JAP&T
TPA-Timişoara

Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2017, *23* (3), 141-147

Journal of Agroalimentary Processes and Technologies

Grape pomace: a potential sustainable resource for natural bioactive compounds recovery

Cristina Gaita[1], Ersilia Alexa[1], Iuliana Popescu[2], Sofia Popescu[1], Monica Negrea[1], Mariana-Atena Poiana[1,*]

[1] *Banat`s University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Faculty of Food Processing, Calea Aradului 119, Timisoara 300645, Romania.*
[2] *Banat`s University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Faculty of Agriculture, Calea Aradului 119, Timisoara 300645, Romania.*

Received: 23 July 2017; Accepted: 29 August 2017

Abstract

The goal of this research was to evaluate the antioxidant properties in terms of total phenolics content and total antioxidant capacity of winery pomace coming out from red grape varieties Burgundy (B) and Pinot Noir (PN), as well as from white grape varieties Italian Riesling (IR) and White Maiden (WM). The B and WM grape pomace was used fresh, while the PN and IR grape pomace was used fresh and also conditioned by drying at 60°C for 24 h, three days in a row for 8 hours. The antioxidant properties were evaluated within whole grape pomace samples and their main fractions, seeds and skins. Our data revealed that the total phenolics content and the antioxidant capacity, expressed by ferric reducing antioxidant power (FRAP) value, of fresh grape pomace samples decreased in the following order: IR >PN >B >WM. As regards the investigated fractions, the highest antioxidant properties were recorded in seeds, especially those coming out from IR and B grape varieties. The conditioning of grape pomace by drying at a moderate temperature of 60°C for 24 h led to the loss of antioxidant properties in a proportion of 17-21% reported to the initial values. These results are useful for the selection of grape pomace to obtain valuable ingredients for developing of high value-added products. Thus, the grape pomace can be used as a sustainable resource for the recovery of high-quality polyphenolic compounds.

Keywords: grape pomace, grape seeds and skins, polyphenolic compounds, FRAP value

1. Introduction

Grapes represent one of the most extensively cultivated crops in the world with a production of 63 million tons worldwide, and approximately 75% of the total grape production is used for winemaking [1]. Approximately 20% of the weight of processed grapes represents the main winemaking by-product, called grape or wine pomace and this by-product mainly consists of skins, seeds, and pulp obtained after pressing operation. Therefore, every year, the wine industry leads to the generation of important quantities of this waste [2].

Wine pomace has been for a long time an undervalued product due to the lack of applications with economic efficiency. Also, it creates a lot of problems for environment, in terms of conditioning, storage or removing. The studies carried out in the last years showed that the grape pomace can be used as a cheap material of health-promoting polyphenolic compounds polyphenols because they are poorly extracted upon vinification [3-5].

The skins and seeds represent about 13% of the amount of processed grapes for wine obtaining and are a rich source of high-quality polyphenols [4, 5].

Grape seed extract is known as a powerful antioxidant that protects the body from premature aging, disease and decay. The pharmacological and nutraceutical benefits derived from grape seed

Corresponding author: *e-mail:* atenapoiana@yahoo.com

polyphenols are because of their free radical scavenging capability. There are a number of studies that reported that grape seed polyphenols help to reduce the risk of cancer and heart disease by inhibiting the oxidation of low-density lipoprotein (LDL) [6]. Teissedre *et al.* [7] indicated that the polyphenolic compounds in grapes and wines inhibited the human low-density lipoprotein oxidation. Antioxidant activity is the common assay used and widely accepted by researchers as an anticancer indicator [8].

Recently, the antioxidant power and health benefits of grape seed extracts have been demonstrated and it leads to their use as dietary supplements and food additives [9].

Besides the antioxidant properties, the polyphenolic compounds within the grape pomace possess antimicrobial activity against fungus, viruses and bacteria and also, act as free radical scavengers [10, 11].

The idea to revalorize the wine pomace is not new and along the time, different alternatives, of which it can be recalled the wine pomace distillation to produce different types of "wine alcohol", the recovery of anthocyanin pigments and grape seeds oil, the use of wine pomace as fertilizer or as animal feed, have been taken into account to exploit the full potential of this by-product. The winemaking by-products have shown many health benefits and multifunctional properties such as natural coloring agents, antioxidant and antimicrobial effects and texturizing properties [12-16].

Nowadays, there are many studies for the valorization of grape pomace as a sustainable resource in the following directions: to obtain dietary supplements, as a valuable ingredient for pharmaceutical or cosmetic applications, as antibacterial and antioxidant agents, for obtaining of value-added products such as natural extracts rich in bioactive compounds with antioxidant properties and also for the recovery of tartaric acid [2, 4, 5, 16].

There is a wide diversity in the types of the grape seed or skin extracts on the basis of total phenolics content, mainly due to the cultivar selection, the conditioning of fresh grape pomace and the extraction procedures and conditions [17].

Taking into account the aforementioned data, the eploitation of grape pomace as main wine industry by-product is important not only for ecological reasons but also because it represents an affordable source of bioactive compounds, especially high-quality polyphenols with various applications in food industry, as potential multifunctional ingredients for developing of high value-added products.

In agreement with the current concerns on this topic, the purpose of this paper is to evaluate the total phenolics content and the total antioxidant capacity of winery pomace and its main fractions seeds and skins coming out from four grape varieties mainly used for winemaking. Additionally, the changes of antioxidant properties in response to grape pomace drying at a moderate temperature of 60°C, three days in a row for 8 hours were investigated.

2. Materials and Methods

Grape pomace samples

The grape pomace used in this study resulted as main by-product in the winemaking process of four grape varieties as follows: Burgundy (B), Pinot Noir (PN), Italian Riesling (IR) and White Maiden (WM). The grape pomace samples were collected from three Romanian wine producers, as follows: B from Recas Winery (Western Romania, Timis County), PN and IR from Teremia Mare Winery (Western Romania, Timis County) while WM was taken from Jidvei Winery (located in the center of Romania, Alba County). B and WM grape pomace samples were used fresh, kept in refrigeration conditioning at 4-5°C for 24 h, while the PN and IR grape pomace samples were previously subjected to conditioning in a forced air oven (Froilabo AC60/France, 1000 W) for 24 h at 60°C, three days in a row for 8 hours, in order to increase their stability against bioactive compounds degradation. The main fractions, seeds and skins, of fresh grape pomace B and WM and dried pomace PN and IR were manually separated and subjected to antioxidant properties investigation.

Extraction procedure

The extracts used for total phenolics content and total antioxidant capacity evaluation were prepared from the whole grape pomace and also, from its main fractions, grapes seeds and skins. The samples were previously grinded and subjected to extraction with a mixture of methanol-water 1:2 (v/v) and the extraction ratio solid:solvent was 1:40 (w/v). The extraction was performed under stirring using the horizontal shaker Heidolph Promax 1020

(Germany) at ambient temperature of 25°C for 1h. After extraction, the mixtures were filtered and the clear fractions were further used for analysis.

Evaluation of total antioxidant activity

The antioxidant activity was evaluated following the spectrophotometric FRAP (ferric reducing antioxidant power) assay [18]. Actually, by this method it was measured the ability of samples to reduce the ferric 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ) complex to ferrous form which is blue-colored in sodium acetate buffer of pH 3.6 at 37°C. This reaction resulted in the change of absorbance monitored at 593 nm. The FRAP values were expressed as µM Fe^{2+}/g d.s. The results were reported as an average value of three determinations.

Evaluation of total phenolics content

The content of total phenolics was evaluated in agreement with the Folin-Ciocalteu colorimetric method [19]. The absorbance was measured at 750 nm using the UV–VIS spectrophotometer Analytic Jena SPECORD 205 and gallic acid as a standard. The results were expressed as mg gallic acid equivalents (GAE)/g d.s. The results were reported as an average value of three determinations.

3. Results and Discussions

The total phenolics content and FRAP value of grape pomace resulting from the winemaking technology of B, PN, WM and IR grape varieties were investigated in the view of their future exploitation as a potential source of natural antioxidant compounds. The evaluation of antioxidant properties is critical for the further use of grape pomace in various nutritional purposes. The dehydration of grape pomace is a required processing technique for increasing its shelf-life for further applications and as a food ingredient. As it can be seen from Table 1, the humidity of the fresh grape pomace samples is located in the range 51-62% (w/w), limiting their chemical and microbiological stability.

The drying of grape pomace represents the most common way for conditioning of grape pomace but the choosing of the most adequate technique is of the great importance because the bioactive compounds are sensitive to heat and oxygen, and may be destroyed during processing and storage.

Table 1. The humidity of investigated grape pomace samples

Grape pomace	Humidity (%)			
	B	PN	WM	IR
fresh	62.44	56.68	54.55	51.29
dried	-	5.61	-	4.08

The total phenolics content registered in the grape pomace samples as well as in their seeds and skins is depicted in the Figures 1 and 2, whereas the FRAP values of invedtigated samples are showed in the Figures 3 and 4.

Our data revealed that the FRAP values and the total phenolics content of fresh grape pomace samples decreased in the following order: IR >PN >B >WM.

As regards the investigated fractions of the fresh B and WM grape pomace, the highest antioxidant properties were recorded in seeds, especially those coming out from B grape variety.

Concerning the dried pomace samples, it can be said that the exposure of grape pomace to a moderate temperature of 60°C, three days in a row for 8 hours, led to the degradation of polyphenolic compounds as an immediate effect of polyphenoloxidase that act during the drying time. The highest content of phenolic compounds has been registered in the IR grape pomace as well as in its fractions.

At the end of drying, the relative losses in FRAP value were 20.27% reported to the initial value for PB grape pomace and 17.53% for IR grape pomace. Also, the total phenolics content registered a decrease of 20-21% in response to grape pomace drying.

Based on these results, it could be noticed that the antioxidant properties of grape pomace exhibited a good stability in response to grape pomace drying at 60°C in the mentioned conditions.

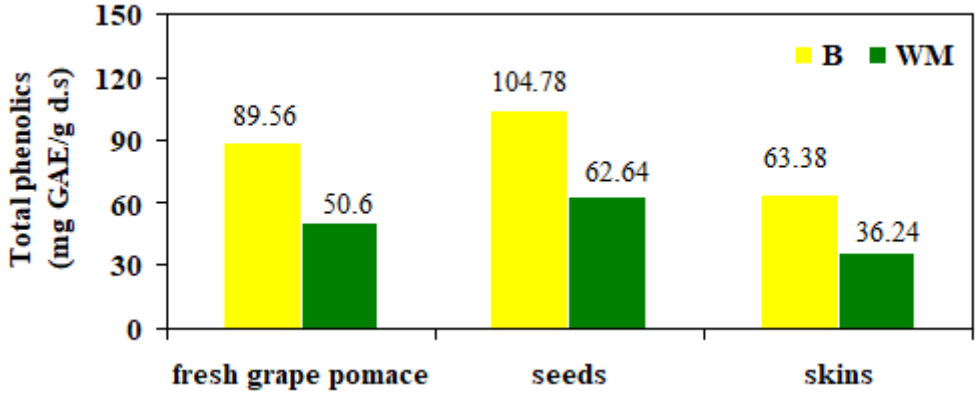

Figure 1. Total phenolics content of fresh grape pomace and of its main fractions

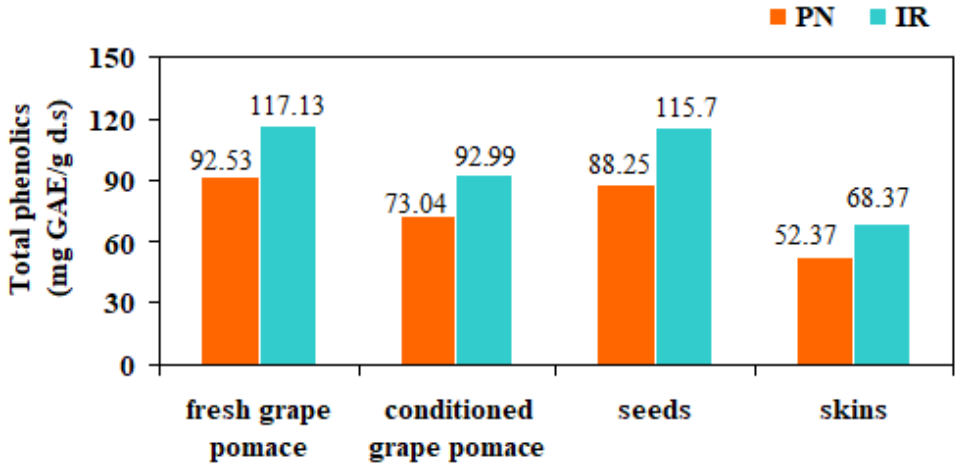

Figure 2. Total phenolics content of fresh and conditioned grape pomace and of its main fractions

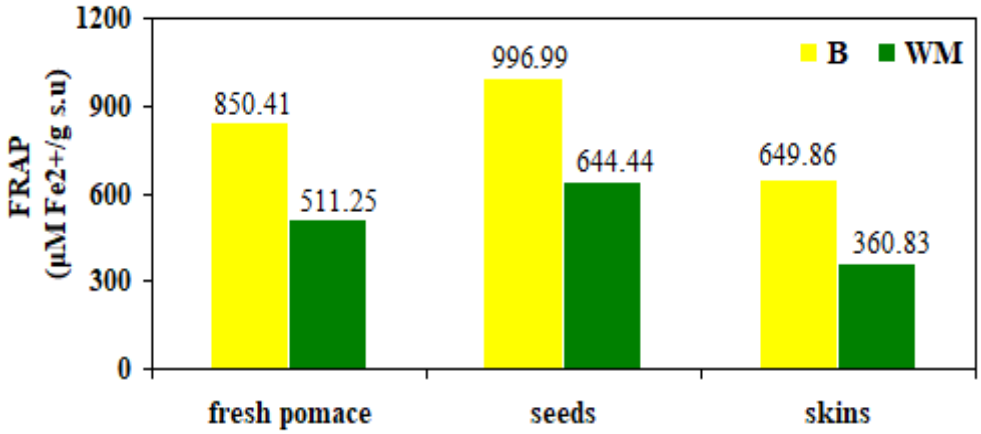

Figure 3. Antioxidant capacity of fresh grape pomace and of its main fractions

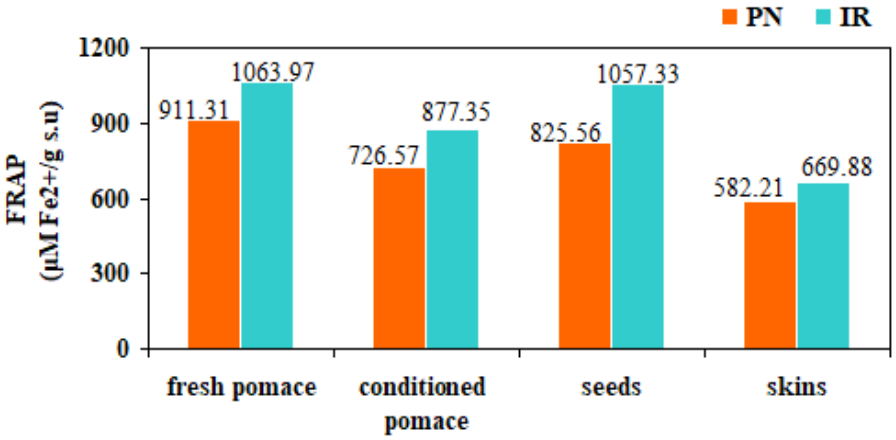

Figure 4. Antioxidant capacity of fresh and conditioned grape pomace and of its fractions

Our results are in agreement with those reported by Larrauri *et al.* [20] in their study about the effect of drying temperature (60, 100, and 140°C) on the polyphenols content and antioxidant activity of red grape pomace peels. Their results have proven that the drying at 60°C did not significantly affect the antioxidant characteristics of the evaluated samples.

The results reported by Tores *et al.* [21] highlighted that the most recommended drying method for conditioning of grape pomace is freeze-drying because it is the least aggressive method in term of protection against degradation of phenolic compounds from grape skins. In its absence, other methods such as vacuum belt drying and oven-drying, at 60°C can be used as preservation methods of polyphenolic compounds from grape pomace [21, 22]. As a result, the method of grape pomace conditioning is of the great importance to preserve its antioxidant properties.

The dried IR grape pomace and its fraction showed higher value than PN grape pomace. The highest content of total phenolics and FRAP value were concentrated in the seeds of both investigated dried pomace samples. These findings were in line with those reported in the study performed by Rockenbach *et al.* [23]. The results of this research state that both, seeds and skins, are rich in natural antioxidants, but there is a greater concentration of phenolic compounds in the grape seeds than in the grape skins.

Also, the research carried out by Ky & Teissedre [24] reported that the grape seed pomace extracts contained higher amounts of polyphenols than the grape skin pomace extracts. The grape seeds also had the highest antioxidant capacity.

The results of the research carried out by de la Cerda-Carrasco *et al.* [25] showed that the white grape pomace samples exhibited higher antioxidant capacities and higher contents of total phenolics and total proanthocyanidins compared with red grape pomace samples, but the latter samples showed much higher anthocyanin levels and color intensities.

It was noticed that the samples with a high polyphenolics content exhibited marked FRAP values. Thus, our results demonstrated that there is a significant correlation between the total phenolics content of investigated grape pomace samples and their main fractions coming out from different grape varieties and their antioxidant activity. These data are in agreement with the previously reported data by Yemis *et al.* [26].

Yilmaz & Toledo [27] reported that the grape seeds and skins contain high amounts of phytochemicals such as gallic acid, catechin and epicatechin and represent valuable raw materials for the production of dietary supplements with antioxidant function.

The results reported in this paper are useful for selection of grape pomace, both whole and its main fractions, to obtain valuable ingredients used in nutraceutical purposes for developing of high value-added products.

4. Conclusions

Our results revealed that the investigated grape pomace samples showed high but diverse contents of polyphenolic compounds and antioxidant

activities. The antioxidant properties of grape pomace exhibited a good stability in response to grape pomace drying for 24 h at a moderate temperature of 60°C. Both, seeds and skins, are rich in natural antioxidants, but there is a greater concentration of phenolic compounds in the grape seeds than in the grape skins. Grape seeds also had the highest FRAP values. The obtained data suggested that grape seeds and skins may be further exploited for recovery of bioactive compounds with proven antioxidant properties. Thus, the grape pomace represents an important potential source of polyphenolic compounds, which could be used in nutraceutical formulations. The knowledge on the antioxidant properties of grape pomace help to developing of a valuable strategy for an efficient and ecologically suitable utilization of winery by-products.

Acknowledgements: This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS/CCCDI-UEFISCDI, project number PN III-P2-2.1.BG-2016-0126, within PNCDI III, contract BG15/2016. The results of this research will be included in the PhD Thesis of Cristina GAITA, PhD student at Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

References

1. Faostat, Food and Agriculture Organization of the United Nations, **2013,** http://faostat3.fao.org/download/Q/QC/E.
2. Laufenberg, G.; Kunz, B.; Nystroem, M., Transformation of vegetable waste into value added products: (A) the upgrading concept; (B) practical implementations, *Bioresource Technology* **2003,** *87(2)*, 167–198.
3. Nerantzis, E.T.; Tataridis, P., Integrated Enology - Utilization of winery by-products into high added value products, *e-Journal of Science & Technology (e-JST)* **2006**, *1(3)*, 79–89.
4. Sanhueza, L.; Tello, M.; Vivanco, M.; Mendoza, L.; Wilkens, M., Relation between antibacterial activity against food transmitted pathogens and total phenolic compounds in grape pomace extracts from Cabernet Sauvignon and Syrah varieties, *Advances in Microbiology* **2014**, *4(5)*, 225–232.
5. Teixeira, A.; Baenas, N.; Dominguez-Perles, R.; Barros, A.; Rosa E.; Moreno, D.A.; Garcia-Viguera, C., Natural bioactive compounds from winery by-products as health promoters: A Review, *International Journal of Molecular Sciences* **2014**, *15(9)*, 15638–15678.
6. Shi, J.; Yu, J.; Pohorly, J.E.; Kakuda, Y., Polyphenolics in grape seeds –biochemistry and functionality, *Journal of Medicinal Foods* **2003**, *6(4)*, 291–299.
7. Teissedre, P.L.; Frankel, E.N.; Waterhouse, A.L.; Peleg H.; German, J.B., Inhibition of in vitro human LDL oxidation by phenolic antioxidants from grape and wines, *Journal of the Science of Food and Agriculture* **1996**, *70(1)*, 55–61.
8. Tsai, T.H.; Tsai, P.J.; Ho, S.C., Antioxidant and anti-inflammatory activities of several commonly used spices, *Journal of Food Science* **2005**, *70(1)*, C93–C97.
9. Nakamura, Y.; Tsuji, S.; Tonogai, Y., Analysis of proanthocyanidins in grape seed extracts, health foods and grape seed oils, *Journal of Health Science* **2003**, *49(1)*, 45–54.
10. Jayaprakasha, G.K.; Selvi, T.; Sakariah, K.K., Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts, *Food Research International* **2003**, *36(2)*, 117–122.
11. Deng, Q.; Penner, M.; Zhao, Y., Chemical composition of dietary fiber and polyphenols of five different varieties of wine grape pomace skins, *Food Research International* **2011**, *44(9)*, 2712–2720.
12. García-Lomillo, J.; González-San José, M.L., Applications of wine pomace in the food industry: approaches and functions, *Comprehensive Reviews in Food Science and Food Safety* **2017**, *16(1)*, 3–22.
13. Arvanitoyannis, IS.; Ladas, D.; Mavromatis, A., Potential uses and applications of treated wine waste: a review, *International Journal of Food Science & Technology* **2006**, *41(5)*, 475–487.
14. Diaz, M.J.; Madejon, E.; Lopez, F.; Lopez, R.; Cabrera, F., Optimization of the rate vinasse/grape marc for co-composting process, *Process Biochemistry* **2002**, *37(10)*, 1143–1150.
15. Dwyer, K.; Hosseinian, F.; Rod, M., The market potential of grape waste alternatives, *Journal of Food Research* **2014,** *3(2)*, 91–106.
16. Djilas, S.; Canadanović-Brunet, J.; Cetković, G., By-products of fruits processing as a source of phytochemicals, *Chemical Industry and Chemical Engineering Quarterly* **2009**, *15(4)*, 191−202.
17. Shrikhande, A.J., Wine by-products with health benefits, *Food Research International* **2000**, *33(6)*, 469–474.

18. Benzie, I.F.F.; Strain, L., Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay, *Analytical Biochemistry* **1996**, *239*, 70–76.
19. Singleton, V.L.; Orthofer, R.; Lamuela-Raventos, R.M., Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent, *Methods in Enzymology* **1999**, *299*, 152–178.
20. Larrauri, J.A.; Rupérez, P.; Saura-Calixto, F., Effect of drying temperature on the stability of polyphenols and antioxidant activity of red grape pomace peels, *Journal of Agricultural and Food Chemistry* **1997**, *45(4)*, 1390–1393.
21. Torres, C.; Díaz-Maroto, M.C.; Hermosín-Gutiérrez, I.; Pérez-Coello, M.S., Effect of freeze-drying and oven-drying on volatiles and phenolics composition of grape skin, *Analytica Chimica Acta* **2010**, *660(1–2)*, 177–182.
22. Vashisth, T.; Singh, R.K.; Pegg, R.B., Effects of drying on the phenolics content and antioxidant activity of muscadine pomace, *LWT - Food Science and Technology* **2011**, *44(7)*, 1649–1657.
23. Rockenbach, I.I.; Gonzaga, L.V.; Rizelio, V.M.; Gonçalves, de Souza Schmidt Gonçalves. A.E.; Genovese, M. I.; Fett, R., Phenolic compounds and antioxidant activity of seed and skin extracts of red grape (*Vitis vinifera* and *Vitis labrusca*) pomace from Brazilian winemaking, *Food Research International* **2011**, *44(4)*, 897–901.
24. Ky, I.; Teissedre, P.L., Characterisation of Mediterranean grape pomace seed and skin extracts: polyphenolic content and antioxidant activity, *Molecules* **2015**, *20(2)*, 2190–2207.
25. de la Cerda-Carrasco, A.; Lopez-Solis, R.; Nunez-Kalasic, H.; Pena-Neira, A.; Obreque-Slier, E., Phenolic composition and antioxidant capacity of pomaces from four grape varieties (*Vitis vinifera* L.), *Journal* of the *Science* of *Food and Agriculture* **2015,** *95(7)*, 1521–1527.
26. Yemis, O.; Bakkalbasi, E.; Artik, N., Antioxidative activities of grape **(***Vitis vinifera***)** seed extracts obtained from different varieties grown in Turkey, *International Journal of Food Science and Technology* **2008**, *43(1)*, 154–159.
27. Yilmaz, Y.; Toledo, R**.,** Major flavonoids in grape seeds and skins: Antioxidant capacity of catechin, epicatechin, and gallic acid, *Journal of Agricultural and Food Chemistry* **2004**, *52(2)*, 255–260.