HPLC METHODOLOGY IN COMPARATIVE CHARACTERISTICS OF WINE TYPE "SAUVIGNON-BLANC"

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***Abstract**: A lot of different sorts of grapes are used for making wine. Nevertheless wine, made from the same sort of grape have their common characteristic features despite differences in production technology and place and conditions, where the grape was grown. We examined two wines type Sauvignon-Blanc. These samples will be called **Proba 1** and **Proba 2** further. In this article we compare sugars and acids composition, mineral composition and aroma-compounds characteristic for this type of wine.*

1. Introduction

Wine – lat. "vinum" – is an alcoholic beverage, which was produced by full or partial fermentation of grape or fruit and berry juice. Number of different brands of wine is great. How can we correctly assess the wine? Degustation can resolve this problem, which makes full organoleptic evaluation of wine.

But very often degustation doesn't answer the question about natural origin of wine. For answer this question it's necessary to complex study of wine using modern methods of analysis.

The most frequent cases of falsification are:

1. non-controlled using of sugars for changing wine features;
2. replacement of ordinary and aged wines;
3. replacement of sorts of grape;
4. modification cheap ordinary wine with using synthetic compounds, essences;
5. artificial flavoring by natural compounds.

O.I.V. suggests some additional indicates for testing of natural origin of wine, such as: Blarez's ratio – ratio between volume fraction of alcohol ethylic and mass concentration; Fonze-Diacon index – ratio between mass concentration of tartaric acid and potassium and; ratio between mass concentration of potassium and sodium, and other indexes [1,2}.

2. Experimental and Results

Two wines type Sauvignon-Blanc were studied as Proba 1, Proba 2.

The degustation grade is:

Proba 1: year 2009 – 9,06/8,9;

Proba 2: year 2008 – 8,96/8,9.

Mineral composition was examined with Inductively Coupled Plasma Atomic Emission Spectrometer ICPE-9000 Shimadzu. The results are presented in table 1.

Table 1
Mineral composition (mg/L)

Nr.	Cd	Cu	Fe	Hg	Sr	Mg	Mn	Zn
1.	0,06	**0,08**	2,4	<0,01	0,02	26,0	0,82	0,08
2.	0,06	**0,54**	2,8	<0,01	0,02	18,2	0,50	0,18

Nr.	P	S	I(µg/L)	K	Na	Al	B	Ba	Ca
1.	7,8	**70**	15,5	82	32,0	**2,40**	2,00	0,02	11,2
2.	9,0	**58**	21,0	88	22,0	**0,92**	1,06	0,02	12,6

From table 1 it is clear that Proba 1 and Proba 2 don't significantly differ from each other, besides low concentration of cooper in Proba 2 and high concentration of Al and S in the same sample.

Acids composition of the samples was obtained with HPLC. Liquid chromatograph LC-20AD by Shimadzu was used for tests [3].

Chromatography conditions:
Chromatography column: Nucleogel 810H
Eluent: 10mM H_2SO_4 + 10% acetonitrile
Flow rate: 0,5 ml/min
Column temperture: 35^oC
Detector: spectrophotometer 210nm, refractometer
Results are presented in table 2.

Table 2
Content of sugars and organic acid, g/l

Sample name	Glucose/ fructose	Glucose/ fructose ratio	Tartric acid	Malic acid	Succnic acid	Citric acid	Lactic acid	Acetic acid	Tartric / (malic+ lactic) ratio
Proba 1	0,44/0,36	1,22 (norm <1)	2,28	0,61	0,51	0,10	0,05	0,10	3,45 (norm 1,2-2,8)
Proba 2	0,49/0,66	0,74	1,49	0,71	0,37	0,21	0,37	0,05	1,96

It's evident from the results of research that Proba 1 does not conform to such indicates as glucose/fructose ratio, which is less than 1 for white dry wine; and ratio concentration of tartric acid to sum of malic and lactic acids is out of norm for Proba 1.

Aroma compounds of the samples were abtained by GC-MS method. Studies were performed on a gas chromatorgaph GC-MS QP-2010 by Shimadzu.

Chromatography conditions:
Column: 5ms
Detection m/z: 29-350
Libraries : NIST 0.8
FFNSC 1.2
Method of sample injection: head-space SPME.
Tables 3 and 4 show chromatograms of samples Proba1 and Proba 2 apropriately.

From the resulting chromatograms it's clear that component composition and ratio of aroma compounds for both samples differ slightly.

Ciclohexanone 2-(1- mercapto-1-methylethyl)-5-methyl- was found in the sample Proba1 (Sinonime: Mangone; p-Mentha-8-thiol-3-one). This compound is a synthetic fragrance (CAS Number:38462-22-5, EINECS:253-953-1, Transport Information:UN 2810, Risk Codes: R22; R50), grade of perception is 60ng/l. 3MH is an chemical analog of the natural thiols of wine type Sauvignon made by several Chinese companies. These compounds have charge of citrus and fruit tones bouquet.

Table 3. „Aromatic structure". Proba1

Peak Report TIC

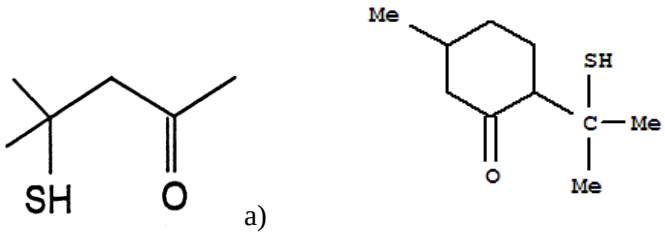
Peak#	R.Time	Area	Area%	Height	A/H	Mark	Name
1	1.514	158819423	25.54	71223366	2.23	MI	Formamide
2	2.078	9116366	1.47	9414495	0.97	MI	Ethyl Acetate
3	2.164	1294353	0.21	1275920	1.01	MI	1-Propanol, 2-methyl-
4	3.159	24695781	3.97	21100864	1.17	MI	1-Butanol, 3-methyl-
5	3.207	4759913	0.77	3539473	1.34	MI	1-Butanol, 2-methyl-
6	4.048	881504	0.14	706193	1.25	MI	Butanoic acid, ethyl ester
7	5.097	1380595	0.22	990620	1.39	MI	1-Hexanol
8	5.210	10845310	1.74	8607456	1.26	MI	1-Butanol, 3-methyl-, acetate
9	5.255	496131	0.08	393091	1.26	MI	1-Butanol, 2-methyl-, acetate
10	7.212	29066641	4.67	22027827	1.32	MI	Hexanoic acid, ethyl ester
11	7.439	5526154	0.89	4140380	1.33	MI	Acetic acid, hexyl ester
12	9.133	2839183	0.46	1681507	1.69	MI	Phenylethyl Alcohol
13	9.951	6571345	1.06	2706221	2.43	MI	Octanoic Acid
14	10.076	529874	0.09	409763	1.29	MI	Butanedioic acid, diethyl ester
15	10.329	203855049	32.79	122485790	1.66	MI	Octanoic acid, ethyl ester
16	11.091	409103	0.07	269832	1.52	MI	Isopentyl hexanoate
17	11.272	781646	0.13	522436	1.50	MI	Acetic acid, 2-phenylethyl ester
18	11.681	412092	0.07	251348	1.65	MI	2(1H)-Naphthalenone, 3,4,4a,5,6,7-hexahydro
19	12.145	175958	0.03	131907	1.34	MI	Decanoic acid, methyl ester
20	12.693	3785798	0.61	2000670	1.89	MI	n-Decanoic acid
21	12.869	143555	0.02	109450	1.32	MI	Cyclohexanone, 2-(1-mercapto-1-methylethyl
22	13.000	9419812	1.52	6918921	1.36	MI	Ethyl 9-decenoate
23	13.110	139603803	22.45	89675961	1.56	MI	Decanoic acid, ethyl ester
24	13.771	645112	0.10	478564	1.34	MI	Octanoic acid, 3-methylbutyl ester
25	15.590	5097004	0.82	3704000	1.38	MI	Dodecanoic acid, ethyl ester
26	15.710	128077	0.02	94567	1.35	MI	Propanoic acid, 2-methyl-, 1-(1,1-dimethyleth
27	15.968	323375	0.05	217966	1.48	MI	Dodecanoic acid, 1-methylethyl ester
28	16.188	149206	0.02	107586	1.38	MI	Pentadecanoic acid, 3-methylbutyl ester
		621752163	100.00	375186174			

Table 4. „Aromatic structure". Proba 2

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	A/H	Mark	Name
1	1.510	155744746	29.10	70467406	2.21	MI	Formamide, N-methoxy-
2	2.076	7761157	1.45	8180632	0.94	MI	Ethyl Acetate
3	2.162	1495653	0.28	1378911	1.09	MI	1-Propanol, 2-methyl-
4	3.157	28436123	5.31	23431950	1.21	MI	1-Butanol, 3-methyl-
5	3.205	6765575	1.26	5404072	1.25	MI	1-Butanol, 2-methyl-, (.+/-.)-
6	4.047	850751	0.16	678744	1.25	MI	Butanoic acid, ethyl ester
7	5.095	1716113	0.32	1204782	1.42	MI	1-Hexanol
8	5.209	13160519	2.46	10338052	1.27	MI	1-Butanol, 3-methyl-, acetate
9	5.254	648966	0.12	510037	1.27	MI	1-Butanol, 2-methyl-, acetate
10	7.212	25113680	4.69	18877748	1.33	MI	Hexanoic acid, ethyl ester
11	7.440	3253661	0.61	2503080	1.30	MI	Acetic acid, hexyl ester
12	9.134	2732641	0.51	1607405	1.70	MI	Phenylethyl Alcohol
13	9.949	6089378	1.14	2697138	2.26	MI	Octanoic Acid
14	10.075	1096880	0.20	774201	1.42	MI	Butanedioic acid, diethyl ester
15	10.329	190491347	35.59	114297871	1.67	MI	Octanoic acid, ethyl ester
16	11.093	323385	0.06	242090	1.34	MI	Isopentyl hexanoate
17	11.271	622421	0.12	434646	1.43	MI	Acetic acid, 2-phenylethyl ester
18	11.682	286013	0.05	176944	1.63	MI	2(1H)-Naphthalenone, 3,4,4a,5,6,7-hexahydro
19	12.146	72488	0.01	54081	1.34	MI	Decanoic acid, methyl ester
20	12.682	2192642	0.41	1226894	1.78	MI	n-Decanoic acid
21	12.857	51205	0.01	43558	1.17	MI	Benzeneethanamine, N-[(pentafluorophenyl)m
22	12.927	203001	0.04	133561	1.52	MI	Undecane, 5-methyl-
23	12.999	2317630	0.43	1659711	1.40	MI	Ethyl 9-decenoate
24	13.106	81318810	15.19	55120372	1.48	MI	Decanoic acid, ethyl ester
25	13.770	574432	0.11	430414	1.33	MI	Octanoic acid, 3-methylbutyl ester
26	15.591	1222820	0.23	874790	1.40	MI	Dodecanoic acid, ethyl ester
27	15.711	557292	0.10	372463	1.50	MI	Propanoic acid, 2-methyl-, 1-(1,1-dimethyleth
28	15.967	82832	0.02	57489	1.44	MI	Dodecanoic acid, 1-methylethyl ester
29	16.188	96591	0.02	70744	1.37	MI	Pentadecanoic acid, 3-methylbutyl ester
		535278752	100.00	323249786			

Natural components which make wine aroma are: 3-mercapto-hexanol (3MH), grade of perception – 60 ng/L; 3-mercapto-hexanol acetat (A3MH), grade of perception – 4 ng/L and 4-mercapto-4methyl-pentanone (4MMP), specific for Sauvignon (grade of perception – 3 ng/L) [4]:



a) b)

Figure 1 .Chemical structure of morcapto compounds specific for Sauvignon:
a) 4-mercapto-4methyl-pentanone (4MMP) (natural compound;
b) Ciclohexanone 2-(1- mercapto-1-methylethyl)-5-methyl- (syntetic compound).

3. Conclusion

From the chemical point of view wine – is the complex multicomponent mixture. We studied two samples, made by different producers from the same type of grape Sauvignon-Blanc. These samples were compared by their mineral composition, and sugar and acids composition.

One of the most important features of wine is its bouquet, which consists of many volatile compounds. Using GC-MS analysis were identified main aroma-components of samples.

For saving the flavor profile of wine it's necessary to reduce the content of quinones and heavy metals (Cu), that can be done by treatment of wine with mixture of PVP (polivinilpirolidon) and PVI (polivinilimidazol).

4. Bibliographie

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