Degradation Degree of Polyphenols Depending on Drying Temperature of the Grape Pomace

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Abstract

Grape pomace provides a rich source of polyphenols, which have the capacity to act as powerful antioxidants. Grape pomace has been used mainly in ruminant feed as a source of fiber and for broiler chickens as a source of antioxidants. It has been proved that the polyphenols from grape pomace are decomposed during the drying process. Taking into consideration the uncertainty of polyphenols thermal degradation, the aim of this study was to identify the polyphenols degree of degradation during the drying step at different temperatures. Red grape pomace (Black Maiden) from the same source was dried at room temperature (20°C) five days and three days at 38°C and 50°C. The total content of polyphenols (TP), tannins (Ta) and anthocyanins (An) was determined by different colorimetric methods. Our data reveal a significant degradation of the TP and An for all the three thermal variants. The Ta and non-adsorbed polyphenols on casein show a high degree of correlation, but with significant differences just between 20 and 38°C. Probably the lack of significant differences after 38°C, is determined by the thermal fragmentation of the polymer chain (Ta), that make this fragments unable to adsorb with casein, an important proportion of Ta being affected by the first shock of heat. The correlation within Ta and non-adsorbed polyphenols on casein is the result of passage of the truncated Ta chains in non-adsorbed polyphenols fraction on casein. The drying and storing process is an important step for grape pomace usage as a feed additive because we demonstrated that the Ta presents a high level of degradation especially between 20 and 38°C.

Keywords: *anthocyanins, grape pomace, tannins, polyphenols*

INTRODUCTION

The grape pomace is a by-product generated during the winemaking process, in a very high quantity made up of seeds, skins and stalk fragments. According to the International Organization of Vine and Wine (OIV) this by-product is about 20% of the total weight of the harvested grapes (*Vitis vinifera*) (Sui *et al.*, 2014).

Studies on the chemical composition of the grape pomace revealed that in addition to its content in protein, fiber, sugars, minerals and fats (Aslanian *et al.*, 2011) it contains high amounts of polyphenols (Alonso *et al.*, 2002; Bonilla *et al.*, 1999), which justify the use of this by-product in animal nutrition (Baumgärtel *et al.*, 2007; Zalikarenab *et al.*, 2007).

Polyphenols accumulate in the solid parts of the grapes, in skins (28-35%) seeds (60-70%)

and stalks (less than 10%) which pass, after the maceration-fermentation process into the grape juice and wine, up to 50% of them, the rest remaining in the grape pomace.

Chemically speaking, phenols are substances, which have in their structure an aromatic ring (benzene) with one or more hydroxyl (-OH), including functional derivatives.

Over 8000 phenolic compounds were identified and according to the similarity of their chemical structures, they were classified into classes and subclasses. The grapes have mainly anthocyanins, flavones, flavonols, stilbenes (resveratrol) and phenolic acids (Dopico-Garcia *et al.*, 2008).

Numerous studies have shown the beneficial effects of polyphenols including the antioxidant, antibacterial, anti-inflammatory and anti-metano-

genic ones, as well as a protective role against the degenerative diseases being able to present thus a great interest in animal health and nutrition (Brenes *et al.*, 2008; Goni *et al.*, 2007; Saurai, 2014).

If fresh, the grape pomace has a high water content (over 60%), which does not allow its preservation for a long time. Likewise, the presence of sugars increases the alteration process of this by-product, thus requiring a preliminary drying to prevent the degradation processes caused by the attack of the microorganisms from the environment.

To dry the grape pomace in well-ventilated areas or by exposure to the sun (Guemour *et al.*, 2010) can be a way to store it, for a better valorization in the animal nutrition. The grape pomace drying may be carried out in such a way that the content of polyphenols to be kept as much as possible. In the published literature, there are few data on the influence of the drying temperature on the content of total polyphenols, anthocyanins and tannins.

It was proven that the content of polyphenols from grapes degrades during the drying process. Similarly, the study carried out by Larrauri *et al.*, (1997) showed that drying the grape pomace at temperatures below 60°C did not cause a very high degradation of the polyphenols, but the 100°C drying caused a rather large decrease of these compounds.

The objective of the hereby study was to identify the degree of degradation of the polyphenols during the drying stage of the grape pomace at different temperatures.

MATERIALS AND METHODS

The fresh grape pomace (FGP) resulted from the red wine grapes (Black Maiden) vinification process was obtained from the Research Station (V. Adamachi farm) of the USAMV Iasi being made up of skins, seeds and stalk fragments.

The grape pomace was dried in different ways: at room temperature (20°C), for five days, or oven drying at 38°C and 50°C respectively for three days.

Given the heterogeneity of this by-product it was tried for the test evidence to be more homogeneous and representative of the whole biomass. The samples from the laboratory experiments were obtained by the fractionation of the aggregate samples, using the quartering method.

To determine the content of polyphenols, the samples were ground in the mechanical laboratory mill Grindomix GM 200 until we obtained a powder with particles of 1mm diameter, according to the SR ISO 6498: 2001 standard.

Chemical reagents. All reagents used throughout the work were of analytical purity. Folin-Ciocalteu reagent (FCR, Merck, Germany) as a chromogenic agent were used; Casein (Merck, Germany) as a adsorbed agent; Sodium carbonate decahydrate (Chemical Company, Romania); Anhydrous Gallic acid (Merck, Germany); concentrated hydrochloric acid (37%) and ethylic alcohol (96%) were purchased from Chemical Company, Romania.

Determination of total polyphenols, tannins and anthocyanins. To determine the total content of polyphenols and tannins, the grape pomace samples were previously degreased to remove the lipophilic substances (oils, acids, vitamins, triglycerides etc.) present in the grape pomace. The extraction of these substances from the vegetal material was performed using the petroleum ether by the Soxhlet classical method.

The total polyphenols (TP%) and tannins (Ta%) in the alcoholic extracts (50%) obtained of the degreased grape pomace samples according to the patent (Bişboacă, 2012) were determined by Folin-Ciocalteu method described by Singleton and Rossi (1965), hereinafter briefly presented.

The degreased grape pomace powder samples (3 g) were extracted with 50 ml of ethanol aqueous solution of 50% (v/v) for 24 hours at thermostat at 45°C with intermittent shaking (Bişboacă, 2012). After 24 hours of thermostating, the extracts were cooled and centrifuged at 5000 rpm for 15 minutes to recover the supernatants (basic sample solution, BSS). To determine the tannins we used the scheme proposed by Tarcau *et al.*, (2013), two milliliters of BSS were sampled in an Erlenmeyer flask (50 mL) where 8 mL of water and 10 mL of acetate were introduced (S1 solution 1). 10 mL were taken from S1 where 50 mg of casein were added and stirred for 45 min, (adsorption of the tannins). After the adsorption on the casein of the tannins and filtration, solution 2 (S2) was obtained. Of each S1 and S2 solution 1 mL was taken to determine the total polyphenols using the Singleton and Rossi, (1965) method, in which 3 mL of Folin-Ciocalteu reagent (FCR) were added, 20.0 mL of Na₂CO₃ 10H₂O solution having a

Tab.1. The content of total polyphenols, tannins and anthocyanins (mean±SD in g/kg) depending on the drying temperature

Drying temperature (°C)	Total polyphenols (g/kg)	Tannins (g/kg)	Non-adsorbed polyphenols on casein (g/kg)	Anthocyanins (g/kg)
20°C	21.68 ± 0.50 ^a	15.34 ± 0.39 ^a	6.19 ± 0.15 ^a	0.42 ± 0.005 ^a
38°C	19.38 ± 0.10 ^b	11.63 ± 0.95 ^b	6.76 ± 0.95 ^b	0.33 ± 0.002 ^b
50°C	17.92 ± 0.46 ^c	11.04 ± 0.34 ^{NS}	6.87 ± 0.17 ^c	0.31 ± 0.002 ^c
Anova* p-value	9.02E-49	1.5E-22	1.16E-08	3.36E-08

Note: Values marked by different letters (a-c) are significantly different (T-test, $p < 0.05$); ^{NS}Non-significant differences; n=3; *Anova single factor ($p < 0.05$)

concentration of 10%. The absorbance was measured after 60 min at a wavelength of 760 nm.

The content of total polyphenols expressed as gallic acid gram equivalents per kg (GAE g/kg), using the standard curve in the range of 0.01 mg to 0.1 mg/mL. The calibration equation for gallic acid was $y = 0.9343x - 0.0103$, being observed a good linearity ($R^2 = 0.9995$).

The differences between the total polyphenols content determined in S1 and S2 (total polyphenols – the non-adsorbed polyphenols on casein) correspond to the concentration of tannins in the samples analyzed. Three determinations for each sample of red grape pomace were performed.

To determine the anthocyanin content of the grape pomace samples, the evaluation method of red grapes (ITV method) was used. In the experiment were used 5 g of dried grape pomace being added 85 mL of 0.1% HCl solution and 15 mL 96% ethanol. After mixing, the samples were soaked for an hour being stirred every 15 minutes at room temperature. The extracts were spectrophotometrically analyzed at 520 nm.

The anthocyanin content was calculated according to the customized ITV method using the following formula: total anthocyanins mg/kg = Anthocyanins (mg/L) × [grape pomace weight + 100/grape pomace weight].

Statistical analysis. Analyses were carried out in triplicate. Means and standard deviations were calculated for total polyphenols, tannins and anthocyanins concentrations.

The obtained results were statistically processed using the single factor ANOVA test ($p < 0.05$), Fisher's test and Student's t-test to highlight the significant differences between the drying temperatures.

RESULTS AND DISCUSSION

The results (Tab.1) showed a significant decrease in TP content, Ta and An ($p < 0.05$) at the same time with the increase of the temperature during the grape pomace drying process.

Using the crude polyphenol extracts to determine the tannins it was found a decrease in the content of Ta and an increase in the content of non-adsorbed polyphenols in the casein extracts. This would imply a degradation of the tannins, depending on the temperature, which may lead to non-adsorbed casein compounds. In support of this assumption the TP values come being determined after the adsorption on casein (Tab. 1).

The results showed a significant reduction in the content of polyphenols especially to the temperature variations: 20°C and 38°C, verified statistically using the F-test and t-test to observe significant differences between the drying temperatures. Thus, in the case of the total polyphenols very significant differences resulted ($p < 0.001$) between the samples dried at 20°C and 38°C and respectively between 38°C and 50°C, allowing to calculate a regression curve

Fig. 1. Regression curve obtained for total polyphenols

(Fig. 1), using the relative values resulted after the determinations of the total polyphenols.

The resulting regression equation ($y = -0.1261x + 24.199$) was used to estimate the percentage of the total polyphenols degradation depending on the temperature (Fig. 2).

The grape pomace dried at 20°C was considered to have the highest concentration (100%) of total polyphenols compared to the other grape pomace samples dried at different temperatures in the oven. Thus, in order to calculate the prediction of thermal degradation, each value obtained from the calculation equation

Fig. 2. Total polyphenols degradation prediction

was reported at the highest amount, that is the maximum amount of total polyphenols obtained in the case of grape pomace dried at 20°C.

The graphical representation (Fig. 2) shows that the degradation of the total polyphenols increases progressively with the temperature increase; the maximum value of degradation is of 17.45% for the temperature of 50°C.

Using the same calculation manner in the case of tannins as well, significant differences could be observed, with a confidence interval of more than 99.9% ($p < 0.001$), only between the drying variations at 20°C and 38°C (Fig. 3).

Fig. 3. Regression curve obtained for tannins

In the drying variants at temperatures of 38°C and 50°C, the values showed a 99% confidence interval ($p < 0.05$), which does not allow to calculate a regression curve in order to estimate the degree of tannins degradation.

Based on the relative values of the tannins resulting in the drying variants at 20°C and 38°C

Fig. 4. The tannins degradation prediction

the regression equation ($y = -0.2062x + 19.46$) was obtained and was used to estimate the degree of tannins degradation (Fig. 4).

Figure 4 shows a gradual degradation of the tannins up to a temperature of 40°C, the maximum value of degradation being of 26.89%, after that the values remain constant.

In the case of the anthocyanins highly significant values ($p < 0.001$) were obtained between the drying temperatures: 20°C and 38°C and respectively 38°C and 50°C; thus were considered all the drying variants to calculate the regression line (Fig. 5) and to estimate the anthocyanins degradation prediction depending on the temperature (Fig. 6).

The graphical representation (Fig. 6) shows a progressive increase in anthocyanin degradation increasing the drying temperature; the maximum degradation value is of 28.23% at a temperature of 50°C.

Fig. 5. Regression curve obtained for anthocyanins

Fig. 6. Anthocyanins degradation prediction

Given the fact that there is no single method to determine the total polyphenols, tannins and anthocyanins, as well as standard methods to dry the grape pomace, the data obtained in our research are difficult to be compared with the few data found in the published literature (Khanal, *et al.*, 2009; Khanal, *et al.*, 2010; Patras *et al.*, 2010), on the effect of the drying temperature on the polyphenols, but we consider they bring supplementary information to this effect.

CONCLUSION

The study found that total polyphenols and anthocyanins content decreases significantly when the drying temperature is increased at 20°C, 38°C and 50°C.

In case of the tannins, very significant differences occurred between the variants drying: 20°C and 38°C. The tannins degradation would lead to non-adsorbed casein compounds, fact supported by the polyphenols increase in the casein extracts.

The hereby study suggests that the optimum temperature to dry the grape pomace in order to preserve the compounds with biologically active properties for use in animal nutrition is 20°C. This aspect is supported by repeating the measurements and the performed statistical calculation.

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