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UTILIZATION OF VITICULTURE WASTES (RESIDUES) – LEAVES AND CANS

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ABSTRACT

Agricultural wastes (and especially those from viticulture) are a largely ignored source of high-value phytochemicals and high value-added industrial products. Experiments for extraction of valuable components from vineyard residues (leaves and cans) were carried out. The extraction was conducted with ethanol-in-water solutions with different concentrations (13, 20 and 40 % vol) at temperatures of 25, 40 and 60^{0}C. It was found that the solvent concentration, as well as the temperature, influence differently both raw materials studied. The leaves have about 3 times higher TPC than the cans. The concentration of the ethanol in the extracting solution also plays an important role concerning the TPC – the best results are obtained with concentration of 40 % vol. The antioxidant capacity of the leaves' extracts is practically not influenced by the extragent concentration. For the cans' extracts the AOC is affected by the ethanol concentration – the best results obtained for 40 % ethanol-in-water solutions. For the leaves'extracts, the TPC values are highest for the lowest temperature. On the contrary, for the cans the temperature increase leads to higher values of TPC for the highest temperature of 60^{0}C. A possible explanation of the fact observed is their different texture leading to different diffusivities. The total polyphenols content and antioxidant activity values found are quite high and characterize the vineyard wastes as a really promising source for food, pharmaceutical and cosmetic industries.

Keywords: *vineyard residues, polyphenols, antioxidant capacity, flavonoids.*

INTRODUCTION

Wine production is among the most important agricultural activities throughout the world [1]. The most competitive wineproducing countries are the United States, Australia and Chile, followed by Italy, Spain, Argentina and South Africa, with France and Germany also producing important quantities of wine [2]. Viticulture as well as winemaking, generate different residues: leaves, cans, grape stalks, lees, etc. The agricultural wastes are a largely ignored source of high-value phytochemicals and value-added industrial products that could contribute to sustainability objectives [3]. The global wine and table grape industry, with annual sales of more than 100 billion US$, generates large quantities of waste that is generally composted or burned for disposal every year [4]. In some European countries, *Vitis vinifera* leaves have been traditionally used as food, both in fresh and brined forms [5]. In Turkey, different kinds of brines are prepared to preserve leaves for future use [6]. These leaves exhibit beneficial to human health effects: they are used to treat hypertension, diarrhea, hemorrhage and varicose veins, inflammatory disorders and to reduce blood glucose levels in diabetics. Leaves of *V.vinifera* have also shown hepatoprotective effect on acetaminophen induced hepatic DNA damage [5]. All these effects can be explained with the content of phenolics, vitamins, resveratrol and other valuable components. Bulgaria is also well known for viticulture and wine production. The important quantities of the residues resting after gathering the crops could find a potential use as a source of valuable compounds with antioxidant activity.

The aim of this study is to evaluate the phenolics content and antioxidant capacity in leaves and branches of grape residues of the cultivar Muscat of Hambourg from the Sofia country region.

EXPERIMENTAL

Materials and methods

For the experiments the leaves and branches of the Muscat of Hambourg, gathered from a local grower during the autumn of 2012 were used. They were dried at ambient temperature and grinded. After that they were extracted with ethanol-in-water solutions with different concentrations: 13, 20 and 40 % vol. with solid-to-liquid ratio 0.05 (1g solid in 20 cm^{-3} solution) for 90 min. The solid-to-liquid ratio and the ethanol concentration range were chosen to make possible the comparison of the data obtained with those of other authors and with our results concerning wine and wine residues. This time was determined in our previous kinetic experiments. The ethanol was 96 % analytical grade supplied by Valerus (Bulgaria). The extracts obtained were analysed for total polyphenols content (TPC), total flavonoids content (TFC) and antioxidant capacity (AOC).

Total polyphenols contents. Total polyphenols content was determined photometrically by the method of Folin-Ciocalteu. Folin-Ciocalteau reactive: (2N solution), $NaCO_3$, (supplied by Sigma) were used. The total polyphenols content was determined using UV-VIS spectrophotometer (SPEKOL 11) at 765 nm wave length and was expressed as gallic acid equivalent (TPC_{GAE}). The details concerning calculations are given elsewhere [7].

Antioxidant capacity determination. For determination of the antioxidant capacity (AOC) of the extracts obtained the $DPPH^+$ method was used [8]. It is based on the discoloration reaction between a nitrogen electron (from $DPPH^+$) and a hydrogen atom of a hydroxyl group (from the antioxidant substance). An inconvenience of this method is its light sensitivity. For this reason, the reaction has to be carried out in dark and in a non-alkaline medium. In our study we applied this method with use of spectrophotometer (SPEKOL 11) at 517 nm wave length. We used the $IC_{50\%}$ parameter as a comparative variable. It represents the amount of antioxidant sample, which inhibits 50 % of the initial concentration of $DPPH^+$. The blank for comparison is methanol.

Reactives: DPPH (2,2-diphenil-1-picrilhydrazil (free radical), 95 %, packed under argon $C_{18}H_{12}N_5O_6$ Alfa Aesar), ethanol, methanol.

Content of flavonoids. The concentration of flavonoids was determined by the reaction with HCl and formaldehyde. It is based on determination of total polyphenols content in the sample, then sedimentation with formaldehyde and once again - determination of total polyphenols content. The total flavonoids' content is the difference between the total polyphenols before and after precipitation. It is expressed in equivalents of gallic acid - mg EGA/g sample.

RESULTS AND DISCUSSION

The total polyphenols content in the samples studied is presented in Table 1. It is evident that the leaves exhibit about 3 times higher TPC than that of the cans. The concentration of the ethanol in the extracting solution also plays an important role concerning the TPC - the best results are obtained with concentration of 40 % vol.

The results for antioxidant capacity of the samples studied are summarized in Table 2. From this table it follows that the antioxidant capacity of the leaves' extracts

Table 1. Total polyphenols content in leaves and cans of Muscat of Hambourg.

Material	Ethanol solution	Leaves - TPC (mg EAG/g)	Cans - TPC (mg EAG/g)
Muscat of Hambourg (MH)	13 %	32.137	7,361
	20 %	38,284	12,279
	40 %	48,81	12,737

Table 2. Antioxydant capacity of leaves and cans of Muscat of Hambourg.

Material	Ethanol solution	IC_{50} (g/L) leaves	IC_{50} (g/L) cans
Muscat of Hambourg	13%	0,392	1,379
	20%	0,379	1,154
	40%	0,381	0,754

Table 3. Total flavonoids content in leaves and cans of Muscat of Hambourg (precipitation) – 13 % ethanol in water solution.

Material		TPC before (mg EAG/g)	TPC after (mg EAG/g)	FC (mg EAG/g)
muscat of Hambourg	leaves	32.137	1,905	30.232
	cans	7,361	0,117	7,244

is practically not influenced by the extragent concentration. For the cans' extracts the AOC is affected by the ethanol concentration – the best results are obtained for 40 % ethanol-in-water solutions. It can be concluded that the AOC is not directly related to polyphenols content in the material. For this parameter it is also valid that the AOC of the leaves is quite higher than that of the cans (about 3.5 times for 13 % EtOH and about 1.9 times for 40 % EtOH).

The total flavonoids' content is presented in Table 3. From the results it is obvious that the polyphenols in the leaves and cans are mainly flavonoids.

In Fig.1 a comparison in TPC, TFC and AOC for the leaves and cans is presented.

Effect of the temperature

To study the effect of temperature on the extracted valuable components, experiments at three different temperatures were carried out: 25^{0}C, 40^{0}C and 60^{0}C. The concentration of the ethanol-in-water solutions was 13 % vol.

The results for the temperature effect on the TPC and AOC in the extracts studied are in Fig. 2. It can be seen that the temperature influences differently the parameters studied for the extracts from leaves and cans. For the leaves'extracts, the TPC values are highest for the lowest temperature. On the contrary, for the cans the temperature increase leads to higher values of TPC for the highest temperature of 60^{0}C. A possible explanation of this observation can be the different texture of the samples leading to different diffusivities of the products. However, the increase in TPC for the cans' extracts is 1.6 times when comparing the extracts for 25 and 60^{0}C - a difference not significant enough to justify an increase of the temperature three times. The values for the antioxidant activity of the leaves are the best at the lowest temperature, and for the cans' extracts – for the highest one. The explanation of this fact can be the same as for the TPC.

As it was already mentioned the grape's leaves are used in the cuisine. This was the reason to obtain extracts after boiling in water and to compare the values of the AOC determined. For AOC the determined $IC_{50\%}$ = 12.5 ml/L (which compares with 10.51 ml/L for the extract with 13 % ethanol at 60^{0}C or with 6.77 ml/L for the extract with 13 % ethanol at 25 ^{0}C). These values are better than those obtained, for example after extraction of dried kiwi wastes (the best results for the AOA are

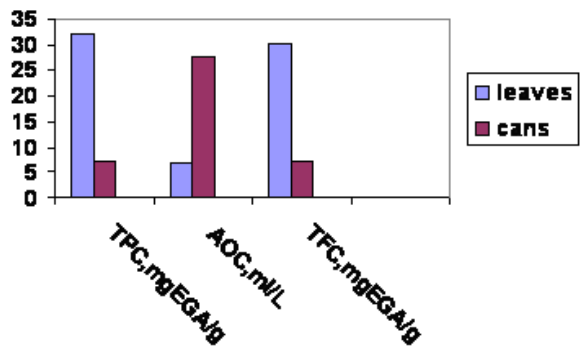

Fig. 1. Comparison between the TPC, TFC and AOC for extracts from leaves and cans of Muscat of Hambourg (13 % vol ethanol, 25^{0}C).

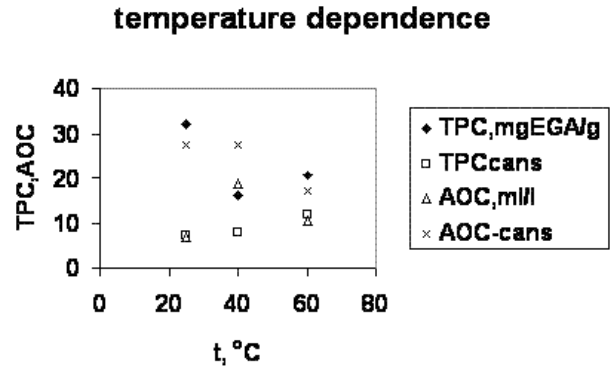

Fig. 2. Effect of temperature on the parameters studied for the cases of leaves and cans (13 % vol ethanol).

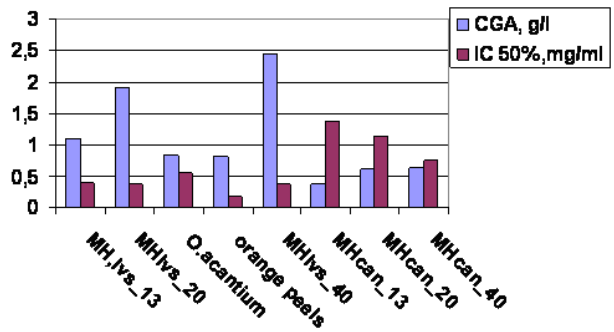

Fig. 3. Comparison of TPC and AOC obtained from different sources.

39.7 ml/L for extraction with 70 % ethanol at 25 ^{0}C [9]).

A comparison between the values of TPC (as concentration of gallic acid) and the AOC of the samples studied and the results of our experiments with other extracts is presented in Fig. 3. It is evident that the values of TPC and AOC, especially for the leaves'extracts, are high and so, they are a really promising source for obtaining of valuable components.

CONCLUSIONS

Experiments for extraction of valuable components from vineyard residues (leaves and cans) were carried out. The extraction was conducted with ethanol-in-water solutions with different concentrations (13, 20 and 40 % vol.) at temperatures of 25, 40 and 60^{o}C. It was found that the solvent concentration and the temperature, influence differently the two raw materials studied. A possible explanation of the fact observed is their different texture leading to different diffusivities. The total polyphenols content and antioxidant activity values found were quite high and characterized the vineyard wastes as a really promising source for food, pharmaceutical and cosmetic industries.

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REFERENCES

1. R. Devesa-Rey, X. Vecino, J.L. Varela-Alende, M.T. Barral, J.M. Cruz, A.B. Moldes, Valorization of winery waste vs. the costs of not recycling, Waste Management, 31, 2011, 2327-2335.
2. M. Hussain, S. Cholette, R.M. Castaldi, An analysis of globalization forces in the wine industry: implications and recommendations for wineries, J. Global Market, 21, 1, 2008, 33-47.
3. H. Das, S.K. Singh, Useful byproducts from cellulosic wastes of agriculture and food industry: a critical appraisal, Crit. Rev. Food Sci. Nutr., 44, 2004, 77-89.
4. Sierra Rayne, Erkan Karacabey, G. Mazza, Grape cane waste as a source of trans-resveratrol and trans-viniferin: High-value phytochemicals with medicinal and anti-phytopathogenic applications, Industrial crops and products, 27, 2008, 335-340
5. C. Dani, L.S. Oliboni, F. Agostini, C. Funchal, L. Serafini, J.A. Henriques, M. Salvador, Phenolic content of grapevine leaves (Vitis labrusca var. Bordo) and its neuroprotective effect against peroxide damage, Toxicology in Vitro, 24, 2010, 148-153.
6. D.D. Orhan, N. Orhan, F. Ergun, Hepatoprotective effect of Vitis vinifera L.leaves on carbon tetrachloride-induced acute liver damage in rats, The Journal of Ethnopharmacology, 12, 2007, 145-151.
7. M. Karsheva, E. Kirova, S. Alexandrova, Natural antioxidants from citrus mandarin peels. Extraction of polyphenols; effect of operational conditions on total polyphenols contents and antioxidant activity, J. Chem. Technol. Metall., 48, 1, 2013, 35-41.
8. Ozlem Yesil-Celiktas et al., Determination of polyphenolic content and antioxidant activity of extracts obtained from Rosmarinus officinalis`calli, J Plant Phys., 164, 2007, 1536-1542.
9. Maria Karsheva, Silvia Alexandrova, Ekatetina Kirova, Julie Bachelart, Extraction de polyphénols et d'autres composés à caractère antioxydant de la peau du kiwi (*Actinidia chinensis*), Revue de génie industriel, 9, 2013, in print.