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NATURAL OR SYNTHETIC SWEETENERS, SOURCE OF WINE ADULTERATION I. STUDIES ON MEDIUM-SWEET WINE ADULTERATION BY ADDING NATURAL SUGARS TO MARKETABLE WINES

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ABSTRACT – Medium sweet and sweet wines are obtained following a series of technological processes. By deviating from these processes and using various practices against the laws in force, sweet wines are likely to be adulterated. The detection of sweet wine adulteration is achieved by chemical polarimetric methods as well as by the TLC-Thin Layer Chromatography method. From the above mentioned methods we chose to detect natural sugars by TLC-Thin Layer Chromatography. In this paper we present the results of a case study on a lot of 16 samples, bought from the supermarket, composed of DOC – CMD medium sweet wines and table wines, bulk wine and wines in PET bottles. The objective of this study was to identify the flaws of the method for the detection of wine adulteration, and to find adulterated medium sweet wines on the market. Therefore, among the 16 studied wine samples we found two counterfeited medium sweet wine samples, in particular a wine obtained by adding sucrose, pointed out by a spot with an Rf of 0.125, specific for the sucrose standard, and a medium sweet wine obtained by adding an unidentified natural sugar source, with Rf of 0.67.

Key words: Adulterations; Sweetener; Sucrose; Fructose; Glucose.

REZUMAT - Îndulcitorii naturali sau sintetici, sursă de falsificare a vinurilor I. Cercetări privind depistarea falsificării vinurilor demidulci prin adaos de zaharuri naturale în vinurile comercializate. Vinurile demidulci şi dulci se obțin prin respectarea unor operațiuni tehnologice. Prin abateri de la aceste operațiuni, folosind diferite practici nepermise de legislația în vigoare, vinurile dulci pot fi falsificate. Depistarea falsificării vinurilor dulci se poate efectua prin metode chimice, polarimetrice, dar şi prin metoda TLC-Thin Layer Chromatography. Dintre metodele menționate s-a ales pentru identificarea zaharurilor naturale metoda TLC-Thin Layer Chromatography. În lucrare sunt prezentate rezultatele cercetărilor privind un studiu de caz, efectuat pe un lot de 16 probe, procurate din comerț, reprezentat de vinuri demidulci DOC – CMD şi vinuri de masă,

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comercializate vrac şi îmbuteliate în PET-uri. În acest studiu s-a urmărit sensibilitatea metodei de analiză în depistarea falsificării vinurilor, precum şi identificarea pe piața de consum a vinurilor demidulci falsificate. Astfel, din cele 16 probe de vinuri analizate, au fost depistate două probe de vin demidulci falsificate, şi anume un vin obținut prin adaus de zaharoză, evidențiat printr-un spot cu Rf-ul de 0,125, caracteristic etalonului de zaharoză, şi un vin demidulce, obținut prin adausul unei surse de zahăr natural neidentificată, cu Rf-ul de 0,67.

Cuvinte cheie: falsuri; îndulcitori; zaharoză; fructoză; glucoză

INTRODUCTION

The practices of counterfeiting wine composition, by using illegal substances, have lately become quite wide spread (Mihalca and Iancu, 2002). Counterfeiters employ these substances because of the low costs, are easy to find and facilitate the achievement of the target desired (Năməloşanu and Antoce, 2005). The progress achieved in the field of chemistry, thanks to the state of the art equipment of the laboratory, determined the performance of wine composition assays under accurate and reproducible conditions which enable adulteration detection (Bulancea and Râșcanu, 2009; Măruțoiu *et al.,* 2005; Pomohaci *et al.,* 2000; Țârdea, 2007; Țârdea *et al*., 2010).

In 2006 the sectorial project financed by the Ministry of Agriculture and Rural Development whose objective was to lay down the methods for determining adulterated products from wine industry, reassessed the classical and modern methods for the detection of adulterated wines. The project aimed at finding the addition of water into wine (dilution), the detection of alcohol addition of wine origin or industrial alcohol, the detection of natural or synthetic sweeteners as well as the detection of colouring agents and artificial flavours (Ranca *et al.,* 2008).

In this paper we present the results obtained by the researchers from Research and Development Institute for Viticulture and Wine making in the case study on the detection of sweet wine adulteration by adding natural sweeteners.

MATERIALS AND METHODS

Sucrose, fructose and glucose were highlighted by means of Thin Layer Chromatography method, employing TLC 60254 silica gel plates. The studied wine samples were processed according to the method for determining the reducing sugars (glucose and fructose), presented in the Compendium of International Methods of Analysis of Wine and Musts – OIV. The mix of solvents used in the migration tank was prepared from 60 mL ethyl acetate, 30 mL isopropyl alcohol and 5 mL distilled water. The control solutions of natural sweeteners had concentrations of 0.5% sucrose, 35% glucose and 35% fructose. For highlighting the spots of sweeteners, the chromatographic plates were sprayed with a developer mix, composed of 5 g urea, 20 mL HCl 2N and ethylic alcohol up to 100 mL. The spots of sucrose and fructose were coloured in blue and those of glucose in light green.

RESULTS AND DISCUSSION

The samples of wine purchased from the supermarkets were grouped together for performing various studies, according to the data shown in *Table 1*.

The wine samples were studied from chemical and organoleptic point of view. The results of the studies conducted were shown in *Table 2.* Thus we observed that wines from the DOC – CMD category showed the physical-chemical parameters specific for natural white wines.

Table 1 - Wines purchased from supermarket

Sample	Mentions made on the label by the supplier			Volume	Package
	Type of wine	Grape variety	Alcohol, %		
Sample 1	Medium sweet white wine (DOC – CMD)	Fetească albă	11.0 vol %	0.75 L	bottle
Sample 2	Medium sweet white wine (DOC – CMD)	Muscat Ottonel	11.5 vol %	0.75 L	bottle
Sample 3	Medium sweet white wine (DOC – CMD)	Muscat Ottonel	12.0 vol %	0.75 L	bottle
Sample 4	Medium sweet white wine (DOC – CMD)	Grasă de Cotnari	11.0 vol %	0.75 L	bottle
Sample 5	Medium sweet bulk white wine (table wine)	-	-	-	-
Sample 6	Medium sweet bulk white wine (table wine)	-	-	-	-
Sample 7	Medium sweet bulk red wine (table wine)	-	-	-	-
Sample 8	Medium sweet white wine (table wine)	-	9.0 vol %	2.0 L	PET
Sample 9	Medium sweet white wine (table wine)	-	10.0 vol %	2.0 L	PET
Sample 10	Medium sweet white wine (table wine)	-	9.5 vol %	2.0 L	PET
Sample 11	Medium sweet white wine (table wine)	-	9.0 vol %	2.0 L	PET
Sample 12	Medium sweet white wine (table wine)	-	9.0 vol %	1.5 L	PET
Sample 13	Medium sweet white wine (table wine)	-	9.0 vol %	2.0 L	PET
Sample 14	Medium sweet white wine (table wine)	-	9.0 vol %	2.0 L	PET
Sample 15	Medium sweet white wine (table wine)	-	8.5 vol %	1.0 L	PET
Sample 16	Medium sweet red wine (table wine)	-	9.0 vol %	2.0 L	PET

Table 2 - Composition features of the wines purchased

Sample	Dose of alcohol, vol %	Total acidity, g/L H_2SO_4	Volatile acidity, g/L CH_3COOH	Non-reducing extract, g/L	Sugars, g/L	SO_2 free, mg/L	SO_2 total, mg/L	Glycerol, g/L	Ash, g/L	Polyphenol, g/L	Dry matter, g/L	Organoleptic testing, notes
Sample 1	12.6	3.6	0.44	23.2	21.0	20	160	11.0	1.56	0.117	43.0	19.67
Sample 2	11.4	4.2	0.45	23.9	11.8	22	128	8.3	2.18	0.237	34.4	19.67
Sample 3	12.0	3.8	0.48	24.6	31.5	16	130	7.47	2.11	0.165	54.9	18.67
Sample 4	11.3	3.5	0.54	21.8	40.0	14	146	7.70	1.92	0.277	59.8	19.00
Sample 5	9.5	3.4	0.71	22.8	31.3	-	56	7.36	1.92	0.177	49.2	17.00
Sample 6	9.4	3.4	0.42	19.6	9.8	27	129	5.75	1.70	0.449	25.1	18.33
Sample 7	11.1	3.2	0.72	22.2	19.4	7	46	6.44	2.11	0.834	38.44	17.33
Sample 8	9.0	2.4	0.57	20.8	9.8	30	76	7.13	1.63	0.086	30.34	15.67
Sample 9	10.0	3.0	0.44	20.4	21.4	25	84	9.66	1.79	0.167	37.9	14.67
Sample 10	9.6	3.3	0.68	27.1	13.3	19	7.9	7.13	1.44	0.084	34.94	16.33
Sample 11	9.0	2.8	0.50	17.4	18.0	19	50	3.91	1.48	0.119	33.76	15.67
Sample 12	9.2	3.1	0.47	20.1	14.5	21	125	8.74	1.82	0.106	34.2	16.33
Sample 13	9.0	2.7	0.69	29.8	12.6	25	117	6.67	2.11	0.104	29.19	16.00
Sample 14	9.1	3.1	0.68	16.9	11.8	29	122	6.44	1.51	0.100	27.97	16.33
Sample 15	8.6	2.9	0.39	18.3	11.9	24	40	10.3	1.62	0.353	30.59	15.67
Sample 16	9.5	3.4	0.71	2.8	31.3	-	56	7.36	1.98	0.177	49.2	15.00

The bulk wine samples 5 and 7 showed the chemical parameters characteristic for natural wine, but these were not accurately stabilized, which is why the values of volatile acidity were quite high, respectively 0.71 and 0.72.

The bulk wine sample from the 6th position corresponded to the physical chemical parameters of a natural wine, except for the big difference between the total non-reducing extract and the dry matter. This aspect was also noticed in the case of wine samples 10, 13 and 16.

As far as alcohol concentration written on the label is concerned, the wine samples 12, 15 and 16 did not correspond to the wine sample study. In the case of 12 and 16 wine samples the alcohol concentrations determined were much higher than the ones mentioned on the label. However, in the case of wine sample 15, the concentration of alcohol was 0.4 vol. % lower compared to the concentration on the label. These inconsistencies raised suspicion of alcohol adulteration in the studied wine samples.

Moreover, we also found some inconsistencies in the case of glycerol concentration. Thus, in the case of samples 9, 12 and 15, the values of the ratio glycerol /alcohol g/L were higher than the allowed mean for natural wines that is 10.9%, 12.09% and 14.98%, which led to the suspicion of glycerol addition.

Table 3 - Chromatograms for samples of wine on the market to detect natural sweeteners

Wine sample	Thin layer chromatographic analysis	Rf value sucrose	Rf value glucose	Rf value fructose
1	Blue spot	-	-	0.28
2	Blue spot	-	-	0.28
3	Blue spot	-	-	0.28
4	Blue spot	-	-	0.28
5	Blue spot	-	-	0.28
6	Blue spot	-	-	0.28
7	Blue spot	-	-	0.28
8	Blue spot Spot unidentified green	-	-	0.28 **0.47**
9	Blue spot	-	-	0.28
10	Blue spot	-	-	0.28
11	Blue spot	-	-	0.28
12	Blue spot Light green spot	-	0.125	0.28
13	Blue spot	-	-	0.28
14	Blue spot	-	-	0.28
15	Blue spot	-	-	0.28
16	Blue spot	-	-	0.28

The sensorial study conducted by means of comparison using different point scales (method B from STAS 12656-88), comprised the granting of grades between 18.67 and 19.67 to wines from DOC - CMD category, grades between 17.00 and 18.33 to bulk wines and smaller grades between 14.67 and 16.33 to wines in PET bottles, as these ones have low grape extract, are watery, astringent and lack specificity.

The next stage was the study of medium sweet wines by Thin Layer Chromatography (TLC), for detecting natural sweeteners. The results and chromatograms obtained are shown in *Table 3 and Fig. 1 and 2.*

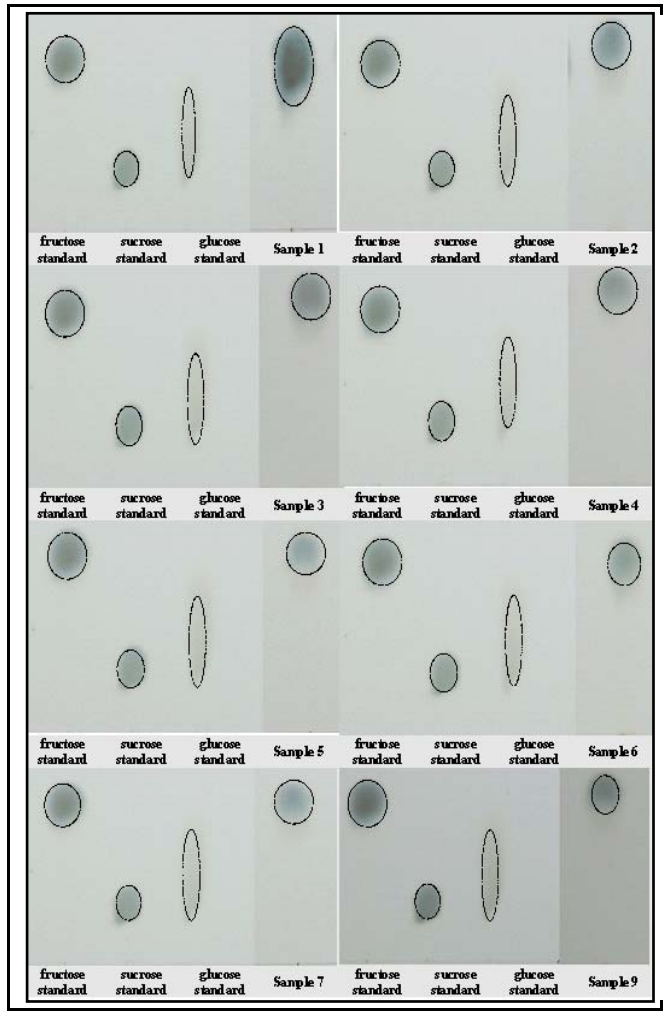

Figure 1 – Chromatograms for wine samples 1, 2, 3, 4, 5, 6, 7 and 9 compared to the sucrose, glucose and fructose standard

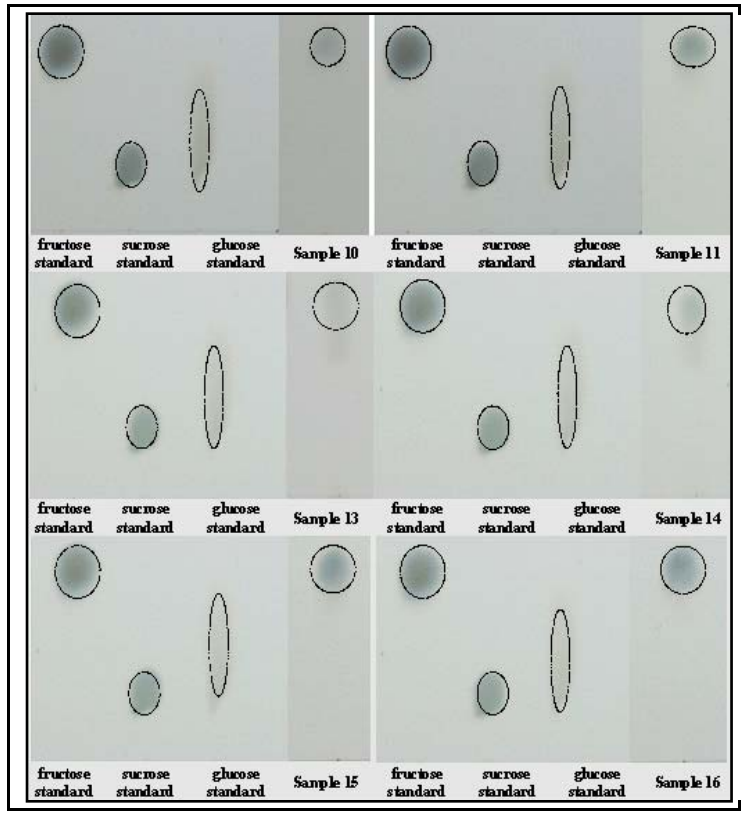

Figure 2 – Chromatograms for wine samples 10, 11, 13, 14, 15 and 16 compared to the sucrose, glucose and fructose standard

The data showed in *Table 3* indicate the fact that all the studied medium sweet wines had chromatograms with blue-colour spots, more or less intense, with Rf of 0.28, specific for the fructose standard.

In the chromatograms of wine samples 8 and 12, showed in *Fig. 3*, besides the fructose spot, other two spots were found, in particular, in sample 8 an unidentified spot with the Rf of 0.47, and in the wine sample 12, a light green spot with Rf of 0.125, specific to the glucose standard. Thus, it is likely that a medium dry wine was used as base for sample 8, because of the fructose spot identified in natural wines to which a sweetener was added, highlighted by the spot with 0.47 Rf.

In the case of sample 12, the presence of glucose spot besides the one of fructose, triggers the conclusion that the medium sweet wine sold was obtained from dry wine to which sucrose was added.

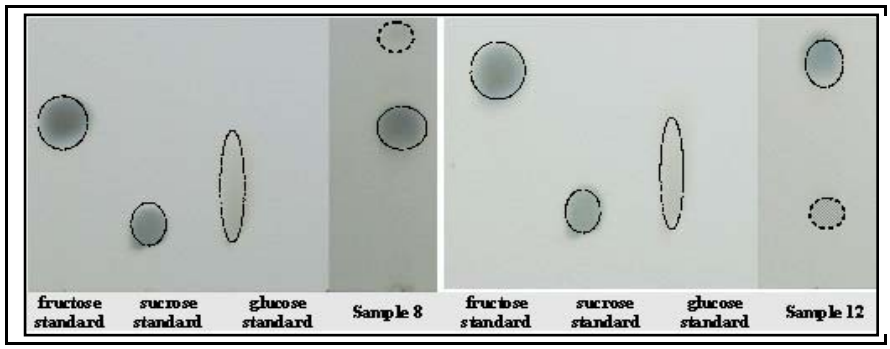

Figure 3 – Chromatograms for wine samples 8 and 12 compared to the sucrose, glucose and fructose standard

This possibility of counterfeiting medium dry and medium sweet wines may be proved by the following experiment. We took three microsamples from a dry wine to which we added sucrose with concentration of 4 g/L, 35 g/L and 67 g/L.

The chromatographic assay was conducted immediately after the addition of sucrose (T^0) and at lengths of time of 30 and 60 days. In the *Table 4 and Fig. 4, 5 and 6* we show the results obtained in this assay.

According to the chromatogram from *Fig. 4*, at T^0, all adulterated wine micro-samples showed only spots of sucrose, with Rf of 0.11, equal to that of the sucrose control specimen.

Table 4 - Analysis of chromatograms obtained in the adulteration of dry wine by sweetening with sucrose

Wine sample	Thin layer chromatographic analysis	Length of time								
		T^o			30 days			60 days		
		sucrose	glucose	fructose	sucrose	glucose	fructose	sucrose	glucose	fructose
Medium dry wine	Blue spot	0.11	-	-	-	-	0.28	-	-	0.28
Medium sweet wine	Blue spot	0.11	-	-	-	-	0.28	-	-	0.28
Sweet wine	Blue spot	0.11	-	-	-	-	0.28	-	-	0.28
	Light green spot	-	-	-	-	0.125	-	-	-	-

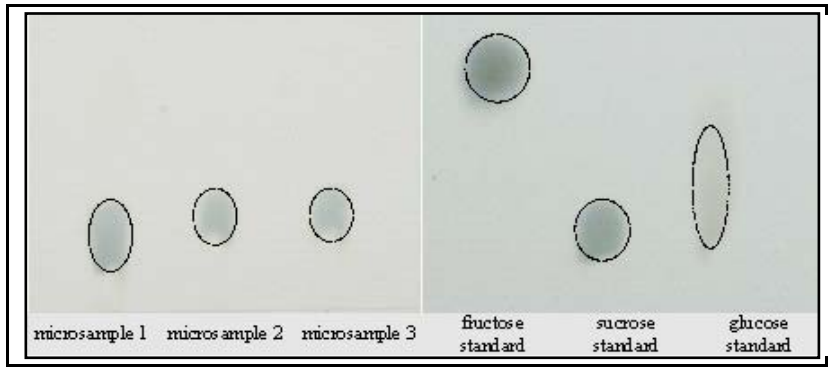

Figure 4 – Chromatogram of adulterated wine microsamples by sweetening with sucrose, by analogy with sucrose, glucose and fructose standard at T^0

After 30 days, we observed the spots of fructose in the adulterated microsamples with addition of 4 g/L and respectively 35 g/L, and in the case of the adulterated wine microsample with 67 g/L sucrose we noticed besides the fructose spot with Rf of 0.28, a glucose spot with Rf value of 125 (chromatogram in *Fig. 5*).

După 60 de zile, în toate microprobele de vin falsificate s-au identificat numai spoturi de fructoză, cu Rf-ul de 0,28 (cromatogram in *Fig. 6*).

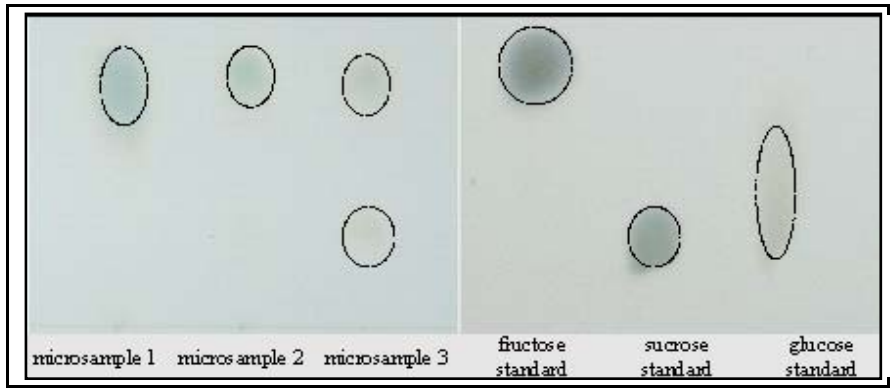

Figure 5 – Chromatogram of adulterated wine microsamples by sweetening with sucrose, by analogy with sucrose, glucose and fructose control samples after 30 days

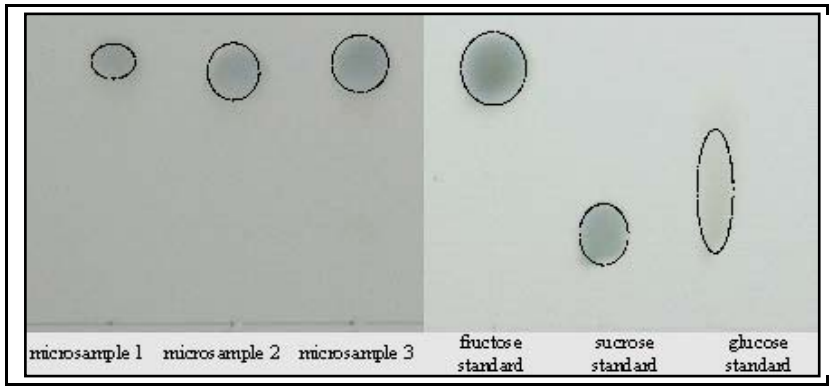

Figure 6 – Chromatogram of adulterated wine microsamples by sweetening with sucrose, by analogy with sucrose, glucose and fructose control samples after 60 days

CONCLUSIONS

The adulteration of sweet table wines may be performed by natural sweeteners (sucrose), using a dry wine as basis and raw material, which in time, due to the invertase from wine, converts sucrose into fructose and glucose. Disloyal commercial operators are selling large amounts of adulterated wines because of the convenient prices and are unfair competitors in relation to the manufacturers of natural wines who comply with the laws in force.

To discourage these practices it is necessary to perform isotope analysis of $^{18}C/^{16}C$, $^{13}O/^{12}O$ and D/H, that also provides the opportunity of information on the origin of non-wine sugars (beetroot, sugar cane etc.).

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