

Nutritional Value and Antioxidant Activity of Caucasian Blackberry (*Rubus Caucasicus* Forche) Growing in the Republic of Azerbaijan

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ABSTRACT

Consumers in developed countries are increasingly understanding the relationship between dietary and functional nutrition and health. Therefore, they prefer more natural and health-promoting products, such as plant extracts and juice concentrates. Blackberries, a member of the *Rubus* genus, are a wild berry common in Azerbaijan that has nutritional, therapeutic, and functional value due to the high bioactivity and bioavailability of its nutritional components. However, information on the phenolic acids, flavonoids, and antioxidant properties of these berries is lacking. The aim of this study was to determine the chemical composition and antioxidant properties of blackberries grown in Azerbaijan. It was established that the mass fraction of soluble dry substances of the Caucasian blackberry is $15.18 \pm 0.24\%$, and the total sugars are $8.72 \pm 0.13\%$. All studied samples of wild blackberries have a low sucrose content ($0.86 \pm 0.08\%$), with glucose ($4.38 \pm 0.1\%$) and fructose ($3.48 \pm 0.20\%$) predominating. The content of the mass fraction of organic acids in the Caucasian blackberry variety is $2.04 \pm 0.12\%$. The pectin content of the studied blackberries is $0.32 \pm 0.05\%$, while the ash content is $0.61 \pm 0.02\%$, and the vitamin C content is 32.75 ± 0.36 mg/100 g. Caucasian blackberries are also rich in anthocyanins (1023.54 ± 4.25 mg/100 g), catechins (102.42 ± 0.48 mg/100 g), and P-active substances (746.36 ± 3.52 mg/100 g). These berries can be used as a dietary, therapeutic and prophylactic product, as well as food supplements and raw materials for various food products.

Keywords: Berries, Blackberries, Nutritional Value, Chemical Composition, Polyphenols, Anthocyanins, Antioxidants.

INTRODUCTION

In recent years, the food industry in the Republic of Azerbaijan has been characterized by a steady trend toward the use of locally grown natural plant materials, including wild fruits and berries, to create food products for preventative and functional purposes. The production of these products is developing dynamically. Although such food products currently represent no more than 5-10% of the market, experts predict that this share will reach 30% in the coming years [1].

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Considering the significant impact of a balanced diet on human health, The World Health Organization predicts that healthy eating and the resulting well-being, through sustainable food production, could prevent 11.0 million premature deaths per year among adults [2].

Food is known to be subject to degradation due to the biochemical and microbiological processes occurring within it. Oxidation produces numerous degradation molecules, which subsequently alter the organoleptic and nutritional properties of foods. Microbial contamination is the primary cause of food spoilage, and oxidation processes are a second significant cause of changes in food [3,4]. Therefore, adding plant-based ingredients with antioxidant properties to foods can positively influence oxidation processes. Currently, the food industry widely uses synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ) due to their ability to inhibit oxidation processes without altering the flavor of food. It is well known that visual perception, and more specifically color, largely determines consumer purchasing preferences [4,5].

During storage, food products undergo color changes, which also reduces the shelf life of the products themselves. This naturally leads to negative consumer attitudes, resulting in decreased purchasing power and, consequently, significant economic losses for food producers. To preserve the original color of food products, the food industry widely uses food colorings. It should be noted that a number of studies have established a link between the consumption of synthetic additives and health problems due to their potential toxicity and carcinogenic effects [6,7].

Consumers in developed countries are increasingly understanding the relationship between dietary and functional nutrition and health, and are therefore opting for more natural and healthful products. In recent decades, the food industry has been constantly searching for new sustainable natural sources of raw materials, such as plant extracts and juice concentrates, which offer alternatives to synthetic additives [8,9].

Among all the natural water-soluble pigments, anthocyanins, found in most berries, are promising food additives that possess both important antioxidant activity and intense color. These compounds (anthocyanins) are a subgroup of flavonoids that impart blue, purple, and red colors to many fruits [10,11].

A recent study reported on the anti-obesity potential of natural bioactive compounds, and in particular, phenolic compounds were found to play a crucial role in combating obesity by regulating appetite [12].

Berries are a group of various red, blue, or purple, small, perishable fruits that are highly valued for their intense color, delicate texture, and unique flavor. These fruits are widely consumed fresh or processed, such as juices, juice concentrates, beverages, jams, preserves, sauces, jellies, etc. [13,14].

Furthermore, a number of researchers recognize the protective effect that berry consumption may have against chronic diseases such as cancer and cardiovascular disease, among others. The biological effects of berries can be attributed to their rich source of bioactive compounds, such as phenolic acids, tannins, stilbenes, flavonoids, and, most importantly, anthocyanins [15,16].

Thus, numerous studies have found a link between anthocyanins and a reduced incidence of cardiovascular disease, diabetes, and cancer. Similarly, several studies have shown improved clinical and biomedical outcomes after consuming anthocyanin-rich fruits [17].

The forests of Azerbaijan are home to numerous species of wild fruit, medicinal, essential oil, spice, ornamental, and other plants used as food, medicine, and as raw materials for the vitamin and pharmaceutical industries. The collection of wild fruits, berries, and nuts plays a significant role in the population's supplementary food supply [18].

One of the wild berries common in Azerbaijan, which has nutritional, medicinal, preventative, and functional value, is the blackberry of the genus *Rubus*, a perennial shrub or subshrub. Blackberries consist of juicy drupes, fused at the base, rarely separated. The seeds are semicircular, round-triangular or ovoid, light yellow, purple or brown, with two narrow ribs. The genus *Rubus* belongs to the rose family (Rosaceae) and contains about 66 species, of which 15 species grow wild in Azerbaijan [19]. The most common species in Azerbaijan are the blood-red blackberry (*Rubus sanguineus* Friv), the blue blackberry (*Rubus caesius*) and the Caucasian blackberry (*Rubus caucasicus* forche), which are used for food and medicinal purposes in fresh and processed form. Jam, compotes, syrup, kissel, liqueurs, marmalade, pastilles, tinctures, cordials, soft drinks, etc. are made from them [19]. Blackberries bear fruit after most other berry crops, significantly extending the shelf life in the growing regions.

Blackberries possess nutritional, biological, and economic value due to the high bioactivity and bioavailability of their nutrients. Therefore, we are conducting comprehensive research into the potential use of blackberry fruits and leaves to create new or enrich existing food products.

Our previous research has established that the key chemical components of blackberries depend on the type of raw material and the soil, climate, and ecological-geographical conditions of the growing region. In terms of vitamin C content, the blood-red blackberry (32.1 ± 1.20 mg/100 g) exceeds the glaucous and Caucasian blackberries (23.76 ± 0.58 mg/100 g and 29.35 ± 0.47 mg/100 g, respectively). The polyphenol complex of all blackberry varieties includes catechins and anthocyanins, with the latter clearly predominating. The blackberry varieties we studied, in terms of anthocyanin content (from 873.60 ± 6.36 to 1283.3 ± 12.5 mg/100 g), can easily compete with such crops as black currant, pomegranate, hawthorn, barberry, etc. [19].

These compounds are responsible for their intense color and are the main factor in their antioxidant potential, which is one of the highest among known berries worldwide. Many studies link the anthocyanin content of blackberries with their wide range of biological activities, such as antidiabetic and antimicrobial [21,22], anti-inflammatory [23] effects, prevention of Alzheimer's disease, cancer [21], neurogenerative and cardiovascular diseases [24,25], inhibition of adipogenesis and inflammation, prevention of oxidative stress and prevention of low-density lipoprotein oxidation [26,27].

Although there are several studies in the scientific literature reporting on the biological effects and anthocyanin content of berries in general and blackberries in particular [13,27], there is no information on the phenolic acids and flavonoids present in blackberries. Therefore, the aim of this study was to determine the chemical composition and antioxidant properties of Caucasian blackberries (*Rubus caucasicus* forche), which grow in various regions of the Republic of Azerbaijan.

MATERIALS AND METHODS

Plant Material. Caucasian blackberry (*Rubus caucasicus* forche) berries were collected in the Lankaran-Astara region (Azerbaijan). The berries were crushed in a fruit crusher and then sifted to remove seeds. A second fine grinding was then performed in a mortar. Sample Preparation. To obtain the extract, 3 g of ground Caucasian blackberry (*Rubus caucasicus* forche) berries were added to 30 ml of acidified

(0.1% HCl) methanol/water (80:20) and then homogenized using an Ultra-Turrax homogenizer at 18,000 rpm for 2 min. The extract was centrifuged at $3,000 \times g$ for 7 min at 4°C, and the supernatant was collected. The precipitate was mixed with 30 ml of acetone/water (70:30) and homogenized using an Ultra-Turrax at 18,000 rpm for 2 min.

Finally, the sample was centrifuged at $3,000 \times g$ for 7 min at 4°C. The supernatants of the two phases were mixed in a round-bottomed flask and evaporated to dryness using an R-205 rotary evaporator (Büchi, Flawil, Switzerland) under reduced pressure (<100 mbar) at 40°C.

Standard and specialized research methods were used in this study:

- Determination of total solids content by thermogravimetric method by drying the test samples to constant weight (GOST 33977-2016);
- Determination of soluble solids by the refractometric method, based on the determination of the refractive index of the test solution using a refractometer and the mass fraction of soluble solids (GOST ISO 2173-2013);
- Determination of sugars using the Bertrand method (GOST 8756.13-87);
- Determination of titratable acids (total acidity) using the titration method (GOST 32114-2013); – determination of pectin substances using calcium pectate (GOST 32223-2013);
- The mass fraction of ash was determined using the generally accepted gravimetric method, after mineralization of the product sample to constant weight in a muffle furnace at 500 °C (GOST 27494-2016);
- Determination of ascorbic acid (vitamin C) using the iodometric method (GOST 34151-2017);
- Determination of P-active substances (anthocyanins, catechins) by the colorimetric method modified by L.I. Vigorova using a KFK-2 photoelectrocolorimeter.

For antioxidant activity, 10 ml of methanol was added to the dried extract, and the mixture was shaken well in a vortex mixer for 2 minutes, then passed through a 0.45 µm Millipore filter and stored at -20°C.

Antioxidant activity

Free radical scavenging activity of the samples was measured according to the methodology described in [27] using the stable radical DPPH. Results were expressed as g Trolox equivalent kg-1 of the DW sample.

Iron-Reducing Antioxidant Capacity

The iron-reducing antioxidant capacity (FRAP) of Caucasian blackberry (*Rubus caucasicus porshe*) extracts was determined using the potassium ferricyanide–ferric chloride method according to the procedure described in [27]. Results were expressed as g Trolox equivalent kg⁻¹ of the DW sample.

Iron (Fe²⁺) chelating activity (FIC) was measured by inhibiting the formation of the Fe²⁺-ferrozine complex after treatment of the test material with Fe²⁺ using the Carter method described in [27]. Results were expressed as g EDTA equivalent kg⁻¹ of the DW sample.

ABTS cation-radical scavenging activity (ABTS•+) was determined as described in [28]. Absorbance values were measured spectrophotometer at 734 nm. Results were calculated using a Trolox calibration curve and expressed as g Trolox equivalent kg⁻¹ of DW sample.

Statistical Analysis

Statistical analysis and comparison of means were performed using the SPSS 19.0 statistical package (SPSS Inc., Chicago, IL, USA). All experiments were performed in triplicate, and data are presented as mean ± standard deviation. Differences in means among polyphenol profiles were analyzed using one-way analysis of variance (ANOVA). Tukey's test was used to compare means; differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Chemical Composition and Nutritional Value of Caucasian Blackberries (*Rubus caucasicus porshe*) The results of studies on the chemical composition of wild Caucasian blackberries grown in the Lankaran-Astara region of the Republic of Azerbaijan for 2022–2024 are presented in Table 1.

Table 1. Average Chemical Composition of Caucasian Blackberries for 2022–2024

Chemical Composition Indicators, Units of Measurement	Chemical Composition
Soluble Solids, %	15.18±0.24
Total Sugars, %, including:	8.72±0.13
Reducing Sugars, of which:	7.86±0.14
- Glucose	4.38±0.11
- Fructose	3.48±0.20
Sucrose	0.86±0.08
Organic Acids, %	2.04±0.12
Sugar Acid Index	4.27±0.10
Pectin Substances, %	0.32±0.05
Ash, %	0.61±0.02
Vitamin C, mg/100g	32.75±0.36
Anthocyanins, mg/100g	1023.54 ± 4,25
Catechins, mg/100g	102,42 ± 0,48
P-Active Substances, mg/100g	746,36 ± 3,52

As shown in Table 1, the mass fraction of soluble solids (15.18±0.24%) and total sugars (8.72±0.13%) of the Caucasian blackberry variety, grown in the Lankaran-Astara region, exceed those of blackberries grown in the Quba-Khachmaz region (12.96±0.31% and 7.74±0.12%), respectively [19]. All studied wild blackberry samples were low in sucrose (0.86±0.08%), with glucose (4.38±0.1%) and fructose (3.48±0.20%) predominating.

In our opinion, the superiority of several chemical composition parameters of Caucasian blackberries grown in the Lankaran-Astara region over those of blackberries grown in the Guba-Khachmaz region depends on the soil, climate, and eco-geographic conditions of these regions [19].

The Lankaran-Astara (southeast) and Guba-Khachmaz (northeast) regions are two unique natural and economic zones of Azerbaijan, representing a kind of “poles” of the country's diversity.

The Lankaran-Astara region has a humid subtropical climate. It receives record amounts of precipitation for the country (up to 1,400–1,600 mm per year), with the maximum falling in autumn and winter. Summers are moderately hot but very humid. The soils are yellow soils (Zheltozems), unique to Azerbaijan, which form under conditions of excessive moisture and high temperatures. The climate of the Guba-Khachmaz region is transitional, ranging from warm temperate to semi-desert along the coast. Precipitation is significantly lower (300–600 mm) and more evenly distributed. Summers are dry and warm. Soils in the region are primarily alluvial meadow, chestnut, and mountain-forest brown soils [29,30].

The content of organic acids in the Caucasian blackberry variety was $2.04 \pm 0.12\%$. The ratio of sugars and acids (sugar-acid index) determines the harmonious taste of fruits and berries. The optimal sugar-acid index is 7.0 [11]. The fruits of the wild blackberry species *Kavkazskaya* have a relatively harmonious taste due to their high sugar content; the sugar-acid index for this species is 4.27 ± 0.10 . Therefore, the *Kavkazskaya* blackberry has a sweet and sour taste. The pectin content in the studied blackberries is $0.32 \pm 0.05\%$, and the mass fraction of ash is $0.61 \pm 0.02\%$.

Regarding the vitamin C content, the *Kavkazskaya* blackberry (32.75 ± 0.36 mg/100 g) is superior to the *glaucus* blackberry (23.76 ± 0.58 mg/100 g) [19].

In terms of anthocyanin (1023.54 ± 4.25 mg/100 g), catechin (102.42 ± 0.48 mg/100 g), and P-active substance (746.36 ± 3.52 mg/100 g), the Caucasian blackberry is also a rich berry. As can be seen, the content of certain chemical components in the studied Caucasian blackberry does not contradict the data of other authors cited in literary sources [19,25-27].

Antioxidant Properties

Due to the complexity of antioxidant compounds and their mechanisms of action, it is common to use various methods to evaluate the in vitro antioxidant properties of food products [27]. This covers all aspects of the antioxidant efficacy of Caucasian blackberry (*Rubus caucasicus porshe*) berries.

One method will provide basic information on antioxidant properties, but a combination of methods describes the antioxidant properties of a sample in more detail. Four different methodologies were used in this study to determine the antioxidant properties of Caucasian blackberry (*Rubus caucasicus porshe*) berries. Table 2 shows the values obtained for the antioxidant activity of Caucasian blackberry (*Rubus caucasicus porshe*) berries using DPPH, ABTS, FRAP, and FIC assays.

Table 2. Antioxidant activity of Caucasian blackberry (*Rubus caucasicus porshe*) berry extracts measured using four different methods: DPPH, ABTS, FRAP, and FIC

Method	Results
DPPH (г TE кг ⁻¹)	$32,02 \pm 0,25$
ABTS (г TE кг ⁻¹)	$20,98 \pm 0,16$
FRAP (г TE кг ⁻¹)	$27,54 \pm 0,31$
FIC (г EDTAE кг ⁻¹)	$0,22 \pm 0,01$

TE, Trolox Equivalent; EDTAE, Ethylenediaminetetraacetic Acid Equivalent.

DPPH and ABTS assays are the most widely used antioxidant methods. The ABTS method is typically used to assess the antioxidant activity of hydrophilic compounds; reactions with ABTS•+ involve electron transfer. The DPPH method is typically used for aqueous/organic extracts with hydrophilic and lipophilic compounds; reactions with DPPH• involve H atom transfer [31].

In the DPPH assay, the results obtained in this study (Table 2) confirm those reported in [32], where the authors reported IC50 values for the DPPH assay of 0.0016 g L⁻¹ and 0.0012 g, respectively. In our study, Caucasian blackberry (*Rubus caucasicus porshe*) berry extract had an antioxidant activity

measured by the ABTS assay (Table 2) of 20.98 ± 0.16 g TE kg⁻¹ DW.

Caucasian blackberry (*Rubus caucasicus porshe*) berries showed a FIC value of 0.22 g EDTA kg⁻¹ of the DW sample. In the FRAP assay, the antioxidant capacity of the analyzed extracts is determined by the ability of the bioactive compounds present in these extracts to reduce Fe³⁺ to Fe²⁺. Caucasian blackberry (*Rubus caucasicus porshe*) berries analyzed in our work (Table 2) showed a FRAP value of 27.54 g TE kg⁻¹ DW. These results were consistent with those obtained in [27], the authors who analyzed the antioxidant properties of blackberries extracted with different solvents.

These authors reported values ranging between 4.81 and 12.97 mol L⁻¹ catechin equivalents kg⁻¹ of extract in the FRAP assay.

There are several studies in the scientific literature that have reported a correlation between polyphenolic compounds and antioxidant activity. These studies have shown that these compounds significantly contribute to antioxidant properties [33].

However, the mechanism of action mediated by the antioxidant activity of these compounds is still not fully understood. It has been reported that these compounds are known for their properties to inhibit lipid oxidation by acting as chain-breaking peroxy radical scavengers and absorbing free radicals or reactive oxygen species such as hydroxyl radicals, peroxy nitrite, and hypochlorous acid. Furthermore, polyphenolic compounds have shown antioxidant activity, mainly due to their redox properties, through various possible mechanisms such as hydrogen donation, transition metal chelating activity, and/or singlet oxygen quenching ability [27].

According to the study by S. R. Khasanova et al., the content of flavonols in the fruits of *Crataegus* soft-skinned is 1.5 times higher and anthocyanins are 4 times higher than in the fruits of *Crataegus sanguinea*. *Crataegus* soft-skinned can be considered a potential raw material source and this species is a promising type of medicinal plant material and a source of anthocyanins [34], and the work [35] showed that the highest level of antioxidants (total content of water-soluble antioxidants - 8.93-10.34 mg/g, anthocyanins - 0.97-1.34 mg/g, polyphenols - 10.23-16.37 mg/g, antiradical activity - 10.18-15.72 mg/g) was noted in the fruits of all the studied elderberry species (*Sambucus nigra* L., *Sambucus racemosa* L., *Sambucus ebulus* L.). Moreover, the fruits of black elderberry and herbaceous elderberry were characterized by higher levels of polyphenols and antiradical activity compared to the fruits of red elderberry.

In the work [36], it was shown that black chokeberry fruits are promising sources of biologically active substances. The main active ingredients of the studied aronia extract are phenolic compounds: phenolic acids, flavonoids and catechins. It is also characterized by high antioxidant activity. Analysis of individual phenolic compounds allowed us to identify compounds of the phenolic acid group (gallic and caffeic acids), proanthocyanidins (catechin and epicatechin), as well as flavonoids – rutin and quercetin. Among the compounds detected, quercetin is predominant (about 170 µg/g); catechin, gallic acid and epicatechin are present in

approximately equal quantities (about 50, 40 and 30 µg/g, respectively) [37].

In [38], the authors established a characteristic high content of cyanidin and peonidin glycosides, especially their galactosides and arabinosides, in cranberries. Lingonberry anthocyanins are represented almost exclusively by cyanidins, with a predominant content of cyanidin galactoside. Blueberries contain fifteen anthocyanins in high concentrations, and, unlike cranberries, the relative minimum concentrations are for peonidin galactosides and arabinosides. The data presented in the cited work show that blueberry extract is rich in anthocyanins, lingonberry extract is rich in procyanidins, and the main phenolic compound in cranberry extract is chlorogenic acid.

It is clear that the high anthocyanin content is associated with antioxidant properties. The antioxidant activity of these compounds has been widely demonstrated. However, this activity largely depends on the chemical structure of the anthocyanins, and not all anthocyanins exhibit similar radical scavenging activity. Generally, the antioxidant activity of anthocyanins is related to the number of free hydroxyls around the pyrone ring: the higher the number of hydroxyls, the higher the antioxidant activity. Anthocyanins, with their 3',4'-dihydroxy groups, can rapidly chelate metal ions, forming stable anthocyanin-metal complexes [39].

However, it should be kept in mind that the antioxidant properties of polyphenolic compounds present in fruits in general and blackberries in particular are difficult to attribute to a specific compound or group of compounds due to their complexity and variability. Therefore, antioxidant activity may be due to the major compounds present in blackberries or to a synergistic effect between the major compounds and minor ones. Another aspect to consider regarding the antioxidant activity of polyphenolic compounds is the extraction process and the extraction methods used.

CONCLUSIONS

Our research shows that Caucasian blackberries (*Rubus caucasicus* porshe) have great potential for use in the food industry as a potential food ingredient for the development of functional foods or as a biopreservative due to:

- High content of polyphenolic compounds, primarily anthocyanins;
- Promising antioxidant and antibacterial properties.

The biological and nutritional value of Caucasian blackberries (*Rubus caucasicus* porshe), grown in various regions of the

Republic of Azerbaijan, allows them to be considered as an additional source of physiologically active substances and a raw material for the production of various non-alcoholic beverages, including energy drinks, and other food products. These results also suggest that foods and preparations made from Caucasian blackberries may be valuable functional ingredients.

Nevertheless, in-depth research is needed to analyze the changes induced by human digestion, which may significantly impact the bioactivity and bioavailability of the biologically active compounds present in Caucasian blackberries (*Rubus caucasicus porshe*). We also believe it would be useful to study the macro- and microelement composition, including the content of toxic metals and anti-nutritional compounds present in these berries.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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The study was performed without financial support.

DATA AVAILABILITY

Manuscript has no associated data.

USE OF ARTIFICIAL INTELLIGENCE

The authors confirm that they did not use artificial intelligence technologies in creating the submitted paper.

AUTHORS' CONTRIBUTIONS

Mikail Maharramov: Theoretical justification; conceptualization; development of research methodology; research management; editing the manuscript.

Sevinj Maharramova: Conceptualization; development of research methodology control, planning and conducting the study; visualization; preparation of the draft article; translation of the article.

Mukhendis Jahangirov: Procurement of materials and raw materials; participation in the study; visualization; preparation of the draft article.

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