International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Qualitative and Quantitative Determination of Total Phenolic Compounds in Georgian Origin Wine Lees

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Abstract

In comparison to the previous century, interest in and demand for natural compounds have grown dramatically in recent years all over the world, particularly for those having anti-inflammatory, anti- microbial and antioxidant effects. It is common knowledge that polyphenols possess these properties. Another well-known fact is that grape-based products like wine are one of the richest sources of polyphenols. Thus, winery byproducts - including wine lees are noteworthy in this context. Wine production is accompanied by quite a significant volume of waste materials, such as grape pomace, seed and wine lees. This was the rationale behind using wine lees to study polyphenols. The current study's goal was to detect the phenolic compounds—biologically active ingredients in wine lees (byproduct) made from commonly planted grape varietals in Georgia and assess their quantitative total. Using the LC-MS/MS method, we detected polyphenols in five samples of wine lees made from different Georgian grape varieties (Saperavi, Rkatsiteli, Kisi) and with different two technologies. The total phenolic content was estimated using the Folin-Ciocalteu reagent by spectrophotometric method. During current studies, 21 individual polyphenols were extracted and identified from wine lees. The study's findings demonstrated that, the qualitative and quantitative concentration of phenolics in wine lees is influenced by the grape variety and winemaking technology. The study's findings suggested that Georgian wine lees, usually wasted product, might be utilized as natural source of biologically active substances.

Keywords: Phenolic compounds, Utilizing waste materials, Wine lees, Natural ingredients, Total Phenolic content

Full length article **Corresponding Author*, e-mail: t.kirvalidze@tsmu.edu

1. Introduction

The protection of natural resources has recently drawn a lot of attention, and it is now more important than ever to create technological organizational systems that save resources and make secondary use of plant raw materials. The European Parliament's resolution (# 52011DC0021, A resource-efficient Europe - Flagship initiative under the Europe 2020 Strategy), which outlines the necessity of resource preservation and offers numerous strategies to assure the achievement of this target [1]. Despite the fact that it is well known that plant-based products have long been used in medicine, there is an increase in demand for these products globally. Therefore, revealing waste materials as a new, green resource which might be a source of compounds with biological activity is a crucial and ground-breaking issue [2-5]. Polyphenols have long been utilized in Pharmaceutical, Cosmetical and food industry [6-10]. However, information on the biological effects of polyphenols has grown significantly in recent decades, and increasingly more study is underway to assess their advantageous effect on the health of humans. In a recent study, Prevención con Dieta Mediterránea (PREDIMED), researchers discovered that those who eat a diet high in polyphenols have lower mortality, cardiovascular disease risk, and diabetes risk. [11-13]. In addition, according to the study, Rutin reduces hypoxia/reoxygenation-induced injury in cardiac cells by up-regulating SIRT1 expression [14]. Also, in many animal models, kaempferol has been shown to protect against

diabetic nephropathy and drug-induced nephrotoxicity [15,16]. Furthermore, according to the researchs, By regulating the p38 MAPK signaling pathway, epicatechin reduces inflammation in lipopolysaccharide-induced severe lung injury in mice [17]. Due to their antimicrobial and antioxidant properties, polyphenols may additionally extend the shelf life of foods without the need of chemical supplements, ensuring high consumer safety standards [18]. Polyphenols are naturally found in various fruits, vegetables, and beverages such as tea and wine [19-22]. Winemaking is one of Georgia's oldest and most lucrative agricultural specialties. Archeologists have found material and historical evidence that backs up claims that the country began producing wine 8,000 years ago. Currently, Georgia is home to about 500 different grape varieties [23,24]. Winemaking is characterized by the production of numerous waste materials, including grape seeds, wine lees and grape pomace. In Georgia, these byproducts-especially wine lees-have not been completely researched for therapeutic or preventive use. Some studies are currently in progress on the analysis and utilization of waste products from the cultivation of grapevines in different areas at The Tbilisi State Medical University. One of them is the research of the biologically active components derived from wine lees, which will be applied for pharmaceutical and cosmetic purposes. During current research, 21 individual polyphenols were extracted and detected from Georgian wine lees: Quercetin-3-O-rutinoside, Quercetin, Apigenine, catechin, Ferulic acid, Gallocatechin, Kaempferol-3-O-galactoside, Kaempferol-3-O-glucoside, p-Coumaric acid, Protocatechuic acid, Delphinidin-3-O-glucoside, Quercetin-3-O-glucoside, Caffeic acid, Malvidin- 3-O-glucoside, Petunidin-3-O-glucoside, Cyanidin-3-O-glucoside, Gallic acid, Kaempferol, Epicatechin gallate, Luteolin-7-O-glucoside, Ellagic acid. Figure 1 shows the chemical structures of several of them. The study's objective is to assess the potential of Georgian wine lees, a currently wasted product, as a source of biologically active ingredients. The aim of the research was to detect and determine the amount of the biologically active chemicals (polyphenols) present in wine lees - byproduct of the wine industry, made from widely distributed grape species in Georgia.

2. Materials and methods

2.1. Chemicals, reagents, samples and their sources

Chemicals and solvents for mobile phase (formic acid, acetonitrile and water) were obtained from Sigma Aldrich and all were HPLC grade in this study, Folin-Ciocalteu's phenol reagent and Gallic acid (>98.0%) from Sigma Aldrich. The lees of Saperavi, Rkatsiteli, and Kisi wine were collected in the local villages of Georgia.

2.2 Instrumentation

The instrumentations used in this study were an Agilent Technologies 6460 triple quad LC/MS Agilent Technologies 1290 infinity, for identification phenolic compounds in the extracts obtained from Georgian wine lees. I9 Hanon instruments UV-VIS Spectrophotometer was used to measure absorbance of samples.

2.3. Sample preparation

Wine lees obtained from three different varieties of grapes (Kisi, Rkatsiteli, Saferavi), which were made by two different technologies (traditional (T) and factory(F)) were collected in different villages of the Kakheti region in Georgia. Through traditional production of wine techniques, a certain volume of grape juice is fermented, vinified, and aged in tanks together with grape skin, grape seed, and grape husks. The basic principle of traditional viticulture is to keep the wine in the tank with the parts of grape both while and following the alcoholic fermentation. Throughout the wine making in factory conditions, process took place without the hard parts of grapes unlike the traditional method. To remove large particles from the lees, we applied a sieve. The sample was then centrifuged for 10 minutes at 3000 rpm to get rid of the liquid (wine). With the use of an ultrasonic bath, the obtained mass extraction was carried out with distilled water for 15 minutes, with 1:100 proportions of distilled water and sample. The supernatant was then collected from the sample after it had been centrifuged for 5 minutes at 3000 rpm. Five times extraction was done and fluids that were generated during this process were mixed. Prior to LC-MS/MS analysis, the mixed extracts were purified via PTFE filters (25 mm 0.45 μm). All varieties of wine lees underwent the same sample-making process. Five analytical objects in all have been prepared and examined [25].

2.4 Qualitative analysis of phenolic compounds in wine lees

Determining phenolic compounds in the samples were conducted by Liquid Chromatography with tandem mass spectrometry. HPLC operating conditions were following: The analysis was conducted with the column set at 30 °C. 0.1% (v/v) formic acid in acetonitrile (solvent A) and 0.1% (v/v) formic acid in water (solvent B) were used for mobile phase, a binary gradient was utilized: 0–5 min, 5%; B; 5–9.0 min, 20% B; 9–13 min, 30%; 13–17 min, 50%; 17–22 min, 80%, 22–26 min, 70%, and 26–30 min, 5%; B. Flow rate 0.8 ml/min. The following mass spectrometry operating conditions were applied: gas and sheath gas temperature were 300°C, flow of gas was 7 L/min and sheath gas - 6.5 L/min, pressure of nebulizer was 635 kilopascal, voltage of capillary 4000 V and nozzle - 500 V. Multiple reaction monitoring (MRM) was the scanning form. MRM transitions for compounds were monitored in positive and negative modes (Table.1). Agilent MassHunter Workstation software Acquisition B.03 was used for evaluating data [26,27].

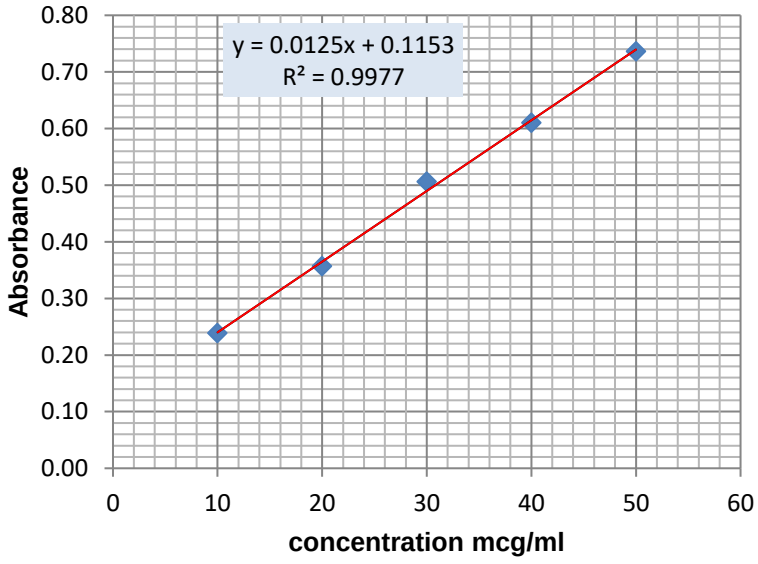
2.5 Total Phenolic content

The Total Phenolic content determination of analytical samples were performed by FolinCiocalteu assay method. Gallic acid standard serial dilutions were prepared in concentrations (10, 20, 30, 40, 50 μg /mL). 5 ml of Folin-Ciocalteu reagent was added to the flasks containing 1 mL of each dilution of standard and samples. After 8 min, 4 mL of 7.5% Na2CO3 solution was added to the mixtures and mixed. The mixtures were held in the dark for 40 min at 25 ◦C, then the absorbance was measured at 760 nm.

Extrapolating from the calibration curve, constructed by Gallic acid solution allowed for the determination of the TPC (Graphic 1). Three sets of measurements were made to estimate phenolic compounds. TPC was expressed as mean ± SD of Gallic acid equivalents [28,29].

3. Results and discussions

The high polyphenol content of wine products is widely recognized. Thus, we made the decision to investigate the phenolic content of a waste product from one of our country's most developed industry business-the wine industry. Three different Georgian grape varieties (Saferavi, Kisi, Rkatsiteli) were selected to identify and quantify the phenolic content present of their wine lees, and to link the grape varietal to the phenolic composition. In our study of samples from Saperavi and Rkatsiteli, we also employed wine lees produced with contrasting technologies, such as the traditional and factory techniques. The analysis of the samples demonstrated that, Saperavi wine lees made by traditional method as well as in factory conditions is rich in a variety of phenolic components, as well as Rkatsiteli wine lees (traditional) has rich phenolic content. However, among the examined samples, Kisi wine lees is the poorest in the composition of phenolic components (Table 2). These results line up with previous researches on the different phenolic composition of wine goods from different origins [30-32]. Similar to our results of different varieties Georgian wine lees investigation, Zhijing Y study had also various phenolic composition in various types of wine lees [33]. Moreover, according to Sacchi K.L. and et al. [34] study, impact of the wine making technique on phenolic composition within wine is confirmed. this result is also supported by Dupas' research on the impact of wine-making method, of which verifies the impact of wine manufacturing techniques on wine composition [35]. This findings correlates with the outcomes of our research on the impact of wine-making techniques on wine lees. Table 2. shows the Phenolic Compounds identified in several samples of Georgian wine lees. Quercetin-3-O-rutinoside, Quercetin, Apigenine, catechin, Ferulic acid, Gallocatechin, Kaempferol-3-O-galactoside, Kaempferol-3-O-glucoside, p-Coumaric acid, Protocatechuic acid, Quercetin-3-O-glucoside, Delphinidin-3-O-glucoside, Malvidin-3-O-glucoside, Petunidin-3-O-glucoside, Caffeic acid, Cyanidin-3-O-glucoside, Gallic acid, Kaempferol, Epicatechin gallate, Luteolin-7-O-glucoside and Ellagic acid have been identified. According to the result, Saperavi wine lees (traditional) contains the most 13 different phenolic compounds among the samples (fig 2). According to the qualitative composition, the Saperavi wine lees (traditional) is the richest in phenolic composition. Furthermore, based on the findings of the analysis, there is no phenolic compound, which is found in all five analytical samples. These findings allow us to formulate the conclusion that the phenolic content of wine lees is influenced by the grape variety. Results of determinations of total phenolic content in wine lees with the Folin-Ciocalteu method using UV/VIS spectrophotometer are listed in table 3. According to the results, In Saperavi wine lees (Traditional) TPC (256.68mcg GAE/ml) is important and also, TPC presented in each analytical samples are noteworthy. Also, wine leeses obtained from the identical grape varieties (Saperavi), made with different techniques, were used to demonstrate the difference quantitatively content of total phenolics.



Graphic 1. calibration curve of Gallic acid

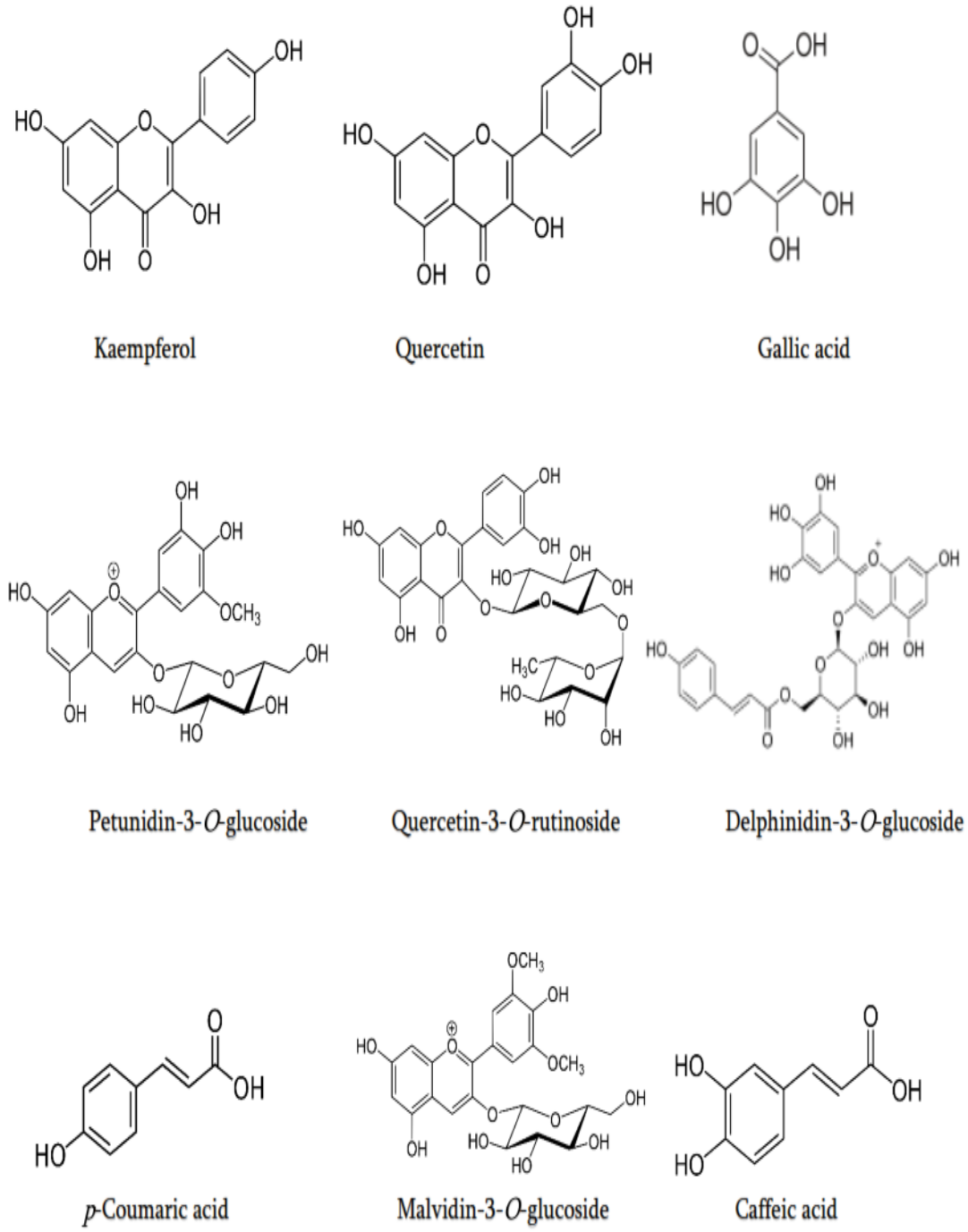


Figure I: Chemical structures of some of polyphenols found in Georgian wine lees

Table 1. LC-MS/MS MRM conditions

Compound Name	Precursor Ion (m/z)	Product Ion(m/z)	Dwell (msec)	Collision energy (V)	Retention Time (RT)(min)	Polarity
Quercetin-3-O-rutinoside	609	301	200	54	14.710	Negative
Quercetin	301	151	200	30	19.290	Negative
Apigenine	269	151	200	15	8.761	Negative
Catechin	289	109	200	34	12.081	Negative
Ferulic acid	196	134	200	24	4.237	Negative
Gallocatechin	305	225	200	20	3.788	Negative
Kaempferol-3-O-galactoside	447	285	200	50	21.538	Negative
Kaempferol-3-O-glucoside	447	255	200	52	11.385	Negative
p-Coumaric acid	163	119	200	22	3.322	Negative
Protocatechuic acid	153	109	200	15	10.13	Negative
Quercetin-3-O-glucoside	463	301	200	42	10.71	Negative
Delphinidin-3-O-glucoside	465	303	200	33	10.56	Positive
Malvidin-3-O-glucoside	493	331	200	36	12.28	Positive
Petunidin-3-O-glucoside	479	317	200	32	11.40	Positive
Caffeic acid	179	134	200	36	13.32	Negative
Cyanidin-3-O-glucoside	447	284	200	36	16.06	Negative
Gallic acid	169	125	200	26	6.655	Negative
Kaempferol	285	186.9	200	42	20.369	Negative
Epicatechin gallate	441	169	200	30	15.537	Negative
Luteolin-7-O-glucoside	285	267	200	30	20.369	Negative
Ellagic acid	302	263	200	20	3.391	Negative

Table 2. Phenolic Compounds detected in Georgian Wine Lees by LC-MS/MS

No	Name of the Compound	Kisi (T)		Rkatsiteli (T)		Saperavi (T)		Rkatsiteli (F)		Saperavi (F)	
		RT [min]	Peak area	RT [min]	Peak area	RT [min]	Peak area	RT [min]	Peak area	RT [min]	Peak area
1	**Quercetin-3-O-rutinoside**	-	-	-	-	14.715	1028	-	-	14.71	185
2	**Quercetine**	-	-	19.290	1997	19.157	8487	19.157	1257	19.290	2292
3	**Apigenine**	8.761	1120	-	-	-		-	-	-	-
4	**Catechin**	-	-	12.214	585	12.081	1744	-	-	12.081	161
5	**Ferulic acid**	4.237	662	-	-	-	-	-	-	-	-
6	**Gallocatechin**	3.788	188	-	-	-	-	-	-	-	-
7	**Kaempferol-3-O-galactoside**	21.538	16	-	-	-	-	-	-	-	-
8	**Kaempferol-3-O-glucoside**	-	-	16.064	46	-	-	16.061	193	-	-
9	**p-Coumaric acid**	3.322	253	-	-	-	-	-		-	-
10	**Protocatechuic acid**	-	-	10.13	784	10.272	1532	10.12	31	10.13	913
11	**Quercetin-3-O-glucoside**		-	-	-	-	-	-	-	10.71	280
12	**Delphinidin-3-O-glucoside**	-	-	10.562	1122	10.693	325741	-	-	10.560	15843
13	**Malvidin-3-O-glucoside**	-	-	12.283	1765	12.283	5402894	-	-	12.289	1079681
14	**Petunidin-3-O-glucoside**	-	-	11.40	2053	11.625	851731	-	-	11.491	61289
15	**Caffeic acid**	-	-	13.32	39	13.460	329	-		13.39	54
16	**Cyanidin-3-O-glucoside**	-	-	-	-	16.06	170	-	-	-	-
17	**Gallic acid**	-	-	6.655	453	6.788	1006	-	-	6.655	243
18	**Kaempferol**	-	-	20.369	86	20.503	121	20.369	17	20.503	115
19	**Epicatechin gallate**	-	-	-	-	15.537	273	-	-	-	-
20	**Luteolin-7-O-glucoside**	-	-	20.369	220	20.36	256	20.369	45	20.36	233
21	**Ellagic acid**	-	-	-	-	-	-	3.391	45	-	-

Table 3. Total Phenolics Content of Wine lees samples

Sample	Total Phenolic Content (TPC (mcg GAE/ml)
Kisi (T)	142.53
Rkatsiteli (T)	121.64
Saperavi (T)	256.68
Rkatsiteli (F)	102.32
Saperavi (F)	160.88

TPC - Total Phenolics Content

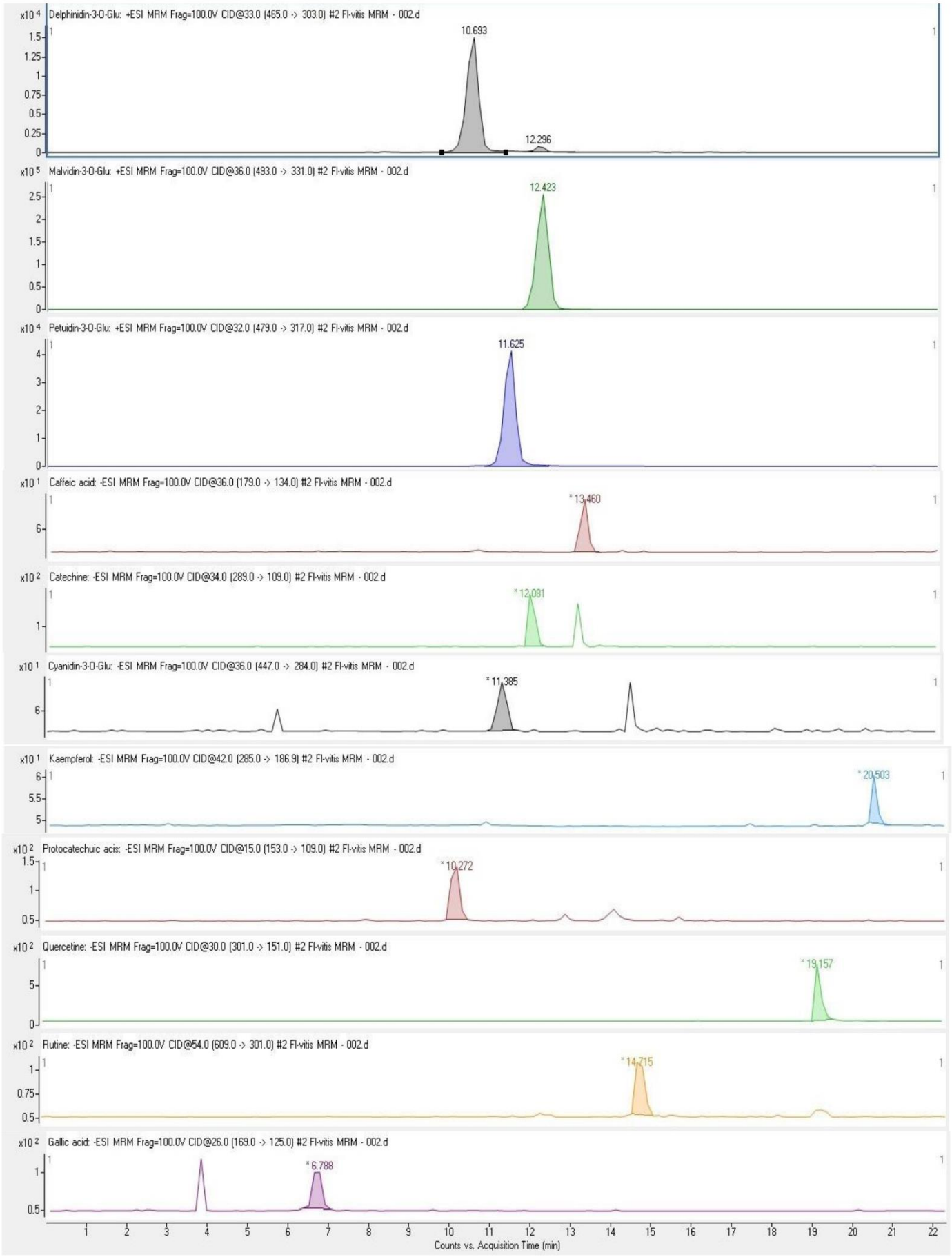


Figure 2. LC-MS/MS chromatogram of Saperavi wine lees (Traditional**)**

4. Conclusions

The present research evaluated the possibility of using Georgian wine lees as an organic source of compounds with biological activity (phenolic compounds). During the study, 13 phenolic compounds were found in Saperavi wine lees and in total 21 phenolic compounds were discovered in all wine lees samples. Additionally, all analytical samples are notable for their Total phenolic content, particularly the traditional Saperavi wine lees. In overall, research results indicates that Georgian wine lees, that's are now considered as wasted byproduct, has possibility become an economical and all-natural source of biologically active substances. The findings also encouraged the necessity of additional studies of wine lees and their capacity role for producing biologically active ingredients.

Acknowledgements

This research No PHDF-22-2101 has been supported by Shota Rustaveli National Science Foundation of Georgia (SRNSFG).

Conflict of Interest

Authors do not have conflict of interest.

Author Contributions

Tamar Kirvalidze, Tamaz Murtazashvili, Lasha Bakuridze, developed the project and the main design concepts. The analysis was done by Tamar Kirvalidze, Koba Sivsivadze, and Malkhaz Jokhadze. The project was supervised by Lasha Bakuridze and Tamaz Murtazashvili. All authors discussed the results and contributed to the final manuscript.

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