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The effect of thawing on the antioxidant activity of the leaves and fruit of the grapevine (*Vitis vinifera*)

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ABSTRACT

Introduction: Grapevine is a valuable source of active health-promoting substances, in particular polyphenols, i.e., flavonoids and phenolic acids, with antioxidant, anti-inflammatory, antibacterial, antiviral and anticancer properties. Grape seeds, peels and leaves also provide a rich source of antioxidants.

The aim of the study was to evaluate the antioxidant activity of aqueous and alcoholic extracts of various parts of the *Vitis vinifera*.

Materials and methods: Fresh and frozen grapevine fruits and leaves of a red variety, cultivated by the authors, were evaluated for antioxidant potential. Extracts were prepared with an ultrasound-assisted extraction at 40 kHz for 15, 30 or 60 min. Methanol, ethanol and isopropanol aqueous solutions, as well as water, were used as extractants. Antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis(ethylbenzothiazoline-6 sulphonic acid) (ABTS) methods.

Results: The antioxidant potential of extracts prepared from both fresh and frozen plant material was observed. The highest activity, evaluated with the DPPH method, was found in methanolic extracts of fresh leaves, obtained after a 60 min extraction. Similarly, a high activity was observed for frozen grapevine leaf extracts. The highest antioxidant capacity, evaluated by the ABTS method, was found in frozen leaves extracted with isopropanol for 60 min. Freezing and thawing markedly increased the antioxidant properties of the extracts.

Conclusions: Extracts from both fresh and frozen parts of the *Vitis vinifera* showed antioxidant activity, and leaf extracts seemed to be particularly valuable. The freezing-thawing process had a beneficial effect on antioxidant potential and should be considered a useful method to increase extraction efficiency of antioxidants to be used to obtain valuable cosmetic, pharmaceutical or food products.

Keywords: *Vitis vinifera*; antioxidants; ultrasound-assisted extraction; DPPH; ABTS.

INTRODUCTION

Vitis vinifera is a plant cultivated around the world and its properties are frequently studied [1]. The most valuable part of this plant is the edible fruit used for both direct consumption in its unprocessed form and for wine production. The beginning of grapevine cultivation in Poland dates back to the 12th century and is still ongoing. With a growing public interest in wine consumption, viticulture is still expanding in this country. In new vineyards, new types of grapevine are cultivated and are characterized, among other things, by a higher resistance to fungi or adverse weather condition [2].

Vitis vinifera belongs to *Vitaceae* family. Its climber, equipped with tendrils, can reach a height of up to 40 m. The colour of the fruit varies from black through red and pink to green or white depending on the variety. In addition to their taste quality, grapes are a valuable source of health-promoting active substances including polyphenols, i.e., flavonoids and phenolic acids with antioxidative, anti-inflammatory, antibacterial, antiviral and anticancer properties. The antioxidative properties of *Vitis vinifera* fruits are mainly due to flavonoids and stilbenes, i.e. the compounds present in grapes at the highest concentration [3, 4, 5]. The most frequently described compound, belonging to the group of stilbenes, is resveratrol (3,5,4'-trihydroxystilben). In grapes, 2 isomers – *cis* and *trans* – were found. These compounds have a positive effect on the plant and are free radical scavengers. Moreover, their anti-inflammatory, antimutagenic, antineoplastic, neuroprotective and chemopreventive properties have been described by others [1, 6, 7]. This compound is also known to prevent the development of cardiovascular diseases and the cardioprotective properties of red wine have been described as the so-called 'French paradox'. This phenomenon is associated with a lower incidence of cardiovascular diseases in France due to the consumption of large amounts of red grape wine, a valuable source of phenolic compounds including the above-mentioned resveratrol [6]. It is not only the fruit of the grapevine that shows antioxidant activity. A valuable source of antioxidants are also grape seeds and skins as well as leaves [1, 7]. The antioxidative properties of the substances contained in plants are mainly beneficial due to the prevention of oxidative stress caused by an excess of free radicals in the body. These are harmful as they can lead to a number of disorders, i.e., metabolic diseases including

diabetes, obesity, and cardiovascular diseases [8]. They can also contribute to the development of neurodegenerative disorders, such as Parkinson's or Alzheimer's disease [9] and mental disorders such as schizophrenia, depression or anxiety disorders [10]. Moreover, oxidative stress can induce carcinogenesis of many organs, including the breast, prostate, colon, skin, eyeball [11], pancreas [12], thyroid [13] and others. It should also be mentioned that oxidative stress can lead to premature aging, including in the skin [14]. In recent years, attention has been focused on finding and using biologically active substances of plant origin, including antioxidants, in the food, cosmetic and pharmaceutical industries [15].

The aim of the study was to evaluate the antioxidant activity of extracts of fresh and frozen parts of the *Vitis vinifera* using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis(ethylbenzothiazoline-6 sulphonic acid) (ABTS) methods.

MATERIALS AND METHODS

The following reagents, all of analytical grade, were used to evaluate antioxidant activity of the extracts: methanol, ethanol, isopropanol, potassium persulphate and L(+)-ascorbic acid, all of which were sourced from Chempur, Piekary Śląskie. As well as this, ABTS and DPPH were sourced from Sigma-Aldrich (USA).

The raw material consisted of fruits and leaves of cultivated red grapes, originating locally from a cultivated area in Szczecin. The fruits and leaves were harvested in September 2017. A part of the raw material was extracted in its fresh form, whereas the rest was frozen at –20°C. Before extraction, the frozen material was thawed to room temperature. All materials were then subjected to ultrasound-assisted extraction for a duration of 15, 30 or 60 min (Sonic, Polsonic, Warsaw). The frequency of the ultrasound was 40 kHz. The following solvents were used as extractants: 40, 70 and 96% (v/v) ethanol, 40, 70 and 99.8% (v/v) methanol, 40, 70 and 99.5% (v/v) isopropanol and distilled water. To obtain extracts, 0.5 g of each plant material and 10 cm^3 of solvent were used. The extracts were analysed immediately after their preparation due to the relative instability of aqueous extracts.

To evaluate antioxidant activity, the DPPH and ABTS methods, based on the reduction of the appropriate radical dissolved in the working solution, were used as described previously [16, 17, 18]. The absorbance of samples was measured at 517 and 734 nm, respectively. Three samples of each extract were prepared and evaluated. The absorbances obtained using both methods were used to determine radical scavenging activity (RSA – %) using the formula:

$$RSA\,[\%] = \left(1 - \frac{A_x}{A_0}\right) \cdot 100\%$$

where A_x = absorbance of the examined sample and A_0 = absorbance of the control sample.

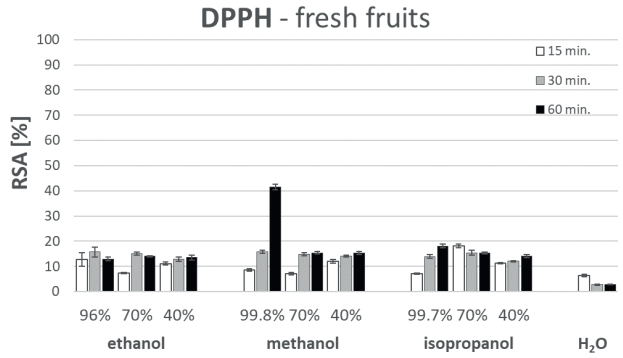
Moreover, the activity of the extracts was also expressed as ascorbic acid equivalents (AAE) in mg ascorbic acid/g of raw material based on a calibration curve prepared using ascorbic acid as a standard. The results are presented as arithmetical means ± standard deviation (SD). The correlation between the results obtained using the ABTS and DPPH methods was also calculated, as well as between the results of extracts obtained from frozen and fresh grape leaves and fruits. Statistical calculations were determined using Statistica 12 (Statsoft) software.

RESULTS

The antioxidant potential of the extracts, determined with the DPPH method, are presented in Table 1 and Figures 1–4. The part of the plant, the solvent used to prepare the extracts, the length of ultrasound-assisted extraction and the form of the material (fresh or frozen) were taken into account. Antioxidant activities were expressed either as AAE (mg ascorbic acid/g of raw material) or as RSA in percent. These activities ranged from 0.10 ±0.01 to 3.12 ±0.02 AAE which corresponded to 2.74 ±0.22 to 90.02 ±0.67% RSA. The highest values were observed in the group of leaf extracts – from 0.88 ±0.07 AAE for aqueous extracts of fresh grape leaves to 3.12 ±0.02 for the methanol extract of the same raw material, both extracted for 60 min. This corresponds to an RSA of 25.50 ±2.15% and 90.02 ±0.67%, respectively. Frozen leaf extracts were also characterized by a high DPPH with a radical reduction capacity from 1.62 ±0.02 to 3.09 ±0.01 AAE. Fruit extracts showed markedly lower activity – below 1.54 ±0.03 AAE. Only samples prepared in concentrated methanol extracted for 60 min, had an activity greater than 1.00 AAE i.e., 1.54 ±0.03 AAE for frozen fruit and 1.44 ±0.03 AAE for fresh, corresponding to 44.36 ±0.89 and 41.54 ±1.00% RSA, respectively. The lowest potential, for most of the raw material, was observed for aqueous extracts – 0.10 ±0.02 AAE for fresh fruit extracts, 0.26 ±0.01 AAE for frozen fruit and 0.88 ±0.07 AAE for fresh leaf extracts. The activities of frozen grape leaf extracts were slightly different as the lowest potential was found for extracts in concentrated isopropanol after a 15 min extraction – 1.62 ±0.02 AAE, whereas for aqueous extract, this was 1.83 ±0.03 AAE. In most of the analysed cases, prolonging the extraction time by up to 1 h led to an increase in activity. The exceptions were the extracts of fresh fruit in 70% and 96% (v/v) ethanol – the highest activity was observed after 30 min of extraction, and extracts in water and 70% isopropanol where the highest potential was found after 15 min. The mean antiradical activity (RSA) of all fresh and frozen fruit extracts was 16.46% and 20.52%, respectively, as compared to that of leaf extracts which were 69.40% and 76.35%, respectively.

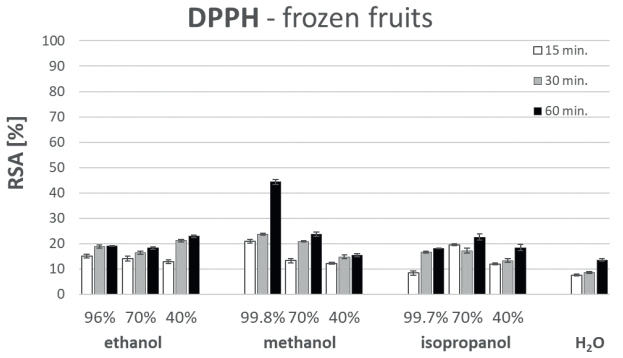
TABLE 1. Antioxidant potential of the extracts of fresh and frozen fruits and leaves of *Vitis vinifera* evaluated with the DPPH method (mean ±standard deviation)

solvent	extraction time (min)	DPPH			
		ascorbic acid equivalents – AAE (mg AA/g of raw material)			
		fruits		leaves	
		fresh	frozen	fresh	frozen
Ethanol 40% (v/v)	15	0.38 ±0.02	0.45 ±0.03	2.32 ±0.02	2.56 ±0.03
	30	0.45 ±0.03	0.73 ±0.02	2.50 ±0.02	2.65 ±0.01
	60	0.47 ±0.04	0.79 ±0.02	2.90 ±0.02	2.94 ±0.01
Ethanol 70% (v/v)	15	0.25 ±0.01	0.49 ±0.04	2.28 ±0.03	2.48 ±0.03
	30	0.52 ±0.02	0.57 ±0.02	2.75 ±0.02	2.75 ±0.02
	60	0.48 ±0.01	0.63 ±0.02	2.94 ±0.02	2.94 ±0.02
Ethanol 96% (v/v)	15	0.44 ±0.09	0.52 ±0.03	2.15 ±0.02	2.39 ±0.02
	30	0.54 ±0.07	0.65 ±0.02	2.71 ±0.02	2.74 ±0.01
	60	0.45 ±0.03	0.66 ±0.01	3.06 ±0.01	3.08 ±0.02
Methanol 40% (v/v)	15	0.41 ±0.03	0.42 ±0.01	2.58 ±0.02	2.12 ±0.02
	30	0.49 ±0.01	0.51 ±0.03	3.00 ±0.02	2.65 ±0.02
	60	0.53 ±0.02	0.53 ±0.02	3.06 ±0.02	2.78 ±0.02
Methanol 70% (v/v)	15	0.25 ±0.02	0.46 ±0.03	2.87 ±0.01	2.65 ±0.01
	30	0.51 ±0.02	0.72 ±0.01	2.94 ±0.01	2.70 ±0.01
	60	0.53 ±0.02	0.82 ±0.03	3.06 ±0.02	2.84 ±0.02
Methanol 99.8% (v/v)	15	0.30 ±0.02	0.72 ±0.03	2.91 ±0.01	2.82 ±0.02
	30	0.55 ±0.02	0.82 ±0.01	2.98 ±0.02	3.05 ±0.03
	60	1.44 ±0.03	1.54 ±0.03	3.12 ±0.02	3.07 ±0.03
Isopropanol 40% (v/v)	15	0.39 ±0.01	0.41 ±0.08	1.86 ±0.03	2.33 ±0.01
	30	0.42 ±0.01	0.46 ±0.02	2.20 ±0.01	2.49 ±0.02
	60	0.49 ±0.02	0.64 ±0.04	3.07 ±0.05	3.09 ±0.01
Isopropanol 70% (v/v)	15	0.63 ±0.03	0.68 ±0.01	1.22 ±0.03	2.82 ±0.02
	30	0.53 ±0.04	0.60 ±0.03	1.59 ±0.02	2.83 ±0.02
	60	0.53 ±0.01	0.78 ±0.04	2.55 ±0.01	2.85 ±0.02
Isopropanol 99.7% (v/v)	15	0.25 ±0.01	0.29 ±0.03	1.34 ±0.02	1.62 ±0.02
	30	0.48 ±0.03	0.58 ±0.01	1.71 ±0.01	2.53 ±0.02
	60	0.63 ±0.03	0.63 ±0.00	2.32 ±0.01	2.58 ±0.03
Distilled water	15	0.22 ±0.02	0.26 ±0.01	0.88 ±0.07	1.83 ±0.03
	30	0.10 ±0.01	0.30 ±0.01	0.94 ±0.04	2.23 ±0.02
	60	0.10 ±0.01	0.47 ±0.02	1.50 ±0.03	2.43 ±0.02



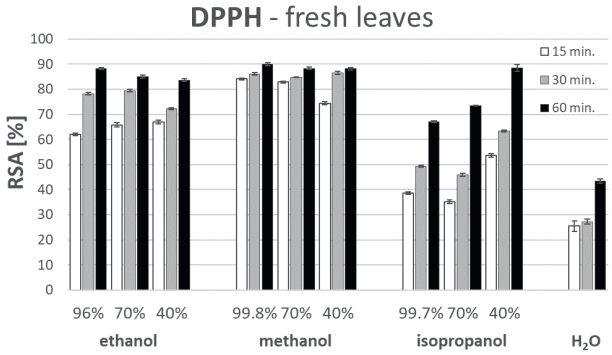
Vertical lines represent standard deviations.

FIGURE 1. Antioxidant potential of fresh fruit extracts evaluated with the DPPH method expressed as radical scavenging activity (RSA – %) – mean ±standard deviation



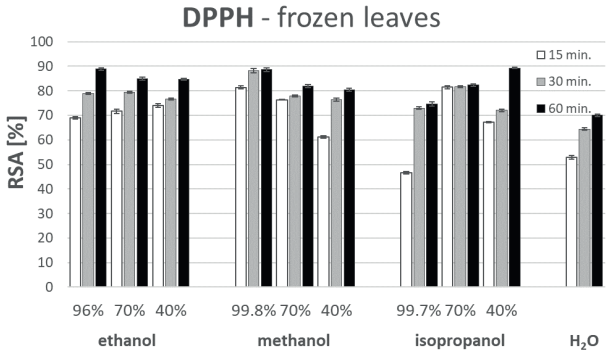
Vertical lines represent standard deviations.

FIGURE 2. Antioxidant potential of frozen fruit extracts evaluated with the DPPH method expressed as radical scavenging activity (RSA – %) – mean ±standard deviation



Vertical lines represent standard deviations.

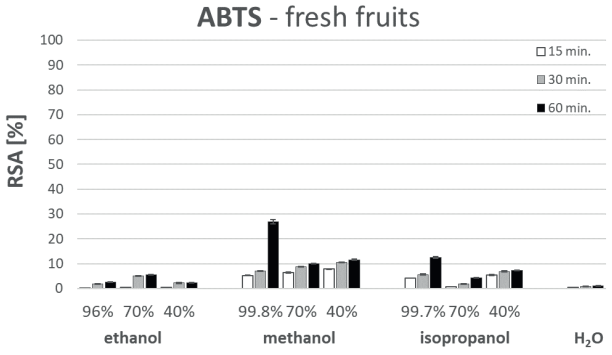
FIGURE 3. Antioxidant potential of fresh leaf extracts evaluated with the DPPH method expressed as radical scavenging activity (RSA – %) – mean ±standard deviation



Vertical lines represent standard deviations.

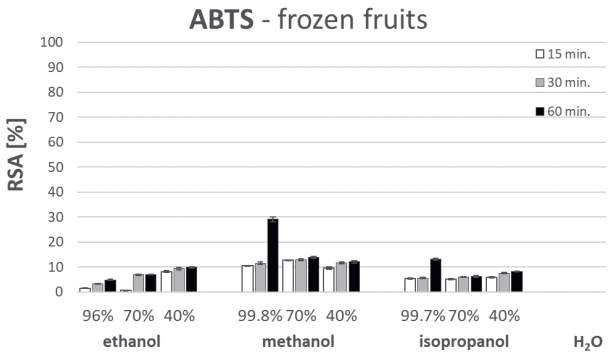
FIGURE 4. Antioxidant potential of frozen leaf extracts evaluated with the DPPH method expressed as radical scavenging activity (RSA – %) – mean ±standard deviation

The activities determined with the 2nd technique, i.e. ABTS, are presented in Table 2 and Figures 5–8 and are expressed, as in the DPPH method, either AAE or RSA. As before, the effects of the solvent used and the extraction time were taken into account. Most of the samples tested showed antioxidant properties except for in the aqueous extracts of the frozen fruit, where no antioxidant potential was found. The activity of the other samples ranged from 0.06 ±0.02 to 26.94 ±0.17 AAE corresponding to 0.22 ±0.09% to 96.73 ±0.60% RSA. Fruit extracts had significantly higher antioxidant properties in concentrated methanol for 1 h than the other samples – 7.48 ±0.23 AAE for fresh fruit and 8.11 ±0.27 AAE for frozen fruit. For leaf extracts, the highest amount of activity was observed for frozen material in 40% isopropanol – 26.94 ±0.17 AAE, in concentrated ethanol – 26.14 ±0.17 AAE and in distilled water – 25.76 ±0.16 AAE. All these results corresponded to an RSA of above 92% and indicated very high antioxidant capacity. It should be noted that all frozen leaf extracts were characterized by a high activity ranging from 6.53 ±0.17 AAE to 26.94 ±0.17 AAE (mean 20.15 AAE) as compared to fresh leaf extracts (highest activity of 12.80 ±0.24 AAE), fresh fruit extracts (highest activity of 7.48 ±0.23 AAE) and frozen fruit extracts (highest activity of 8.11 ±0.27 AAE). Contrary to this, the antioxidant potential of the fresh fruit extracts in 96% ethanol obtained after a 15 min ultrasound-assisted extraction varied from 0.06 ±0.02 AAE to 0.13 ±0.00 AAE, whereas that of the aqueous extracts was 0.10 ±0.02 AAE. Taking into account the solvent used to prepare the extracts, the highest activity of fruit extracts was observed if concentrated methanol was applied as an extractant, whereas for leaf extracts, the most optimal solvent was 40% (v/v) isopropanol. The average RSA of all extracts studied and evaluated with the ABTS method, was 5.54% and 8.89% for fresh and frozen fruit extracts and 20.30% and 77.23% for fresh and frozen leaf extracts.



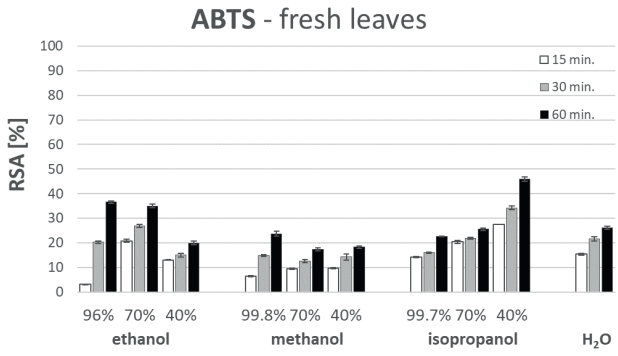
Vertical lines represent standard deviations.

FIGURE 5. Antioxidant potential of fresh fruit extracts evaluated with the ABTS method expressed as radical scavenging activity (RSA – %) – mean ±standard deviation



Vertical lines represent standard deviations.

FIGURE 6. Antioxidant potential of frozen fruit extracts evaluated with the ABTS method expressed as radical scavenging activity (RSA – %) – mean ±standard deviation

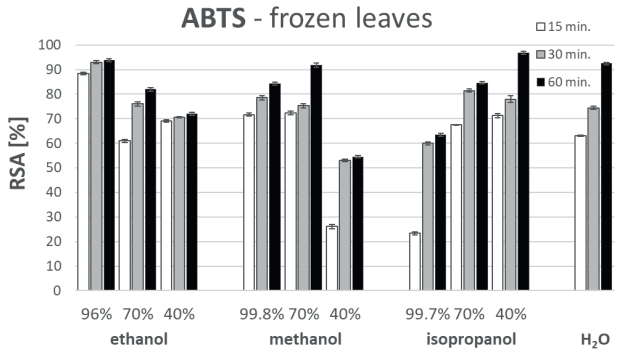


Vertical lines represent standard deviations.

FIGURE 7. Antioxidant potential of fresh leaf extracts evaluated with the ABTS method expressed as radical scavenging activity (RSA – %) – mean ±standard deviation

TABLE 2. The antioxidant potential of the extracts of fresh and frozen fruits and leaves of *Vitis vinifera* evaluated with the ABTS method (mean ±standard deviation)

ABTS					
solvent	extraction time (min)	ascorbic acid equivalents – AAE (mg AA/g of raw material)			
		fruits		leaves	
		fresh	frozen	fresh	frozen
Ethanol 40% (v/v)	15	0.10 ±0.02	2.30 ±0.09	3.64 ±0.02	19.25 ±0.17
	30	0.63 ±0.07	2.62 ±0.12	4.20 ±0.21	19.66 ±0.09
	60	0.67 ±0.04	2.75 ±0.09	5.56 ±0.19	20.05 ±0.15
Ethanol 70% (v/v)	15	0.13 ±0.00	0.20 ±0.02	5.81 ±0.18	17.00 ±0.17
	30	1.39 ±0.02	1.94 ±0.06	7.48 ±0.19	21.18 ±0.26
	60	1.57 ±0.05	1.98 ±0.02	9.74 ±0.22	22.83 ±0.21
Ethanol 96% (v/v)	15	0.06 ±0.02	0.42 ±0.04	0.88 ±0.04	24.62 ±0.15
	30	0.49 ±0.02	0.93 ±0.02	5.66 ±0.15	25.89 ±0.17
	60	0.70 ±0.02	1.37 ±0.04	10.20 ±0.15	26.14 ±0.17
Methanol 40% (v/v)	15	2.19 ±0.05	2.67 ±0.14	2.71 ±0.06	7.29 ±0.24
	30	2.92 ±0.02	3.28 ±0.11	3.97 ±0.35	14.79 ±0.15
	60	3.24 ±0.04	3.39 ±0.13	5.09 ±0.13	15.17 ±0.15
Methanol 70% (v/v)	15	1.79 ±0.04	3.58 ±0.02	2.62 ±0.08	20.18 ±0.21
	30	2.46 ±0.02	3.64 ±0.10	3.51 ±0.17	20.96 ±0.23
	60	2.78 ±0.04	3.87 ±0.10	4.48 ±0.20	25.55 ±0.25
Methanol 99.8% (v/v)	15	1.47 ±0.02	2.95 ±0.04	1.79 ±0.08	19.97 ±0.19
	30	1.95 ±0.04	3.22 ±0.13	4.12 ±0.09	21.87 ±0.25
	60	7.48 ±0.23	8.11 ±0.27	6.59 ±0.27	23.49 ±0.17
Isopropanol 40% (v/v)	15	1.51 ±0.06	1.62 ±0.07	7.67 ±0.00	19.86 ±0.23
	30	1.93 ±0.05	2.10 ±0.06	9.55 ±0.20	21.72 ±0.37
	60	2.06 ±0.02	2.28 ±0.04	12.80 ±0.24	26.94 ±0.17
Isopropanol 70% (v/v)	15	0.21 ±0.00	1.44 ±0.05	5.70 ±0.19	18.81 ±0.02
	30	0.52 ±0.02	1.69 ±0.02	6.07 ±0.10	22.69 ±0.17
	60	1.23 ±0.02	1.75 ±0.11	7.12 ±0.13	23.56 ±0.13
Isopropanol 99.7% (v/v)	15	1.17 ±0.00	1.51 ±0.06	3.94 ±0.08	6.53 ±0.17
	30	1.57 ±0.09	1.58 ±0.04	4.48 ±0.07	16.72 ±0.17
	60	3.47 ±0.10	3.68 ±0.10	6.30 ±0.04	17.67 ±0.19
Distilled water	15	0.10 ±0.02	0.00 ±0.00	4.29 ±0.10	17.55 ±0.06
	30	0.21 ±0.04	0.00 ±0.00	6.03 ±0.25	20.74 ±0.20
	60	0.30 ±0.04	0.00 ±0.00	7.27 ±0.19	25.76 ±0.16



Vertical lines represent standard deviations.

FIGURE 8. Antioxidant potential of frozen leaf extracts evaluated with the ABTS method expressed as radical scavenging activity (RSA – %) – mean ±standard deviation

Figure 9 presents statistically significant correlations between the activities determined by the DPPH method vs. the ABTS method for extracts of fresh and frozen fruits as well as of frozen leaves. The highest correlation was found between the activities of fresh ($r = 0.773$, $p < 0.001$) and frozen ($r = 0.759$, $p < 0.001$) fruit extracts, and the lowest for frozen leaf extracts ($r = 0.643$, $p < 0.001$). Moreover, the relationship between the activities of fresh vs. frozen material was also evaluated for both applied methods (Fig. 10, 11). For the DPPH method, a higher correlation was found for fruit ($r = 0.867$, $p < 0.001$) than for leaf ($r = 0.698$, $p < 0.001$) extracts (both fresh and frozen), whereas in the ABTS method, a significant correlation was only found in fruits (both fresh and frozen) – $r = 0.901$, $p < 0.001$.

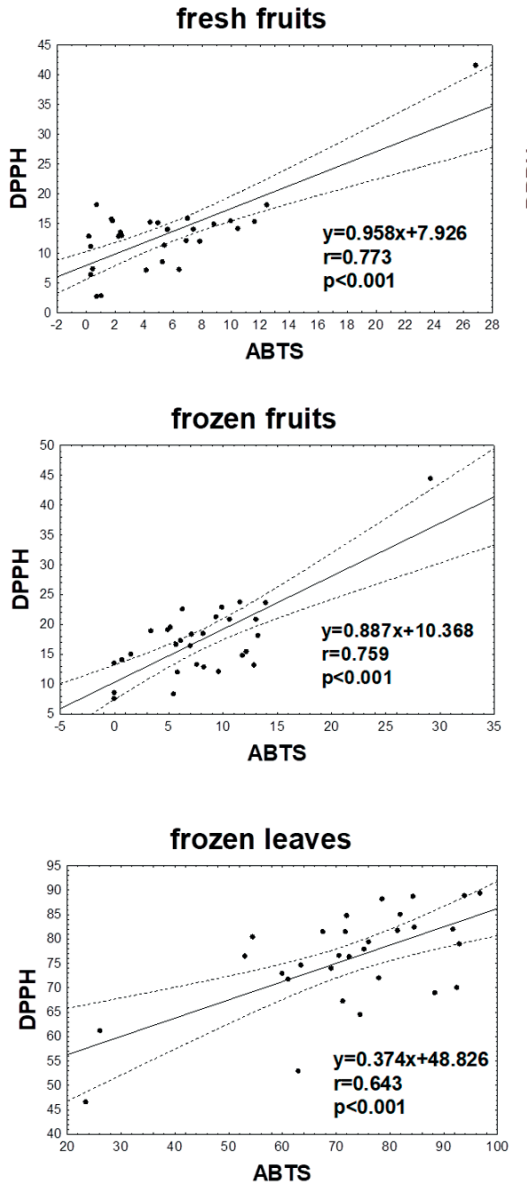


FIGURE 9. Correlations between the results of antioxidant activities obtained by different methods and expressed as radical scavenging activity (%) for individual parts of the *Vitis vinifera*

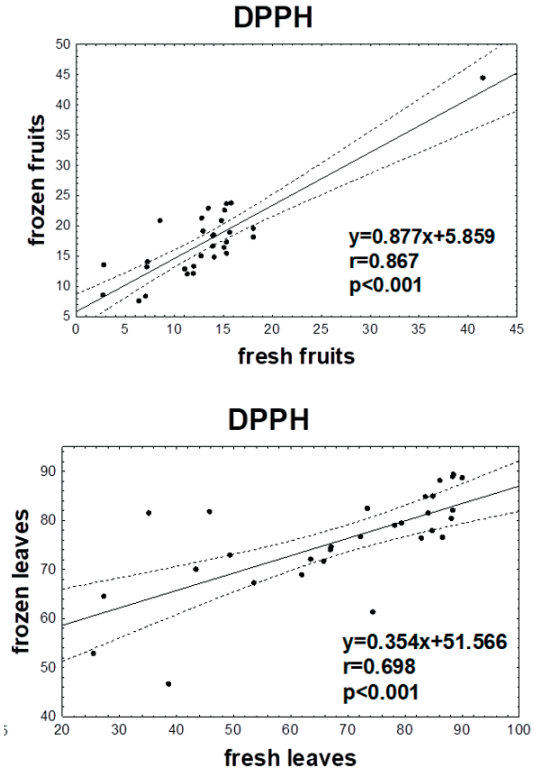


FIGURE 10. Correlations between the results of antioxidant activities of fresh and frozen material expressed as radical scavenging activity (%) and obtained by the DPPH method

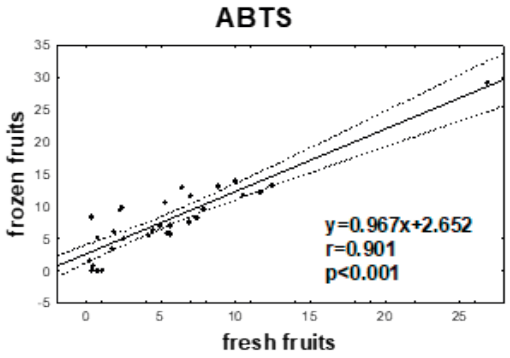


FIGURE 11. Correlations between the results of antioxidant activities of fresh and frozen fruits expressed as radical scavenging activity (%) and obtained by the ABTS method

DISCUSSION

In our study, the properties of extracts from different fresh and frozen parts of the grapevine were evaluated. Grapevine leaves are used to aid digestive discomfort and for their anti-inflammatory and antibacterial properties, whereas the fruit is mainly used to make wine. The antioxidant activity of these raw materials is largely dependent on a high content of polyphenols, flavonoids, organic acids and anthocyanins [19, 20, 21, 22, 23]. In our study, the analysed extracts of individual parts of the grapevine were characterized by different antioxidant activity, mostly depending on the type of raw material used. Taking into account the results obtained by both methods, the leaves, both fresh and frozen, had an activity (RSA) which was usually greater than 80%, and as such seemed to be most valuable. In contrast, the antioxidant activity of fruit extracts did not usually exceed 30% (Fig. 1, 2, 3, 4). Similar results were observed for dried vine leaves by Farhadi et al. They found the antioxidant potential determined by the DPPH method was 82% on average. The authors analysed different grapevine varieties using a 20 min ultrasound-assisted extraction and concentrated methanol to prepare the extracts [19]. In our study, the duration of extraction was similar (15 and 30 min), and a comparable high RSA of leaf extracts was observed. However, Farhadi et al. found greater antioxidant activity for fruit extracts where the results exceeded 90% [19]. It should be noted that they studied freeze-dried fruits. This method of fruit preservation may increase antioxidant potential and the content of bioactive compounds. This was demonstrated by Rutkowska et al. when analysing the antioxidant activity of freeze-dried wild rose fruits [24]. The diversified capacity to scavenge free radicals by *Vitis vinifera*

leaf extracts was found by Katalinic et al. who analysed raw material harvested during 3 vegetation periods. Antioxidant activity ranged from 32–69% and the highest levels were found for leaves harvested in October [7]. It should be added that, in our study, the leaves were also characterized by high antioxidant activity and were also harvested in October.

The antioxidant activity of grapes and the individual parts of the fruit, i.e. the skin, seeds and pulp, were also evaluated by Shrikanta et al. Fruits were frozen, then thawed, separated into individual parts and then freeze-dried. The extracts were prepared in 80% (v/v) ethanol by shaking in a water bath for 30 min at 60°C. The authors, using the DPPH method, found a higher antioxidant activity in the pulp than in the seeds and peel of the fruit [21]. Rockenbach et al. observed a higher ability in the skin than the seeds to scavenge free radicals. This was probably caused by a higher content of polyphenols and flavonoids in the fruit skin [1]. Extracts of grapevine leaves, due to their content of valuable antioxidants, may also have a beneficial effect in toxic liver damage caused by excessive alcohol consumption. Pari and Suresh added grapevine leaf extract enriched with 70% (v/v) ethanol to the diets of rats. They found that this extract had a beneficial effect on the liver by regulating enzyme activity, antioxidant concentration, by reducing lipid peroxidation and was beneficial for alcohol-induced oxidative damage. As a result, in animals treated with the grapevine leaf extract, a decreased activity of liver enzymes, such as aspartate aminotransferase, alanine aminotransferase, gamma-glutamyltransferase or alkaline phosphatase, was observed. Moreover, the activity of antioxidants such as vitamin E, C or glutathione had increased significantly [23].

The solvent used for extraction can also affect the isolation of active substances from the raw material and thus increase their antioxidant potential [24, 25, 26]. In our study, antioxidative properties were dependent on the solvent used. In the case of leaves, the highest results were obtained for samples prepared in either 96% ethanol or 40% isopropanol, whereas 99.5% methanol proved to be the most beneficial in fruit extraction. The activity of aqueous extracts was relatively low (Tab. 1, 2). Jayaprakasha et al. compared the antioxidant effect of grape seeds using 4 solvents: acetone, ethyl acetate, methanol and water as well as mixtures of ethyl acetate-water in different proportions. They evaluated the antioxidant potential of extracts with the linoleic acid peroxidation method. The extracts prepared in a mixture of ethyl acetate and water at different ratios showed the most beneficial antioxidant activity. Moreover, they were characterized by the highest concentration of flavonoids and proanthocyanidins [27]. Aouey et al. found a high content of valuable components in extracts using 80% (v/v) ethanol. They used dry extracts of the grapevine leaf as a raw material and observed an increased content of resveratrol, quercetin, catechin, flavonoids, flavonols, anthocyanins, gallic acid and epicatechin [20].

Freezing plant material is a common method of storage, especially for short-term raw materials. Freezing involves reducing the temperature of the product to as low as –30°C in a fast and controlled manner. This method of storage may affect the quality of the raw material [28, 29]. Analysing the results of our study, it can be observed that in the case of the ABTS method, the freezing of fruits and leaves seems to be beneficial as the activities of frozen material were higher – in some cases as much as sevenfold (Tab. 2). Freezing can lead to an increase in the amount of active compounds including polyphenols in the extracts. Moreover, during freezing, some damage in cell walls is likely to occur resulting in increased penetration of active substances into solvent and, as a consequence, a higher antioxidant potential can be observed. After freezing strawberries for 6-months, Kolniak found a higher total amount of polyphenol content in frozen fruits than in their fresh counterparts [29].

The fruits and leaves of vine proved to be a valuable source of antioxidants. However, antioxidative activity may vary depending on the part of the plant used as well as the method of preparation and the storage of extracts. Therefore, it is important to assess the effect of the above-mentioned factors in order to select the most appropriate way to obtain extracts to be useful in various industries.

CONCLUSION

Extracts prepared from both fresh and frozen leaves and the fruit of the *Vitis vinifera* showed antioxidant activity. Leaf extracts seemed to be particularly valuable. The results suggest their usefulness as a source of antioxidants to be applied in different branches of industry. Moreover, the positive effect of methanol applied as an extractant on the antioxidant potential of the extracts should be also mentioned. Additionally, a beneficial effect of freezing raw materials was also observed as in most cases, higher antioxidant activities were found in the extracts of the frozen parts of *Vitis vinifera*.

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