraction method. The organoleptic analysis of the wines has been performed by a commission of specialists, according to a methodology authorized on the national level.

Experimental

Material and method

Based on the natural genetic variability 15 yeast strains have been identified, belonging to the following species S. oviformis (2 strains), S. cerevisiae var. ellipsoideus (3 strains), S. cerevisiae (3 strains), S. carlsbergensis (3 strains), S. italicus (1 strain), S. dairensis (1 strain) and Schizosaccharomyces pombe (2 strains). The prelevation of the biologic material was performed from various locations, from white and red grapes cultivated in the vineyards of Panciu. The identification of new isolated yeast strains was performed by using the classical microbiological methods as describe Lodder (1974) [11] and by multitest system API -20. In order to confirm the taxonomical identification of strains, a molecular technique has been used. The chromosomal DNA profiles were compared by pulsed field electrophoresis. [1-3] The TAFE - Beckman system for vertical gel electrophoresis was used and the chromosomal bands were visualised in U.V. light after ethidium bromide staining. Following some repeated enologic tests only 6 valuable

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strains were selected (S. italicus -symbol T_1; S. cerevisiae var. ellipsoideus - symbol T_2; S. cerevisiae - symbol T_6; S. carlsbergensis -symbol T_3; S. carlsbergensis - symbol T_4; S. carlsbergensis - symbol T_5). The control variant - symbol C, was a grape juice sample fermented with commercial yeasts used in the wine industry. They were used in wine making at a pilot scale from Feteasca regalã, a cultivar much extended in the region of Panciu. Healthy grapes yielded in 1995 have been used for obtaining a grape juice with 178 g/L sugar and an acidity of 7, 8 g/L $C_4H_6O_6$. The climatic conditions of the year were considered normal for wine making. The routine analyses were accomplished: the alcoholic strength, free SO_2, glycerol, the total and volatile acidity, the extract, the total *N*, according to the O.I.V. standards [23]. Volatile compounds were isolated by solvent extraction. 75 ml of wine (containing internal standard IS - 2-ethyl hexanol) were extracted with 15 mL solvent of pentane:dichloromethane (Merck, Darmstadt, Germany) 2:1 in a separation funel. Internal standard solution in absolute ethanol (Merck, Darmstadt, Germany) was previously prepared by adding 20 µL (16.2 mg) of 2-ethyl hexanol to 4 mL of ethanol. From this solution 10 µL were introduced in the wine sample. The solvent extract was placed in a 25 mL round bottom flask, then concentrated to about 1 mL under a gently nitrogen flow, at 25 °C. This 1 mL volume was further concentrated in a 2 mL vial under nitrogen flow to about 100 µL. From this solution 1 mL was injected in splitless injection mode.

Chromatographic conditions

A Carlo Erba GC 8000 gas chromatograph equipped with split/splitless injector was used. The separation of volatiles was achieved on a Supelcowax 10 fused silica capillary column (Supelco, Belefonte, USA) of 60 m × 0.32 mm i.d. × 0.5 µm film thickness. The oven temperature was programmed from 35 °C (5 min) to 250 °C (20 min) with 5 °C/min. Hydrogen was used as carrier gas with an inlet pressure of 60 Kpa. The temperature of the injection port was 250 °C and the detector (FID) temperature was set at 260 °C. For data aquisition a Spectra Physics DP700 integrator was used. Compounds identification was performed on the same capillary column installed in a FISONS INSTRUMENTS MD800 gas chromatograph/mass spectrometer equipped with split/splitless injector, using the same oven temperature program with helium as carrier. Data acquisition was performed with MassLab software for the mass range from 10 to 350 a.m.u. with a scan rate of 1 scan/sec. The ionization energy was set at 70 eV. Recordered spectra were compared with NIST (National Institute for Standardization, USA) and Wiley (6th edition - 220.000 spectra) mass spectral libraries. Compounds identification was confirmed by Kovats retention time indices on Carbowax 20M capillary columns. Standard substances were also used for compound confirmation.

Results and discussion

By performing the routine chemical analyses and the degustation, the obtained results are presented in table I.

It is obvious that the obtained wines are normal, with composition indices that are characteristic to the zone and to the cultivar.

The alcoholic strength is placed around the same value (10.5% vol. alcohol). The total nitrogen content ranges between 1.316 and 1.400 g/L.

As regards the glycerol, its values are placed between 5–6 g/L for the strains T_2, T_3, T_6, and even over 6 g/L (T_1, T_4). The T_5 strain produces a glycerol content under 4 g/L.

The values of the dry extract recommend the wines in the range of the white table ones (15–17 g/L).

A clearer distinction between the yeast strains can be made by taking into account the values of the volatile acidity. As regards this parameter, we found out that the T_2 and T_3 strains produce a superior wine comparing to the control (0.36 mg/L CH_3COOH comparing to 0.4 mg/L CH_3COOH). The rest of the strains are producing a volatile acidity of a value over 0.5 mg/L CH_3COOH.

Table I. The results of the routine chemical analyses and the degustation.

	*Wine identification**						
	T_1	*Control*	T_2	T_3	T_4	T_5	T_6
Total acidity (mg/L)	6.75	6.6	6.3	6.7	6.37	5.92	5.45
Volatile acidity (mg/L CH_3COOH)	0.58	0.4	0.36	0.36	0.5	0.54	0.59
Alcohol % vol as ethanol	10.5	10.5	10.5	10.5	10.5	10.5	10.5
Solid substance (g/L)	16	17	17	16	15	17	14
Glycerol (g/L)	6.67	5.98	5.75	5.80	6.21	3.91	5.75
Total nitrogen content (g/L)	1.316	1.344	1.372	1.330	1.400	1.358	1.372
Grades at degustation	15.6	17.6	18.3	18.8	16.6	16.2	16.8
Classification	VII	III	II	I	VI	V	IV

*The wines received the symbol of the strain used for the grape juice fermentation.

Generally, these values are concordant with the grades obtained by the organoleptic assessment (Place I - T_3; Place II - T_2; Place III - the control).

By using the chromatographic analysis (Fig. 1) 52 compouds were identified and their quantity was evaluated by the method of the internal standard. We point out that this analysis has been used for the first time in order to characterize the aroma of the wine obtained from the indigenous cultivar *Feteasca regalã*. In the table II are presented the identified compounds, the Kovats retention indices and the coresponding concentration calculated according to the formula:

$$C_x = (1000 \text{ mL}/75 \text{ mL})\ m_{IS}\ A_x/A_{IS}$$

where:

C_x – represents the concentration of compound X in µg/L;

m_{IS} = 40.5 µg, is the quantity of internal standard always added in wine before extraction;

A_x – is peak area corresponding for X compound;

A_{IS} – is peak area for internal standard in each chromatogram.

It is noticeable that the T_2 and T_3 strains lead to the obtaining of a wine characterized by superior concentrations of some majoritary compounds resulted by the fermentation (2-pentanone, *n*-propanol, iso-butanol, isoamylic alcohols, ethyl caproate, *n*-amyl alcohol, ethyl lactate, 1-hexanol, ethyl caprylate, gamma butyrolactone, caproic acid, beta phenyl ethyl alcohol, diethyl malonate, caprylic acid, capric acid), by contraries to the wine obtained by the fermentation with other yeasts. Also the ethyl acetate is present in higher concentrations in the T_2 and T_3 wines, as a consequence of the higher biological activity of the yeast strains [20].

The strains T_1, T_4, T_5 and T_6, though are leading by fermentation to superior concentrations of the compounds mentioned before comparing to the control sample, produced also higher concentrations of acetic acid. These concentrations of acetic acid are similar with those determinated by classical titration method (see volatile acidity in Tab. I).

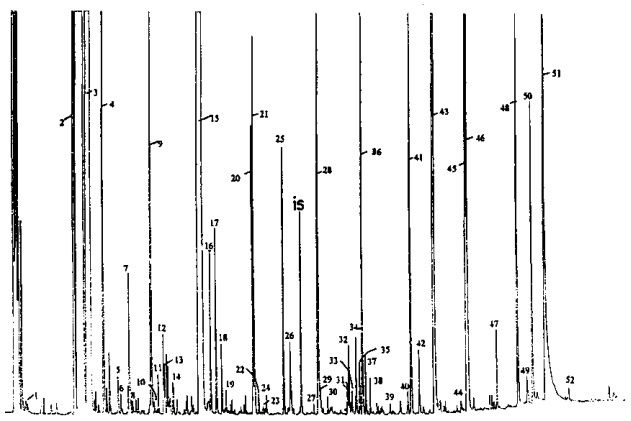

Fig. 1. Typical chromatogram of the volatile compounds isolated from wines obtained from the indigenous cultivar *Feteasca Regalã*. For peak identification see table II.

Among the identified compounds, delta 3-carene, camphor, iso-menthol and alpha terpineole are present as compounds originating in the grape aroma. Their concentration presents small variations, because the monoterpenes are not changed by the yeast metabolism during fermentation and this enables the characterization of wines made from different grape varieties [16]. Acetaldehyde could not be determinated due to its high volatility.

Conclusions

From the 6 selected strains, T_2 (*S. cerevisiae var. ellip - soideus*) and T_3 (*S. carlsbergensis*) proved to be especially valuable, this being validated by the chemical, chromatographic and organoleptic analysis. All these analyses confirmed that different strains from the same yeast family have the same fermentation metabolites, but in different concentrations.

The chromatographic analyses point out that *Feteascã regalã* cultivar has some specific aroma compounds resulted from the grapes. The main compounds issue from the fermentation where the two strains lead to the obtaining of wines of superior qualities comparing to the control sample. However, this that cannot put into value the authenticity of the cultivar in the specific conditions for the Panciu zone.

References

1. Blondin, B.; Vezinhet, F. Identification de souches de levures oenologiques par leurs caryotypes optenus en electrophorese en champ pulse; *Rev. Fr. Oenol.* **1988**, *28*, 7-11.
2. Carle, G. F.; Olson, M. V. An electrophoretic karyotype for yeast. *Proc. Natl. Acad. Sci.* **1985**, *82*, 3756-3760.
3. Coarer, M.; Poulard, A.; Daniel, P. Caractérisation des genres et espèces de levures oenologiques en champ pulse. Compte rendu I.T.V. Oenologie 1990, 1991; pp 165-166.
4. De la Calle Garcia, D.; Magnaghi, S.; Reichenbacher, M.; Danzer, K. *HRC-J. High Resolut. Chromatog.* **1996**, *19*(5), 257-262.
5. Etievant, P. X. *Connaiss. Vigne Vin* **1987**, *21*(4), 247-265.
6. Etievant, P. X.; Maarse, H.; Van den Berg, F. *Chromatographia* **1986,** *21*(7), 379-386.
7. Gassiot-Matas, M.; Comellas-Riera, L.; Rabada-Baiges, J. *Afinidad* **1983,** *40*(385), 207-212,
8. Gelsomini, N.; Capozzi, F.; Faggi, C.; *J. High Resolut. Chromatogr.* **1990**, *13*(5), 352-355,
9. Guntert, M.; Rapp, A.; Takeoka, G. R.; Jennings, W. *Z. Lebensm. Unters Forsch.* **1986,** *182*(3), 200-204.
10. Kallio, H. *J. Chromatogr. Sci.* **1991**, *29*(10), 438-443.
11. Lodder, J. The yeasts, a taxonomic study, North Holland Publising Company, Amsterdam, 1974.
12. Macici, M. *The Romanian wines*, Ed. Academiei, Bucuresti, 1990.
13. Noij, T.; Van-Es, A.; Cramers, C.; Rijks, J.; Dooper, R. *J. High Resolut. Chromatogr. Chromatogr. Commun.* **1987,** *10*(2), 60-66.
14. Rapp, A. *J. Anal. Chem.* **1990,** *337*, 777-785.
15. Rapp, A.; McNamara, K.; Hoffman, A.; Proceedings of 18th International Symposium on Capillary Chromatogr., Riva del Garda (Italy), 20-24 May, 1996.

Table II. The main volatile compounds identified in wines with their Kovats retention time indices and the corresponding concentration (in μg/L).

Compound		*Kovats Retention*	*Compound concentration (μg/L or ppb) Sample index*						
No	*Name*	*Indices*	T_1	*C*	T_2	T_3	T_4	T_5	T_6
1	acetaldehyde	703	–	–	–	–	–	–	–
2	ethyl acetate	854	2270	2209	4270	4340	3153	4105	3757
3	ethanol	908	–	–	–	–	–	–	–
4	2-pentanone	989	1791	1406	1931	1809	1237	1627	1590
5	delta-3-carene	1036	86	102	122	111	92	114	108
6	ethyl butyrate	1052	45	144	72	72	38	60	58
7	n-propanol	1069	255	225	472	358	119	221	241
8	2-methyl 3-buten-2-ol	1075	54	43	65	67	38	41	50
9	iso-butanol	1099	2704	1665	2677	3597	1569	3005	2056
10	tiglic aldehyde	1104	8	10	30	28	21	26	26
11	3-pentanol	1120	76	75	98	84	111	73	76
12	2-pentanol	1135	173	178	217	183	279	164	167
13	isoamyl acetate	1142	127	149	139	151	129	190	155
14	ethylbenzene	1147	88	116	158	144	115	146	141
15	2-methyl and 3-methyl iso-butanol	1210	26393	18376	31724	33206	24117	30617	29454
16	ethyl caproate	1239	292	325	387	358	248	128	341
17	n-amyl alcohol	1259	414	370	462	412	399	369	369
18	styrene	1273	113	140	166	149	123	153	150
19	n-butyl acetate	1285	48	44	64	56	49	64	41
20	ethyl lactate	1367	676	540	725	769	555	620	557
21	1-hexanol	1371	870	620	964	881	639	779	807
22	trans 3-hexen-1-ol	1385	38	43	41	39	28	33	34
23	acetonyldimethylcarbinol	1396	55	1024	31	146	31	981	186
24	cis 3-hexen-1-ol	1404	64	54	59	52	43	48	50
25	ethyl caprylate	1451	701	596	720	678	547	668	683
26	acetic acid	1470	585	397	352	373	480	545	601
IS	2-ethyl hexanol (internal standard)	1505	–	–	–	–	–	–	–
27	ethyl 3-hydroxybutyrate	1542	24	20	23	27	18	14	16
28	camphor	1560	2022	1947	2078	1966	1851	1984	2042
29	ethyl 2-hydroxy 4-methyl pentanoate	1562	90	56	70	70	67	30	73
30	iso-butyric acid	1587	73	49	48	63	36	37	42
31	n-butyric acid	1661	96	98	89	89	47	51	54
32	ethyl decanoate	1678	170	132	164	157	133	118	152
33	iso-menthol	1684	84	79	73	71	73	70	70
34	gamma butyrolactone	1690	210	189	244	295	139	177	259
35	iso-valeric acid	1697	136	175	162	130	88	81	149
36	diethyl succinate	1702	1296	1134	1281	917	840	955	1259
37	ethyl 9-decenoate	1708	186	168	147	136	147	150	149
38	alpha-terpineole	1716	95	83	117	92	91	75	87
39	ethyl 4-hydroxybutyrate	1795	22	49	35	60	16	40	28
40	beta-phenethyl acetate	1862	93	85	86	89	80	75	84
41	caproic acid	1873	1640	1595	2060	2006	1318	1721	1629
42	N-(3-methylbutyl) acetamide	1890	144	136	199	192	135	116	129
43	beta phenyl ethyl alcohol	1926	9139	6766	11263	11357	9354	8544	10742
44	iso-propyl myristate	1964	24	22	36	25	25	23	23
45	diethyl malate	2059	942	952	1202	1293	910	823	1036
46	caprylic acid	2062	4460	4620	4687	4857	3606	4584	4207
47	diethyl 2-hydroxy-pentanedioate	2115	160	201	212	254	149	161	232
48	capric acid	2182	1300	1109	1383	1582	1046	1071	1174
49	ethyl-2-hydroxy-3-phenylpropanoate	2196	78	71	108	110	95	79	97
50	9-decenoic acid	2209	1010	1048	864	890	866	986	852
51	ethyl succinic acid	2231	1813	1529	2464	1329	1433	1226	1983
52	ethyl oleate	2305	45	48	54	64	27	87	38

16. Rapp, A. Wine Analysis, Springer, Berlin, Chapter 3, 1988.
17. Sârghi, C.; Zironi, R. Aspecte inovative ale Enologiei moderne, Sigma, Chisinău Eds., 1994; p 54.
18. Shimoda, M., Shibamoto, T., Noble, A.-C. *J. Agric. Food Chem.* **1993**, *41*(10), 1664-1668.
19. Takeoka, G.; Jennings, W. G. *J. Chromatogr. Sci.* **1984**, *22*(5), 177-184.
20. Usseglio-Tomasset, L. Chimie Oenologique. Technique et Documentation, Lavoisier, 1989; pp 244-247.
21. Uzochukwu, S. V. A.; Balogh, E.; Tucknot, O. G.; Lewis, M. J.; Ngoddy, P. O. *J. Sci. Food Agric.* **1994,** *64*(4), 405-411.
22. Vernin, G. *Parfums, Cosmet Aromes* **1986**, *68*, 83-90.
23. *** *Recueil des méthodes internationales d'analyse des vins et des mouts*, O.I.V., 1990.