Study of phenolic compounds and lipids of grape pomace

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Abstract. The article is devoted to the research of the makeup of phenolic compounds and fatty acids of grape pomace. The phenolic compounds were identified in skin and seed extracts and in extracts of skin-and-seed mixtures; the fatty acids – in grape oil generated by direct pressing. It was established that anthocyanins were present mainly in the skins. Low concentrations of ten components of the anthocyanin complex were identified in the Saperavi seeds. Maximum amounts of anthocyanins were found in the Saperavi skins. The concentration of quercetin distinguished by a PP-vitamin activity was by 1.5 to 2.0 times higher in the skin-and-seed mixtures, especially of Roesler grapes, than in the skin itself. Maximum amounts of flavan-3-ols, hydroxy-cinnamic and hydroxy-benzoic acids and oligomeric procyanidins, as well as the highest antioxidant activity were observed in the skin-and-seed mixture. The highest value of the correlation factor was observed in cases of interaction of antioxidant activity and concentration of procyanidins (r = 0.83), antioxidant activity and concentration of anthocyanins (r = 0.78), and antioxidant activity and concentration of flavan-3-ols (r = 0.75). Among the flavan-3-ols, it was (+)-D-Catechin that prevailed in grape seeds, with its concentration in the Pinot Noir extract (OAO APF Fanagoria) reaching 468 mg/dm³. Maximum concentration of Epigallocatechin-gallate was observed in the Saperavi and Pinot Noir seeds. As regards the concentration of hydroxy-cinnamic acids in the seeds, n-coumaric acid (Ancefolla, Saperavi) stood apart among the others; gallic acid (Saperavi, Ancellotta) came forward among the hydroxy-benzoic acids. In the reviewed samples of grape seeds, procyanidins of groups B₁, B₂ and B₃ distinguished by high antioxidant activity prevailed. Prevalence of linoleic and oleic acids was established for grape oil extracted from the seeds of such red grape varieties as Cabernet Sauvignon, Pinot Noir and Saperavi. Maximum concentrations of oleic acid were found in the Pinot Noir and Riesling seeds. Palmitic and stearic acids were also available in rather high concentrations in the grape oil.
1 Introduction

Grape pomace (marc) obtained in the process of industrial processing of grapes at primary wineries by crushing fresh or fermented must, is considered as winemaking waste. Its quantity varies between 17 and 22%, depending upon the grape variety and the processing technology (averagely, 10 to 15 kg/100 kg of processed grapes). The mechanical makeup of pomace is represented by grape juice, skins, pulps, and seeds. The chemical composition of grape pomace of white and red varieties is variegated; it includes components of various nature – polyphenols, polysaccharides, nitrogenous matters, vitamins, major and minor nutrients; and lipids contained in seeds. Their sustainable use will make it possible to produce various food products, including with functional properties [1, 2, 3, 4, 5], and antiradical and antioxidant activity [6, 7, 8, 9, 10] – viz., polyphenol concentrates, grape oil, soft drinks, dietary fibres, etc. [11, 12, 13, 14, 15]. Because of that, acquisition of new knowledge is required about grape pomaces with maximum amounts of the listed biologically valuable components in order to develop the technologies for concrete new kinds of products with due account given to their localization in different elements of the pomace.

The purpose of this work was to gain new knowledge on the chemical composition of phenolic compounds and lipids in grape pomace.

2 Study objects and methods

As the study objects, the following dried substances were used:
- skins of red grape varieties derived from different Krasnodar wineries: Merlot (JSC Divnomorye), Cabernet Sauvignon (CJSC Slavprom), and Roesler (OAO APF Fanagoria);
- seeds of Pinot Noir (APF Fanagoria); Syrah, Merlot and Ancellotta (all from JSC Divnomorye); Pinot Noir, Merlot, Saperavi and Cabernet Sauvignon (all from LLC Olimp).

The phenolic compounds were extracted from the pomace and seeds at the temperature of 20–24° for 5 months by an aqueous alcoholic solution with 70% alcohol by volume. The mass concentration of the phenolic compounds was determined by high-performance chromatography method with the use of the Agilent Technologies (model 1100) chromatographic system with a diode matrix detector. For separation purposes, we used a 2.1 x 150 mm Zorbax SB-18 chromatographic column, filled with silica gel with bonded octadecylsilyl phase with 3.5 μm sorbent particles. The elution was performed in gradient mode. The eluent flow rate was 0.25 ml/min. To build the gradient, the following were used: solution A – methanol; solution B – an 0.6% aqueous solution of trifluoroacetic acid. The volume of the input sample was 2 μl. The chromatograms were registered by optical absorption of the eluate at the following wave lengths: 280 nm for gallic acid, (+)-D-Catechin, (-)-Epicatechin, and procyanidins; 313 nm for derivative hydroxy-cinnamic acids; 371 nm for quercetin; 350 nm for glycosides of quercetin; and 525 nm for anthocyanins. To register trans-resveratrol, a fluorometric detector was used at absorption wavelength of 280 nm and emission wavelength of 320 nm. The components were identified by comparison of the spectral performances and peak retention times in the reviewed samples and in standard calibration solutions. The quantitative contents of individual components were calculated with the use of calibration curves of dependance of peak responses upon substance concentrations drawn by solutions of individual substances. The value of antioxidant activity expressed in terms of TROLOX (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxic acid – a synthesized analogue of gallic acid) was performed on the “Colour-Yauza-01-AA” chromatograph [16]. The statistical data processing was performed with the use of Microsoft Excel software.
The grape oil was generated from ground grape seeds by direct pressing according to [17] method. The fatty and acid composition was identified by gas-liquid chromatography at the Crystal-2000M chromatograph. A 30-metre-long spiral stainless-steel gas chromatographic column was used. Pro analysis compressed nitrogen was used as the carrying gas. A sample of the analyzed product was injected into the chromatographic column with a microsyringe through the evaporator by piercing the rubber membrane. The components were identified by the retention time.

3 Results and discussion

Table 1 presents the results of the study of phenolic compounds in the skins and skin-and-seed mixture.

Table 1. Compositional makeup of polyphenolic compounds in aqueous alcoholic extracts of grape skin and skin-and-seed mixture.

| Polyphenols | Mass concentration of polyphenols in extracts, mg/dm³ |
|-------------|-------------------------------------------------------|
|             | Skins | Seeds + skins |
|             | Merlot | Rosler | Cabernet Sauvignon | Superavi | Rosler | Superavi |
| Delphinidin-3-O- glycoside | 23.5 | 22.7 | 27.8 | 36.2 | 22.0 | 37.1 |
| Cyanidin-3-O-glycoside | 10.6 | 8.8 | 13.3 | 14.8 | 8.6 | 15.0 |
| Peonidin-3-O-glycoside | 12.4 | 10.6 | 12.8 | 14.0 | 12.2 | 15.0 |
| Petunidin-3-O-glycoside | 1.2 | 1.6 | 2.4 | 3.7 | 2.0 | 3.5 |
| Malvidin-3-O-glycoside | 156 | 154 | 166 | 178 | 157 | 178 |
| Delphinidin-3-O-(6'-acetyl-glycoside) | 16.5 | 15.4 | 18.7 | 21.7 | 15.2 | 22.4 |
| Cyanidin-3-O-(6'-acetyl-glycoside) | 1.2 | 1.2 | 2.0 | 2.5 | 1.2 | 2.5 |
| Peonidin-3-O-(6'-acetyl-glycoside) | 3.4 | 4.0 | 5.2 | 5.4 | 4.3 | 5.0 |
| Petunidin-3-O-(6'-acetyl-glycoside) | 1.7 | 1.5 | 2.1 | 2.2 | 2.2 | 2.3 |
| Malvidin-3-O-(6'-acetyl-glycoside) | 2.0 | 1.9 | 2.5 | 2.7 | 2.4 | 3.0 |
| Delphinidin-3-O-(6'-n-coumaroyl-glycoside) | 2.1 | 1.7 | 3.1 | 4.8 | 3.1 | 5.0 |
| Petunidin-3-O-(6'-n-coumaroyl-glycoside) | 0.7 | N/A | 1.1 | N/A | 2.1 | N/A |
| Malvidin-3-O-(6'-n-coumaroyl-glycoside) | 2.3 | 3.6 | 2.8 | 2.7 | 4.3 | 3.0 |
| Total anthocyanins | 233.6 | 227.0 | 259.8 | 288.7 | 236.6 | 291.8 |
| Flavones | | | | | |
| Quercetin | 42.8 | 43.7 | 46.5 | 51.5 | 88.0 | 52.0 |
| Flavan-3-ols | | | | | |
| (+)-D-Catechin | 332 | 345 | 374 | 412 | 867 | 940 |
| (-)-Epicatechin | 138 | 147 | 154 | 166 | 450 | 564 |
The performed studies showed that the concentration of anthocyanins, the main colouring agents of grape fruit, in the skin-and-seed mixture was practically identical to skin anthocyanins, i.e. grape pomace anthocyanins are concentrated in the skins. Maximum quantity of anthocyanins was found in Saperavi skins. At the same time, it is worth noting the absence of petunidin-3-O-(6'-n-coumaroyl-glycoside) in Roesler which may have been explained by the genetic peculiarity of this grape variety.

The concentration of quercetin, a mighty antioxidant with a PP-vitamin activity, in the skin-and-seed mixture, especially of Roesler grapes, was by 1.5 to 2.0 times higher than in the skins, and varied depending on the study object.

Seeds made a valuable contribution to the aggregate accumulation of flavan-3-ols, hydroxy-cinnamic and hydroxy-benzoic acids, and in particular oligomeric procyanidins, whose maximum values were found in the skin-and-seed mixture. The availability of the above listed compounds was important for the formation of antioxidant activity: its maximum value was observed in the skin-and-seed mixture.
With the help of correlative analysis module, the existence of a statistically relevant relation between the antioxidant activity and a component of the phenolic complex. It is worth noting that the maximum value of the correlation factor was observed in cases of interaction of antioxidant activity and concentration of procyanidins ($r = 0.83$), antioxidant activity and concentration of anthocyanins ($r = 0.78$), and antioxidant activity and concentration of flavan-3-ols ($r = 0.75$).

In light of the obtained results, research of the compositional makeup of seeds of various grape varieties is of great importance. The performed studies (Table 2) showed nearly a total absence of anthocyanins in grape seeds. None of the samples let us identify petunidin-3-O-glycoside, malvidin-3-O-glycoside, malvidin-3-O-(6'-acetyl-glycoside), or delphinidin-3-O-(6'-n-coumaroyl-glycoside). Anthocyainins were detected in small quantities in the seed extracts of Ancellotta and Saperavi grapes, characterized by coloured juice.

Table 2. Monomeric and oligomeric polyphenols in aqueous-alcoholic extracts of grape seeds.

| Polyphenols                  | Mass concentration of polyphenols in seed extracts, mg/dm³ |
|------------------------------|-----------------------------------------------------------|
| JSC Divnomorye               |                                                           |
| Pinot Noir                   | Syrah           | Merlot         | Ancellotta     | AFF Fanagoria  | Pinot Noir     | Merlot        | Cabernet Sauvignon | Saperavi     |
| Anthocyanins                 |                                                           |
| Delphinidin-3-O-glycoside    | -              | -              | 0.2            | -              | -              | -             | 0.4              |
| Cyanidin-3-O-glycoside       | -              | -              | -              | -              | -              | 0.2           |
| Peonidin-3-O-glycoside       | -              | -              | -              | -              | 0.2            |
| Delphinidin-3-O-(6'-acetyl-glycoside) | -              | -              | 0.6            | -              | -              | -             | 1.0              |
| Cyanidin-3-O-(6'-acetyl-glycoside) | -              | -              | -              | -              | -              | 0.7           |
| Peonidin-3-O-(6'-acetyl-glycoside) | -              | -              | 0.4            | -              | -              | 1.0           |
| Petunidin-3-O-(6'-acetyl-glycoside) | -              | -              | -              | -              | -              | 0.4           |
| Petunidin-3-O-(6'-n-coumaroyl-glycoside) | -              | -              | -              | -              | -              | 0.3           |
| Malvidin-3-O-(6'-n-coumaroyl-glycoside) | -              | -              | 0.4            | -              | -              | 0.6           |
| Total anthocyanins           | -              | -              | 1.6            | -              | -              | 4.8           |
| Flavones                     |                                                           |
| Quercetin                    | 1.0            | -              | 2.2            | -              | 1.2            | -             | 1.9              | 1.8          |
| Flavan-3-ols                 |                                                           |
| (+)-D-Cachetin               | 452            | 413            | 347            | 386            | 468            | 457           | 361              | 432          | 448          |
| (-)-Epicaetin                | 391            | 312            | 334            | 377            | 388            | 376           | 341              | 392          | 312          |
| (-)-Epicachetin-gallate      | 53             | 31             | 33             | 43             | 54             | 57            | 34               | 50           | 55           |
| Hydroxy-cinnamic acids       |                                                           |
| Caftaric acid                | 0.8            | -              | -              | 1.2            | 1.4            | -             | -                | 0.8          |
Polyphenols

|                        | JSC Divnomorye | LLC Olimp         |
|------------------------|----------------|-------------------|
|                        | Pinot Noir | Syrah  | Merlot | Pinot Noir | APF Fanagoria | Pinot Noir | Merlot | Cabernet Sauvignon | Saperavi |
| Cautaric acid          | 0.3        | -      | -      | 0.5        | 0.5          | -          | -      | 0.4                  |          |
| n-Coumaric acid        | 131        | 118    | 121    | 134        | 142          | 135        | 127    | 135                  | 141       |
| Hydroxy-benzoic acids  |            |        |        |            |              |            |        |                      |          |
| Gallic acid            | 291        | 287    | 282    | 317        | 322          | 315        | 294    | 318                  | 343       |
| Syringic acid          | 129        | 118    | 132    | 138        | 147          | 154        | 129    | 142                  | 151       |
| Oligomeric procyanidins|            |        |        |            |              |            |        |                      |          |
| Procyanidin B1         | 341        | 310    | 287    | 326        | 334          | 337        | 318    | 322                  | 337       |
| Procyanidin B2         | 225        | 218    | 210    | 241        | 231          | 236        | 225    | 225                  | 230       |
| Procyanidin B3         | 114        | 100    | 100    | 112        | 116          | 117        | 98     | 100                  | 121       |
| Procyanidin B5         | 57         | 48     | 41     | 60         | 61           | 61         | 54     | 56                   | 64        |
| Procyanidin B7         | 68         | 54     | 60     | 58         | 66           | 70         | 57     | 65                   | 70        |

The presence of quercitin was identified in the seed extracts of Pinot Noir, Ancellotta (highest concentrations), Cabernet Sauvignon, and Saperavi.

According to contemporary views, cachenins are deemed as several derivatives varying in the stereoisomeric orientation of substituents with two chiral carbon atoms in the structure of a flavan-3-ol nucleus [18, 19]. There exist derivatives of catechin and gallo catechin; the latter can occur in the seeds of hybrid grape varieties. Out of the four possible stereoisomers, only two can occur in native form, viz.: (+)-D-catechin and (-)-epicatechin. (-)-Epicatechin-gallate is a compound ester of (-)-epicatechin and gallic acid. One of the strongest natural antioxidants, (-)-epigallocatechin-gallate, is available in red wines and grape seeds.

Among the flavan-3-ols, (+)-D-Cachetin prevailed in grape seeds whose concentration in the Pinot Noir extract (Fanagoria) reached 468 mg/dm³. The maximum concentration of epigallocatechin-gallate was found in Saperavi and Pinot Noir seeds, which proved their high antioxidant characteristics.

Phenolic acids – hydroxy-cinnamic and hydroxy-benzoic ones – also reveal antioxidant properties. Gallic acid showed the highest activity [20, 21]. Among hydroxy-cinnamic acids, n-coumaric acid (Ancellotta, Saperavi) excelled by concentration; as for hydroxy-benzoic acids, gallic acid (Saperavi, Ancellotta) stood out among the rest.

Polymeric procyanidins localized in seeds and partly in skins of grapes [22, 23] are deemed one of the most important and structurally diverse groups of polyphenols. In the analyzed grape seed samples, highly antioxidant procyanidins of B₁, B₂ и B₃ groups prevailed. Thus, it can be argued that grape seeds are a valuable source of antioxidants and a good primary material for the production of food products, including beverages with a high biological value.
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