

VALORISATION OF GRAPE MARC FROM PIETROASA VINEYARD FOR OBTAINING NEW DRY FEED INGREDIENTS AS A POTENTIAL POLYPHENOLS SOURCE

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Abstract

The paper relates to obtaining new feed ingredients based on dry grape marc rich in polyphenols, using the grape marc from Pietroasa vineyard. In the study presented herein, was also determined the content of total polyphenols (TP) and the antioxidant activity of 4 samples from white and red grape marc varieties before (frozen samples control) and after drying. The grape marc investigated in this study was obtained in 2017 from 'Riesling Italian' and 'Tamaioasa Romaneasca' (white sample) and from 'Merlot' and 'Burgund mare' (red sample). The samples drying were carried out by two processes also made in light and dark by dehydration and by drying in the oven for 24 hours at 50-60°C, and the polyphenol content was compared. The TP content is correlated with the dehydration method for three of the 4 analyzed samples. The high content of TP 4928.3 mg/100g GAE equivalent (Folin-Ciocalteu method), was found in the 'Merlot' grape marc variety dried by dehydration at dark ($p < 0.05$). The antioxidant activity determined by the DPPH method was approximately similar (7.5 mM TRE) for all sample and drying methods. Antioxidant properties of new feed ingredients were based on the drying methods of the grape marc varieties.

Key words: polyphenols, feed ingredients, DPPH, grape marc.

INTRODUCTION

Viticulture is one of the main agricultural activities, with a production of 210 Mton of grapes worldwide, of which 15% are addressed to the winemaking industry. The International Vine and Wine Organization (OIV) showed that 279 million hl of wine were produced globally in 2014 (Martínez et al., 2016). According to Teixeira et al. (2014), this socio-economic activity involves the generation of significant quantities of solid waste up to 30%, w/w of material used, grape marc (Nerantzis et al., 2006) and vine shoots (Matei et al., 2016) are the most abundant.

The food value of grapes is due to the chemical compounds and their form accessible to the human body: sugars, minerals, vitamins, polyphenolic compounds, flavours and organic acids, all of which have a positive effect on human health (Dejeu, 2011). As a result of the winemaking process, the grape marc (skins and seeds) and lees (sediment solids) is the solid

part that is separated. Grape marc, as the most abundant by-product of the winemaking process (dry 15%, wet up to 25–45%) (Nerantzis et al., 2006), allows multiple uses: by distillation it is possible to obtain brandy; the seed produces oil for food use (Fiori et al., 2007); or biodiesel from it (Fernández et al., 2010); as a result of the extraction of seeds, compost is obtained for the organic fertilization of the soil (Alexandrescu and Băjescu, 1960); and the extraction of polyphenols with antioxidant properties (Pinelo et al., 2005). Traditional applications of grape marc without any pre-treatment care were for animal feed formulations or compost production. Scientific works carried out on the characterization of the chemical components of grape waste by-products have allowed looking for different applications in trying to obtain high added value ingredients.

The Pietroasa - Buzau Development Research Center for Viticulture and Winemaking is a subsidiary of the University of Agronomic

Sciences and Veterinary Medicine since 2005. The Pietroasa area is one of the principle regions of Romania where the quality of the grapes determines the quality of the wine.

The quality of grapes is influenced by the variety, as well as weather conditions during the growing season, soil minerals and acidity (Cubadda, 2004). Grape varieties cultivated in Pietroasa vineyard may accumulate great quantities of polyphenol compounds, which assure wines with high chromatic indices and a harmonious chemical composition, presented in the study performed by Drăghici and Răpeanu (2011). Grape marc is a potential source of polyphenols and natural antioxidants. Cellulase digested grape pomace showed significantly higher polyphenolic contents in the Folin-Ciocalteu's assay, and it also showed significantly higher reductive activities in DPPH radicals, compared to the gluco-amylase digested and the not digested groups (Kabir, 2015).

Thus, in this study aims to determine the content of total polyphenols and the antioxidant activity of dry sample of white and red grape marc varieties from Pietroasa vineyard, to obtaining new feed ingredients.

MATERIALS AND METHODS

Raw materials

According to EC 955/2014, which establishes the waste list under Directive 2008/98/EC of the European Parliament and of the Council, grape marc has received code no. 02 07 01 - wastes from washing, cleaning and mechanical processing of raw materials. It consists in solid residues from fermentation or unfermented yeasts (skins, seeds, bunch of clove and must). From 100 kg of grapes result 25 kg of grape marc (of which 50% skin, 25% seeds, 25% stems) (Llobera and Cañellas, 2007).

Grape marc freeze (control)

The grape marc investigated in this study was obtained in 2017 from white sample: 'Riesling Italian' and 'Tamaioasa Romaneasca' and from red sample: 'Merlot' and 'Burgund mare'. Part of the marc sample of the grape varieties analysed was stored in the freezer and represents the control sample.

The wine cellar of Pietroasa has long functioned as one of the best endowments in the

country, benefiting since 1940, from a vintage centre equipped with modern equipment imported from Italy.

The wine collection that started at that time now counts about 100,000 bottles, the oldest in the 1940s Vinum Universitas variety wines are made from Italian Riesling and Feteasca Neagră. The ecological, pedological and climatic conditions meet the requirements of these varieties and provide them with the resources to reach full maturity, sometimes going well beyond this stage.

Grape marc dehydration

Samples of grape marc taken in study were dried at darkness and light by dehydration.

The wet grape marc is distributed in pre-sterilized Petri dishes and is introduced into the dehydrator (Gorenje FDK24DW Food Dehydrator) at 50-60°C for 24 h until it reaches a humidity of 5% (Figure 1).



Figure 1. Grape marc dehydration in Gorenje FDK24DW Food Dehydrator

Grape marc dried by oven

Drying the grape marc in the oven: the wet fermented grape marc is evenly distributed in a thin layer in Petri dishes (Figure 2) and is introduced at 50°C for 24 hours in the oven for drying (Drying Oven/Incubator Biobase BOV-D30).

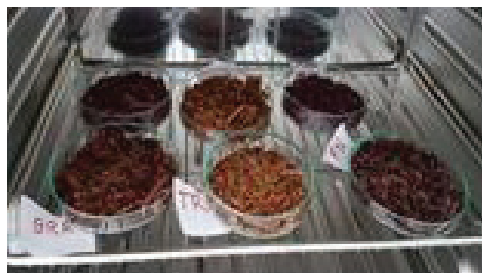


Figure 2. Grape marc dried in Drying Oven Biobase (BOV-D30)

Extraction of polyphenols and determination of total polyphenol

The polyphenols were extracted from the marc grapes in 80% acetone at 37°C for 20 hours with continuous stirring. Total content of polyphenols of the extract was determined by the Folin-Ciocalteu method, adapted to a microscale (Arnous et al., 2001). In short, 10 µl diluted 1/9 sample mixed with 790 µL of distillate water and 50 µl of Folin-Ciocalteu reagent in 1.4 mL cuvettes.

After 1 minute, 150 ml of Na₂CO₃ were added to the reaction medium and stored in the dark at room temperature for 2 hours. The absorbance was read at 750 nm using a Specord 250 spectrophotometer (analytical Jena) and the total concentration of polyphenol was calculated from a calibration curve using gallic acid as standard. The results were expressed as milligrams gallic acid equivalent (GAE) 100 g⁻¹ product (Barbulescu et al, 2007).

Antioxidant activity determination

Antioxidant activity was measured in terms of hydrogen donation or radical scavenging capacity, using the stable radical, DPPH (Brand-Williams et al., 1995) as described by (Garcia et al., 2012). An aliquot of 25 µl of diluted sample was added to 975 µl of DPPH solution (60 µM in MeOH), then vortexed and the absorbance was read at 515 nm at t = 0 and t = 30 min. The results were expressed as mM trolox equivalents (TRE). The DPPH assay provides a quick way to evaluate potential antioxidants (Barbulescu et al, 2007).

Data analysis

Samples were assayed in 4 repetitions and results are given as averages ± SD.

Statistical analysis was performed using ANOVA Microsoft Excel 2010 and multiple comparison procedures were performed to test the effect of drying method to content of total polyphenols and the antioxidant activity.

RESULTS AND DISCUSSION

Content of total polyphenols (TP)

Given the absorbance values obtained from the samples of grape marc on methanolic extract solutions that reacted with the reagent (Folin-Ciocalteu) and compared with the standard

GAEs solutions, the TP content is shown in Figure 3.

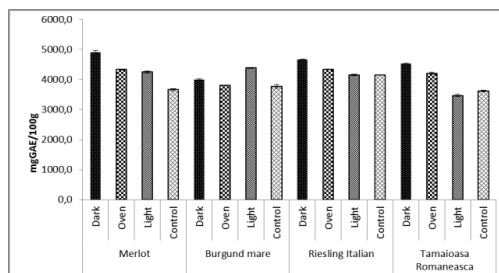


Figure 3. Total polyphenolic content (TP) (mg GAE/100g) in methanolic extracts from grape marc varieties dried by dehydration and in oven-dried. Error bars show the standard deviation

The TP content of grape marc extracts varied between varieties and between drying method, ranged from 3471.1 to 4918.8 mg GAE/100g. According to the drying method, we observe the highest average values for drying in the dark by dehydration (4528.7 mg GAE/100g) followed by drying in the oven (4175.7 mg GAE/100g), as for in the light by dehydration (4068.3 mg GAE/100g) and the lowest average values for the freezed sample (control) (3803.6 mg GAE/ 100g).

‘Merlot’ grape marc has a higher TP amount (4918.8 mg GAE/100g) for drying in the dark by dehydration than ‘Tamaioasa Romaneasca’ grape marc drying in the light by dehydration (3471.1 mg GAE/100g) ones.

The average values of the polyphenol content for all the drying methods showed that the lowest obtained were those of the white grape marc ‘Tamaioasa Romaneasca’ variety (3956.6 mg GAE/100g).

Antioxidant activity

Grape marc antioxidant activity showed that the examined varieties and drying method of sample were able to scavenge the DPPH radical (Figure 4). Three of four dried grape marc varieties by dehydration at light (‘Burgund mare’, ‘Riesling Italian’ and Merlot) displayed the highest antioxidant activity; values were: 7.66, 7.66- and 7.64-mM TRE, respectively.

The average values for all the drying methods of grape marc variety displayed the highest antioxidant activity for ‘Riesling Italian’ compared to ‘Burgund mare’ (7.57 and 7.35 mM TRE, respectively).

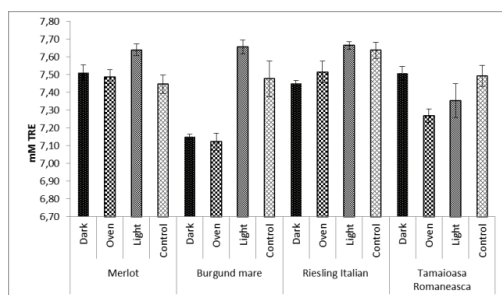


Figure 4. Antioxidant activity (mM TRE) of grape marc varieties dried by dehydration and in oven-dried. Error bars show the standard deviation.

However, the values of antioxidant activity for the grape marc showed that are no significant differences between varieties, average value was 7.46 mM TRE.

The samples studies by Lafka et al. (2007), were dried at $60 \pm 0.5^\circ\text{C}$ in an air-circulating tray dryer (Apex SSE17M, London, England), ground to fine powder in a Brabendergrinder and stored at 20°C for further experimentation.

The antioxidant activity of winery waste was significant influenced by the drying temperature. Drying a 80°C reduced the antioxidant activity of winery waste of 21% and for 100°C of 33%, compared to drying a 60°C .

According to Larrauri et al. (1997), the antioxidant activity of samples dried with air at 100°C was reduced by 28% and, at 140°C by half, with respect to drying at 60°C .

Correlation between drying grape marc method and TP content

The grape marc 'Merlot' and 'Riesling Italian' varieties showed a higher TP content, which was most closely correlated with the dried method of dehydrating (correlation coefficient: $r = 0.97$, $p < 0.05$ for grape marc 'Merlot' variety and $r = 0.92$, $p = 0.5$ for grape marc 'Riesling Italian' variety) (Figure 5).

Drying of winery waste before extraction was performed at a temperature of 60°C , as increasing the temperature above 60°C significantly reduced the yield of extracted phenols (Lafka et al., 2007).

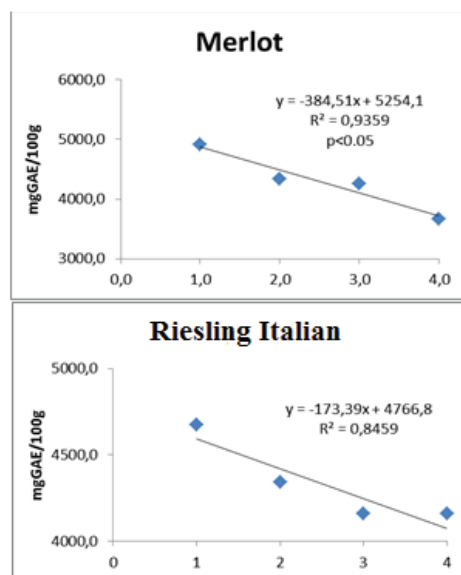


Figure 5. Correlation between drying grape marc dehydrated method (1-dark, 2-light, 3-oven) and 4-frozen (control), and TP content (mg GAE/100g)

Correlation between drying grape marc method and antioxidant activity

Grape marc 'Riesling Italian' variety displayed also the highest antioxidant activity, which was most closely correlated with the dried method of dehydrating (correlation coefficient: $r = 0.91$) (Figure 6).

Correlation between TP content and antioxidant activity for dried grape marc varieties

A good correlation was found for the total polyphenols and antioxidant activity for grape marc 'Riesling Italian' variety ($r = 0.94$ and p value equal to 0.05).

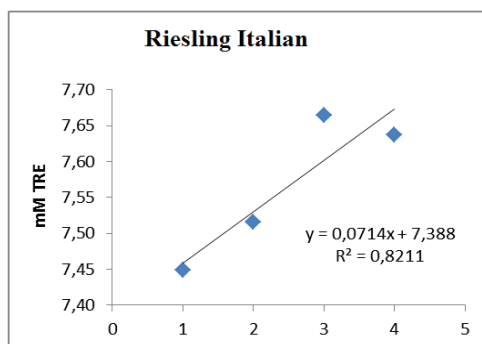


Figure 6. Correlation between drying grape marc dehydrated method (1-dark, 2-light, 3-oven) and 4-frozen (control), and antioxidant activity (mM TRE)

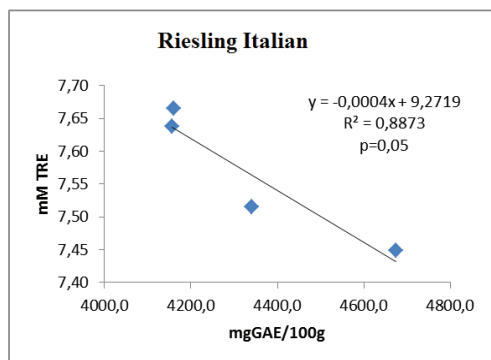


Figure 7. Correlation between TP content (mg GAE/100g) and antioxidant activity (mM TRE) for dried grape marc varieties

CONCLUSIONS

It was observed that the TP content of grape marc extracts varied between varieties and between drying method, ranged from 3471.1 to 4918.8 mg GAE/100g;

‘Merlot’ grape marc have a higher TP amount (4918.8 mg GAE/100g) for drying in the dark by dehydration;

The average values of the polyphenol content for all the drying methods showed that the lowest obtained were those of the white grape marc ‘Tamaioasa Romaneasca’ variety (3956.6 mg GAE/100g);

Also, it was observed that the average values for all the drying methods of grape marc variety displayed the highest antioxidant activity for ‘Riesling Italian’ compared to ‘Burgund mare’ (7.57- and 7.35-mM TRE, respectively);

The grape marc ‘Merlot’ and ‘Riesling Italian’ varieties showed a increased TP content, which was most closely correlated with the dried method of dehydrating compared to frozen samples;

Grape marc ‘Riesling Italian’ variety presented also the highest antioxidant activity, which was most closely correlated with the dried method of dehydrating;

A good correlation was found for the total polyphenols and antioxidant activity for grape marc ‘Riesling Italian’ variety.

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