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STUDY OF RED WINE PRODUCED IN THE TUTOVA AREA, ROMANIA

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Abstract. Dark red and very sweet common grape grows in most rural areas in the Tutova Area. From these grapes a dark red wine is made. It is a semi-dry to dry wine with a high content of energizing substances. As this kind of wine is drunk by some 300,000 locals, we undertook to conduct research on this wine. The research contained phytochemical studies to determine the active principles groups constituent of the wine; determination of microelements constituents via atomic absorption spectrophotometry, performed with Wizard application, AA-6200 Shimadzu equipment; determination of resveratrol content, via HPTLC thin-layer chromatography, by means of Wincats application, CAMAG equipment. The findings of the research revealed the presence of flavonoids, vitamins and anti-oxidant products is by far superior to many other kinds of domestic as well as European wines.

Key words: Tutova, common grape, wine, spectrophotometry, chromatography.

1. Introduction

Plantavorel Piatra-Neamt launched a programme aiming at researching red and purple wines produced in various area of Romania with a view to using such wines in preparing tonic beverages with enhanced phytotherapeutic properties. The quality of the wine used in tonic beverages is the only selecting criterion.

Wine is a hydro alcoholic solution, which contains very many substances that differ from the point of view of their chemical structure but having a well-defined role with a well-known qualitative and nutritive value. The phytotherapeutic properties of wine are given by the group of active principles and microelements contained by ripe grapes.

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The currently available literature points to the following groups of active principles and microelements contained by red and purple wines:

1. FLAVONOIDS

Flavonoids are a group of crystalline chemical compounds to be found in plants, discovered in 1936 by the Nobel Prize Laureate Albert Szent Gyorgyi. He later called them vitamin P. Since its discovery, scientists have isolated over 4,000 flavones. Flavonoids are sometimes called *bioflavonoids*, a term often used to describe biologically active flavonoids. Recently, interest in flavonoids has grown significantly a greater attention owing to their impressive antioxidant properties. Some of these compounds seem to be more efficient than common antioxidants, e.g. vitamins C and E via their effect such as protecting low-density lipoprotein (LDL) from oxidation. Research have revealed that bioflavonoids reduce the level of cholesterol, providing further protection against cardiovascular diseases, having antiviral, anticarcinogenic, anti-inflammatory, and anti-histaminic qualities.[2, 10]

Dr C. Kandaswani's and Dr G. Middleton's research proved how efficient some flavonoids are in preventing and treating various cancer forms. [2, 10]

2. POLYPHENOLS

A large class of active principles is represented by phenolic compounds, which are widely spread substances in plant regnum, abundance probably exceeded only by glucidic substances. Phenolic compounds have very different structures whose complexity takes the most unexpected shapes. They appear in plant metabolism ranging from the simplest phenols, e.g. *hydroquinone* or *pirocatehol*, to high molecular weight macromolecules (higher lignin). Polyphenolic compounds in plants may be formed from of one or more free or condensed benzenic cycles or of mixed and heteroclite benzenic cycles.

Phenolic compounds may diversify according to the number of hydrophile groups grafted on the nucleus (plant polyphenols) or by the formation of other functional groups such as metoxiles, carboxiles, aldehyides, ketones, etc.

Phenolic compounds can be found in small quantities in wine [2, 11]. These compounds contribute to defining the organoleptic characteristics, the hygiene-sanitary value, and mainly, the typical characteristics of wines. Due to their bactericide and anti-oxidant properties, phenolic compounds protect especially the colour and the taste of red wines.

The class of phenolic compounds also contains phenolic acids, i.e. organic combinations, whose molecule contains the functional carboxyl (-COOH) and hydroxyl (-OH) group linked to the aromatic nucleus. This category also includes hydroxibenzoic and hydroxicinnamic acids. [11]

Grapes, especially the purple ones, and oak wood contain some more complex polyphenols, which belong to the stilbenic family, whose benzenic cycles are linked by an ethane $–CH_2-CH_2–$ or ethenic $–CH=CH$ bridge. Resveratrol is one of these stilbenic compounds, produced by the grape vine in response to mould attack (Langcake, 1981) and is located in the grape skin, and later extracted while making the wine in red in 1-3 mg/l quantities. The health benefits of resveratrol have been recently revealed. [2,11]

3. ANTHOCYANS

Anthocyans are the pigments that give the red, purple or bluish colour of the flowers, fruit, and seed skin. They are characterised as benzopyril, due to the formation of pyroxane cation. [2]

In red wines, they amount 2,200 – 250 mg/l and such quantities decrease during the first year of storage, to 200 mg/l. [11]

4. AMINO ACIDS

The literature shows that there are 32 amino acids identified in wine. The wine content of amino acids varies largely depending on the species of grapes the wine was made of, the wine making technology, and the micro organisms that performed the various fermenting stages. Amino acids are uniform molecules with a general formula $R-CH(NH_2)-COOH$, where R may be an atom of hydrogen (in the case of glycine, for instance) or hydro carbonate chain. In the case of multifunctional amino acids on R radical, there are other acid-like groups grafted, e.g. (-COOH), basic ($-NH_2$) or neutral (-OH) (serine, tyrosine or thyronine, -SH in cystine methionine). In wines, the total amino acid quantity varies between 0.01-0.2 g/l. [3,10]

5. CARBOHYDRATES

Carbohydrates play a very important role in the taste of wines. Hence, dry wines contain 2-3g/l sugar and are easily felt when tasted. The types of carbohydrates are very important as the tasting impression of wine changes according to such type. Dry wines generally lack glucose whereas fructose, pentose is between 0.2-0.3g/l. [3, 11]

6. VITAMINS AND ENZYMES

The vitamin content of wines is generally low but in terms of quality, they contain all the vitamins necessary to life, playing thus the role of growth factor indispensable to yeast and bacteria.

Among the vitamins found in wines, a sizeable amount is group B vitamins (thiamine, riboflavin, pyridoxine, and mesoinositol), vitamin PP, panthothenic, acid and biotin. [11].

1. Materials and methods

Materials:

- Red wine in the Tutovei area, location: Epureni, Vaslui; wine maker: Vasile Marcu
- Specific reactive substances to determine acids, flavones, total polyphenols, polyphenolcarboxylic acids, amino acids, anthocyans, proanthocyans, vitamins, fructosans, reactive substances listed in Farmacopeea Romana, volume X.

Equipment:

- Atomic Absorption Spectrophotometer AA – 6200 Shimadzu/2006, Wizard application;
- Thin-layer Chromatograph HPTLC CAMAG, Wincats application.

As a result of physical-chemical tests, the main physical and chemical characteristics of wine were determined: dry substance, pH, density, alcohol content, and acidity [5].

The phytochemical studies revealed the main chemical compound groups which form the active principles of wine: flavones [5], polyphenols [1,7], amino acids [6], anthocyans [4], proanthocyans [6], vitamins (vitamin C) [5], fructosans [6], antioxidant activity [7].

The experimental results are given in Table 1.

Table 1. Quantitative tests on the main physical and chemical characteristics of some active principle groups in the dark red (black) Tutova – Epureni, Vaslui wine.

No.	ACTIVE PRINCIPLE	TUTOVA RED WINE (% g/l)
1	Dry substance	2.94
2	pH	3.80
3	Density	0.9952
4	Alcohol level, %, m/V	10.62
5	Acidity	14.6;0.86
6	Flavones expressed as rutoside, g/l	0.017
7	Polyphenols-carboxylic acids, expressed as cafeic acid	0.00199
8	Total polyphenols, expressed as gallic acid	0.056
9	Amino acids, expressed as glutamic acid	0.136
10	Anthocyans expressed as cyanidol chloride	0.106
11	Proanthocyans, expressed as cyanidol chloride	1.42
12	Vitamin C	0.312
13	Total fructosans, expressed as fructose	0.1701
14	Antioxidant activity	77.63

Acidity = mval/l or g/l sulphuric acid, or g/l acetic acid.

The Romanian regulations provide a maximum content of 24 mval/l – 1g H_2SO_4 l/wine for red wines.

Microelements analysis

Microelements tested: K, Na, Ca, Cu, Zn, Fe, Mg, Mn, Co, Pb.

Method applied for the test: Atomic absorption AA -6200 Shimadzu, Wizard program. Experimental results are given in Table 2 and Figure 1, respectively.

Table 2. Experimental results for K element.

No	*Action*	*Sample*	*True Value*	*Conc. (ppm)*	*WF*	*VF*	*DF*	*CF*	*Actual Conc.*	*Actual*	*%RSD*	*SD*
0	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
1	STD 1	10.0000		0.1778							1.5474	0.002
2	STD 2	5.0000		0.1043							3.5645	0.003
3	STD 3	3.0000		0.0642							2.0369	0.001
4	STD 4	1.0000		0.0210							5.8284	0.001
5	RED WINE		4.5661	0.0850	1.0	1.0	250.0	1.0	1141.5	ppm	0.3328	0.000

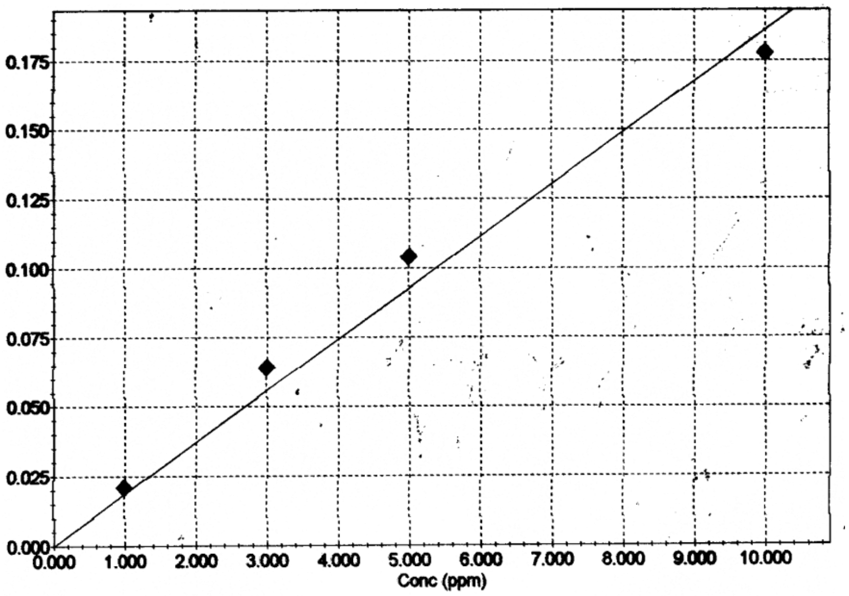

Figure 1. Element K Standard curve.

The other elements were determined in a similar manner.
Results are given in Table 3.

Table 3. Content of wine elements (ppm)

No	*Element*	*True Value*	*Conc. (ppm)*	*WF*	*VF*	*DF*	*CF*	*Actual Conc.*	*Actual*	*%RSD*	*SD*
0	*1*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
1	*Na*	8.1488	0.0093	1.0	1.0	1.0	1.0	8.1488	ppm	0.0000	0.000
2	*Ca*	3.4789	0.4044	1.0	1.0	11.2	1.0	38.963	ppm	1.3289	0.005
3	*Cu*	0.3305	0.0315	1.0	1.0	1.0	1.0	0.3305	ppm	0.0000	0.000
4	*Zn*	-0.0987	-0.0478	1.0	1.0	1.0	1.0	-0.098	ppm	15.2209	0.007
5	*Fe*	1.6921	0.1246	1.0	1.00	1.00	1.0	1.6921	ppm	4.6535	0.005
6	*Mg*	0.1768	0.3463	1.0	1.0	1120	1.0	198.01	ppm	0.5570	0.001
7	*Mn*	1.2967	0.2674	1.0	1.0	1.0	1.0	1.2967	ppm	1.0315	0.002
8	*Co*	1.2967	-0.0020	1.0	1.0	1.0	1.0	1.2967	ppm	1.0315	0.002
9	*Pb*	1.2967	-0.0020	1.0	1.0	1.0	1.0	1.2967	ppm	1.0315	0.002

Determination of resveratrol-content in wine via thin-layer chromatography

Resveratrol phytoalexin was first discovered in grape skin and then in wine.

Resveratrol is a phytoalexin of the antibiotic class produced by some plants as a defence mechanism against infection.

Resveratrol (3,5,4,-trihydroxystilbene) is a polyphenolic phytoalexin. It is a stilbenoid, derivative of stilbene and is produced by plants with the help of stilbene enzyme. There are two isomers: CIS-(Z) and TRANS-(E). The *trans* isomer may be isomerised under the form *cis* when heated or exposed to ultraviolet radiation.[10]

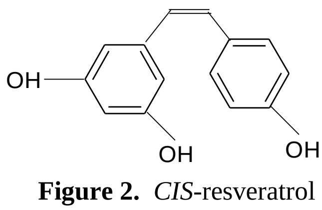

Figure 2. *CIS*-resveratrol

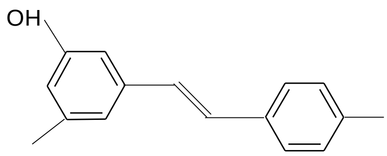

Figure 3. *TRANS*-resveratrol

Resveratrol is found in grapes, wine, grape juice, bilberries, and blackberries.

Phytotherapeutic properties of resveratrol:
- 50 times stronger antioxidant than vitamin E and C together;
- lowers total cholesterol level in blood;

- controls osteoporosis;
- administration of resveratrol increases sirtuin activity, the enzyme which prolongs life by 70-80%.[9]

METHOD APPLIED – HPTLC Chromatography CAMAG/winCATS

Analysis Report .

Method C:/CAMAG/winCATS/Data/ chromatographic photos
Research/Dosage RESVERATROL
- red wine, FIC 25 cme

Validated Design

STATIONARY STAGE

Chromatographic plate (X x Y) 10.0 X 10.0 cm
Material silica gel plate 60 F 254
Manufacturer E. MERCK KGaA

MOBILE STAGE

Solvent: Chloroform: ethyl acetate: formic acid = 25:10:1
Position in solvent 87.0 mm
Solvent volume 36.0 ml
Temperature 21
Time 15 min.
Simple application: CAMAG Linomat IV
Volume syringe used 100 µl
Band layer start at: 12.0 mm
Band width: 8 mm
Space between bands: 9 mm

Table 4. Solutions applied

No.	*Position applied*	*Value applied*	*Name of samples*	*Sample quantity*
0	*1*	*2*	*3*	*4*
1	16.0 mm	2 µl	Resveratrol 1	330 mg
2	33.0 mm	3 µl	Resveratrol 2	500 mg
3	50.0 mm	4 µl	Resveratrol 3	660 mg
4	84.0 mm	12 µl	Red wine 4	

The double developing camera CAMAG 10 cm saturated was used with 30-minute developer.

In Figure 4 the chromatoplate presented, where 5 bands can be noticed, of which bands 1, 2, 3 are resveratrol standards corresponding to the resveratrol quantity applied: 2µl, 3µl, 4µl, and bands 4 and 5 are the red wine samples.
Chromatoplate processing was conducted by means of CAMAG TLC Scanner 3 “Scanner 3” S/N 070823 equipment.
Band length used: 318 nm
Lamp: D2/W
Measuring technique: Absorption

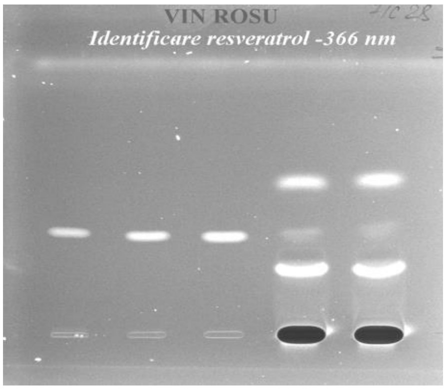

Figure 4 Chromatoplate resveratrol analysis

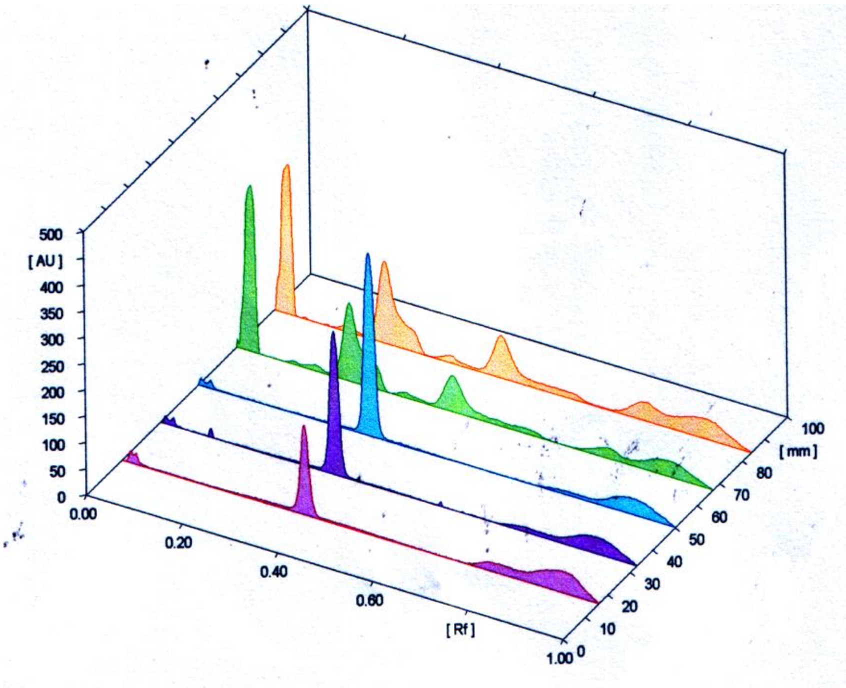

Figure 5. Chromatoplate spatial representation

Figure 5 shows the chromatoplate assessment at 318 nm wave length. In this figure the five bands on the chromatoplate can be seen in space as well as the location of resveratrol at *Rf*=0.33-0.38.

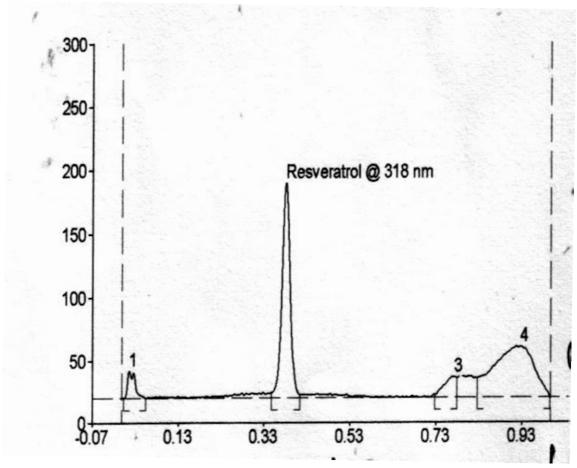

Figure 6. Standard 1 quantity, depending on peak height

Table 5

No.	*Start RF*	*Start Height*	*Max Rf*	*Max Height*	*Max %*	*End Rf*	*End Height*	*Area*	*Area%*	*Assigned substance*
0	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
1	0.00	0.2	0.02	22.2	8.91	0.06	0.3	326.2	4.85	Unknown
2	0.35	4.3	0.38	169.8	68.05	0.42	2.8	2755.9	40.95	Resveratrol
3	0.73	2.7	0.78	16.8	6.72	0.78	16.0	413.1	6.14	Unknown
4	0.83	16.0	0.93	40.7	16.32	1.00	0.5	3234.8	48.07	Unknown

Thus, for band 1, as shown in Table 5 and Figure 6, respectively, resveratrol in Standard 1 is located at the Rf within 0.35 nm start 0.38 nm maximum and ends at 0.429.

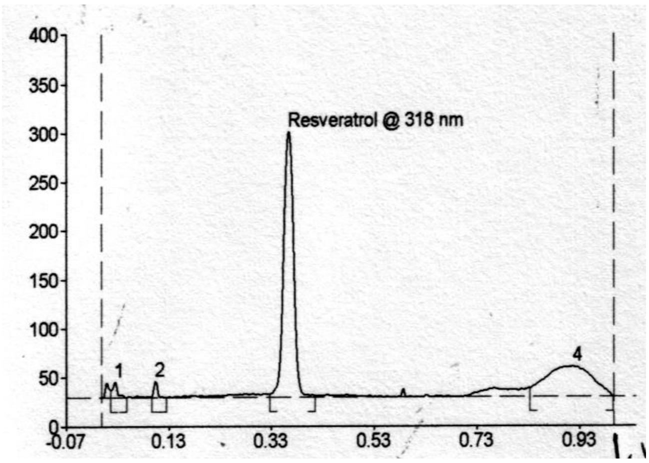

Figure 7. Standard 2 quantity depending on peak height

Table 6

Nr.crt	*Start RF*	*Start Height*	*Max Rf*	*Max Height*	*Max %*	*End Rf*	*End Height*	*Area*	*Area%*	*Assigned substance*
0	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
1	0.02	7.9	0.03	15.7	4.68	0.05	0.4	141.1	1.98	Unknown
2	0.10	0.5	0.11	16.5	4.94	0.13	0.4	91.0	1.28	Unknown
3	0.33	3.8	0.37	271.9	81.15	0.42	1.9	4524.0	63.42	Resveratrol
4	0.84	8.9	0.92	30.9	9.23	1.00	0.2	2377.9	33.33	Unknown

Band 2 represents standard 2 where standard 0.3µl was applied. Resveratrol is located, as shown in Figure 7 and Table 6, respectively, exactly at the same Rf ca as in the standard 1 case.

Band 3 on the chromatoplate represents standard 3 with 0.4 μl applied. Resveratrol, peak 3, as shown in graphic representation, i.e. Figure 8, Table 7, is located at the same Rf. The resveratrol peak height and area increases proportionally with the standard quantity applied. Band 1-2755.9, band 2 -4524.0, band 3 – 6408.8. Peak height: band 1=169.8 mm; band 2=271.9 mm; band 3 =346.3mm, and peak area: band 1=2755.92; band 2=452,404; band 3=6408.78.

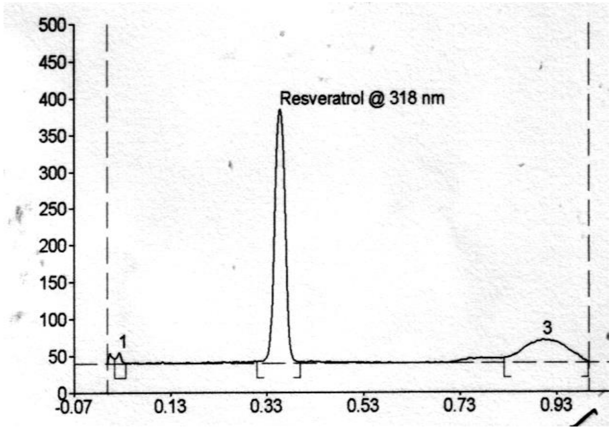

Figure 8. Standard 3 quantity depending on the peak height

Table 7

Nr.crt	*Start RF*	*Start Height*	*Max Rf*	*Max Height*	*Max %*	*End Rf*	*End Height*	*Area*	*Area%*	*Assigned substance*
0	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
1	0.02	7.5	0.03	15.3	3.89	0.04	1.1	136.5	1.52	Unknown
2	0.31	2.9	0.36	346.3	88.14	0.40	2.2	6408.8	71.32	Resveratrol
3	0.82	6.9	0.91	31.3	7.97	1.00	0.7	2440.1	27.16	Unknown

Band 5 on the chromatoplate represents the wine sample applied. In Figure 9 and Table 8, respectively, resveratrol peak 3 can be noticed to be located at Rf – start 0.33 – Rf-maximum 0.37 and ends at Rf=0,42. Still in this table, it can be noticed that peak area 5 is of 413.5 representing 2.22% of the total peak areas.

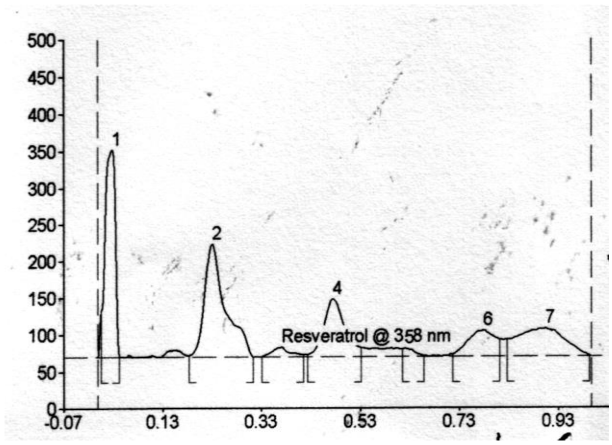

Figure 9. Sample Resveratrol depending on height

Table 8

No.	*Start RF*	*Start Height*	*Max Rf*	*Max Height*	*Max %*	*End Rf*	*End Height*	*Area*	*Area%*	*Assigned substance*
0	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
1	0.01	63.1	0.03	282.9	46.04	0.04	1.5	4983.1	26.73	Unknown
2	0.18	2.8	0.23	153.8	25.03	0.32	0.3	5445.8	29.22	Unknown
3	0.33	0.8	0.37	13.2	2.15	0.42	3.1	413.5	2.22	Resveratrol
4	0.42	3.8	0.48	79.2	12.90	0.53	12.6	2835.4	15.21	Unknown
5	0.62	10.6	0.62	11.3	1.83	0.66	1.2	220.8	1.18	Unknown
6	0.72	3.3	0.78	35.8	5.83	0.81	23.5	1637.4	8.78	Unknown
7	0.83	23.2	0.91	38.2	6.22	1.00	2.0	3104.0	16.65	Unknown

Assessing each band sequences

Table 9 shows the resveratrol sequences on each band in Tables 5, 6, 7, and 8. Rf, the maximum height of resveratrol peaks, the maximum area of resveratrol peaks, and the sample quantity used.

The graphic representation of the simple quantity depending on resveratrol peak height, Figure 10, and the sample quantity depending of peak areas, Figure 11, reveal a linear curve regression against the peak height and against the peak area:

$Y=-3.355+0.5356* X$; $r=0.99732$ $sdv=3.49$

$Y = -931.4+11.06* X$; $r=0.99935$ $sdv =2.03$

Table 9

No	*Track*	*Rf*	*Amount*	*height*	*X(calc)*	*Area*	*X(calc)*	*Sample*
0	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
1	1	0.38	330.00 ng	169.76	-	2755.92	-	-
2	2	0.37	500.00 ng	271.91	-	4524.04	-	-
3	3	0.36	660.00 ng	346.27	-	6408.78	-	-
5	5	0.37	-	-	30.98 ng	-	121.57	Red wine

The resveratrol found and calculated in red wine is 30.98 ng as compared to peak height and 121.57 ng against the peak area.

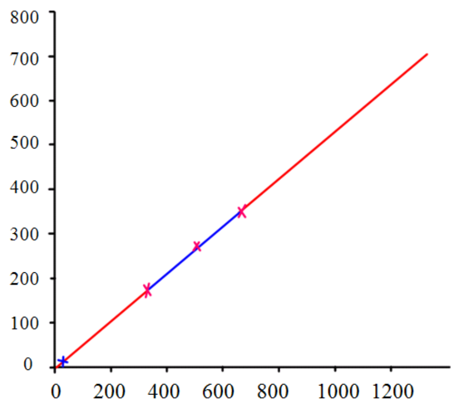

Figure 10 Standard quantity depending on peak height

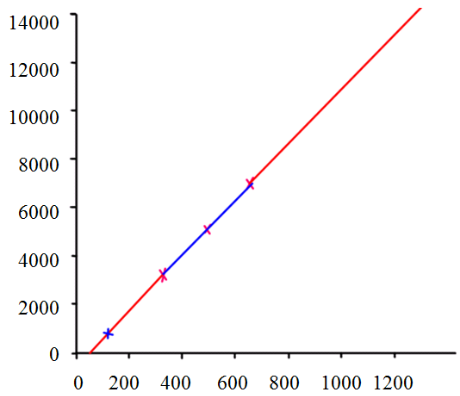

Figure 11 Standard quantity depending on peak area

Conclusions

The Tutova wine analysis revealed that it does not contain heavy metals, but it contains large amounts of K, Ca, and Fe. All the other metals are below legal limits.

Anthocyans and proanthocyans are within the limits set by wine quality regulations.

There is a significant quantity of vitamin C, hence its remarkable antioxidant activity, i.e. 77.63%.

In the wine under study, even after the age of one year, there is some 2.5 mg/l resveratrol, which allowed out to infer that the resveratrol content is higher in fresh wine.

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