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Original Research

Tocopherol contents and antioxidant activity in grape pomace after fermentation and alcohol distillation

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Abstract: The wine industry in Georgia produces vast amounts of grape pomace that is currently mostly wasted, while only a minor amount is used for distilling alcohol. The study was carried out on the grape pomace from the three most widely used grapevine sorts (*Vitis vinifera* var. *Rkatsiteli, V. vinifera* var. *Saperavi, V. labrusca* var. *Isabella*) in Georgia, and quantities of tocopherols and antioxidants were evaluated. The antioxidant activity was assessed by diphenyl-picrylhydrazyl (DPPH) and measurement of visible light absorption at 515 nm, and tocopherol was measured by absorption at 470 nm via a spectrophotometer. The results indicated that the grape pomace contains considerable tocopherols and antioxidant activity. However, the antioxidant activity had slightly been decreased. These results suggest that grape pomace can be an economically attractive resource for the pharmaceutical and food industries. Utilization of grape pomace for producing pharmaceutical and cosmetic goods with tocopherol and antioxidants can solve two problems: it can recycle waste and develop new profitable businesses in biotechnology.

Key words: Antioxidants; Tocopherols; Grape pomace; Rkatsiteli; Saperavi; *Vitis labrusca* var. *Isabella*.

Introduction

Grapes are the most cultivated fruit worldwide and are valuable products for pharmaceutical, cosmetic, and food industries (1). Grapes are harvested to make wine, and wine-making leaves millions of secondary products and waste (2). This massive amount of waste – mostly pomace – accumulates for years and poses a growing problem to the environment (3). Simultaneously, grape pomace is an interesting source of fiber with antioxidant activity and a valuable by-product from wineries (4). It can be used as animal food (5) and may produce food supplementary with antioxidant activity (5).

Grape pomace contains 10-30% of polyphenols, sugars, alcohol, tannins, and other valuable products (6), showing high antioxidant activity (7, 8) together with high contents of A-tocopherol, which can be used as a preserving agent of food oil (9). The polyphenol content of *Vitis vinifera*'s underused bioresources such as stem, seeds, and grape pomace reveals anticancer effects (8). Grape seed extract enhances the anti-inflammatory potential (10) and prevents risks for stroke (11). Grape seed can be used as a herbal treatment option for alopecia by inhibiting the 5-alpha-reductase enzyme and reducing hair loss (12); the potent antioxidant activity positively affects bone healing (13).

Wine-making is Georgia's priority field. The National Wine Agency of Georgia reports that 271 000 tons of grapes were collected in 2019 and used mostly for wine production (14). However, if wine production is thrown away without any use or, to a minor extent, the secondary products are used as a good source for ethanol extraction by private entrepreneurs.

This work aimed to evaluate the potential of secondary products of wine production as valuable products for medicinal applications, namely, tocopherols and antioxidants. We investigated the most widespread three sorts of grapevine (*Vitis vinifera* var. *Rkatsiteli, V. vinifera* var. *Saperavi, V. labrusca* var. *Isabella*) in Georgia and determined tocopherols and total antioxidant activity separately in the pomace's skin and seeds after fermentation. We determined the same compounds in the pomace after additional fermentation and ethanol extraction.

Materials and Methods

Plant material

We used the pomace from the three most cultivated grapevine sorts in Georgia: *Vitis vinifera* var. *Rkatsiteli*, *V. vinifera* var. *Saperavi*, and. All experimental material was supplied from the private vineyard (property of one of the authors – Maia Akhalkatsi, the village of Shilda, Kvareli region, Georgia). The sap was squeezed out from berries to obtain unfermented seeds and berry skin of the experimental plants. Residual seeds and berry skin were dried at room temperature and ground for analysis. Fermented seeds and berry skin were received from the mentioned person after preparing wine by the "Kakhetian" method. Part of the pomace from all grapes were mixed and used to distill alcohol – the residual after-distillation pomace (hereafter Chacha); we dried this latter material without separating skin and seeds

Table 1. Pomace samples and their abbreviations.

No	Sample	Code
1	*V. vinifera* var. *Rkatsiteli* pre-fermentation skin	RP
2	*V. labrusca* var. *Isabella* post-fermentation skin	ISF
3	*V. vinifera* var. *Saperavi* post-fermentation skin	SSF
4	*V. vinifera* var. *Rkatsiteli* post-fermentation skin	RSF
5	*V. labrusca* var. *Isabella* post-fermentation pips	IPF
6	*V. labrusca* var. *Isabella* pre-fermentation pips	IP
7	*V. vinifera* var. *Saperavi* pre-fermentation pips	SP
8	*V. vinifera* var. *Saperavi* post-fermentation pips	SPF
9	*V. vinifera* var. *Saperavi* pre-fermentation skin	SS
10	*V. vinifera* var. *Rkatsiteli* post-fermentation pips	RPF
11	*V. labrusca* var. *Isabella* pre-fermentation skin	IS
12	*V. vinifera* var. *Rkatsiteli* pre-fermentation pips	RS
13	Mixed waste after distilling alcohol	MW

and used it in further analyses. Overall, there were 13 samples (Table 1) in which we determined tocopherol contents and total antioxidant activity.

Tocopherols

Tocopherols were determined by the method described in Fillipovich *et al*. (1982). 20- 25 ml pure ethanol was added to 500 mg ground material to extract tocopherols; the procedure was performed three times at room temperature. 20 ml of 60% potassium hydroxide were added to the combined extract, and it was saponified on a water bath for two hours. Distilled water was used to wash the combined extract until alkaline residuals were entirely removed. Later water was removed with Na_2SO_4, after which the received solution was evaporated on a water bath, cooled, and then added to the mix of alcohol-nitric acid (1 ml of concentrated HNO_3: 5 ml of 96° alcohol). The solution was boiled for three minutes until it turned dark red. Extinction of the extract was measured at 470 nm by a spectrophotometer (Spekol 11, Karl Zeiss, Germany).

The total antioxidant activity (TAA)

The TAA was measured by the modified method using diphenyl-picrylhydrazyl (DPPH) (15). 200mg of experimental powder was extracted two times with 96° ethanol. The extract was evaporated on a water bath, and the remaining sediment was dissolved in 10 ml of the water-alcohol mixture. 0.01 ml of the obtained solution was added with 4 ml of 40 µM DPPH solution. After 30 minutes of incubation in the dark, the optical density was measured at 515 nm by the spectrophotometer (Spekol 11, Karl Zeiss, Germany).

Statistical analyses

We performed usual descriptive statistics (means, standard deviations, etc.) and one-way ANOVA using Statistix9 (Analytical Software, Tallahassee, Fl., U.S.A.). Means were compared with Tukey's post hoc pairwise comparison test at $\alpha = 0.05$.

Results

Total antioxidant activity distributed unevenly among samples of the three grape sorts so that among 13 tested samples, there could be ten groups distinguished with significantly different total antioxidant activity (Figure 1). In general, fermentation could reduce antioxidants (e.g., in *V. vinifera* var. *Saperavi* pips and berry skin as well as in *V. vinifera* var. *Rkatsiteli* berry skin). However, fermentation could also increase antioxidant activity in *V. labrusca* var. *Isabella* pips and berry skin, and even more strongly in *V. vinifera* var. *Rkatsiteli* berry skin.

Overall, red grapes (*V. labrusca* var. *Isabella*, *V. vinifera* var. *Saperavi*) were somewhat richer in tocopherols than the white *V. vinifera* var. *Rkatsiteli*. Finally, total antioxidant activity in the mixed waste after alcohol distillation appeared to be among the lowest levels (the black bar in Fig. 1).

Tocopherols were also distributed unevenly among the samples of the studied grape sorts, and nine groups with significantly different tocopherol contents could be distinguished (Figure 2). The highest tocopherol

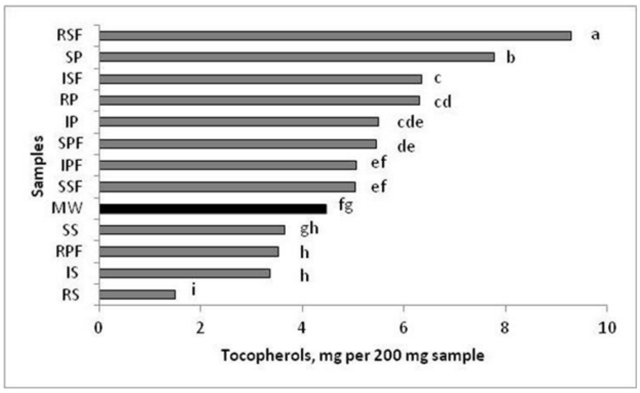

Figure 1. Total antioxidant activity in the samples of different grapes: IP: *V. labrusca* var. *Isabella* pips(crude), IPF: *V. labrusca* var. *Isabella* pips fermented; IS: *V. labrusca* var. *Isabella* berry skin (crude); ISF: *V. labrusca* var. *Isabella* berry skin-fermented; MW: mixed waste (distilled); RP: *V. vinifera* var. *Rkatsiteli* pips (crude); RPF: *V. vinifera* var. *Rkatsiteli* pips fermented; RS: *V. vinifera* var. *Rkatsiteli* berry skin (crude); RSF: *V. vinifera* var. *Rkatsiteli* berry skin-fermented; SP: *V. vinifera* var. *Saperavi* pips (crude); SPF: *V. vinifera* var. *Saperavi* pips fermented; SS: *V. vinifera* var. *Saperavi* berry skin; SSF: *V. vinifera* var. *Saperavi* berry skin fermented. The letters right to the bars show the homogeneous groups (Tukey's post-hoc pairwise comparisons test at $\alpha = 0.05$).

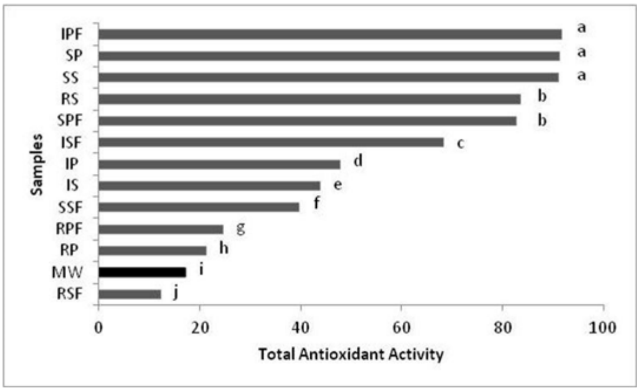

Figure 2. Tocopherols in the samples of different grape sorts: IP: *V. labrusca* var. *Isabella* pips (crude), IPF: *V. labrusca* var. *Isabella* pips fermented; IS: *V. labrusca* var. *Isabella* berry skin (crude); ISF: *V. labrusca* var. *Isabella* berry skin-fermented; MW: mixed waste (distilled); RP: *V. vinifera* var. *Rkatsiteli* pips (crude); RPF: *V. vinifera* var. *Rkatsiteli* pips fermented; RS: *V. vinifera* var. *Rkatsiteli* berry skin (crude); RSF: *V. vinifera* var. *Rkatsiteli* berry skin-fermented; SP: *V. vinifera* var. *Saperavi* pips (crude); SPF: *V. vinifera* var. *Saperavi* pips fermented; SS: *V. vinifera* var. *Saperavi* berry skin; SSF: *V. vinifera* var. *Saperavi* berry skin fermented. The letters right to the bars show the homogeneous groups (Tukey's post-hoc pairwise comparisons test at $\alpha = 0.05$).

content was found in the fermented pips of *V. vinifera* var. *Rkatsiteli*, followed by crude *V. vinifera* var. *Saperavi* berry skin. Fermentation could both increase (e.g., berry skin of *V. labrusca* var. *Isabella* and *V. vinifera* var. *Rkatsiteli*) and reduce (e.g., *V. vinifera* var. *Rkatsiteli* pips) tocopherol contents in the samples. Red and white grapes did not differ significantly in the contents of tocopherol. Interestingly, alcohol distillation reduced the tocopherols moderately in the mixed sample(the black bar in Figure 2).

Discussion

Tocopherols are a class of lipophilic methylated phenols with vitamin E activity. The major source of production of these substances is vegetable oil. Despite Georgia having a good resource basis for tocopherols' commercial production, such an industry does not exist in this country. On the other hand, the wine industry's waste can be a very cheap raw material for producing tocopherols commercially. Tocopherols have plenty of uses. The latest research shows that tocopherols can act as chemically protective agents against hepatotoxicity (16). Tocopherols can be used as topical anti-inflammatory agents against infectious skin diseases (17). Tocopherol can considerably reduce the risk of cardiac attacks (18). There is evidence that tocopherols can have an anticarcinogenic activity (19). Overall, tocopherols are characterized by antioxidant and anti-inflammatory effects by which these substances can protect cells from oxidative stress (20). A strong antioxidant activity illustrates grape pips, and grape pomace contains a large number of antioxidants (21).

The wine was prepared by the "Kakhetian" method. After mixing the pomace from all grapes, they were used to distill alcohol, and it's waste without separating skin and seeds was collected as one of the samples for assessment. Twelve more samples containing pip and berries skin were carried out, and all were used in tocopherols measurements and antioxidant activity assessment.

Data shows that grape pomace contains tocopherols in measurable quantities. Our results are in line with these data, but we also found that the antioxidant activity reduces strongly after distilling alcohol. Nevertheless, experiments show that there is still sufficient antioxidant activity in an alcohol extract from grape pomace that can be used as a resource for pharmaceutical production. These products are in wide medicinal use as pills, ointments, etc. Extracts from grape pips have antioxidant effects and can show hepatoprotective activity (22) and a protective effect against oxidative stress (23). There is evidence that grapeseed oil is an excellent agent to heal colitis in colorectal colitis patients (24). It is also used as an anti-inflammatory and antioxidative agent at renal failures (25). Our data confirm that grape pomace contains antioxidants in significant quantities, and we recommend the industries and businesses to tap this potential for producing valuable pharmaceuticals with antioxidant effects.

Finding new natural sources for producing biologically active compounds is one of the priorities in healthcare. At the same time, there is a problem of recycling biotechnological waste, which can be considered to be such a natural raw material for subsequent utilization. This recycling method can be cost-benefit as it offers cheap (or even free) raw natural materials and utilizes waste. Grape pomace in wine-making countries is precisely such a case, which can be recycled and help develop small and medium-size industries. Our experiment showed that secondary products after wine production, which are utilized for ethanol extraction, still contain valuable materials for the medicinal, cosmetic, and food industry.

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