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CONTENT IN BIOACTIVE COMPOUNDS AND ANTIOXIDANT CAPACITY OF FLOURS OBTAINED FROM WINEMAKING BY-PRODUCTS

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Abstract

Grape pomace is particularly noted for its content of fiber, phenolic compounds, unsaturated lipids and sterols, vitamins and antioxidants. In this paper are presented results of the performed research for determination of vitamin C, total polyphenol content and antioxidant capacity in case of flours (grape pomace flour, grape seed flour, grape skin flour) obtained from winemaking by-products. There were taken into the study, winemaking by-products from three grape varieties: Blauer Zweigelt, Burgund Mare and Fetească Regală. Vitamin C content of flours obtained from winemaking by-products varied in the range of 18.25-25.75 mg/100 g FW and total polyphenol content varied in the range of 57.45-258.75 mg gallic acid equivalent (GAE)/g FW. Due to the content in bioactive compounds, the obtained flours have an antioxidant capacity (9.98-46.12 mg trolox equivalent (TE)/g FW). The highest value of the antioxidant capacity was recorded in the case of grape seed flour, this one being followed by the grape pomace flour, on the last place being the grape peel flour. Due to the complex biochemical composition, the flours obtained from winemaking by-products, can be considered as functional ingredients.

***Key words**: winemaking by-products, polyphenols, vitamin C, antioxidant capacity.*

INTRODUCTION

Vinification is an important agro-industrial activity, in countries such as Italy, Spain and France, but also in Romania. Following the winemaking process, significant quantities of winemaking by-products (grape stalk, grape skins, grape seeds, traces of pulp) are produced. It is estimated that the production of 100 L of white wine results in about 30 kg of winemaking by-products (Mendes et al., 2013). Grape pomace represents a mixture of skins, seeds and traces of grape pulp, resulted after the wine is obtained. At the international level, there is a great interest for the increasing of the added value of industrial cultures, both for economic reasons and for the protection of the environment, associated with the tendency to obtain new food products and ingredients with nutritional and functional properties (García-Lomillo et al., 2017; Gil-Sánchez et al., 2018).

Grape pomace is particularly noted for its content in fiber, phenolic compounds, unsaturated lipids and sterols, vitamins and antioxidants (Kalli et al., 2018). According to the international performed studies (Brenes et al., 2016), the content of bioactive compounds of grape pomace depends on the variety of grapes, the area where the culture is located, the conditions of fertilisation, the soil and the period of grape harvest. Also, research conducted by Cappa et al. (2015), showed that more than 70% of the phenolic compounds in grapes remain in the grape pomace. Therefore,

it is very important their recovery from this food matrix and use them in the composition of food products, as preservatives, antioxidants, colorants or fortifying agents. Sousa et al. (2014) evaluated the biochemical composition of the flour obtained from grape pomace (*Vitis vinifera* L.), *Benitaka* variety, grown in the semiarid region of Northeast Brazil. The results showed that this flour obtained from these residues had below neutral pH (3.82), moisture (3.33 g/100 g), acidity (0.64 g of citric acid/100 g), noting the content in ash (4.65%), total dietary fiber (46.17%) carbohydrate (29.2%), protein (8.49%), lipids (8.16%), vitamin C (26.25 mg/100 g), and anthocyanins (131 mg/100 g). Also, these authors mention that the minerals iron (Fe), potassium (K), zinc (Zn), manganese (Mn), and calcium (Ca) were present in higher concentrations. Flours achieved from winemaking by-products (black seed flour, black pomace flour) by Catană et al. (2017) are noted by their content in ash (2.80-6.61%), protein (10.53-10.85%), fat (8.49-15.36%), total fibre (58.86-66.06%), K (1102.35-3406.67 mg/100 g), Ca (476.62-988.45 mg/100 g), Mg (146.55-223.75 mg/100 g), Fe (3.97-8.67 mg/100 g), total polyphenols (200.15-322.75 mg/100 g) and antioxidant capacity (40.75-51.25 mg Trolox Equivalents/g).
In this paper are presented results of the performed research for determination of vitamin C, total polyphenol content and antioxidant capacity in case of flours (grape pomace flour, grape seed flour, grape skin flour) obtained from winemaking by-products.

MATERIALS AND METHODS

Samples

The winemaking by-products from three grape varieties *(Blauer Zweigelt, Burgund Mare* and *Fetească Regală),* were provided by the National Research & Development Institute for Biotechnologies in Horticulture Ștefănești Argeș. Experiments were performed within the Pilot Experiments Plant for Fruits and Vegetables Processing from the National Research & Development Institute for Food Bioresources. Till processing, winemaking by-products were stored under refrigeration (3°C). The winemaking byproducts were subjected to dehydration process in a convection dryer at temperature of 50ºC to a moisture which allows their milling and conversion into flours and, at the same time, their stability in terms of quality. Milling of dried semi-finished products was performed by using Retsch mill. The achieved functional ingredients (flours) were packed in glass containers, hermetically sealed, protected by aluminum foil against light and stored in dry and cool areas (temperature of maximum 20ºC), till to the biochemical analysis. Figures 1-3 show flours obtained from grape pomace, resulted from the three studied grape varieties: *Blauer Zweigelt, Burgund Mare* and *Fetească Regală*.

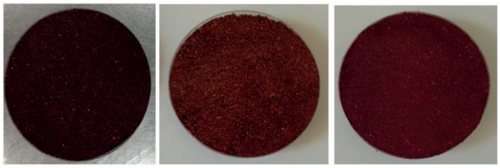

Figure 1. Flours achieved from grape pomace, *Blauer Zweigelt* variety (FTS_BZ - grape pomace flour; FSS_BZ - grape seed flour; FCS_BZ - grape skin flour)

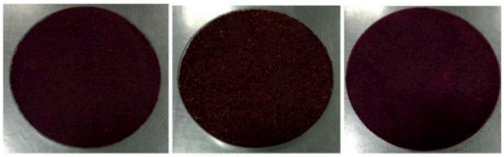

Figure 2. Flours achieved from grape pomace, *Burgund Mare* variety (FTS_BM - grape pomace flour; FSS_BM - grape seed flour; FCS_BM - grape skin flour)

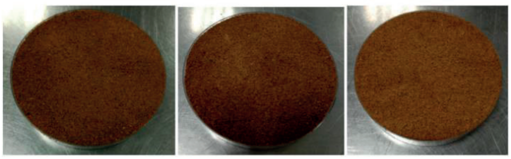

Figure 3. Flours achieved from grape pomace, *Fetească Regală* variety (FTS_FT- grape pomace flour; FSS_FT - grape seed flour; FCS_FT - grape skin flour)

Methods

Vitamin C content

Determination of vitamin C was performed by high performance liquid chromatography (HPLC) (Accela, Thermo Scientific) coupled with high resolution mass spectrometry (HRMS) (LTQ OrbitrapXL Hybrid Ion Trap-

Orbitrap MassSpectrometer, Thermo Scientific) using hippuric acid as internal standard.
LC conditions: Column (Hypersil GOLD aQ, 150 x 2.1 mm, 3 μm); Column temperature: 40°C; Sample temperature: 4°C; Mobile phase A: 990 mL water: 10 mL 1M ammonium formate (aq): 1 mL formic acid; Mobile phase B: 990 mL methanol: 10 mL 1M ammonium formate (aq): 1 mL formic acid; Flow rate: 0.400 mL/min; Injection volume: 25 μL;
MS conditions: Analyzer: Fourier Transform Mass Spectrometry (FTMS); Resolution: 60000; Ionization mode: Electrospray ionization in negative ion mode (ESI-); Specific ions were: *m/z* = 175.02438 (for vitamin C) and *m/z* = 178.05051 (for hippuric acid). In Figure 4 is presented the calibration curve of vitamin C, achieved in the concentration range 1500-10000 μg/L.

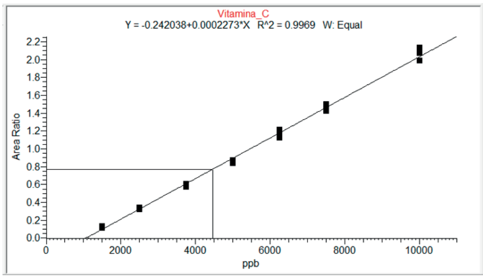

Figure 4. Calibration curve of vitamin C

Total polyphenol content
Total polyphenol content was conducted according to Horszwald & Andlauer (2011), with some modifications (concerning extract volumes of the used sample and reagents, using UV-VIS Jasco V 550 spectrophotometer), based on calibration curve of gallic acid achieved in the concentration range of 0-0.20 mg/mL (Figure 5).

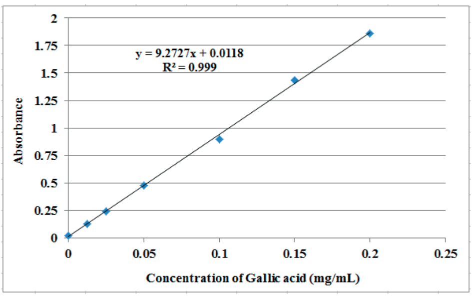

Figure 5. Calibration curve of gallic acid

The extraction of phenolic compounds was performed in methanol: water 50:50 (v:v), and the absorbance of the extracts was determined at a wavelength λ = 755 nm. Results were expressed as mg of gallic acid equivalents (GAE) per g flour.

Antioxidant capacity
The 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging radical assay was conducted according to Horszwald & Andlauer (2011), with some modifications (concerning extract volumes of the used sample and reagents, using UV-VIS Jasco V 550 spectrophotometer). The reaction was performed in dark for 30 min (at ambient temperature) and after this time the absorbance was read at 517 nm. It was achieved the calibration curve Absorbance = f (Trolox concentration), in the concentration range of 0-0.4375 mmol/L (Figure 6). Results were expressed as mg Trolox Equivalents per g flour.

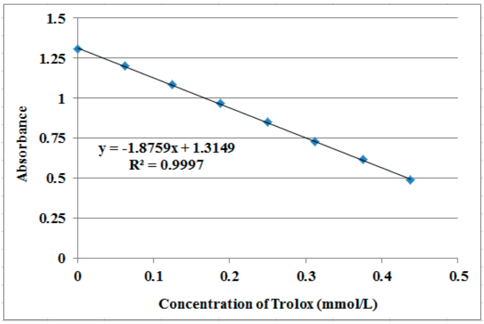

Figure 6. Calibration curve of Trolox

RESULTS AND DISCUSSIONS

Vitamin C content
Vitamin C content of the flours obtained from winemaking by-products varied in the range of 18.25-25.75 mg/100 g (the minimum value was registered in case of grape pomace flour *Burgund Mare* variety, and the maximum one in case of grape skin flour *Fetească Regală* variety) (Figure 7).Vitamin C content of these powders is comparable with that reported by Sousa et al. (2014) for grape pomace flour (26.25 mg/100 g) and higher than that reported by Nayak et al. (2018), in case of *Cabernet* grape pomace (22.8 mg/100 g). Concerning the type of flour obtained from winemaking by-

products, the highest value of the vitamin C content was registered in the case of grape seed flour, this one being followed by grape pomace flour and, on the last place being grape skin flour.

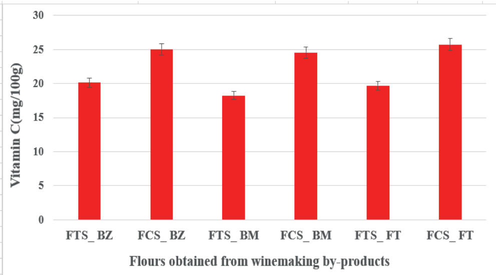

Figure 7. Vitamin C content of the flours obtained from winemaking by-products

Vitamin C plays a role in many processes as a cofactor for enzymes involved in processes and effects important for cancer transformation: antioxidant defence, transcription, and epigenetic regulation of gene expression (Granger & Eck, 2018). Vitamin C is an antioxidant that may scavenge reactive oxygen species preventing DNA damage and other effects important in cancer transformation (Pawlowska et al., 2019). Vitamin C is also reported to exert beneficial effects in the immune system and inflammation, which is crucial in fighting precancerous and cancer cells by the host (Ang et al., 2018). It is important to mention that vitamin C is needed for the repair of tissues in all parts of the body (Devaki et al., 2017).

Total polyphenol content

The flours achieved from winemaking by-productsare also noted by total polyphenol content (Figure 8).Total polyphenol content of flours obtained from the winemaking by-products taken into study varied in the range of 57.45-258.75 mg GAE/g (the minimum value was registered in the case of grape skin flour *Fetească Regală* variety, and the maximum one, in the case of grape seed flour, *Burgund Mare,* variety). Concerning the type of flour obtained from winemaking by-products, the highest total polyphenol content was registered in the case of seed flour, this one being followed by grape pomace flour, on the last place being skin flour. Results are according to those obtained by Muncaciu et al. (2017), who studied two grape varieties *Fetească neagră* (variety for red wine) and *Italian Riesling* (variety for white wine).

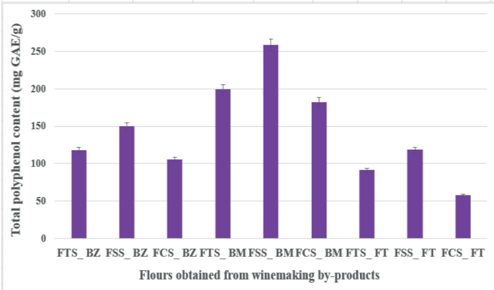

Figure 8. Total polyphenol content of the flours obtained from winemaking by-products

In results obtained by Muncaciu et al. (2017), grape seed flour presented the highest total polyphenol content (103.62 mg epicatechin/g DW in he case of grape seed flour, *Italian Riesling* variety and, respectively, 94.40 mg epicatechin/g DW in the case of grape seed flour, *Fetească neagră* variety).
Concerning the grape variety, in the case of experiments carried out, powders obtained from winemaking by-products belonging to the *Burgund Mare* variety, presented the highest values of the total polyphenol content: 199.87 mg GAE/g in the case of grape pomace flour; 258.75 mg GAE/g in the case of seed flour; 182.55 mg GAE/g in the case of skin flour.
It is also worth noting that the total polyphenol content of grape pomace flour, obtained from winemaking by-products belonging to the three grape varieties taken into study (*Blauer Zweigelt, Burgund Mare, Fetească Regală)* is higher than that reported by other authors: 3.64 mg GAE/g (*Barbera* variety) and 16.06 mg GAE/g (*Chardonnay* variety) (Marchiani et al., 2016); 55.80 mg GAE/g (*Quebranta* variety) and 49.67 mg GAE/g (*Torontel* variety) (Solari-Godiño et al., 2017); 41.14 mg GAE/g (*Cabernet Sauvignon* variety) (Urquiaga et al., 2015).
Polyphenols are an important class of compounds that have antioxidant, anti-inflammatory (Chedea et al., 2018), anti-aging (Kostyuk et al., 2018) and anti-cancer (Cipolletti et al., 2018) effects, as well as the prevention of different diseases (Figueira et al., 2017; Kujawska et al., 2018).

Costabile et al. (2019) evaluated the acute effects of the consumption of a drink rich in polyphenols from red grape pomace on glucose/insulin and triglyceride responses to a standard meal in healthy individuals, and the relationship between plasma levels of phenolic metabolites and metabolic parameters. These authors have shown that red grape pomace consumption acutely reduced postprandial insulin levels and improved insulin sensitivity. This effect could be likely related to the increase in gallic acid levels. The drink rich in polyphenols from red grape pomace added to the regular diet, it could contribute to the increasing of the daily intake of polyphenols, with potential health benefits. Lu et al. (2019) have shown that grape polyphenol extracts have a great influence on the recovery of gut microbiota after antibiotics and high-fat diet treatment.

Antioxidant capacity

Due to their content in phenolic compounds, flours achieved from grape pomace (grape pomace flour, grape seed flour, grape skin flour) have antioxidant capacity (Figure 9).

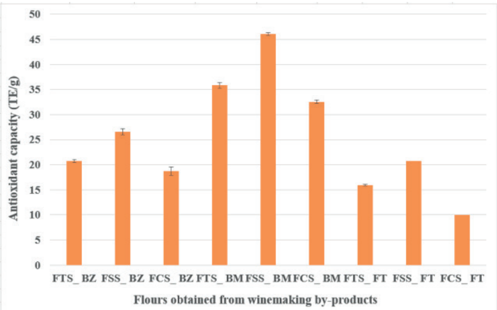

Figure 9. Antioxidant capacityof the flours obtained from winemaking by-products

Antioxidant capacity of the flours obtained from winemaking by-products taken into study, varied in the range of 9.98-46.12 mg TE/g (the minimum value was registered in the case of grape skin flour, *Fetească Regală* variety, and the maximum one, in the case of seed flour, *Burgund Mare* variety). Concerning the type of flour obtained from winemaking by-products, the highest value of the antioxidant capacity was registered in the case of seed flour, this one being followed by grape pomace flour, on the last place being grape skin flour. As regards as the grape variety, in the case of experiments carried out, powders obtained from winemaking by-products belonging to the *Burgund Mare* variety, presented the highest values of the antioxidant capacity: 35.17 mg TE/g in the case of grape promace flour; 45.60 mg TE/g in the case of grape seed flour; 32.10 mg GAE/g in the case of grape skin flour. Between the total polyphenol content and antioxidant capacity of the powders obtained from winemaking by-products, there is a linear correlation ($y = 5.5381x + 2.5929$, $R^2 = 0.9999$) (Figure 10).

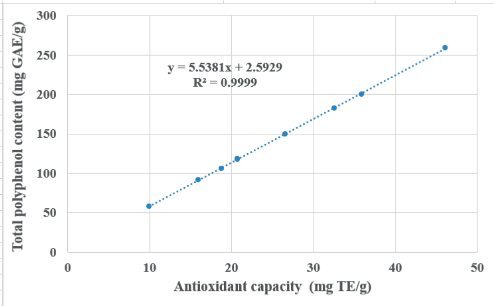

Figure 10. Correlation between the total polyphenol content and antioxidant capacity in case of flours obtained from winemaking by-products

The results presented are consistent with those reported by Catană et al. (2017), which also obtained a linear correlation between the total polyphenol content and the antioxidant capacity values, in case of the flours achieved from tomato waste and winemaking by-products.

CONCLUSIONS

Flours (grape pomace flour, grape seed flour, grape skin flour) obtained from winemaking by-products are noted for their vitamin C content (18.25-25.75 mg/100 g) and total polyphenol content (57.45-258.75 mg GAE/g). Also, due to their content in bioactive compounds, these powders have antioxidant capacity: 9.98-46.12 mg TE/g.

Flours obtained from winemaking by-products can be regarded as functional ingredients and can be used to fortify food products (bakery and pastry products, especially) in order to increase the nutritional value and their antioxidant capacity.

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