



Full Length Research Paper

Chemical Composition and Antioxidants of 14 Varieties of White Grape spread in Western Georgia

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Abstract

We have studied 14 samples of dry fruits, titrated acidity, active acidity of 5 species of white grapes (Tsolikouri, Tsitska, Klarjula, Krakhuna and Kutaturi) spread in three regions of Western Georgia - Adjara, Imereti and Samegrelo, and with the help of a spectral method there has been defined the following: the total phenols of Folin-Ciocalteu, the total flavonoids of aluminum chloride, the catechins of vanilla and the antioxidant activity using DPPH reagents. Due to the grape variety there has been found differences between the quantity content of biologically active compounds and the antioxidant activity, dependent on it in a directly proportional correlation. Such varieties of grapes as the Tsolikouri and the Tsitska, grown in Opcha (Imereti) and Keda (Adjara) are distinguished by their high antioxidant activity.

Keywords: DPPH Method, Tsolikouri, Tsitska, Antioxidant Activity, Common Phenols, Catechins, Flavonoids.

Introduction

Different zones of Georgia are significantly distinguished by grape yields, quality, and therefore they determine the type of wine produced. During the centuries the regions of Western Georgia-Imereti, Adjara and Samegrelo have been forming and creating Georgian wine culture [1]. The quality indicators of grape and wine are determined by various factors. Among them, the grape variety is of particular importance, but no less important is the soil-climatic factor, the amount of precipitation and the air temperature. The soil-climatic factor refers both to the grape and the chemical composition of wine made from it. Grape phenolic compounds and their transformation products affect the formation of wine type (wine taste, bouquet, color, transparency, stability) and determine its antioxidant activity and the medicinal and preventive effect on various diseases [2,3,4,5,6]. They are presented in the form of hundreds of compounds and are mainly localized in the skin, stems and seeds of grape.

The aim of the work is to study the chemical and biochemical characteristics of the white grape varieties (the Tsolikouri, the Tsitska, the Krakhuna, the Klarjuri and the Kutaturi) prevalent in different regions of western Georgia, and to determine their antioxidant activity; to define their dependence on the location of grape distribution. According to the area of grape cultivation in Georgia, the Tsolikouri takes the second place after the Rkatsiteli. It is distinguished by high agricultural and technological characteristics. High-quality and naturally semisweet wines, characterized by a large body, alcohol and acidity of normal content, as well as high tasting indicators, are made from it on the basis of European and Imeretian traditional methods. The Tsitska is predominantly spread in western Georgia (Upper and Middle Imereti), where it is a main industrial grape variety. For viticulture the most important are the old varieties – the Klarjuri and the Krakhuna, which are preserved in small quantities in the villages of Guria-Adjara mountain range [8] due to the influence of vine phylloxera and other fungal diseases.

Materials and Methods

Study was conducted at Batumi Shota Rustaveli State University's Agrarian and membrane technology institute, at the department of Chemical Analysis and Food Safety, and at West Georgia Chromatography Center (Grant AP/96/13 Georgia National Science Foundation). Biochemical and chemical analysis was conducted using different physico-chemical and instrumental methods. Separation-identification and quantitative analysis was conducted using pH-meters (Mettler Toledo). Refractrometer -Misco, Spectrometer -Cuvette Changer (Mettler Toledo UV5A). Chemicals – stability radical- 2,2-Diphenil-1-picrilhydrazyl (Aldrich-Germany), AlCl₃, Folin Ciocalteu reagent (preparation), Standarts –Gallic acid (Sigma), Quercetin (Loba-chemie). C18 Cartridge Solid Phase Extraction (SPE) Waters Sep-Pak C18 (500 mg). Waters Acrodisc LC PVDF Filter 13 mm 0,45µm.

Five samples of grape varieties were taken for analysis in October - November 2016-2017 including the Tsolikouri in Adjara, Imereti and Samegrelo, the Tsitska in Adjara and Imereti, the Klarjuri and the Kutatura in agro service center of vine and fruit cattery (Adjara). After the removal of stems, all five varieties of grapes (each 1 kg) were placed in a homogenizer, diluted with ethyl alcohol and stored in a refrigerator at a temperature of -400 ° C (Table 1).

Samplers filtration Cartridge Solid Phase Extraction (SPE) Waters Sep-Pak C18 (500 mg) (Condition 2 ml methanol, equilibration 2 ml water, load 2 ml samplers, wash 2ml water + 0,1 % formic acid and elute 4 x 1 ml acetonitrile+0,1 % formic acid). All samplers before chromatography filtration (0,45µm).

Total acidity- Method OIV-MA- AS313-01 [12], Actual acidity - pH -Method OIV-MA-AS313-15[13], total sugars concentration in grape by refractometry - Method OIV-MA-AS2-02 [14].

Table 1. Samples of grape

№	variety	Region	egionR	Village	Grape
1	Tsolikouri	Adjara	Kobuleti	Gvara	G.1
2	Tsolikouri	Adjara	Keda	Kokotauri	G.2
3	Tsolikouri	Imereti	Bagdadi	Ofcha	G.3
4	Tsolikouri	Samegrelo	Martvili	Bandza	G.4
5	Tsolikouri	Samegrelo	Martvili	Najachao	G.5
6	Tsolikouri	Samegrelo	Martvili	Muchurcha	G.6
7	Tsolikouri	Samegrelo	Martvili	Lechaidravo	G.7
8	Tsolikouri	Samegrelo	Martvili	Nagvazao	G.8
9	Tsolikouri	Samegrelo	Martvili	Vedidkari	G.9
10	Tsitska	Imereti	Bagdadi	Ofcha	G.10
11	Tsitska	Adjara	Kobuleti	Gvara	G.11
12	Klarjula	Adjara	Kobuleti	Gvara	G.12
13	Krachuna	Adjara	Kobuleti	Gvara	G.13
14	Kutatura	Adjara	Kobuleti	Gvara	G.14

Determination of Catechins and Procyanidine contents- Swain and Hillis Spectral method [10], 1 ml samplers added 3 ml 1 % Vanillin reagents (1g Vanillin added Sulfur acid (70 % solution). Blanc all solution without samplers. After 15 min Determination is conducted at 750 nm. Flavonoids - aluminum chloride colorimetric method- [11]. Determination of Total Flavonoids Total flavonoid content (TFC) was determined by the aluminum chloride colorimetric method as previously described. Wine samples (0.5 mL) were mixed with 2 mL of distilled water and 150 µL of a 5% NaNO₂ solution. After 5 min, 150 µL of 10% AlCl₃ were added and, after 6 min, 2 mL of a 1 mol/L NaOH solution were also added. The final volume was brought to 5 mL with distilled water. Finally, the absorbance was measured at 510 nm. Results were expressed as mg catechin (or Ruthin) equivalent per liter (mg CE/L)[14].

Determination of total phenolic compounds using Folin-Ciocalteus spectral methods [9]. Extraction of the samples was conducted using 80% ethil alcohole, 0.5 or 1.0 ml-s of the extract is transferred to 25 ml volumetric flask, and 5.0 ml of water is added, with 1.0 ml of Folin-Ciocalteu reagent. After 8 minutes at 25⁰C, 10.0 ml of 7% Na₂CO₃, flask is then filled with water and left at room temperature for 2 hours determination is conducted at 750 nm. As control, 1 ml of extragent is used. After the values are obtained, calculations are performed using calibration curve of Gallic Acid. The Formula used to determine phenols is provided below:

$X = (D K V F) * 1000 / mX$ – amount of phenols mg/kg; D – optical density; K – coefficient; F – factor of dilution ;V – volume of extract in ml;m – mass of the raw material used for extraction in grams.

Determination of antioxidant activity using DPPH (2,2-Diphenyl-1-pic rylhydrazil) methods[7,8]. For determination of antioxidant activity-radical retention to the 1 ml of the sample 3 ml of DPPH extract (0.1 mM DPPH-0.004 g/100mL in ethil alcohol) and after 30 minutes optical density was evaluated on spectrophotometer. DPPH and 96% ethyl alcohol were used as blanks. Formula used to determine activity of free radical inhibition (DPPH) is provided below: $In \% = A_c - A_s / A_c * 100\%$, Ac indicates absorption of DPPH/Alcohol solution and As indicates absorption of the extract.

Results and Discussion

The largest fruits of the Tsolikouri variety were among the samples taken from the agro-service center of Kobuleti, the smallest fruits were collected in Keda, and the grapes harvested in Samegrelo (Lekhindrao) had comparatively small bunches. Collected in Imereti and Kobuleti, the Tsitska fruits differ in the technical characteristics of samples taken in other regions. The fruits of the Klarjuli and the Krakhuna, harvested in Kobuleti, differ significantly from those which, by their weight, are almost one and a half times larger than the industrial varieties of the Tsitska and the Tsolikauri from the regions of Imereti and Samegrelo (Table 2).

Table 2. The technical indicators of white grape varieties harvested in different regions

Sample name	The technical indicators of white grapes						
	Color of berry	Shape of berry	taste	Mass of bunch, gr	Height of bunch, cm	Width of bunch, cm	Mass of berry, gr
G.1	Green	Round	sweetish	552,03	22	18	3,07
G.2	Greenish-amber	Round	sourish	199,92	17	12	2,0
G.3	Greenish-amber	Round	sweet	188,65	13,83	9,16	2,0
G.4	Green	Round	sweetish	139,7	12,75	9,5	2,4
G.5	Amber	Round	sweet	185,0	17,5	11	2,6
G.6	Green	Round	Sweetish	130,66	14,5	10,16	2,52
G.7	Greenish-amber	Round	sweet	104,75	14,75	9,87	2,5
G.8	Yellowish-green	round	sourish	151,66	22,7	12,16	2,4
G.9	Green	round	Sweetish	169,83	24,16	10,5	2,46

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G.10	Amber	round	Sweet	225,25	16,66	12,33	2,3
G.11	Green	round	Sweetish-sourish	258,83	16,8	10	2,94
G.12	Green	round	Sweet	364,95	17	12	3,31
G.13	Green	round	Sourish-sweetish	345,94	15,5	12	3,08
G.14	Greenish-amber	round	sourish	144,85	9,25	7	1,97

The high content of sugar in the ripe fruit and low acidity are important components for wine production. Almost all samples, collected in Samegrelo (21.0 to 23.8%) had a high content of dry substance; this indicator was relatively low in the grapes grown in Kobuleti territory - 19.0%, in Keda and Imereti it is average from 20.0 to 21.3%. Among the technological characteristics of the wine grapes, the most important ones are the titrated acidity and the dry substance. These figures are different for the Tsolikouri samples harvested in different climatic conditions. The titrical acidity varies between 0,23 - 0,76%. The grapes, grown in Imereti zone are characterized by a high content of dry substance (acidity - 0,23%, dry substance - 21,3%). The Tsolikouri of Kobuleti is distinguished by high active acidity (pH 3.15), while an active acidity indicator of the Imeretian Tsolikouri juice is relatively low (pH 4.2).

Table 3. Biochemical indicators of white grape varieties harvested in different regions

Samplers	Physical and chemical indicators of grape.		
	Dray compounds, %	Titric acidity, %	Actual acidity, pH
G.1	19	0,62	3,15
G.2	20,0	0,74	3,72
G.3	21,3	0,23	4,20
G.4	23,6	0,45	3,76
G.5	23,2	0,43	3,95
G.6	21,2	0,61	3,63
G.7	23,8	0,51	3,65
G.8	21,9	0,76	3,46
G.9	21,0	0,62	3,66
G.10	21,2	0,34	3,86
G.11	20,3	0,85	3,22
G.12	19,6	0,99	2,98
G.13	19,8	0,90	3,35
G.14	19,4	0,73	3,09

As for the Tsitska, the Klarjuri, the Krakhuna and the Kutatura varieties, the grapes with comparatively dry substance content is the Tsitska, harvested in Imereti and Adjara regions - 20.3 - 21.2%, while this content in the Klarjuri, the Krakhuna and the Kutatura grapes is almost identical - 19.4 - 19.8%. The titrical acidity varies between 0,34 - 0,99% and the active acidity - pH 2,98 - 3,86 (Table 3). Samples taken in Imereti (village Opcha) are distinguished with a high content of common phenols (1748,98 mg / kg), catechins (1147.73 mg / kg) and flavonoids (453,92 mg / kg). The quantity of the samples taken in Keda are close to the above given ones: the common phenols (1578,00 mg / kg), catechins (1006.7 mg / kg) and flavonoids (420,80 mg / kg). Although they belong to different regions territorially, the climatic conditions and the location above sea level are similar. The sample, taken in Kobuleti, are distinguished by relatively high content of flavonoids, what is probably caused by the fact that concentration of flavonoids in grapes increases depending on how much they are exposed to the sun's rays.

Despite the fact that according to technical and biochemical data, the sample of Samegrelo (Vedidkari) did not lag behind other samples, it is distinguished by a lower level of all types of phenolic compounds. It is interesting, that the content of biologically active compounds in the sample of the Tsitska, taken in Imereti (Opcha), is higher compared to the samples of the Tsitska taken in Adjara. All samples maintain the ratio of 3: 2: 1 between common phenols, catechins and quantitative content of flavonoids (Table 4). All samples maintain the ratio 3: 2: 1 between the quantitative content of common phenols, catechins and flavonoids (Table 4). The concentration of catechins in the vineyards grown in moist conditions is higher than in dry and sunny places. It can be observed on the Tsitska's sample, collected in Adjara. Determining antioxidants with the DPPH method, it has been found out that the samples of Imereti region are distinguished with a high antioxidant content. There is some correlation between phenolic compounds and antioxidants (Table 4).

The grape samples of the Tsitska, harvested in Adjara and Imereti regions, are distinguished by high quantity of common phenols - 1410,0 - 1748,98 mg/kg, Catechine 1001,5 - 1147,73 mg/kg, Flavonoids 540,0 - 481,5 mg/kg, respectively, their antioxidant potential is high as well - 56,04 - 65,2 %. During the determination of the antioxidant activity (required to achieve 50% identification) it was necessary to dilute samples - 1:25 (fruit: extragent).

Table 4. Total number of phenol, catechin, flavonoid compounds and the antioxidant activity of the grape fruit of the Tsitska, the Klarjuli, the Krakhuna and the Kutatura , harvested in different regions.

№ Grapes	Total phenols, mg/kg	Catechine, mg/kg	Flavonoids mg/kg	Antioxidant activity dilution factor 1:25 %
G.1	1347,58	964,67	449,50	56,27
G.2	1578, 00	1006,7	420,80	60,20
G.3	1748,98	1147,73	453,92	65,2
G.4	1135,55	828,00	339,70	58,00
G.5	988,70	778,5	317,90	52,50
G.6	1137,0	827,7	340	58,4
G.7	1098,3	799,8	337,2	57,0
G.8	998,9	779,8	325,89	54,0
G.9	976,56	750,8	315,7	45,5
G.10	1582,68	1052,82	540,0	62,49
G.11	1410,0	1001,5	481,5	56,04
G.12	1280,56	918,43	420,0	51,27
G.13	1265,92	954,97	346,9	49,42
G.14	902,91	772,0	196,5	53,35

Conclusion

There have been studied the technical characteristics, the dry substance, the titrated acidity, the active acidity of 14 samples of 5 species of white grape - the Tsolikouri, the Tsitska, the Klarjuli, the Krakhuna and Kutaturi, grown in the three regions of western Georgia: Adjara, Imereti and Samegrelo. Among the technological characteristics of the wine grapes, the most important ones are the titrated acidity and the dry substance. These figures are different for the Tsolikouri samples harvested in different climatic conditions. The titrated acidity varies between 0,23 - 0,76%. The grapes, grown in Imereti zone are characterized by a high content of dry substance (acidity - 0,23%, dry substance - 21,3%). The Tsolikouri of Kobuleti is distinguished by high active acidity (pH 3.15), while an active acidity indicator of the Imeretian Tsolikouri juice is relatively low (pH 4,2). The quantitative content of common phenols, catechins and flavonoids and their antioxidant activity have also been determined. It has been established that the best data is represented by the grape varieties of the Tsolikouri grown in Imereti (Opcha) Total phenols 1748,98 mg/kg, Catechine, 1147,73 mg/kg, Flavonoids 453,92 mg/kg and Adjara (Keda), Total phenols 1578, 00 mg/kg, Catechine, 1006,7 mg/kg, Flavonoids 420,80 mg/kg, what is due to the location of the distribution and climatic conditions.

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