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The bio-flavanoid concentrate of Vitis vinifera L. 'Red Aladasturi'

Introduction

Flavanoids are the largest group of phenolic compounds, and owing to their high biological activity, they are often referred to as bioflavanoids. Deficiency of flavonoids in the human body manifests with the following symptoms: the general weakness and chronic fatigue, nasal hemorrhage, reduced immunity recurrent colds and infections, the formation of hematomas and vesication, the reduction in vascular conductance and elasticity, pains in the upper and lower extremities during movement, and so on (Kurkin et al., 2013; Yilmaz, Toledo, 2004; Gvinianidze et al., 2019).

There is extensive literature on high antioxidant activity of bioflavonoid-rich coloured grape seed and skin hydrophilic extract and red and white wine produced from it, as well as on inactivation of free radcals (Demrow et al., 1995; Gvinianidze, Gvinianidze, 2018). In 2011, the VITAL (Fred Hutchinson Cancer Research Center, Seattle, Washington) published a study on prostate cancer, and 35.239 men aged 50–76 volunteered for this study. It was found that patients regularly consuming grape-seed hydrophilic extracts were 41% less likely to suffer from prostate cancer than patients taking other drugs such as chondroitin, coenzyme Q10, fish oil, ginseng, ginkgo biloba, garlic, and glucosamine and palmetto (Zharskaya et al., 2014).

Vitis vinifera L. 'Red Aladasturi' is a Georgian, aboriginal, late-ripening, industrial cultivar, mostly common in the viticulture and winemaking zones of Imereti and Guria. Grapes ripen in late October and early November, and in full maturity, sugar content reaches 19.5–24.5%, and titrable acidity varies in the range of 8.0–9.3 g/dm³ (Ketskhoveli et al., 1960).

It has been established that grape raw materials grown in different micro-zones differ in their sensory characteristics, uvological and chemical composition, as well as in antioxidant, antiradical and antimicrobial properties (Darra et al., 2012; Kvesitadze et al., 2019). Secondary resources accrued from the processing of coloured grapes (in

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the form of skin and stone), by the contents of biologically active compounds have barely analogs in the autotrophic organisms, and they are not of less value products than wine itself. Only 9–12% of the total amount of phenolic compounds is contained in grape juice and pulp, accounting for 75–81% of the total mass of raceme, while the remaining 88–91% of phenolic compounds is mostly localised in the skin and stone, the mass of which is only 18–25% of raceme. This clearly shows how rich the biologically active compounds are in the solid parts of colored grapes, as well as how big is their role in the production of powerful antioxidant polyphenolic concentrates. Accordingly, research in this field is of high relevance.

The aim of the study was to investigate a polyphenolic complex and antioxidant activity of secondary resources remained after the initial processing of *V. vinifera* 'Red Aladasturi' grapes growing in Imereti and different micro-zones, as well as to explore the possibilities of using them for the production of drastic, antioxidant polyphenolic concentrates. The solid parts of colored grapes, with the content of biologically active compounds are the best raw materials for the production of therapeutic extracts and concentrates to treat various pathologies (Gvinianidze et al., 2017, 2018; Morandi Vuolo et al., 2019).

Materials and methods

Object of study

Research covered the raw materials of *Vitis vinifera* 'Red Aladasturi' grape from different vineyards of the Imereti viticulture and winemaking zone, particularly: sample N1 – Lifnari vineyards (Rokhi Village, Baghdati district, 120–160 m above sea level), sample N2 – Sviri vineyards (Sviri Village, Zestafoni district, 230–250 m above sea level) and sample N3 – Bagineti vineyards (Bagineti Village, Vani district, 580–600 m above sea level).

Research also covered hydrophilic extracts of grape skin and stone thickened by the vacuum of 'Aladasturi' coloured grapes raw materials, as well as the concentrates produced from their composition.

Research Methods

For research, there were used gravimetric, extractive, spectral and chromatographic methods (Singleton et al., 1999; Palomino et al., 2000; Giusti, Ronald, 2001; Mensor et al., 2001; Kammerer et al., 2004; Prior et al., 2005; Gómez-Alonsoet al., 2007; Rajha et al., 2013; Benmeziane et al., 2016; Gvinianidze et al., 2018). In test samples, we determined: the moisture and solid matter contents by heat-gravitational (GOST 28561-90) and refractometric methods.

Quantitative analysis of total phenols

Quantitative analysis of total phenols was performed spectrophotometrically, by Folin-Ciocalteu reagent. In particular, we extracted the crushed test samples with 75– 81% ethyl alcohol at the temperature of 72–75°C and under conditions of periodic stirring for 6–7 hours. 1 ml of extract obtained, we placed into a 25 ml flask and added 0.5 ml of H_2O , 1 ml of Folin-Ciocalteu reagent, and settled for 8 minutes at room temperature, then we added 10 ml of 7% Na_2CO_3 , filled the flask with H_2O , and settled it for 2 hours at room temperature.

The determination was carried out at 750 nm. As a control, we took 1 ml of the appropriate extracting agent and went through the same process. Calculation of the data obtained from the determination was carried out on the calibration curve of gallic acid.

The total phenol content shall be calculated in accordance with the formula:

$$\mathbf{X} = (\mathbf{D} \times \mathbf{K} \times \mathbf{V} \times \mathbf{F}) \times 1000 \ /m,$$

where X – the total phenol content, mg/kg β ; D – optical density; K – gallic acid conversion factor; F – solubility; V – the total volume of extract, ml; m – raw materials mass taken for extraction, g.

Antioxidant activity

Antioxidant activity in test samples was determined by one of the most common methods – DPPH method. DPPH is a rapid, simple and accurate test method for determining antioxidant activity. DPPH – ($C_{18}H_{12}N_5O_6$ M = 394.33) is a stable free radical with maximum absorption at 515–517 nm, and purple-violet coloration of its methanol extracts changes to bright yellow as a result of the recovery. The reaction occurs in accordance with the following pattern:

DPPH. + AH \rightarrow DPPH-H + A. DPPH. + R. \rightarrow DPPH-R,

where AH is an antioxidant and R is a free radical.

Quantification of total flavonoids was carried out with $AlCl_3$ reagent by spectral method – test sample was extracted with 80% ethyl alcohol at the temperature of 70–75°C. 1 ml of extract obtained from the total volume was placed into a 10 ml flask, then we added 5 ml of H₂O, 0.3 ml of 5% NaNO₂ was settled for 5 minutes, and then we added 0.3 ml of 10% $AlCl_3$ and settled for 6 minutes, then we added 2 ml of 1N NaOH- R and the determination was performed at 510 nm. As a control, we took 1 ml of the appropriate extracting agent and then went through the same process.

Calculation of the data obtained from the determination was carried out on the rutin calibration curve. The total flavonoid content shall be calculated in accordance with the formula:

$$X = (D \times K \times V \times F) \times 1000 /m;$$

where X – the total flavonoid content, mg/kg; D – optical density; K – rutin conversion factor; F – solubility; V – the total volume of extract, ml; m – raw materials mass taken for extraction, g.

Monomeric anthocyanins

The course of the pH-differential method for quantification of monomeric anthocyanins was as follows: we take test sample from 1 to 5 grams and carry out extraction with 45% ethyl alcohol. The volume of extract was reduced to 50 or 100 ml according to the extraction quality. From the total volume of extract, we take in two test-tubes 1 ml of extract in each, and add 4 ml of buffer solution in each. In one test-tube, we add 0.025 M of potassium chloride, and in the other test-tube, we add 0.4 M of sodium acetate, and 20 minutes later, we determine the optical density of the test solutions at 520 nm and 700 nm.

Quantification of leucoanthocyanins and catechins by spectral method Quantification of leucoanthocyanins and catechins by spectral method – extraction of test sample was carried out with 80% ethyl alcohol at the temperatures of 70–75°C. 1 ml taken from the total volume of extract was added with 3 ml of vanillin reagent and, 3 minutes later, we determine the optical density of red test sample at 500 nm. As a control, we shall take 1 ml or 3 ml of vanillin reagent. Calculation of the data obtained from the determination was carried out on the (+)catechin calibration curve. The catechin content shall be calculated in accordance with the formula: $X = (D \times K \times V \times F) \times 1000$ /m; where X – the catechin content, mg/kg; D – optical density; K – 35.0 (+) catechin conversion factor; F – solubility; V – the total volume of extract, ml; m – raw materials mass taken for extraction, g.

Results and discussion

Vitis vinifera 'Red Aladasturi' is a late-ripening colored grape cultivar with a very special aroma that reaches full maturity in the second half of November, and the range of aromatic compounds in it increases in proportion with the increase in the sugar content (Ketskhoveli et al., 1960). The area of our concern was represented by polyphenolic compounds, and we were less interested in the sugar and aroma compound contents. Accordingly, the grape raw materials

were taken during the period of their technical maturity, while phenolic compounds were present in grapes to the extent possible. Grape samples were taken on 16 October 2018. The analysis of the uvological characteristics of individual samples of grape raw materials is given in table 1.

	Chamataniatia	_		Samples	
	Characteristic	S	N1	N2	N3
	Juice and flesh		78.60	79.67	79.83
Parts of the cluster	Grape	stalk	4.71	4.74	4.69
of grapes [%]		skin	11.87	10.85	10.82
		stone	4.48	4.44	4.39
Number of seeds in	the grain			1-4	
Solid remains (grape	e stalk + grape sł	tin + grape stone)	21.06	20.03	19.90
Structural indicator			3.74	3.98	4.02

The study of the uvological characteristics of selected samples showed that structural indicators of all three samples of grapes (the ratio of flesh and juice to solid waste), at both stages of the grape harvest, were almost similar (relatively smaller for sample N1, and relatively larger for sample N3), indicating small differences in the quantitative phenolic complex contents in these samples (Gvinianidze et al., 2018).

We processed samples of grapes raw materials according to the following pattern: (1) identifying qualitative indicators of grapes raw materials; (2) passing grapes raw materials through the DMCSI-type grape clustercomb divider; (3) pressing-out the comb-less must in a basket press and separation of juice; (4) vacuum sublimation drying of juice-less sweet pomace with an initial moisture content of 45–65% to a final moisture content of 9–10%; (5) separation of the 'Aladasturi' cultivar's skin and stone dried to the moisture content of 9–10%, using tea sorting machine; (6) crushing separately the skin and stone in a micro-mill (TP2 Hammer Mill) until the fraction of 50–100 μ m. The crushed grape-stone was extracted by two different methods.

The first method (Grape-stone I – extract): as an extracting agent for extraction of the grape-stone micropowder, we have selected a complex hydrophilic solvent – ethanol containing 40% volumetric alcohol, which was diluted with mineral drinking water "Borjomi" whose pH = 3.6-6.3 and mineralisation is in the range of 7-14 g/dm³. This mineral water contains sodium (potassium) hydrogen carbonate and boric acid. Preliminary experiments have demonstrated that the extracting agent of ethanol diluted with mineral water can successfully replace the extracting agent diluted with water of ethanol containing 40% volumetric alcohol, which is oxidised by hydrochloric acid.

B.A.C. [mg / 100 g/ dry	Stages of superfluid extraction							Total	
weight basis]	1	2	3	4	5	6	7	8	
			San	nple N1					
Phenolic compounds	131.6	977.66	782.9	395.9	344.6	114.1	137.5	95.1	2979.3
Flavonoids	290.8	505.6	421.9	310.3	243.6	144.6	219.4	89.0	2225.2
Flavan-3-ols	120.6	293.7	414.4	284.9	192.5	104.2	100.4	84.5	1594.2
Leukoanthocyanins	_	123.4	253.0	148.37	-	_	-	-	524.7
			San	nple N2					
Phenolic compounds	123.8	943.0	762.1	382.7	332.4	184.9	129.5	87.9	2946.3
Flavonoids	289.6	500.2	418.1	308.7	243.4	146.4	219.6	91.8	2217.8
Flavan-3-ols	118.0	287.6	406.0	279.0	188.4	101.8	97.2	82.6	1560.6
Leukoanthocyanins	_	130.6	257.7	153.2	_	_	_	-	541.5
			San	nple N3					
Phenolic compounds	132.4	953.3	764.2	388.7	343.9	201.9	142.0	99.8	3026.1
Flavonoids	292.9	501.1	421.8	310.5	247.7	149.9	219.3	98.5	2242.7
Flavan-3-ols	119.7	288.6	403.8	271.1	190.4	105.6	101.1	86.6	1566.9
Leukoanthocyanins	-	114.3	249.1	147.9	-	-	-	-	511.3

 Tab. 2. Biologically active compounds of the grape-stone fluid extract

We have determined experimentally the mass ratio of the extracting agent and the grape-stone microdispersed powder, which is 5 l/kg. We have also determined experimentally the extraction parameters: temperature 54–57°C, duration 180–210 minutes, pulsation 4 sec⁻¹ and the pulsation amplitude 2–3 mm. Grape-stone ethanol extract at the initial stage, at the temperature of 4–5°C, is subject to sedimentation for 7–9 hours, removal from sediment and filtration with a wine filter with plates.

Tab. 3. Biologically active compounds and antioxidant activity of grape-stone extracts with 61-63% of solid matter content

Composition of hydrophilic			AOA [%]			
extracts	lille	Phenolic compounds	Flavonoids	Flavan-3-ols	Leukoanthocyanins	(F = 100)
	N1	3043.76	2293.94	1643.90	567.20	51.50
Sample	N2	3014.78	2276.10	1597.70	585.90	50.60
	N3	3181.23	2308.65	1603.80	549.80	52.30

The second method (Grape-stone II – extract): extraction of a bioflavanoid complex from the grape-stone micro-powder was carried out using a supercritical super-fluid extractor (SFE – 100-2-C10) produced by Water Corporation, where the extracting agent was present together with CO_2 ethyl alcohol. For maximal extraction of the bioflavanoid complex, we have determined experimentally the optimal fluid

extraction parameters: pressure –95 bar, CO_2 delivery rate – 6.5 kg/h. In addition, the extraction quality was also affected by 72% ethanol as co-solvent, whose ratio to CO_2 was 21–22%. Grape-stone fluid extract at the initial stage, at the temperature of 4–5°C, is subject to sedimentation for 7–9 hours, removal from sediment and filtration with a wine filter with plates. The data of the studies of biologically active compounds of the grape-stone superfluid extract are shown in table 2. We have blended the grape-stone extracts obtained by both methods at a ratio of 1:1. The filtered extract contained 5.2–6.3% of solid matters, and it was concentrated using a vacuum-rotary evaporator at the temperature of 54–57°C to the solid matter content of 63%.

The composition of the concentrated grape-stone hydrophilic exracts was pumped over into the enameled collecting tank, from which test samples have been taken for the analysis on the biologically active compound content and antioxidant activity (Tab. 3). From the crushed grape skin, we obtained a hydrophilic liquid extract rich in bioflavonoids in accordance with the following technological scheme (grape skin extract): to effectively carry out extraction of anthocyanins from the grape skin, we processed the grape skin micropowder in advance to 0.4% with potassium metabisulphate.

As an extracting agent, we selected 36–45% volumetric ethanol processed by 2% citric acid. The optimal ratio of microdispersed raw materials and the extracting agent we determined experimentally at 3 l/kg.

We determined experimentally the extraction optimal parameters: temperature 54–57°C; duration 180–210 minutes; the extraction mass pulsation 4 minutes; the amplitude 5 mm. Prior to sedimentation and filtration, the obtained grape skin ex-

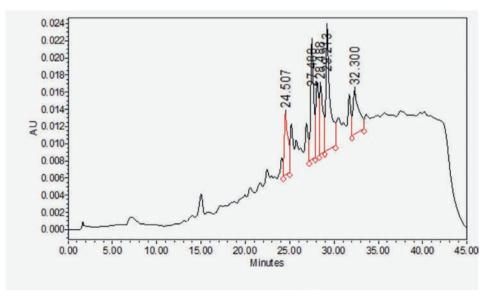


Fig. 1. Chromatogram of anthocyanins (sample N1)

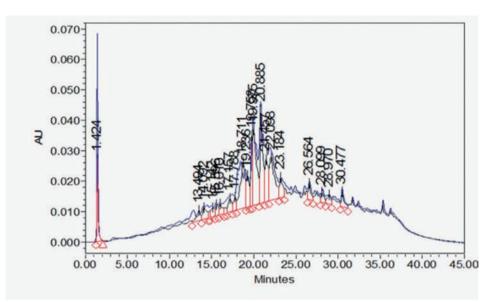


Fig. 2. Chromatogram of flavonoids (sample N1)

tract was processed by potassium bicarbonate (KHCO₃ – Potassium bicarbonate) for correcting 0.7-0.9 g/dm³ excessive acidity.

Grape skin				active compour on dry weight ba		AOA [%]
hydrophilio extract	2	Phenolic compounds	Flavonoids	Flavan-3-ols	Leukoanthocyanins	(F = 100)
	N1	3178.5	646.9	1295.9	2106.1	46.6
Sample	N2	3098.8	396.0	1484.5	1302.4	45.3
	N3	3265.3	520.6	1667.8	1954.8	47.1

Tab. 4. Biologically active compounds and antioxidant activity of grape-skin hydrophilic extracts

The obtained extract, at the temperature of 4–5°C, is subject to sedimentation for 7–9 hours, removal from sediment and filtration with a wine filter with plates. The composition of the filtered grape skin exracts contained 4.5–5.2% of solid matters, and it was concentrated using a vacuum-rotary evaporator at the temperature of 54–57°C to the solid matter content of 61–63%, and then we assessed biologically active compounds and antioxidant activity (Tab. 4). Figure 1 illustrates the chromatogram of anthocyanins of extract containing 61–63% of solid matters of the micro-dispersed skin of Lifnari's 'Red Aladasturi' cultivar, and figure 2 illustrates the chromatogram of flavonoids.

We have blended the obtained grape-stone ethanol and fluid extracts containing 61–63% of solid maters at an equal ratio (1:1:1) and assessed biologically active compounds and antioxidant activity in this composition (Tab. 5).

sona	matter	content						
Samı	mla	Biologically active compounds [mg / 100 g on dry weight basis]						
num		Phenolic compounds	Flavonoids	Flavan-3-ols	Anthocyanins	Leukoanthocyanins	AOA [%] (F = 100)	
le	N1	3089.8	1746.3	1529.1	2131.9	572.5	51.4	
Sample	N2	3044.7	1651.8	1562.6	1332.7	591.0	50.3	
Sa	N3	3210.6	1714.2	1625.9	2011.8	554.9	52.2	

Tab. 5. Biologically active compounds of grape-stone and skin ethanol and fluid extracts with 61-63% of solid matter content

The second stage of concentration was implemented by method of vacuum-sublimation or lyophilization to 74–75% of the solid matter content and pumped over into the enameled collecting tank, from which test samples have been taken for the analysis. The results of the assessment of biologically active compounds and antioxidant activity of bio-flavonoid liquid concentrate 'Red Aladasturi' are shown in table 6.

Biologically active compounds		Sample		
[mg / 100 g on dry weight]	N1	N2	N3	
Phenolic compounds	3401.8	3351.1	3533.3	
Flavonoids	1921.2	1808.4	1886.7	
Flavan-3-ols	1682.2	1719.0	1788.9	
Anthocyanins	2348.3	1467.9	2213.2	
Leukoanthocyanins	578.9	597.1	560.6	
Dry matter [%]	74-75	74-75	74-75	
AOA, (F = 100) [%]	56.6	55.31	57.45	

Tab. 6. Biologically active compounds and antioxidant activity of Vitis vinifera L. 'Red Aladasturi'

The studies have shown that the bio-flavonoid concentrates containing 74–75% solid matters of 'Red Aladasturi' obtained from different samples of colored grapes are slightly different from each other in the biologically active compound contents, but all three samples produce the bio-flavonoid concentrates with high antioxidant activity.

Conclusion

It has been studied that the grape-stone and skin hydrophilic extracts of 'Aladastur' colored grape cultivar's raw materials taken in the separate viticulture and winemaking micro-zones of Imereti and the liquid bio-flavonoid concentrates are characterised by high antioxidant activity (N1 – 56.60%; N2 – 55.31% and N3 – 57.45%).

The bio-flavanoid liquid concentrates obtained from sample N1 are characterised by a high anthocyanin content, while the conentrates obtained from sample N2, are characterised by a high leucoanthocyanin content, and the bio-flavanoid liquid concentrates obtained from sample N3 are characterised by the content and antioxidant activity of phenolic compounds and flavan-3-ols. Anthocyanins in samples of 'Red Aladasturi' cultivar are localised in the grape skin.

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Conflict of interest

The authors declare no conflict of interest related to this article.

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Abstract

This paper dwells on the uvological characteristics of cultivar Vitis vinifera L. 'Red Aladasturi' grape raw materials growing in the viticulture and winemaking zone of Imereti (Georgia), as well as biologically active compounds and antioxidant activity of hydrophilic extracts and liquid concentrates of its solid matters (stone and skin). Research also covered hydrophilic extracts of grape skin and stone thickened by the vacuum of 'Red Aladasturi' grapes raw materials, as well as the concentrates produced from their composition. For research, there were used gravimetric, extractive, spectral and chromatographic methods. We processed samples of grapes raw materials according to the following pattern: identifying qualitative indicators of grapes raw materials; passing grapes raw materials through the DMCSI-type grape clustercomb divider; pressing-out the combless must in a basket press and separation of juice; vacuum sublimation drying of juiceless sweet pomace with an initial moisture content of 45-65% to a final moisture content of 9-10%; separation of the 'Red Aladasturi' cultivar's skin and stone dried to the moisture content of 9-10%, using tea sorting machine designed by G. Lominadze; crushing separately the skin and stone in a micro-mill (TP2 Hammer Mill) until the fraction of 50-100 µm. we have blended the obtained grape-stone ethanol and fluid extracts containing 74-75% of solid maters at an equal ratio (1:1:1) and assessed biologically active compounds and antioxidant activity in this composition. It has been established that the bio-flavanoid liquid concentrate 'Red Aladasturi' is strong antioxidant (55.31-57.45%), and one tablespoon or 8-9 ml of it contains 110-127 mg of flavanoids, which is 105-110% of a full day of rations per person per day.

Key words: anthocyanins, antioxidant activity, grapes, phenolic compounds, Vitis vinifera 'Red Aladasturi'

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Koncentrat bio-flawonoidów z *Vitis vinifera* L. 'Red Aladasturi' Streszczenie

W artykule omówiono właściwości odmian winogron Vitis vinifera L. 'Red Aladasturi', rosnących w strefie uprawy winorośli i winiarstwa w Imereti (Gruzja), a także związki aktywne biologicznie i aktywność przeciwutleniająca ekstraktów hydrofilowych oraz płynnych koncentratów z ich ciał stałych (pestka i skórka). Badania obejmowały również hydrofilowe ekstrakty ze skórki winogron i pestek, zagęszczone przez sublimaty surowca z gron "Aladasturi", a także wytwarzane z nich koncentraty. Do badań wykorzystano metody grawimetryczne, ekstrakcyjne, spektralne i chromatograficzne. Próbki surowców winogronowych przetwarzano według następujacego szablonu: identyfikacja wskaźników jakościowych surowców winogronowych; przepuszczanie surowców do produkcji winogron przez dzielnik kombajnu do zbioru winogron typu DMCSI; wyciskanie moszczu w prasie koszowej i oddzielanie soku; sublimacja próżniowa – suszenie słodkich wytłoków bez soku, o początkowej zawartości wilgoci 45-65% do końcowej zawartości wilgoci 9-10%; oddzielenie skórek i pestek odmiany 'Red Aladasturi', wysuszonych do wilgotności 9-10%, za pomocą maszyny do sortowania herbaty, zaprojektowanej przez G. Lominadze; oddzielne mielenie skórek i pestek w mikro-młynie (TP2 Hammer Mill), do frakcji 50-100 µm. Zblendowano otrzymany etanol z pestek winogron i płynne ekstrakty owoców, zawierające 74–75% substancji stałych w równym stosunku (1: 1:1) i oceniono w tym składzie związki aktywne biologicznie oraz aktywność przeciwutleniająca. Ustalono, że płynny koncentrat bio-flawanoidów 'Red Aladasturi' jest silnym przeciwutleniaczem (55,31-57,45%), a jedna łyżka stołowa lub jego 8-9 ml zawiera 110-127 mg flawanoidów, co stanowi 105-110% pełnej racji żywnościowej dziennie na osobę.

Słowa kluczowe: antocyjany, aktywność przeciwutleniająca, winogrona, związki fenolowe, *Vitis vinifera* 'Red Aladasturi'

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