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A liquid bio-flavanoid concentrate "Red aladasturi"

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A B S T R A C T

This paper dwells on the uvological characteristics of "Aladasturi" colored grape variety 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). The amount of phenolic compounds was determined using Folin Chocalteu reagent. The flavonoid, catechin and anthocyanin contents were determined by spectral methods, and antioxidant activity was determined by DPPH method. Research resulted in the establishment of the dynamics of changes in the biologically active compound content in test samples of "Aladasturi" variety growing in certain zones of Imereti region according to its growing areas. „The bio-flavanoid concentrate "Red Aladasturi" containing 64-75% of solid matters, which was thickened by vacuum-sublimation method constitues a composition of the following components: 33% grape-stone ethanol extract, 33% grape-stone super-fluid extract and 33% grape-skin hydrophilic extracts. 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 52-57 mg of flavanoids, which is 109-112% of a full day of rations per person per day.

Keywords: "Aladasturi", Colored grapes, Phenolic compounds, Anthocyanins, Antioxidant activity, Flavanoids.

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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, the reduction in 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 the movement, and so on [1-3].

There is an extensive literature on high antioxidant activity of bioflavpnoid-rich colored grape seed and skin hydrophilic extract and red and white wine produced from it, as well as on inactivation of free radcals [4, 5].

In 2011, the VITAL (Fred Hutchinson Cancer Research Center, Seattle, Washington) published studies on prostate cancer, with 35 239 males aged 50-76 voluntarily taking part in the study. Patients who received systemic hydrophilic extracts of grape juice were found to be 41% less likely to have prostate cancer than those receiving other drugs such as chondroitin, coenzyme Q10, fish oil, ginseng, ginkgo biloba, garlic, and glucosamine and palmetto [6].

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 [6].

The aim of the study was to investigate a polyphenolic complex and antioxidant activity of secondary resources remained after the initial processing of "Aladasturi" grape variety growing in Imereti

and different micro-zones, as well as to explore the pssibilities of using them for the production of drastic, antioxidant polyphenolic concentrates, because 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 [7-9].

"Aladasturi"" is a Georgian, aboriginal, red, late-ripening, industrial variety, 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 8-9.3 g /dm^3 [10].

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 [11,12].

Secondary resources accrued from the processing of colored grapes (in 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 localized 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.

Materials and methods

Object of study

Research covered the raw materials of "Aladasturi" grape variety from different vineyards of the Imereti viticulture and winemaking zone, particularly: sample No. 1 - Lifnari vineyards (Rokhi Village, Baghdati district, 120-160 m above sea level), sample No. 2 - Sviri vineyards (Sviri Village, Zestafoni district, 230-250 m above sea level) and sample No. 3 - 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" colored 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 [13-22].

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 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 ^{0}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_20, 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:

X = (D K V F)1000 /m,

where X - the total phenol content, mg/kg;

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 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 DPPH-H + A. DPPH. + R. DPPH-R,

where AH is antioxidant and R is a free radical.

Quantification of **total flavonoids** was carried out with AlCl3 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 H_2O, 0.3 ml of 5% $NaNO_2$ was settled for 5 minutes, and then we added 0.3 ml of 10% AlCl3 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 K V F) · 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.

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 - 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 K V F) · 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

"Aladasturi" is a late-ripening colored grape variety 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. 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.

Table 1.*Uvological characteristics of individual samples of grape raw materials*

Characteristics		October 16, 2018		
		Sample N1	Sample N2	Sample N3
Parts of the cluster of grapes, %	Juice and flesh	78,60	79,67	79,83
	Grape stalk	4,71	4,74	4,69
	Grape skin	11,87	10,85	10,82
	Grape stone	4,48	4,44	4,39
Number of seeds in the grain		1-4		
Solid remains (Grape stalk +Grape skin +Grape stone)		21,06	20,03	19,90
Structural indicator		3,74	3,98	4,02

The study of the urological 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 No. 1, and relatively larger for sample No. 3), indicating small differences in the quantitative phenolic complex contents in these samples [22].

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 comb-less must in a basket press and separation of juice;
- Vacuum sublimation drying of juice-less sweet pomace with an initial moisture content of 45-65% to a final moisture content of 9-10%;
- Separation of the "Aladasturi" variety'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.

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 Borjomi and mineralization is in the range of 7-14 g/dm^3. 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 oxidized by hydrochloric acid.

- 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 0C, 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 0C, is subject to sedimentation for 7-9 hours, removal from sediment and filtration with a wine filter with plates.

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 0C, 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 0C 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 (see Table 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 0C; duration - 180-210 minutes; the extraction mass pulsation - 4 minutes; the amplitude - 5 mm.
- Prior to sedimentation and filtration, the obtained grape skin extract was processed by potassium bicarbonate (KHCO3 - Potassium bicarbonate) for correcting 0.7-0.9 g/dm3 excessive acidity.

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

B.A.C., mg / 100 g. on dry weight basis	Stages of superfluid extraction								Total
	1	2	3	4	5	6	7	8	
Sample 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
Leukoanthocya-nins	-	123,4	253,0	148,37	-	-	-	-	524,7
Sample 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
Leukoanthocya-nins	-	130,6	257,7	153,2	-	-	-	-	541,5
Sample 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
Leukoanthocya-nins	-	114,3	249,1	147,9	-	-	-	-	511,3

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

Composition of hydrophilic exracts	Biologically active compounds , mg / 100 g. on dry weight basis				AOA, % (F=100)
	Phenolic compounds	Flavonoids	Flavan-3-ols	Leukoanthocya-nins	
Sample N 1	3043,76	2293,94	1643,9	567,2	51,5
Sample N 2	3014,78	2276,10	1597,7	585,9	50,6
Sample N 3	3181,23	2308,65	1603,8	549,8	52,3

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

Grape skin hydrophilic extract	Biologically active compounds , mg / 100 g. on dry weight basis				AOA, % (F=100)
	Phenolic compounds	Flavonoids	Flavan-3-ols	Leukoanthocyanins	
Sample N 1	3178,5	646,9	1295,9	2106,1	46,6
Sample N 2	3098,8	396,0	1484,5	1302,4	45,3
Sample N 3	3265,3	520,6	1667,8	1954,8	47,1

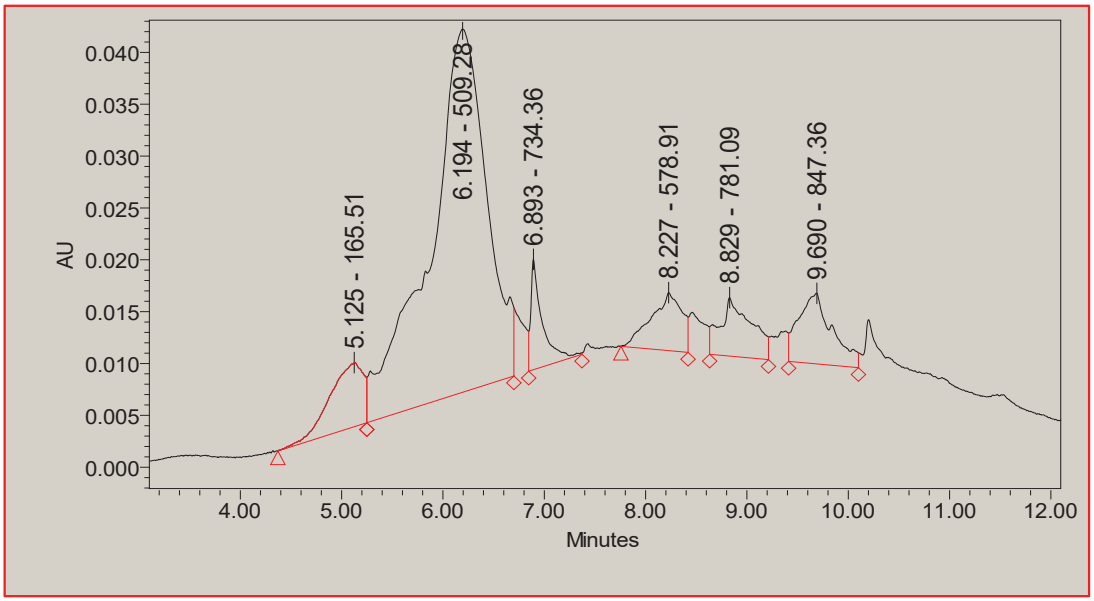

Fig. 1. *Chromatogram of anthocyanins containing extract (61-63%) of Aladasturi peel (sample N1), m/z 509.28 [M-2H+H2O] Malvidin-3-O-Glucoside*

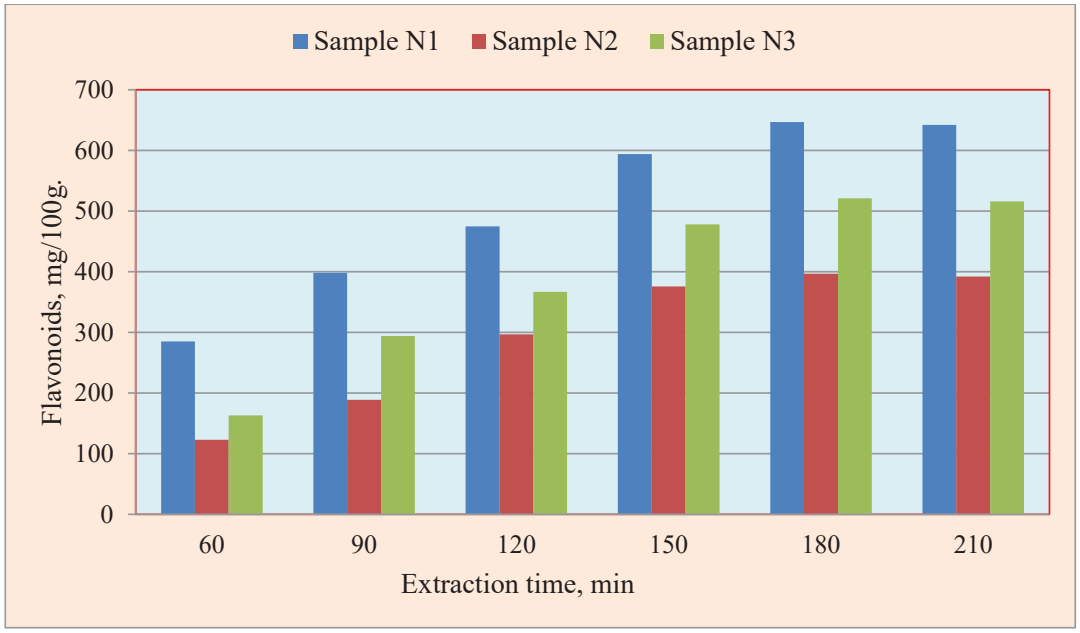

Fig. 2. *Diagram of the extraction of flavonoids from grape skin*

- The obtained extract, at the temperature of 4-5 0C, 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 0C to the solid matter content of 61-63%, and then we assessed biologically active compounds and antioxidant activity (Table 4). Figure 1 illustrates the chromatogram of anthocyanins of extract containing 61-63% of solid matters of the micro-dispersed skin of Lifnari's "Aladasturi" variety, and Figure 2 illustrates diagram of the extraction of flavonoids from grape skin.

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 (Table 5).

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.

The studies have shown that the bio-flavonoid concentrates containing 74-75% solid matters of 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 variety's raw materials taken in the separate viticulture and winemaking micro-zones of Imereti and the liquid bio-flavonoid concentrates are characterized by high antioxidant activity (N1-56.6%; N2-55.45% and N3-55.45%).

The bio-flavanoid liquid concentrates obtained from sample No. 1 are characterized by a high anthocyanin content, while the conentrates obtained from sample No. 2, are characterized by a high leucoanthocyanin content, and the bio-flavanoid liquid concentrates obtained from sample No. 3 are characterized by the content and antioxidant activity of phenolic compounds and flavan-3-ols.

Anthocyanins in samples of "Aladasturi" variety are localized in the grape skin.

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

Sample number	Biologically active compounds , mg / 100 g. on dry weight basis					AOA, % (F=100)
	Phenolic compounds	Flavo-noids	Flavan-3-ols	Antho-cyanins	Leukoantho-cyanins	
Sam. N 1	3089,8	1746,3	1529,1	2131,9	572,5	51,4
Sam. N 2	3044,7	1651,8	1562,6	1332,7	591,0	50,3
Sam. N 3	3210,6	1714,2	1625,9	2011,8	554,9	52,2

Table 6. *Biologically active compounds and antioxidant activity of "Red Aladasturi"*

Biologically active compounds , mg / 100 g. On dry weight	Sample N 1	Sample N 2	Sample N 3
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), In, %	56,6	55,31	57,45

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