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RECOVERY OF TOTAL POLYPHENOLIC COMPOUNDS FROM DIFFERENT GRAPE SKINS VARIETIES, UNDER ENZYMATIC TREATMENT

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Abstract
Grape (Vitis vinifera L.) is an excellent source of phenolics, particulary in the skin, and the seeds. In the first part of this study, we compared the total phenolic compounds (determined by Folin-Ciocalteu method) from grape skins of seven different varieties (Chasselas dore, 1001, Royal, Noah, Moldova, Concord and Othella). Regarding to total phenolic compounds the highest level of these bioactive compounds were recorded in the case of Chasselas (0.875 GAE/g dry matter), followed by Moldova (0.69 GAE/g dry matter), while The smallest amount in the case of Noah varieties (0,027 GAE/g dry matter). In the second part, we recorded the total phenolic compound from skin grape under different enzymatic treatmenst. The amount of extractable phenolics in grape skins were increase as a result of the pectinase treatment. Cell-wall degrading enzymes are potential tools to release phenolics and other bioactive components from the cell-wall matrix of grape skins.
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Key words: grape skin, total phenolic compounds, cellulase, pectinase, enzymatic treatment

INTRODUCTION

Grape (*Vitis vinifera* L.) is an excellent source of phenolics, particulary in the skin, and the seeds. Zoecklein et al., 1990, reported the distribution of total phenolic compounds (expressed by gallic acid equivalent (GAE)/mg/kg berries) in the red grape. The phenolic compounds are primarily located in the seed and skin of the berry (Fig. 1).

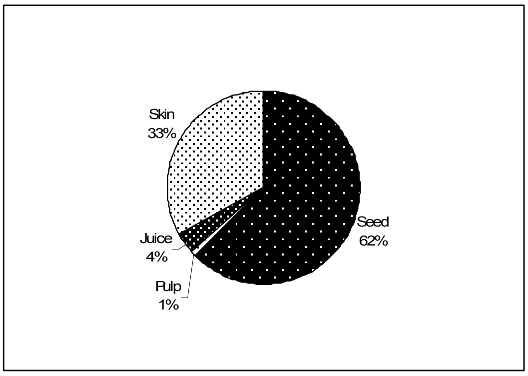

Fig.1 The procent of phenolic compounds (GAE/mg/kg berries) in the red grapes (Zoecklein et al., 1990)

Phenolic compounds in wine are usually subdivided into two groups: flavonoids and non-flavonoids. Total phenolic compound ranged from 1850 – 2200 mg/L for red and 220 – 250 mg/L for white wines. White wine as usually made from free-run juices, so they have much lower resveratrol and others phenolic compound than the red ones, due to minimal skin contact time. The mainly phenolic compounds from skin grapes are: proanthocyanidins, ellagic acid, myricetin, quercetin, kaempferol, trans-resveratrol (Pastrana-Bonilla et al., 2003; Hernandez-Jimenez et al., 2009).

Phenolic compounds have attrached much interest recently, because they have a variety of beneficial biological properties. They are potent antioxidant (Mitić et al., 2010), and exhibit various other physiological activities including anticarcinogenic (Agarwal et al., 2000), antimicrobial (Baydar et al., 2004), anti-inflammatory (Xia et al., 2010) and antihypertensive activities (Peng et al., 2005). High phenolic consumption is associated with reduced risk of chronic diseases like cardiovascular diseases (Leifert and Abeywardena, 2008).

Different treatments, including addition of enzymes (specially pectic enzymes), have been proposed to enhance degradation of grape berry cell walls and extraction of aroma and phenolic compounds into the wine (Doco et. al., 2007). Polyphenolic compounds (flavonoid, phenolic acid and stilbene) in grapes, musts and wines have been found in different concentrations, those are depending on the grape variety, environmental conditions in vineyard, and the wine processing techniques (Vinci et al., 2008). In their research study, Arnous and Meyer (2009), analysed a grape skin (after acid hydrolysis) of three wine grape cultivars, *Vitis vinifera* L., Cabernet Sauvignon, Merlot, and Shiraz regarding to composition in structural polysaccharide elements. They reported that in the red wine grape skins are present 57−62 mol % homogalacturonan, 6.0−14 mol % cellulose, 10−11 mol % xyloglucan, 7 mol % arabinan, 4.5−5.0 mol % rhamnogalacturonan I, 3.5−4.0 mol % rhamnogalacturonan II, 3 mol % arabinogalactan, and 0.5−1.0 mol % mannans.

After, wine production significant amounts of process waste are produced. These by-products may, however, have functional value due to their high content of phenolic compounds and other bioactive compounds. The aims of this paper were to compare the total phenolic compounds from different grapes varieties originating from Romania (nobles and hybrids) and also, to compare the yield of total phenolic compounds from skin grapes using different enzymes treatments in order to enhance the availability of phenolics.

MATERIALS AND METHODS

Grape varieties

Grape samples were obtained from a commercial market from Oradea, Romania, in 2010. The grapes were identified in the Oenology Laboratory, from Environmental Protection Faculty, University of Oradea. The varieties and their characterization are shown in Table 1. From all varieties used in this study, the Chasslas and Moldova are nobles, and others are hybrids.

Experimental procedure

Grape samples were manually separated into juice, skins and seed. The skins were dried in on oven at 60^0C for few days. The dry skins were grounded and the powders were treatment with different enzymes. Cellulase from *Trichoderma viride* were purchased from Sigma Aldrich and Pectinase were purchased from Gamma ChemieGMBH, Munchen, 1 g of skin grapes varieties were homogenized with 10 ml distilled water, pH 7 (this represent the martor). The enzymatic treatment of skin grapes are shown in Table 2.

Table 1

The characterization of different grapes varieties from Romania

Grapes	Characterisation
1.Chasselas dore	It is ripe in the second part of august. The grapes are medium sized and round. When they are ripened, the membrane becomes golden, sometimes with spots which are the color of rust. It can be used to obtain table wine.
2. 1001	The grapes are medium sized, cone shaped, similar to the Cabernet Sauvignon breed, with grain which are round shaped and black, they have a thin membrane with a hard core and a pleasant taste. It is very productive, able to produce about 4 – 4.5 kg of grapes for every stump. The wine however is weak and with a low acidity. It is resistant against plasmopara fungi, uncinula fungi, botrytis cinerea fungi, it is practically unaffected by *Agrobacterium radiobacter*. It has a tolerance for low temperatures up to -30° C.
3. Royal	It is a cultivary which comes from the *Vitis labrusca* species. The membrane has a green - blue color at maturity, with a yellow - green pulp. The grain is larger than the Noah's and it is less adhesive to the membrane. It has the disadvantage that the grapes are easily detached. It has a "foxiness" taste, which makes it not suitable for wine.
4. Noah ***(Vitis lambrusca)***	The grapes are cylindrical or cone shaped, medium sized, with round grains of a white - green color, a meaty core and a strawberry flavor. It is easily shaken, which makes harvesting difficult. Because of the thick membrane and the meat core it is difficult to use for wine. The wine has an ether like taste.
5. Moldova	The cluster has a cylindrical shape or is cone shaped, with a middle density of the grain and a weight of approximately 385 g. The largest ones can weigh up to 1 kg. The grapes are large (2,5 x 1,9 cm), oval, of a dark violet color and they are covered in a pronounced layer of epicuticular wax. The membrane is thick, dense and rough., the pulp is meaty and the taste is simple.
6. Concord **(*Vitis lambusca*)**	The grapes are medium sized. The grain is large, spherical, dark blue, covered in an intense layer of epicuticular wax. The grain has a consistent membrane, which can be easily detached from the content of the core. The specific "strawberry" aroma makes it

	easy to recognize by its taste. It is recommended for the production of juices and for consumption while fresh. It is resistant against plasmopara fungi, uncinula fungi, botrytis cinerea fungi, it is practically unaffected by *Agrobacterium radiobacter*. It has a tolerance for low temperatures up to -30° C.
7. Othello	The grapes are large, winged, with black meaty black grains which are strawberry flavored. The wine has an ether like taste. It is not resistant against illnesses.

Table 2

Enzymatic treatment of grape skin

Grape varieties	Cellulase 0. 1% (ml)	Pectinase (ml)	Cellulose + Pectinase (ml)
Grapes skin powder (1g:10 ml distilled water)	0.5	0.5	0.25:0.25

Samples were stirred on a water bath at 120 rpm, at 30^{0}C. After 18 hours enzyme treatments, the samples were centrifuged at 6000 rpm, for 20 minutes, and supernatant was used for determination of total phenolic compounds by Folin-Ciocalteu assay.

Total phenolic compounds by Folin-Ciocalteu assay

This method combined 100 µl skin grape extract, 2000 µl distillated water and 200 µl Folin-Ciocalteu reagent; then mixed well using a Vortex. The mixture was allowed to react for 3 minutes, and then 1 ml of 15% Na_2CO_3 solution was then added and mixed well. The samples were incubated at room temperature, in the dark for 2 hours. The absorbance was taken at 765 nm using a spectrophotometer (Schimadzu UV-Vis). The standard curve was linear, between 0.1-1 mg/ml gallic acid.

The results were expressed in gallic acid equivalents (GAE; mg/g dry skin grape). Adequate dilution was needed if the absorbance value measured was over the linear range of the standard curve.

Statistical Analysis

All data were expressed as mean ± standard deviation (SD) of two replications for each grape skin extracts tested. The data obtained from the antioxidant activity tests were analyzed statistically by the one-way analysis of variance (ANOVA) and Tukey's Multiple Comparison Test for comparison of means. The probability level of less than 0.05 was accepted as significant.

RESULTS AND DISCUSSION

The results obtained under the enzymatic treatments of different varieties grape skins are shown in Fig. 2. Regarding to total phenolic compounds (determinated by Folin Ciocalteu assay) of different skin grape varieties, the highest level of these bioactive compounds were recorded in the case of Chasslas (0.875 GAE/g dry matter), followed by

Moldova (0.69 GAE/g dry matter). Regarding to statistical analysis, it was obtained significantly differences between Chasslas varieties and all other varieties. Among the grape hybrids used in this study, the largest amount of total phenolic compounds were recorded in the case of Concord (0.364 GAE/g dry matter), Royal (0.308 GAE/g dry matter),followed by Othello (0.254 GAE/g dry matter), and 1001 (0.249 GAE/g dry matter).

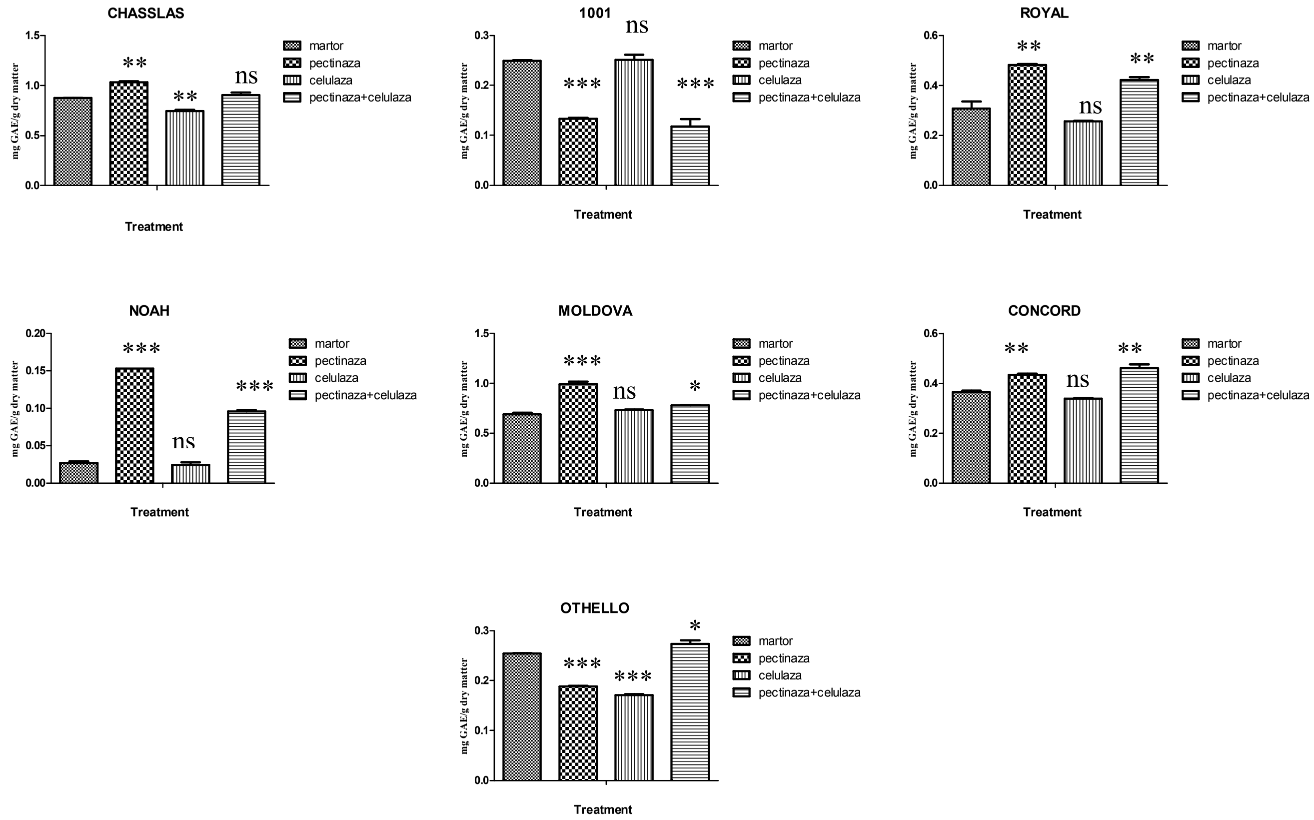

Fig. 2. Total phenolic compounds (GAE/ g dry matter) after enzymatic treatment of grape skins. The data were expresed as means ± standard deviation (n=2) and evaluated by one-way ANOVA. Tukey's Multiple Comparison Test. * significant, ** distinctly significant, *** very significant; ns – no significant

The smallest amount of total phenolic compounds were obtained in the case of Noah varieties (0,027 GAE/g dry matter).

The amount of extractable phenolics in almost grape skins were increase very significant ($p<0.001$) as a result of the pectinase treatment. For example, in the case of Noah varieties, the increase of total phenolic was most pronounced, i.e. from 0.027 GAE/g dry matter to 0.153 GAE/g dry matter. On the other hand, the cellulose treatment of grape skins did not enhance the amount of phenolic compounds.

Only, in the 1001 and Othello varieties skin grapes, the total phenolic content were decrease after the enzymatic treatment.

Suutarinen et al., 2004, studied the effect of enzymatic cocktails treatment on vegetable in order to enhance the availability on the phenolics and vitamins. They obtained significant increase of phenolic compounds in the case of onion peels, carrot peels after enzymatic treatments.

CONCLUSION

Cell-wall degrading enzymes are potential tools to liberate phenolics and other bioactive components from the cell-wall matrix of grape skins. The enzyme treatment, especially, pectinase treatment, was found to increase the total phenolic compounds from the skin grapes. The enzymatic treatment can be combinated to processing technologies in order to eliberated aroma and phenolic compounds into the wine.

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