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HPTLC Profile of Phenolic Compounds Presents in Extracts Obtained from Different Varieties of White and Red Grape Pomace

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ABSTRACT

Grape pomace is used in different areas in the food and food supplement, cosmetic, pharmaceutic industries, and for compost or animal food. The chemical composition is different between white and red grapes varieties, depending also on the sort of wine obtained. Grape pomace contains various chemical compounds from the following classes: phenolics, proteins, minerals, lipids. Our goal was to show the HPTLC fingerprint for quality evaluation and total phenol content for the quantitative evaluation of extracts obtained from 4 varieties of grape pomace (Feteasca regala, Riesling, Burgund, Cabernet Sauvignon). The extracts were obtained by UAE and MAE. The HPTLC fingerprints of the extract shown that there are 4 compounds present in all samples: rutin, hyperoside and, chlorogenic and caffeic acids. In all samples was observed the presence of resveratrol. Total phenol content expressed as gallic (GAE) acid equivalents per 100ml extract were between 4.75% and 13.08%.

Keywords: Grape pomace HPTLC fingerprint, polyphenols, red grape pomace, white grape pomace

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INTRODUCTION

Grapes (*Vitis vinifera*) are one of the oldest cultures and one of the most important horticulture crops (This et al., 2006). According to Statistics Unit of the International Organisation of Vine and Wine (OIV) - 2019 STATISTICAL REPORT ON WORLD VITIVINICULTURE in 2018, 7.4mha were cultivated with *Vitis vinifera*, 50% of the world vineyard being represented by 5 countries (Spain, China, France, Italy and, Turkey). The whole production of grapes is divided into three parts: 57% of wine grape, 36% of table grape, 7% of dried grape. In the same report, Romania is in the 10th place with 191000 ha and 5.1 million hl of wine produced. Grape pomace in the main by-product of the winemaking industry and consists of stalks and seeds, fragmented skin, pulp remains broken cells (Luchian et al., 2019).

Grape pomace is approximately 20% of the grapes (by weight) (Laufenberg et al., 2003) and the composition depends on the grape varieties: red wine pomace is a by-product that has been fermented, while white pomaces are removed before alcoholic fermentation.

Grape pomace is considered a waste of the wine industry, but for pharmaceutic, food supplement, food, and cosmetic products is a very important raw material, because of chemical composition in various compounds, including polyphenols. These compounds are considered one of the most important categories of natural chemical components of extracts because of the biological properties as an antioxidant, anti-aging, metal-chelating (Antoniolli et al., 2015).

Therapeutic properties of grape pomace are associated with chemical composition, and therefore is important to determine the qualitative and quantitative composition of extracts obtained from waste of different varieties of grape (Zhu et al., 2019).

In Romania, there are approx. 15 main grape varieties cultivated for wine industry. We have selected four of them, 2 white varieties–*Feteasca regala*, *Riesling* and, 2 red varieties –*Burgund, Cabernet Sauvignon,* for polyphenol qualitative and quantitative evaluation. Table 1 presents the wine regions of cultivation and EU growing zone type for the four varieties (Antoce et al., 2013).

Table 1: Wine region and EU growing zone type

Wine region	**Grape varieties**				**EU growing type**
	Cabernet Sauvignon	***Burgund***	***Feteasca Regala***	***Riesling***	
Hills of Vallachia and Oltenia	X	X	X	X	C II
Hills of Moldova	X		X	X	C I
Transylvanian plateau			X	X	B
Hills of Crisana and Maramures	X	X	X	X	C I
Hills of Banat	X	X	X	X	C I
Hills of Dobrogea	X		X	X	C II
Danube Terraces	X	X	X	X	C II

These work aims to show the HPTLC fingerprint and total phenolic content of grape pomace extracts obtained by microwave and ultrasonic-assisted extraction from four varieties of grape, cultivated in Romania.

MATERIALS AND METHODS

Raw material: Cabernet Sauvignon, Burgund, Feteasca Regala, and Riesling grape pomace were obtained from Vineyards from the Hills of Vallachia and Oltenia region. Voucher specimens are deposited in the Plant Material Storing Room.

Sample preparation:

Microwave-Assisted Extraction: raw material was extracted under the influence of microwave energy using 40% ethanol (v/v) with parameters as follows: raw material/ solvent rate 1/10, pressure 220 W, time 10min. The obtained extracts were evaporated to volume and frozen until analysis.

Ultrasonic-Assisted Extraction: raw material was extracted under the influence of microwave energy using 40% ethanol (v/v) with parameters as follows: raw material/ solvent rate 1/10, time 15min, temperature 18- 59^{0}C, pressure 78-165 W, amplitude 80%. The obtained extracts were evaporated to volume and frozen until analysis.

HPTLC Analysis - according to TLC Atlas - Plant Drug Analysis (Wagner and Bladt, 1996) the determination of characteristic fingerprint of extracts obtained from grape pomace was performed as follow: samples (3-7µL) (extracts – MAE - T1 Cabernet Sauvignon, T3 – Burgund, T5 Feteasca Regala, T7 Riesling; UAE – T2 - Cabernet Sauvignon, T4 - Burgund, T6 - Feteasca regala, T8- Riesling) and reference substances (1-5µL) solutions were loaded as 10mm band length in the 20 x 10 Silica gel 60F254 TLC plate. The samples were spotted using Hamilton- Bonaduz, Schweiz syringe. The analysis was made with CAMAG LINOMAT 5 instrument. As reference substances were spotted 10^{-3}M T9- rutin, T10- hyperoside, T11- chlorogenic acid, T12- caffeic acid, T13- resveratrol (Sigma-Aldrich).

Polyphenolic compounds: the mobile phases (A) consisted in ethyl acetate-acetic acid-formic acid-water 100:11:11:27 (v/v/v/v) and (B) consisted in chloroform: ethyl acetate: formic acid 2.5:1:0.1 (v/v/v). The TLC twin-chamber was pre-saturated with the mobile phase for 30 min at ~20°C. The plate was developed in the mobile phase up to 90mm. After development, plates were dried and derivatized in for A system in Natural Product followed by PEG4000 reagent. The fingerprints were evaluated at UV with WinCats and VideoScan software for A system and in visible light for B system.

Total phenol content - Total phenol content was determined with Folin - Ciocalteu method (European Pharmacopoeia 6,0). Briefly, 1 ml of extract was transferred to a 25 ml volumetric flask, 10ml of water and 1 ml of Folin Ciocalteu regent was added. The volume was made to 25 ml with 5% sodium carbonate (w/v). The blend was left at room temperature for 30 minutes. Then the absorbance of the samples was read at 760 nm with a UV/VIS spectrophotometer (Helios λ, Therno Electron Corporation). Distilled water was used as a blank. Total phenol content was determined from the extrapolation of the calibration curve (*y=1,474x-0,438,* R2 = *0.992)*, which was obtained for gallic acid (Sigma Chemical Co., St. Louis, USA) The results were expressed as milligrams of gallic acid equivalents (GAE) per 100ml extract.

RESULTS AND DISCUSSIONS

High-performance thin-layer chromatography is a technique used for quantification (qualitative and quantitative) of compounds from complex mixtures. It is, also, a simple, cost-efficient, rapid method of analysis that allows investigation of multiple different samples (Attimarad et al., 2011).

HPTLC fingerprints (fig no.1, fig no.2) of extracts obtained from grape pomace revealed the presents of compounds presented in table no 2.

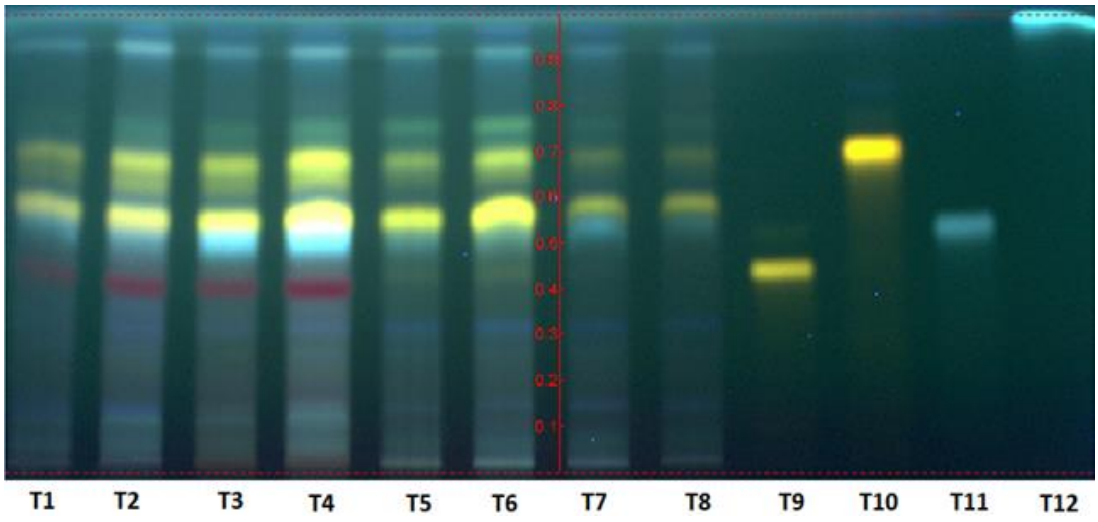

Figure 1: HPTLC fingerprint (polyphenolic compounds) of grape pomace extracts

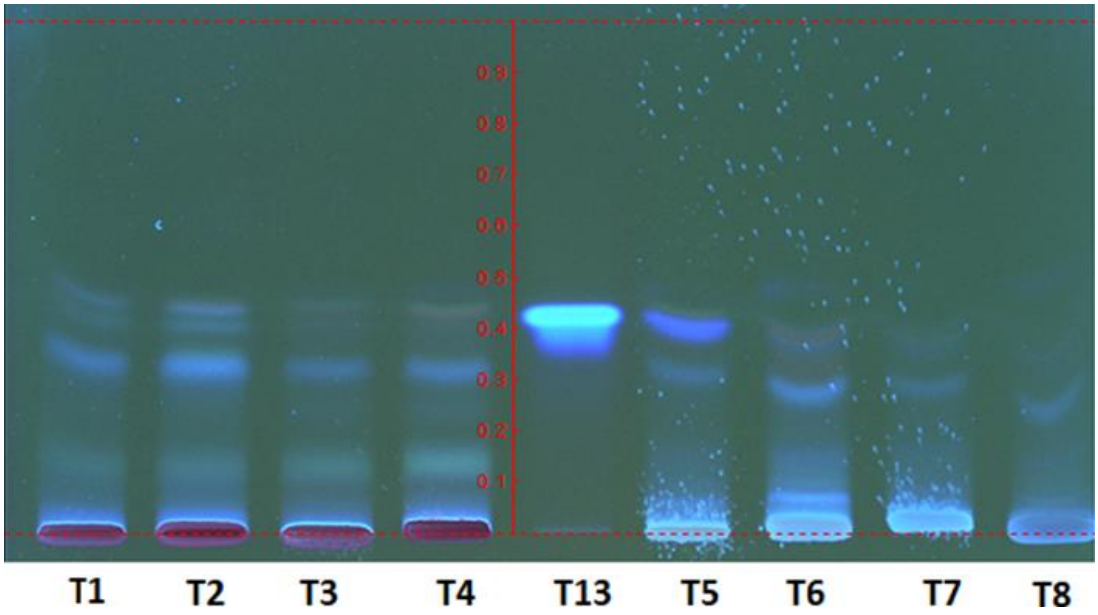

Figure 2: Identification of resveratrol in grape pomace extracts

According to Wagner and Bladt, blue fluorescent spots are attributed to phenolic acids, yellow-orange spots to flavonoids, and purple one to anthocianins.

The few HPTLC studies performed until now, to determine the chromatographic fingerprints of grape pomace reveal the presence of compounds from the classes of flavonoids, phenolic acids, and anthocyanins (Bernardi et al., 2019).

The main difference between red and white grape pomace extracts is the presents of anthocyanins as purple spots in both red extracts, regardless of the extraction method used UAE or MAE. It is also important that in all extract's resveratrol is present, showing that a part of the compound remains in the pomace, and only a certain amount of resveratrol is transferred to wine in the wine making process (Feijoo et al., 2008).

Even the chromatographic fingerprint seems to be similar for all the extracts, total phenolic content shows important differences.

Table 2: Compounds identified in grape pomace extracts

Gape variety	Grape pomace extract		Compound identified	
	Microwave-assisted extraction (MAE)	Ultrasonic-assisted extraction (UAE)		
Cabernet Sauvignon	T1	T2	purple spot – attributed to anthocyanins (Rf ~0.4)	T9 – **rutin,** orange fluorescent spot (Rf~0.45) T10 – **hyperoside** – orange fluorescent spot (Rf~0.7) -orange spot at Rf~0.6, mostly due to flavonoid glycosides T11 - **chlorogenic acid** -blue fluorescent spot (Rf~0.55) T12 – **caffeic acid** – blue fluorescent spot (Rf~0.97) -blue fluorescent spot at Rf~0.9, as quinic acid (chlorogenic acid derivates) T13 – resveratrol – blue fluorescent spot (Rf~0.4)
Burgund	T3	T4		
Feteasca Regala	T5	T6		
Riesling	T7	T8		

Table no 3 shows the total phenol content of the extracts expressed as gallic (GAE) acid equivalents per 100ml of extract.

Table 3: Total phenol content of grape pomace extracts

Extract	mg (GAE)/100ml extract
Cabernet Sauvignon MAE	13.50
Cabernet Sauvignon UAE	10.75
Burgund MAE	4.90
Burgund UAE	9.60
Feteasca Regala MAE	8.53
Feteasca Regala UAE	4.75
Riesling MAE	8.94
Riesling UAE	13.08

The highest content in TPC was observed in extracts of grape pomace - *Cabernet Sauvignon* (red variety), the results being in concordance with those obtained in a Brazilian study of pomace phenolic composition from different grape varieties (Yu and Ahmedna, 2013).

The other extracts have lower TPC content, in the following order: *Riesling*> *Burgund*>*Feteasca Regala.*

Grape pomace utilisation in different areas, as food and food supplement, pharmaceutical and cosmetic industry is especially due to the polyphenols composition.

One of the most important biologic activity of grape pomace is antioxidant properties attributed to polyphenolic composition (Veskoukis et al., 2012), being used in food supplement and pharmaceutical products as well as in food ones. The antimicrobial properties of extracts are used preponderant for food preservation potential and medicinal purpose to prevent oxidation of lipids suppress the growth of pathogenic bacteria (Yu and Ahmedna, 2013).

CONCLUSION

Different varieties of grape pomace extracts have different content of polyphenolic compounds, depending on the method of extraction. The HPTLC fingerprint can be important information in the evaluation of grape pomace extracts, especially when are part of the final products (food, cosmetic, food supplement).

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