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Physicochemical Characterization and Antioxidant Activity of Jara Honey Produced in Western Georgia

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Abstract: The purpose of this research article was to study the physicochemical characteristics of semi-wild Jhar honey grown in Western Georgia. Jara honey is produced in the alpine and sub-alpine forest zone of high mountain Adjara, which is distinguished by the variety of honey plants [1]. The physicochemical characteristics were examined concerning the Alimemtarius Code and EU regulations: moisture content, total carbohydrate, free acidity, pH , electrical conductivity, microelements (Li, Na, K, Mg, Ca), color, total phenols, total phenolic acids, total flavonoids, proline, diastase activity, protein and microscopic study of pollens. Using the UPLC-MSB method, grayanotoxin-III was identified in the semi-wild Jara honey samples. The findings demonstrated that honey has significant concentrations of phenols, phenolic acids, and flavonoids. A directly proportional relationship was established between the quantitative content of phenolic compounds and the antioxidant activity of honey. This article is the first study of the characteristics of Jara honey produced in Western Georgia.

Keywords: honey; antioxidant activity; physicochemical; melissopalynology; grayanotoxin

1. Introduction

Honey is the most recognized and famous natural food produced by bees (Apis mellifera) from nectar and honeydew [2]. The predominance of one of these sources leads classification of honey as either blossom honey or honeydew honey [2]. Its historical, cultural, and economic significance makes it the major beekeeping product [3]. Honey is an aromatic, sweet natural product with high nutritional value that has been used by humans since time immemorial. [4].

Due to its taste and nutritional value, honey has been commonly consumed as a food for thousands of years [5]. It is used as a component in the food industry [6] and also serves as a food preservative [5,7]. Moreover, honey is also used in medicine [9] and in the production of cosmetics [10] due to the numerous positive properties that can be attributed to the various bioactive molecules it contains [3].

Honey has a very complex composition [2]. It contains more than 200 compounds [6]. It is mainly composed of sugars (70–80%) [11], namely 38% fructose, 31% glucose, 10% other sugar types [12], and water (10–20%) [12]. The uniqueness of this food product resides in the rich spectrum of compounds that do not make up the bulk of the mass. For instance, honey also contains proteins, organic acids (such as acetic acid and gluconic acid, etc.) [6], lipids, carotenoids, minerals [13], and enzymes [11,14]. More importantly, bioactive components that are crucial in determining specific and individual characteristics of honey, such as flavonoids, phenolic acids, and vitamins (ascorbic acid, niacin, etc.), are also present in minor quantities [13,15–17]. Honey can originate from single or multiple plant species [18]. Accordingly, many different types of honey are available on the global

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market. Following its origin, honey is divided into blossom, honeydew, monofloral, and multifloral varieties based on the source of the honey [18–21].

Although Georgia is a small country, it is a very important region for the development of beekeeping, where it is possible to produce high-quality honey of various origins [22]. In this regard, the uniqueness of Georgia is determined by its geographical location, climatic conditions, and rich and varied vegetation [23,24]. Georgia mainly produces five types of honey in large quantities. These are acacia honey, blossom honey, alpine honey, linden honey, and chestnut honey [25]. In Georgia, we also have traditional Georgian semi-wild jara beekeeping. Jara honey is produced in the alpine and sub-alpine forest zones of the high mountains of Adjara, which are distinguished by the variety of honey plants [23]. A Jara is a hollowed log cut in two. It is mainly carved from the Linden tree (Tilia begoniifolia Steven), which is chosen for its lightweight and lack of specific smell to avoid disturbing the bees [17,23,26]. Jara is positioned on trees in the forest, and beekeepers no longer interfere in the development of the bee colony, allowing for the completely natural and unmanaged production of Jara honey. There is also no resource management for honey bees. The use of pesticides and antibiotics [27]. The obtained honey is matured in natural pine. Jara honey is collected once a year, at the beginning of autumn [23,24].

It is also worth noting that the flowering of honey plants in the highlands of Adjara is preceded by the flowering of Rhododendron, which contains a toxin, in cold and late spring conditions. Accordingly, there is a high probability that bees collect Rhododendron nectar and the toxin gets into the Jara honey, eventually reaching the human body [20,28–30].

Our research aimed to study the physical and chemical characteristics of jara honey, as a virtually "unknown" product. Content of Biologically Active Compounds and Antioxidant Activity. We also determined the content of pollens, and based on the obtained results, we created a natural and distinctive product - Jara honey.

2. Materials and Methods

2.1. Materials

2.1.1. Samples – Honey samples were provided by beekeepers and collected from three different municipalities - Adjara, in particular, Keda - Jara Honey 1-9), Shuaxevi - Jara Honey 10-13), Khulo - Jara Honey 14-18 (Figure 1. Jara Map).

Honey samples were provided by private beekeepers. The harvest took place in the fall of 2018 (in September) (Table 1).

Table 1. A sample of Jara honey taken for analysis.

Samples	Samplers code	Sampling Place (municipality)	Height above mean sea level, m	Coordinates
Jara Honey 1	JH - 1	Keda - Gobroneti	607	41° 39′ 8.5″ N, 42° 2′ 18.6″ E
Jara Honey 2	JH - 2	Keda - Zesopeli	536	41° 37′ 14.2″ N, 41° 57′ 44.6″ E
Jara Honey 3	JH - 3	Keda - Tskhmorisi	523	41° 38′ 22″ N, 42° 2′ 50″ E
Jara Honey 4	JH - 4	Keda - Zendidi	336	41° 36′ 10″ N, 41° 55′ 55″ E
Jara Honey 5	JH - 5	Keda - Zundaga	400	41° 34′ 42.4″ N, 41° 49′ 32.9″ E
Jara Honey 6	JH - 6	Keda – Namonastrevi	820	41° 34′ 13″ N, 42° 3′ 10″ E
Jara Honey 7	JH - 7	Keda - Silibauri	490	41° 34′ 46.3″ N, 42° 0′ 47.1″ E
Jara Honey 8	JH - 8	Keda - Medzibna	640	41° 33′ 51.9″ N, 41° 58′ 29″ E
Jara Honey 9	JH - 9	Keda – Merisi	700	41° 34′ 54″ N, 42° 0′ 18″ E
Jara Honey 10	JH – 10	Shuakhevi - Instkirveli	1385	41° 42′ 57.4″ N, 42° 14′ 9.3″ E
Jara Honey 11	JH - 11	Shuakhevi - Khabelashvilebi	766	41° 42′ 28″ N, 42° 10′ 45.7″ E
Jara Honey 12	JH - 12	Shuakhevi - Kidzinidzeebi	1040	41° 35′ 6.1″ N, 42° 11′ 37.4″ E
Jara Honey 13	JH - 13	Shuakhevi - Karapeti	1334	41° 33′ 8.1″ N, 42° 15′ 24.5″ E

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Jara Honey 14	JH - 14	Khulo - Skhalta	800	41° 35′ 4.84″ N, 42° 19′ 46.21″ E
Jara Honey 15	JH - 15	Khulo – Kvatia	1090	41° 34′ 40″ N, 42° 24′ 17″ E
Jara Honey 16	JH - 16	Khulo - Pushrukauli	1180	41° 33′ 41″ N, 42° 27′ 0″ E
Jara Honey 17	JH - 17	Khulo - Rakvta	1350	41° 34′ 11″ N, 42° 28′ 58″ E
Jara Honey 18	JH - 18	Khulo - Bardnali	570	42° 36′ 22″ N, 42° 42′ 50″ E

Figure 1. Location of sample origins on the regional map (https://www.jarahoney.com/).

We stored honey samples in hermetic containers (4-5 ° C). In the case of impurities, we passed the honey through a grid with a diameter of 0.5 mm.

2.1.2. Chemicals and Instruments

Measurements of absorbance were performed using a Mettler Toledo UV-5 model UV-VIS spectrophotometer. The chemicals and reagents used were of analytical grade and were sourced from Sigma and Merck Chemical Company. A Waters HPLC (Water 2414 Refractive Index and Waters 2489 UV/Visible detector) and Waters Acquity UPLC/H Class (PDA Detector) were used for identifying compounds. A UKA Technic professional light microscope was used for melissopalynological analysis.

2.2. Methods

The research examined the quantitative and qualitative content of carbohydrates, total phenols, flavonoids, phenolic acids, and cations using modern instrumental research methods such as HPLC, UPLC-MS Acquity H Class, pH/Ion meter S220 and S230 Conductivity, Mettler Toledo UV5, UV/Visible Scanning Spectrophotometer and the characteristics provided by the Regulations on Honey Codex Alimentarius (Codex Standard for Honey, 2001): determination of moisture, electrical conductivity, dry content, pH, free acidity, diastase activity, proline, pollen content, and antioxidant activity.

The honey samples were analyzed for physicochemical characteristics. According to the EU Regulation (Council EU, 2001) and Codex Alimentarius (2001):

The refractometric method was used to determine the moisture, or conversely the soluble solids in honey [31,32].

pH/Ion and free acidity meter S220 and S230 Conductivity (Seven Compact, Mettler Toledo) [33].

Determination of electrical conductivity - Electrical conductivity was using a conductometer Mettler Toledo [34,35].

Determination of diastase activity –Standard Schade method and proline[36,37].

2.2.1. Color intensity - was determined using the Pfund scale (Lovibond 2000 & 1000 comparator), using the following equation[38,39].

$$Pfund = - 38.70 + 371.39\ Abs$$

2.2.2. Bioactive compounds and antioxidant activity

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2.2.2.1. Total content of phenolic compounds (TCPC), Determination to the Folin-Ciocalteu method (in terms of gallic acid); [40,41].

2.2.2.2. Total content of flavonoids (TCF), by the spectral method (AlCl3 reagent in terms of rutin) [42,43].

2.2.2.3. Total content of phenolic acids (TCPA), Phenolic acids were determined according to the method of Mazza, Fukumoto, Delaquis, Girard, and Ewert with some modifications[44,45].

2.2.3. Antioxidant activity assay with DPPH- 2,2-Diphenyl-1-picrylhydrazyl - DPPH 0.1 N 50% inhibition mg, with a sample [32,40].

2.2.4. Carbohydrates - the sugar content is determined by Waters HPLC (High Pressure Liquid Chromatography) with RI-detection [34,35].

2.2.5. Cations - the minerals are determined by the Waters 1515 isocratic HPLC Pump (High-Pressure Liquid Chromatography) with Waters 432 Conductivity detection. Column - IC-Pak C M/D; Mobile phase: 0.1 mM EDTA/3 mM HNO3 [35,46].

2.2.6. Protein – Based on the Kjeldahl method, determining the nitrogen and the conversion factor yields the result of the crude protein.[18,46].

2.2.7. Pollen Analysis - The pollen analysis was made according to the method of

Louveaux et al. slides were prepared from each sample and photographed under a light microscope. The identification of the pollen grains is based on their size and shape [8,34,35].

2.2.8. - Grayanotoxin-III Analysis - Liquid chromatography-tandem mass spectrometry (Waters, UPLC Acquity, QDa Detector) was used for the identification of grayanotoxin-III. The analytical column was an Acquity UPLC BEN C18. GTX-III was eluted using a mobile phase consisting of a 50:50 water/methanol solution containing 1% acetic acid at a flow rate of 0.3 mL/min over 8 minutes [30,32].

3. Results

3.1. Moisture content (%) - One of honey's most crucial properties is its water content because it affects the substance's viscosity, specific gravity, maturity, crystallization, flavor, preservation, shelf life, and palatability [47]. It is a crucial element in determining the quality of honey [48]. It depends on several variables, including bee species, floral supply, honey harvesting season, honey maturation level (total dehydration), and meteorological factors [18,29].

Excessive water content in honey can lead to the growth of microbiological reactions and the development of the fermentation process, which negatively affects the qualitative characteristics of honey [4]. The moisture level in this inquiry ranged from 14.3% to 18.6%. (Figure 2), Specifically, the average rate in the Keda samples is 17.78%, in the samples of Shuakhevi it is 16.47%, and in the samples of Khulo, it is 15.9%. The water content in the honey samples taken for analysis is less than 20%, which is within the norm stipulated by the standard (International Honey Commission). This also indicates the maturity of the analyzed honey samples.

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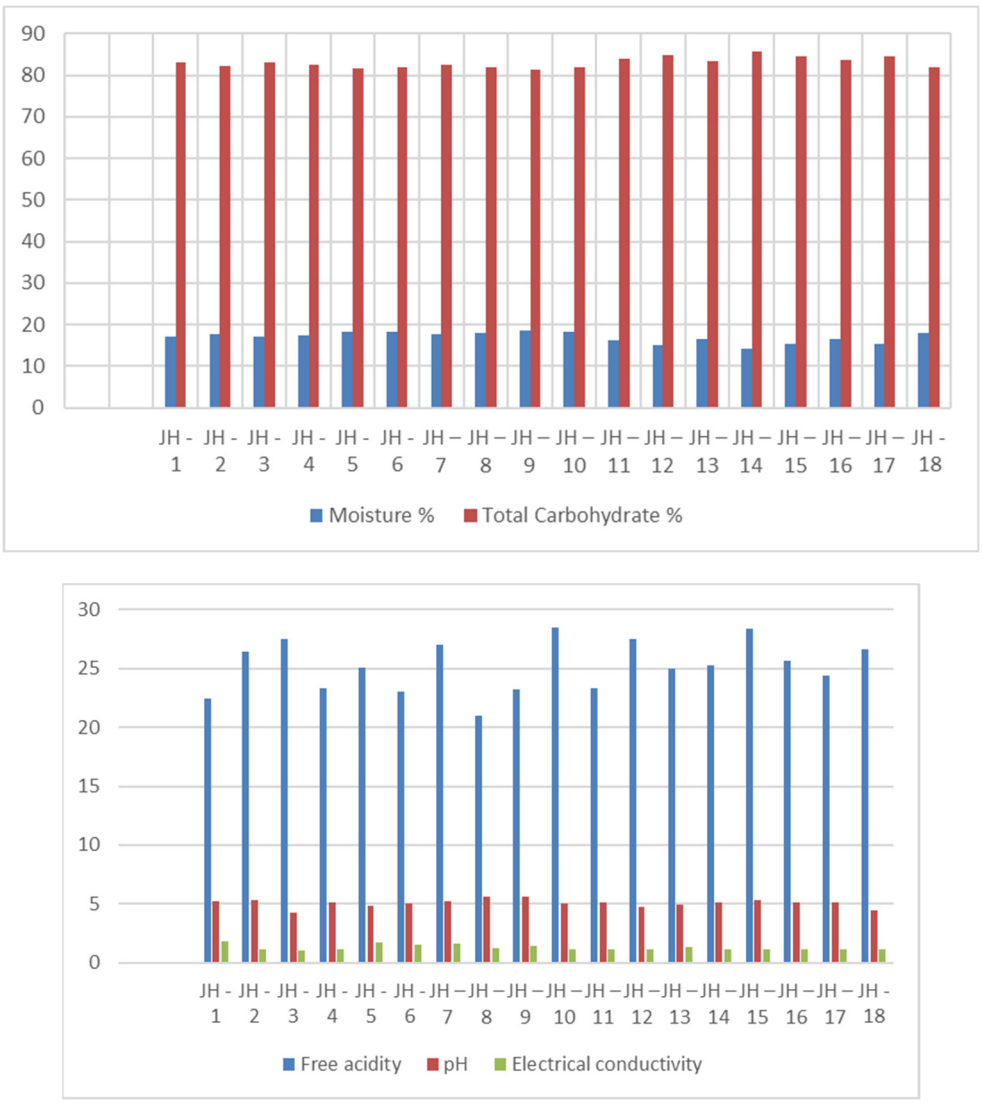

Figure 2. Jara honey characteristics of water, dry substances, free acids, pH, and Electrical conductivity in western Georgia.

3.2. Sugars **-** Sugar is a crucial component of honey [49], primarily existing in the form of monosaccharides and disaccharides. The total content of monosaccharides (fructose and glucose) is about 75%, and 10-15% consists of disaccharides [50,51].

The qualitative and quantitative content of sugars in honey is primarily determined by its botanical and geographical origin. However, the amount of sugars can also be slightly influenced by factors such as weather, processing, and storage conditions (13, 52). One of the important characteristics when evaluating the quality of honey is the ratio of fructose and glucose, as well as glucose and water. When the fructose-to-glucose ratio is less than 1.0, crystallization occurs more quickly, and it decreases when the ratio is greater than 1.0 [53,54,56].

When confirming the naturalness of honey, the concentration of sucrose is also important because a higher-than-permissible content serves as an indicator of adulteration [57].

The high-pressure liquid chromatography method was used to identify and quantify the individual components of sugars in Jara honey. Based on standard calibration, the quantitative results for each honey sample are shown in Figure 2. Among the compounds identified (fructose, glucose, sucrose, and maltose), the dominant compounds were fructose and glucose. Based on standard calibration, the quantitative results for each honey sample are shown in Figure 3.

Comparing the obtained results, the concentration of fructose was 44.5% - 56.4% and that of glucose was 27.18% - 37.8% (Figure 4). In particular, the fructose content of Keda's honey samples ranges from 45.175% to 52.58%, with an average of 48.79%. Glucose concentrations ranged from 37.8%

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to 34.97%, with an average of 32.67% glucose. In the samples from Shuakhevi and Khulo, the fructose content is higher compared to the honey from Keda, at about 51% (average value), the average glucose concentration in Shuakhevi honey is 31.32%, while it is 32.21% in Khulo samples.

In the samples taken for analysis, considering the average value, fructose is the dominant sugar (Mass share of fructose in total sugars (average of 23 indicators): in Keda samples - 52.58%, in Shuakhevi samples - 60.37%, in Khulo 2 samples - 61.42%) and it is followed by glucose: in Keda samples - 37.06%, in 23 samples of Shuakhevi - 37.64%, in samples of Khulo - 38.33% (23 of the mass share of total sugars average rate). (Figure 4).

Sucrose identified in identified in samples: JH-2, JH-3, JH-10, JH-13, JH-16, and JH-17, and the contents of the honey samples ranged from 0.065% to 0.403%. Maltose was identified only in 2 samples: JH-12 and JH-15. The maltose content was 2.006% and 1.8%. The sucrose and maltose contents are significantly lower than the fructose content as well as the glucose content (Figure 4) (Figure 3). According to international standards, the sucrose content should not be higher than 5% [58,63].

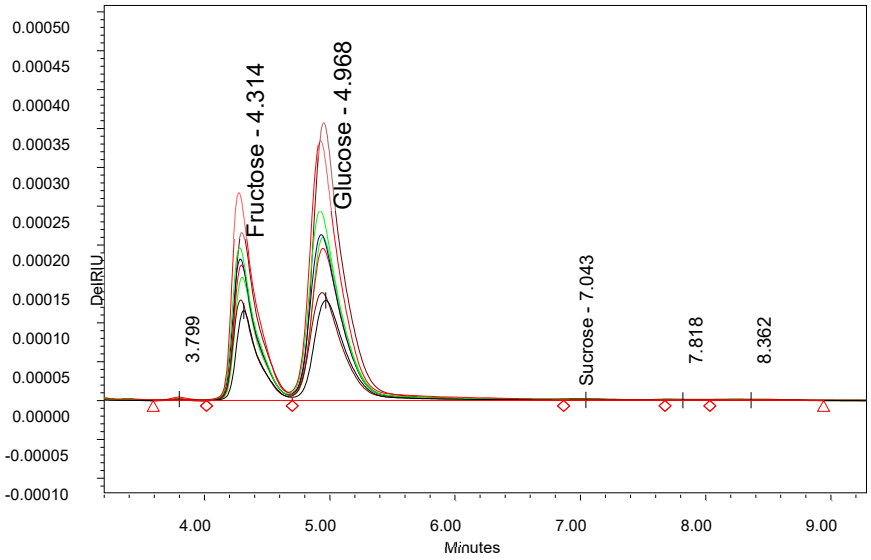

Figure 3. Chromatograms of a sugar Jara honey samples.

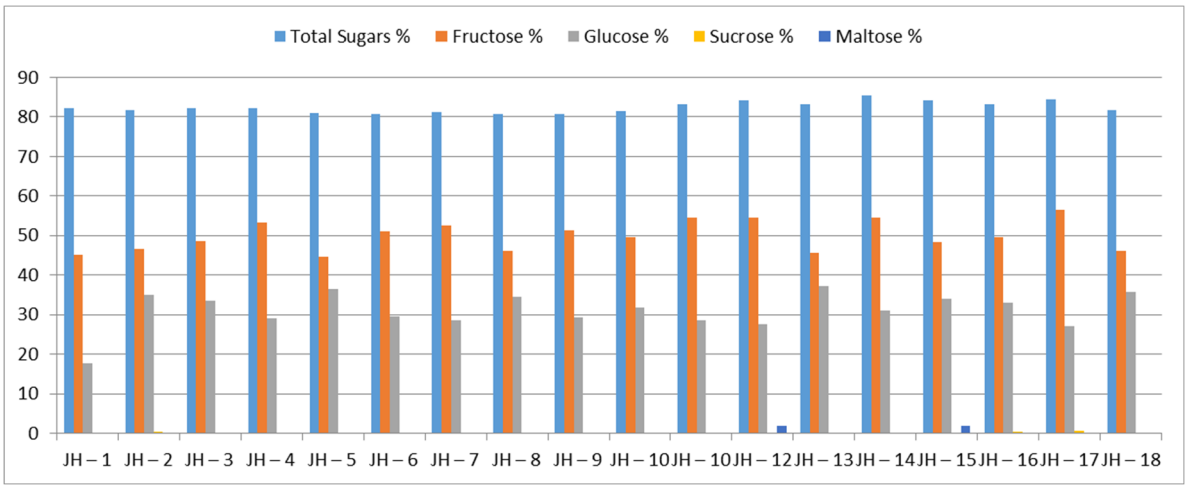

Figure 4. Quality and quantity of Jara honey carbohydrates in Western Georgia.

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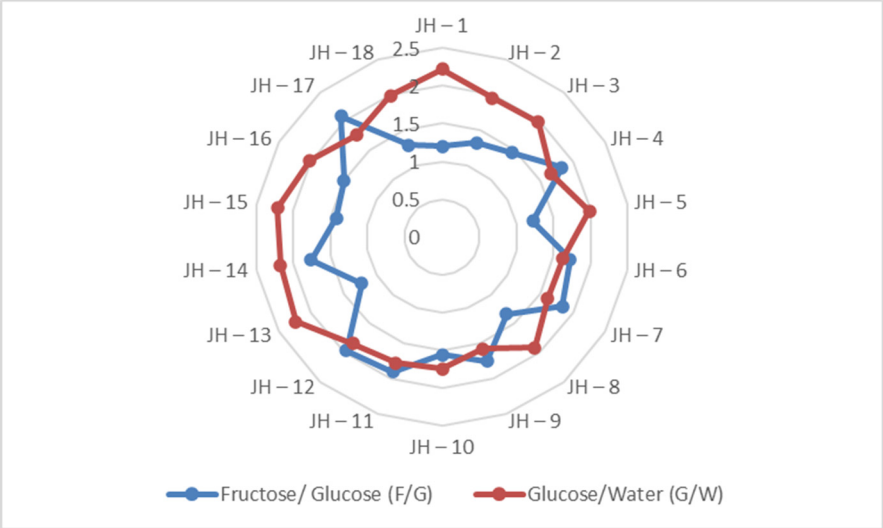

Figure 5. Fructose/glucose and glucose/water ratio in jara honey.

Carbohydrates, especially glucose and fructose, are the main components of honey and are essential for crystallization. Glucose is considered the crystallizing sugar because of its reduced solubility. Certain monofloral honey (such as citrus) naturally crystallizes neatly and uniformly, but most commercial honey is considered to be of low quality.

The G/F ratios of the Jara honey samples in the present study varied between 1.2 and 2.08. The glucose-fructose ratio in Jara honey samples is greater than 1, and the average value is 1.6 in the Keda samples, 1.63 in the Shuakhevi samples, and 1.58 in the Khulo samples

As for the glucose/water ratio in honey samples, it ranges from 158 to 2.22 in the Keda samples, from 1.75 to 2.24 in the Shuakhevi samples, and from 176 to 2.21 in the Khulo samples. Jara honey is characterized by a high ratio of F/G and G/W (more than 1.2) 3.3. Free acidity meq/kg - The acid content of honey is relatively low, but it is crucial for the taste of honey. The acidity of honey is determined by titration and is expressed in milliequivalents per kg [59]. Less than 0.5% of the solids in honey are organic acids; however, these acids have a significant impact on flavor [18], since free acidity is a key indicator of microbial deterioration of honey [60]. According to the EU Regulation (Council EU, 2001) and Codex Alimentarius (2001), the maximum amount of free acidity that can be present in honey is 50 meq/kg [47]. The results of the examination of free acidity are shown in Figure 2. The free acidity content ranged from 20.96 to 28.5 meq/kg in Jara honey varieties, with a mean value of free acidity lower than the permitted threshold.

From the analysis results, the average acidity rate in Jara honey ridge samples is 24.23 units, while the average rates in Shuakhevi and Khulo samples are almost similar to 26.06 and 26.08 meg/kg.

Hence, the low free acid values obtained in the current work are a good indicator of conservation. These results showed that there was no unwanted fermentation.

3.4. pH - The pH is one of the most important characteristics of honey [61], as it may influence honey texture, stability, and shelf life [62]. In particular, it prevents the development of microbiological processes. [63].

The pH values between 3.4 and 6.1 indicate the freshness of honey samples [64]. All of the investigated Jara honey samples were acidic and were within the limit (pH 4.23 to 5.63) (Figure 2) and within the standard limit (pH 3.40–6.10) [65], ensuring the freshness of the honey samples. In particular, the average value of pit in Honey Ridge samples is 5.214, in Shuakhevi samples - 4.96, and in Khulo samples -5.02.

3.5. Electrical conductivity (mS/cm) - Electrical conductivity is a very important property of honey [66]. It is greatly influenced by the concentration of organic acids and proteins, as well as by the ash content and active acidity [50]. Electrical conductivity generally falls within the range of 0.39-0.76 mS/cm [94], Accordingly, the electrical conductivity index, along with other parameters, serves as the main marker for confirming the botanical origin of honey [67].

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The bright color of honey usually points to a lower conductivity compared to dark-colored honey [68].

Values of electrical conductivity in the investigated honey samples ranged from 1.071 to 1.706 µs/sm (Table 2). The EC values in the investigated honey samples were not within the recommended range (below 0.8 mS/cm) [50].

The conductivity values of Jara Honey Ridge samples range from 1,071 to 1,706, with the highest values observed in 4 samples: JH 5 - 1,706, JH 6 - 1,552, JH 7 - 1,606, and JH 9 - 1,447 µS/cm. In Shuakhevi, the electrical conductivity ranges from 1.1008 to 1.3119 µS/cm, while in Khulo samples, the indicator is almost similar, ranging from 1.122 to 1.66.

3.6. Cations - Honey contains a variety of macro and micro minerals that are minor constituents of honey, presented in the range of 0.02–1.03% [69]. These elements mainly include K, Na, Mg, Ca, P, Mn, Fe, Li, Co, etc. [13,24]. The ash content of honey is the principal source of trace elements [70].

The qualitative and quantitative content of ash is an important feature of honey, influenced by both botanical and geographical origin [13,70]. A high degree of ash content in honey is a confirmation of a high concentration of pollen. [100,101]. Minerals affect the color of honey and are found in greater quantities in dark honey compared to light honey [60].

Also, it is possible to detect falsification based on ash content. When bees are fed sugar syrup, the ash content is also low.

In the Jara Honey, there were identified microelements such as Li, Na, K, Mg, and Ca (Figure 6), with concentrations ranging from 2613.5 to 5568.4 ppm. Analysis of the results is presented in Table 4. Based on the obtained results, it can be concluded that K is the dominant element, with its content ranging from 2174.86 to 5074.36 ppm.

This mineral is the most quantitatively important in honey, accounting for around 89% of the total mineral content.

The average potassium ion concentrations in the honey samples from Keda (3546.72 ppm) and Shuakhevi (3501.46 ppm) are similar. However, the concentration of potassium ions in the ridge samples varies significantly. For instance, in the first, second, third, and sixth samples, the concentration ranges from 2239.88 ppm to 2998.04 ppm, while in the fourth, fifth, and eighth honey, it ranges from 4702.58 ppm to 48603 ppm, with a difference of up to 36 ppm. In Shuakhevi honey samples, there is no significant variation in the indicators (3259.64 ppm to 3856.74 ppm). Similar to the ridge samples, the potassium content in Khulo honey varies. For instance, JH 16 (2356.68 ppm) and JH 18 (2395.72 ppm) have almost identical concentrations. However, compared to these samples, the potassium content is twice as high in the fourteenth sample of Khulo honey, reaching 5074.36 ppm.

The second most abundant mineral in all samples was Ca, ranging from 141.28 – 795.06 ppm. The average content of Mg and Na varied significantly among samples. The concentrations found in the samples ranged from 41.6 to 262.34 ppm for Mg and from 2.4 to 33.58 ppm for Na. The concentration of Li was the lowest (8.22 – 19.44 ppm).

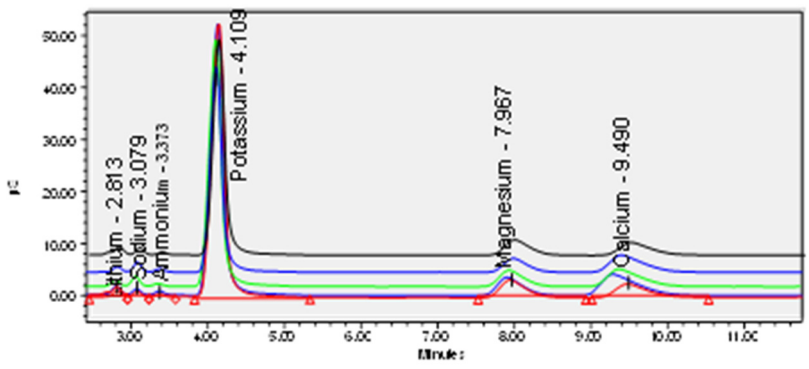

Figure 6. Chromatograms of the Cations Jara honey samples.

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3.7. Color intensity– Honey color is an important sensory characteristic ranging from colorless to dark brown [71]. The honey color completely depends on the honey plants from which the nectar is taken. The color range can also vary depending on the geographical origin [17,71]. There are often cases when there is a difference in the order of honey color in the international market, for example, in the European market, there is a higher demand for honey with a dark color and strong aroma, whereas in America, they prefer light-colored honey with a light aroma [38]. The color intensity of honey also determines the concentration of biologically active secondary metabolites in nectar [72], and it is directly correlated with the antioxidant activity of honey [73].

Only one of the possible seven color classes (Pfund scale) was found in the Jara honeys (Figure 7), dark amber. The color of the samples taken for analysis is in the range of 122.44 - 294.81 mm, according to the scale, it exceeds 144 mm. That is a dark amber.

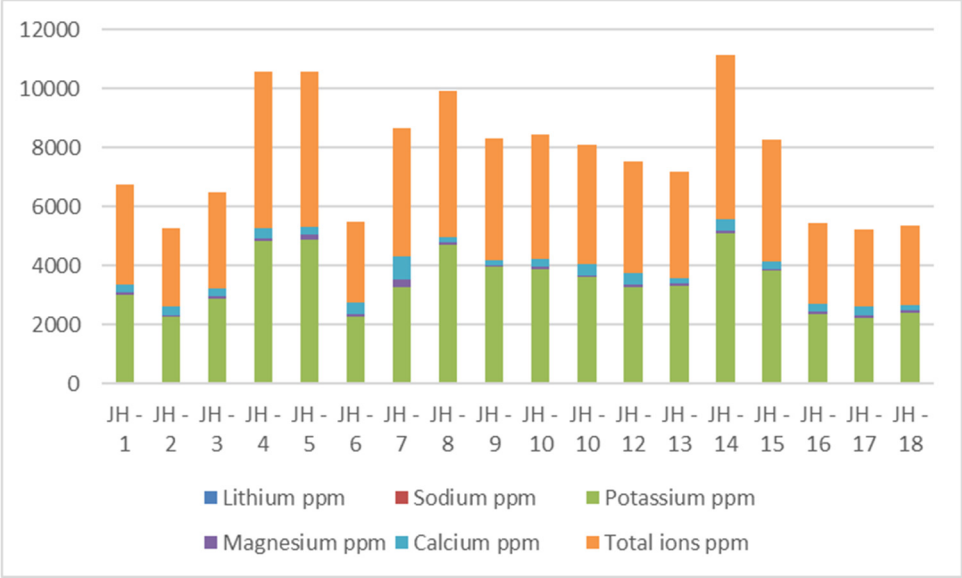

Figure 7. The Cations of Jara Honey in Western Georgia.

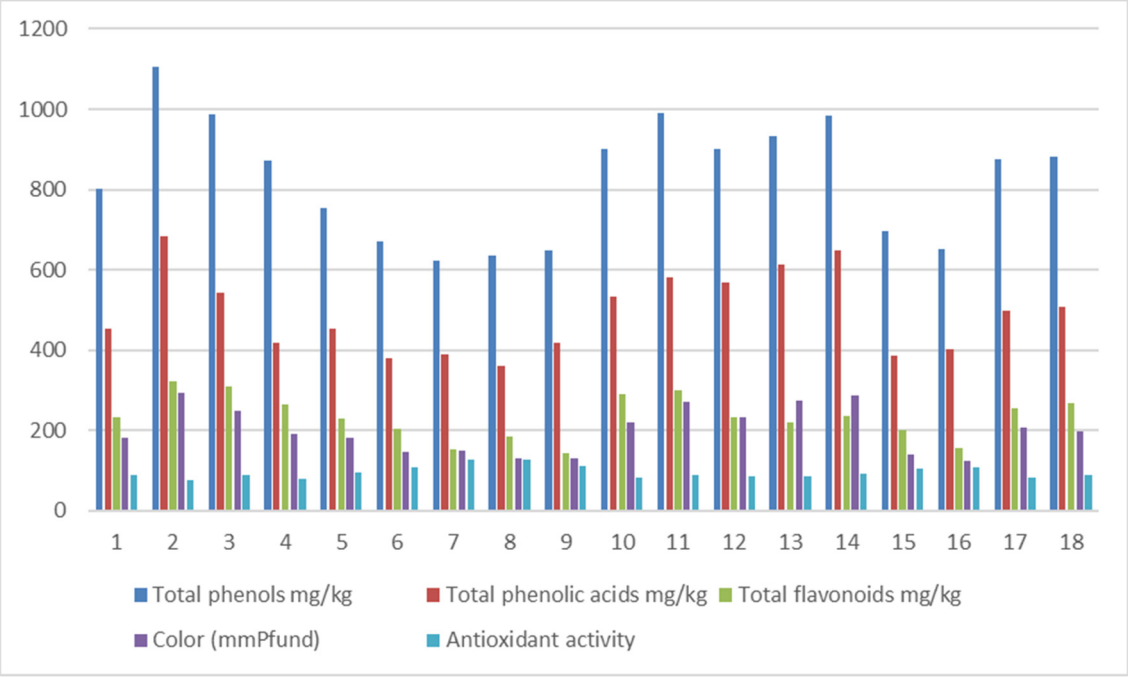

Figure 8. Total phenols. phenolic acids, flavonoids, color, and antioxidant activity of Jara honey.

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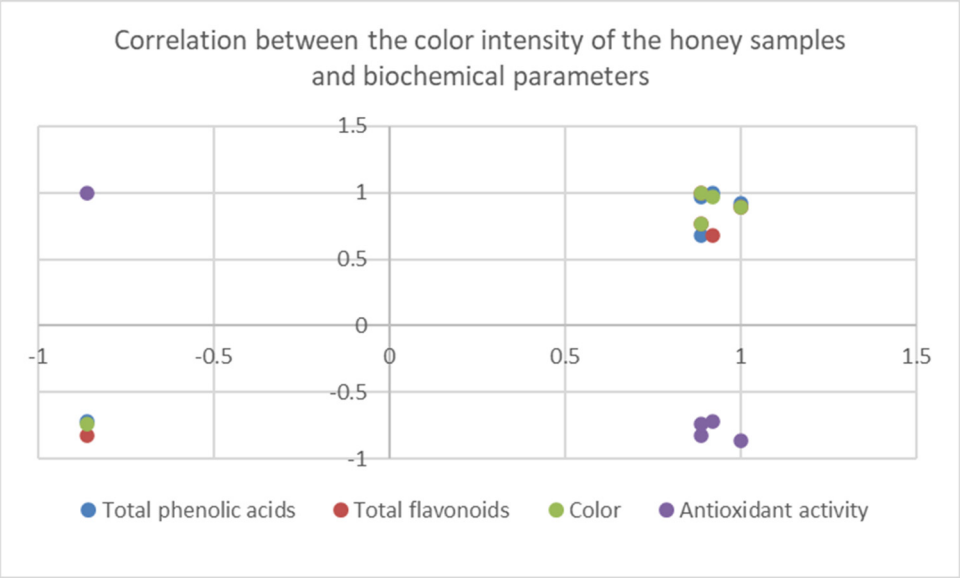

Figure 9. Correlation between the color intensity of honey samples and biochemical parameters.

3.9. Phenolic compounds - Phenolic compounds are one of the most significant chemicals in honey [22]. They are powerful natural antioxidants that are biologically active secondary metabolites from plants that operate at the molecular level [74]. Phenolic compounds, as secondary plant metabolites and natural antioxidants, significantly determine the biological activity of honey [8,22]. In particular, these compounds are responsible for the antioxidant activity of honey [8,75], as they can bind or neutralize free radicals. [76]. The content of phenolic compounds, both qualitatively and quantitatively, depends entirely on the type of honey plants [38,76,77].

The main indicators of honey's botanical origin are polyphenols, which also have a strong medicinal and dietary value (113,16). Honey is utilized as an essential source of phenolic compounds in the human diet due to its abundance of phenolic compounds [22,78].

The total amount of phenols in the honey samples taken for analysis ranges from 622.34 to 1105.56 mg/kg: According to the phenol content, the samples from Keda (788.31 mg/kg) and Khulo (817.51 mg/kg) have similar phenol levels. However, in Keda honey, there is a noticeable difference between the samples. In particular, the phenol content, including JH 6-9, averages 643.73 mg/kg, with a relatively high content observed in the third sample (987.19 mg/kg) and the second sample (1105.56 mg/kg). A similar difference is observed in the samples of Khulo Jara honey. The average total phenols content in Shuakhevi honey is relatively high at 930.72 mg/kg.

3.10. Flavonoids are low molecular weight phenolic compounds based on the flavan nucleus [79], responsible for the aroma and antioxidant potential of honey [48]. Their biological effects span a wide range, including antibacterial, anti-inflammatory, anti-allergic, and antithrombotic actions [80]. According to the floral and geographic sources of the honey, flavonoid profiles typically vary greatly [38,79,81]: JH 2 (321.9 mg/kg), JH 3 (307.9 mg/kg), JH-11 (299.7 mg/kg), and JH-10 (291.4 mg/kg). They were relatively lower in JH-18 (267.1 mg/kg), JH-4 (265.8 mg/kg), and JH-17 (255.3 mg/kg).

3.11. Phenolic acids (aromatic carbonic acids) are a subclass of the most numerous and ubiquitous groups of secondary plant metabolites [79]. Phenolic acids are classified as derivatives of cinnamic and benzoic acids [71]. Their chemical structure is simple C6-C [58]. Honey contains a wide range of phenolic acids [28) Phenolic acids are not only present in honey, but they also might designate some kinds of honey [79,82].

The phenolic acids content in the Jara honey samples ranged from 359.8 to 682.4 mg/kg and accounted for around 45 - 65 % of the total phenolic content. The amount of phenolic acids (according to the average indicator) is much higher in Shuakhevi samples, amounting to 573.40 units, while it is almost equal in Keda (455.25 mg/kg) and Khulo (488.42 mg/kg) samples, similar to the content of total phenols.

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Phenolic acids content was highest in honey samples: JH-2 (682.4 mg/kg), JH-13 (612.4 mg/kg), and JH-14 (648 mg/kg), whereas samples JH1 had the lowest content (453,3 mg/kg).

3.12. Antioxidant activity by the DPPH method – Honey is a well-known abundant source of both enzymatic (glucose oxidase and catalase) and non-enzymatic (L-ascorbic acid, flavonoids, and phenolic acids) antioxidants, which have been shown to have health-promoting anti-oxidative properties [12]. Consuming honey is a successful strategy for boosting total plasma antioxidants and reducing capacity in people [22,83]. The determination of antioxidant activity in food products by the free radical - DPPH method is a highly adapted method. The method is simple and completed in a short time [84]. 2,2-Diphenyl-1-picrylhydrazyl is a stable compound at room temperature and rapidly recovers in solution in the presence of antioxidants. The violet color of the radical disappears or turns yellow, so the absorption index also decreases at 517 nm. [85]. The antioxidant activity of the analyzed sample is calculated by the difference in absorbance values. In particular, the lower the difference index, the higher the antioxidant activity of the analyzed sample. The smaller mass sample achieves 50% inhibition of the DPPH radical [86]. This method is also actively used to determine the antioxidant activity of honey because honey is rich in secondary metabolites - phenolic compounds [32,63].

Among Jara's honey samples, those with a total phenol content of 801.61 to 1105.56 mg/kg have a relatively high antioxidant activity, so less of the honey mass is required to inhibit 50% of the radical, namely 75.1 to 90, 68 mg, While 622.34 - 752.95 mg/kg of total phenols are inhibited by 94.75 - 128.08 mg of honey. In the presented samples the relatively high activity (75.1 mg, 50% inhibition of honey 0.1 mm DPPH) stands out among the honey grown in Keda municipality - JH–2, which contains a large amount of total phenols (1105.56 mg/kg), total phenolic acids (682.4 mg/kg), and total flavonoids (321.4 mg/kg) (Figure 8).

A decrease in the amount of phenolic compounds (total phenols, total phenolic acids, and total flavonoids) in honey also leads to a decrease in antioxidant activity. The amount of phenolic compounds in Jara honey 7 is the lowest (total phenols 622.34 mg/kg, total phenolic acids 388.7 mg/kg, and total flavonoids 151.0 mg/kg), and more honey is required for 50% inhibition of 0.1 mm DPPH (128.08 mg) (Figure 8).

A directly proportional relationship was established between the quantitative content of phenolic compounds, color, and the antioxidant activity of honey (Figure 8).

3.13. Proline - The amino acid composition of honey is completely dependent on the botanical origin of honey [8], and therefore its qualitative and quantitative content is successfully used as an indicator of the naturalness and quality of honey. The mass share of amino acids in honey is about 1%. Its composition includes glutamic acid, aspartic acid, glycine, threonine, histidine, glutamine, proline, and others. But among them, there is more proline, which is mainly formed when the nectar is processed. Its content depends on the time of nectar processing by the bees and, accordingly, on the origin of the honey. It is about 50-85% of the total mass of amino acids, and its concentration is different in different honeys [8]. The regulation defines the content of proline in honey, and it should not be less than 180 mg/kg [37]. The proline content of Jara honey samples is presented in Figure 10.

The proline content of Jara honey samples ranged from 761.28 to 1372.29 mg/kg (Figure 10). According to the average values of the obtained results, the proline content is almost similar: in Keda samples, it is 1019.40 mg/kg, in Shuakhevi honey, it is 1086.43 mg/kg, and in Khulo samples, it is 1123.37 mg/kg. The lowest proline concentration was measured in JH-2 (761.28 mg/kg) and JH-3 (790.62 mg/kg) in the honey samples from Keda. In the other honey, the proline content is higher than 800 mg/kg. The highest proline concentration was in JH-18 (1372.9 mg/kg). The highest amount was observed in five samples: JH-5 (12498.39 mg/kg), JH-7 (1311,06 mg/kg), JH-12 (1234.9 mg/kg), JH-13 (1208.16 mg/kg), and JH-18 (1372.29 mg/kg). Table 10 shows that all proline values for honey were well above the 180 mg of proline per kilo of honey standard.

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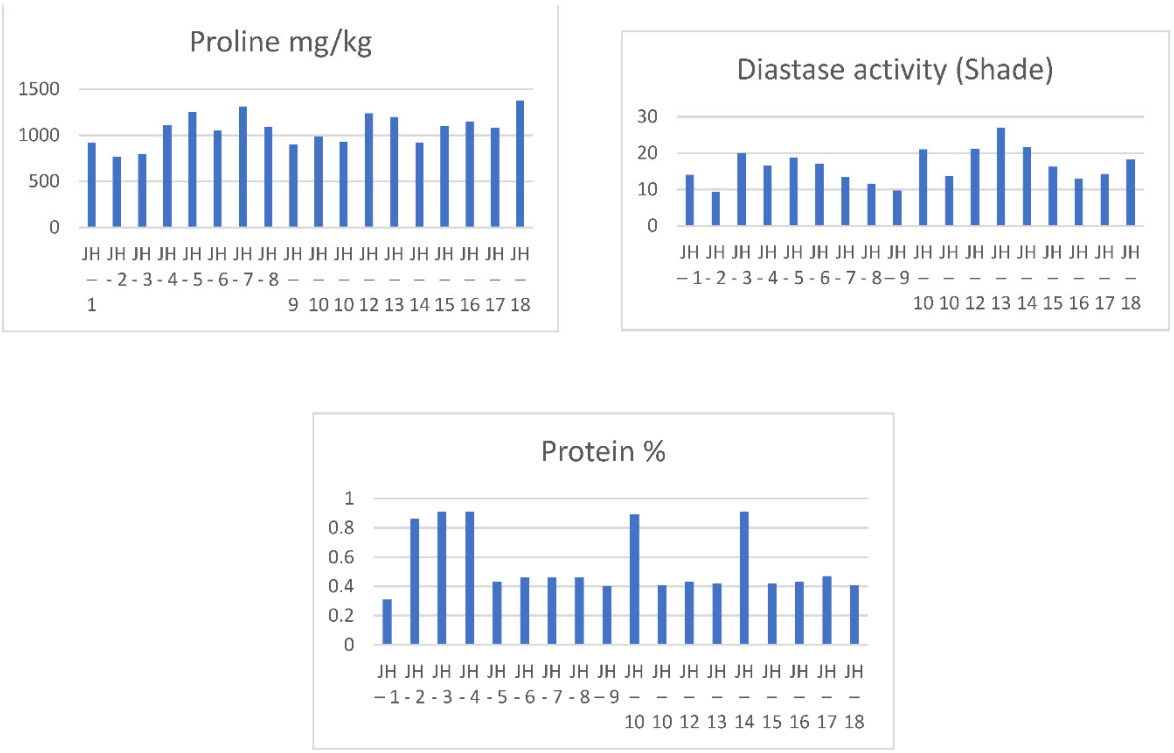

Figure 10. Proline content, Diastase activity, and Protein content of Jara Honey.

3.15. The activities of enzymes - The activities of enzymes are the basis for evaluating the quality of honey [88]. The enzymatic composition of honey includes glucosidases, α and β amylases, α and β glucosidases, as well as proteases. Honey differs from each other in the composition and quantity of enzymes, as their content is completely dependent on both the nectar collection period and the physiological age of the bee colony.

α and β amylases, or diastases, are the enzymes that occur in relatively large quantities in honey, and their content depends on their botanical and geographical origin. [88]. A diastase catalyzes the breakdown of starch into maltose [36,37,44].

Diastases are sensitive to heating, and their activity decreases at high temperatures. Therefore, its value is used as a marker of age and an indicator of high-temperature treatment in storage conditions [36].

Diastase activity is a honey quality parameter used to determine if honey has been extensively heated during processing [86,89,90]. According to the Honey Quality and International Regulatory Standards, the diastase activity must not be less than or equal to 8 [65].

Active enzymes are very sensitive to high temperatures and will lose their activity when they exceed a certain temperature [36,37].

In all 18 samples of Jara honey, the characteristic diastase activity is much higher than 8, from 9.56 to 27.0 (Figure 10).

3.16. Protein – Proteins are one of the main constituents that perform critical functions in food systems [91], and therefore they are the most important marker for confirming the origin and naturalness of honey [92].

Protein in honey can come from either plants or animals. Animal protein is produced by the bee itself and is composed of salivary gland secretions as well as by-products gathered during nectar collection or honey maturation, whereas plant origins are derived from nectar and pollen gathered in the field [18]. Floral honey has a protein value of between 1.0 and 1.5%, whereas honeydew honey has a protein content of around 3.0% [87].

The nitrogen content of Jara honey samples ranged from 0.31 to 0.91% (Figure 9).

3.17. Melissopalynology, or pollen analysis – Honey color, aroma, taste, and therapeutic-prophylactic properties depend on the flower's nectar, and the composition of the latter depends on

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those entomophilic plants that blossom during the period of honey collection [93]. The biologically active compounds of the plant (flower) are present first in nectar and then in honey, which determines flower biological activity. [81,94,95].

Several markers are used to determine the botanical origin of honey, among which it is important to determine the morphological structure and concentration of honey pollen. If the honey is obtained entirely or mainly from the nectar of a specific plant or plant flower, it must have physico-chemical, organoleptic, and morphological characteristics that are characteristic only of honey obtained from the nectar of a specific plant (monofloral honey) or plant flower (polyfloral honey) [96].

Honey consists of pollen grains collected by honeybees; hence, pollen taxonomy is the prerequisite to compare the pollen present in honey samples with special reference to melissopalynological. The taste, smell, and color of honey change according to the nectar of the flowers [97].

The flower origin of Jara honey was determined by melissopalynological analysis. Pollen types were identified by comparison with reference slides of pollen collected directly from the plants in the study and reference images of pollen and apicultural plants in the literature [98,99].

Pollen types were identified by comparison with reference slides of pollen. Pollen grains were identified and quantified by applying microscopy to preparations taken from the honey samples **(Appendix A)**. About 500 pollen grains were counted from each sample. All measurements have been repeated to ensure significant precision.

The proportion of each type of pollen was calculated as a percentage of total pollen. The pollen concentration in honey is regulated according to European and international standards. according to the pollen content, pollen is divided into 4 groups: dominant pollen (more than 45%), secondary pollen (16-45%), significant minor pollen (3–15%), and minor pollen (less than 3%). In general, honey is considered monofloral if the dominant pollen content exceeds 45%, and if there is no dominant pollen content, the honey is considered polyfloral. However, the number of dominant pollens is different for different kinds of honey; the average rate of dominant pollens is 94.5% for chestnut honey, 38.6% for rhododendron honey, 28.1% for acacia honey, and 22.9% for lime honey, etc. [100–102].

For identification of Jara honey's botanic origin, beekeepers used information about Jara`s location.

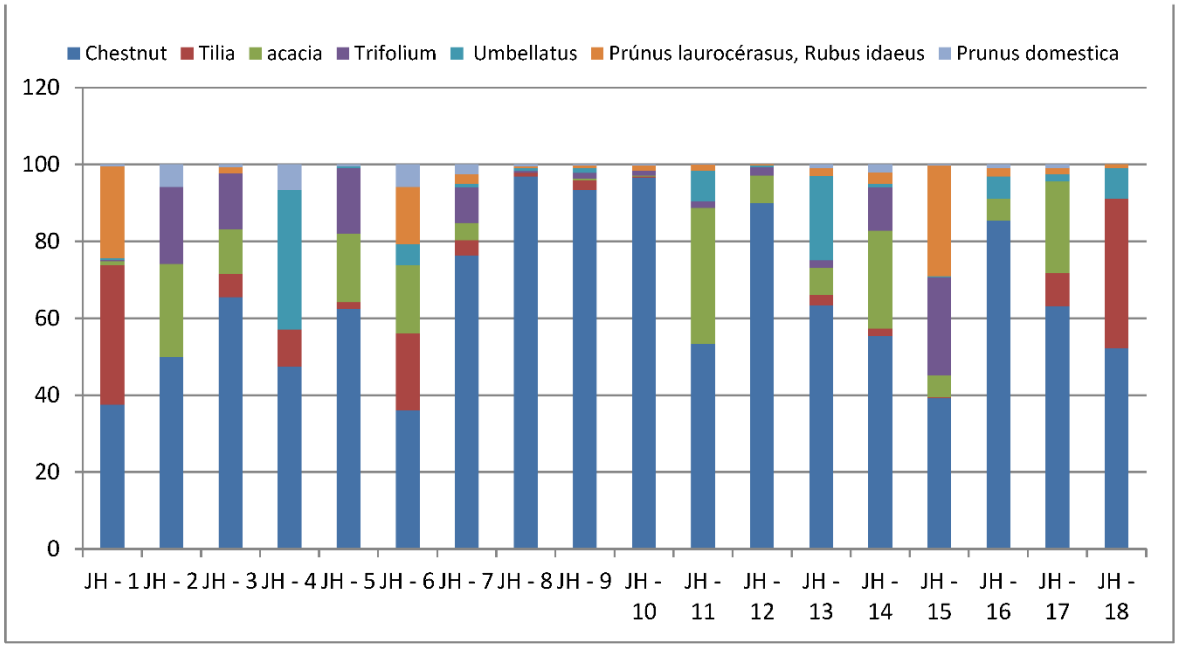

Figure 11. Pollen types in the Jara honey.

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The results of qualitative pollen analysis indicate the diversity of resources utilized by honeybees in the region of investigation. Pollen analysis of Jara Honey showed that out of 18 analyzed samples, the following were identified: Chestnut, Tilia, Acacia, Juglans regia, Prunus laurocerasus, Malus domestica, Pyrus communis L., Prunus domestica, Trifolium pratense, Taraxacum, Solidago virgaurea, Rubus idaeus, Rhododendron, and another pollen (Figure 10).

In 15 samples of honey, the consistent amount of chestnut pollen was more than 45%; for example, in Qeda`s sample, it was 47.5–96.89%, 53.28–96.6 in Shuakhevi, and 46.19–80.47% in Khulo.

Interestingly, in 3 samples (JH 1, JH 6, and JH 15), chestnut pollen doesn't show up as dominant. However, their consistency in a secondary pollen group is higher than that of Tilia, Acacia, and Trifolium pratense (Figure 10).

Depending on the melissopalynology analysis result, we can conclude that Jara`s honey can be classified as multi-floral honey because of its botanic origin. Depending on melissopalynology analysis results, we can conclude that Jara`s honey can be classified as multi-floral honey, but in 7, 8, 9, 10, 12, and 16 samples of honey, the concentration of chestnut flower pollen is high (73.36% to 96.89%).

Grayanotoxin III consistency enhances the treatment function of honey. It`s identified in samples (JH 1, JH 6, JH9, JH10, JH11, JH13, JH14, JH15, JH16, and JH17) from three different municipalities (Keda, Shuaxevi, and Khulo). The consistency of rhododendron pollen is higher in 9, 13, and 16 samples. These samples belong to the important minor pollen group (JH9 – 4.45%, JH13 – 4.88 %, and JH9 – 4.26%), in other cases, their consistency is lower than 3% (Figure 10).

3.18. The identification of grayanotoxin-III – for grayanotoxin-III identification, ultra-performance liquid chromatography (UPLC) mass (MS), and a photodiode array (PDA) detector were used in the Jara honey samples.

The molecular weight of GTX-III is 370 g/mol, appearing at m/z 369 in negative ion mode.

A compound 1 (Figure 12) has a retention time of 8.359 min, m/z 369 [M-H]+, ʎ max 289 nm; according to the obtained results and compounds mass database METLIN (https://metlin.scripps.edu) substance 1 is grayanotoxin-III. - $C_{20}H_{36}O_6$ Negative FABMS: m/z = 369.26 [M-H+], Molecular Weight: 370 g/mol.

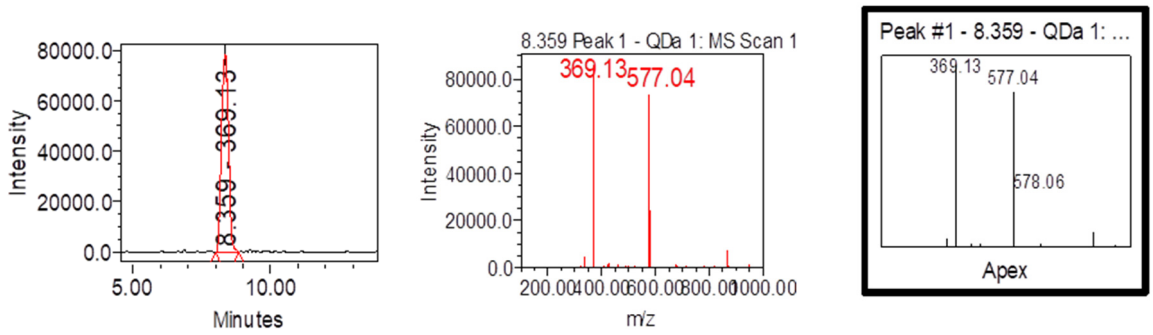

Figure 12. Chromatograms of a Flower of Rhododendron, scan ESI-MS m/z: 369 [M-H⁺].

For comparing chromatography analyses, Rhododendron`s flower and mad honey samples (Figures 12 and 13) were used, where the consistency of tocsin was much higher than in Jara honey's samples. An almost equal quantity of grayanotoxin-III was in the 9th, 13th, and 19th samples (Figures 14, 15, and 16). It`s proportional to rhododendron pollen. In other samples of Jara honey, grayanotoxin-III is left as a small portion, and the consistency of pollen is much lower (minor pollen (<3 %) (Figure 11).

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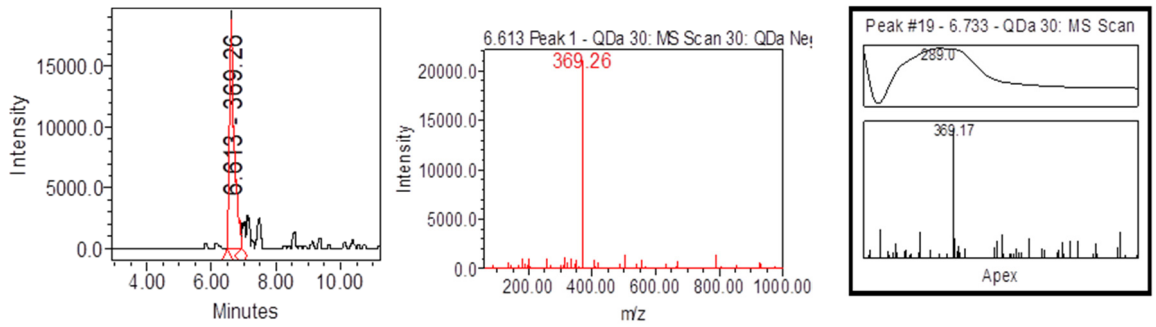

Figure 13. Chromatograms of a Mad honey, scan ESI-MS m/z: 369 [M-H⁺].

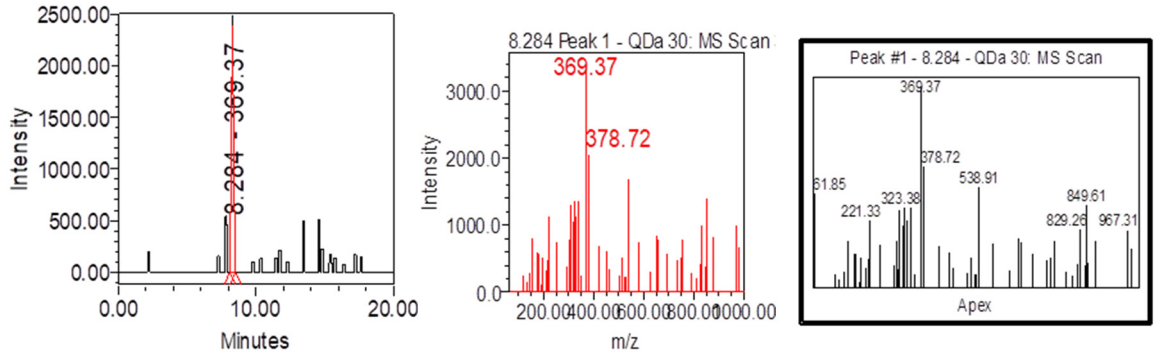

Figure 14. Chromatograms of a Jara honey 13, scan ESI-MS m/z: 369 [M-H⁺].

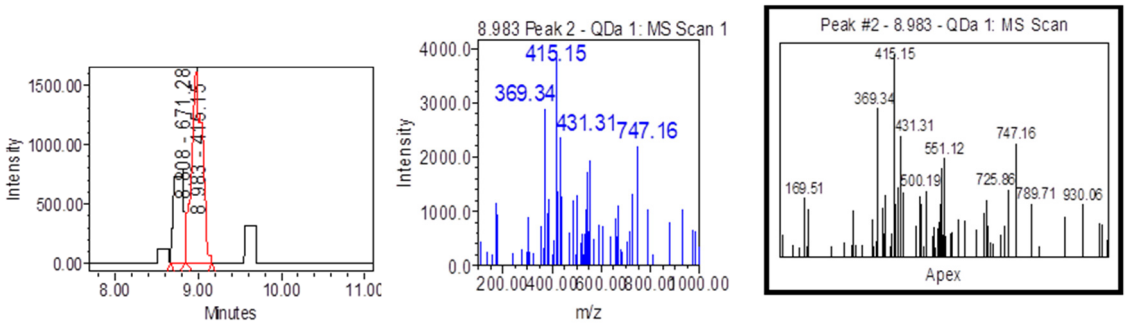

Figure 15. Chromatograms of a Jara honey 9, scan ESI-MS m/z: 369 [M-H⁺].

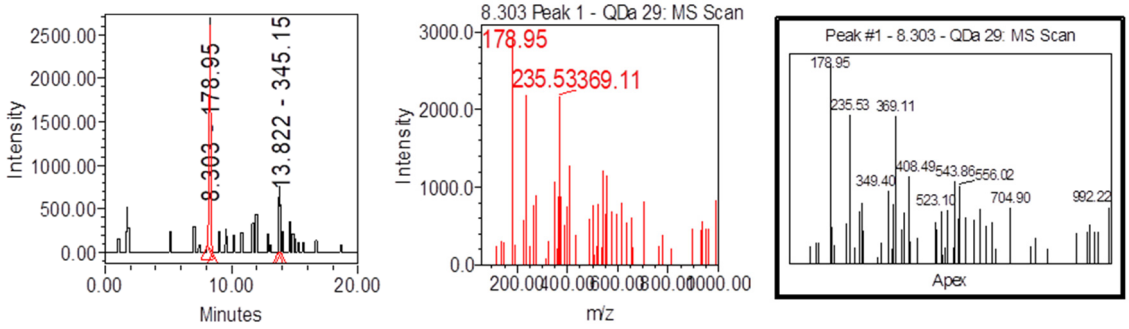

Figure 16. Chromatograms of a Jara honey 16, scan ESI-MS m/z: 369 [M-H⁺].

4. Discussion

All 18 samples of Jara honey taken for the analysis are dark in color, have a bitter-sweet taste, and have an extremely strong and pleasant aroma. This is the first survey about Jara honey`s physicochemical and biochemical characteristics. West Georgia's Jara honey is high quality and rich in bioactive compounds. The physical and chemical characteristics of honey are according to

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European standards. Honey's moisture levels varied and were compatible with honey codices. The average value between total free acidity content, pH, and conductivity (Figure 8) showed a positive linear correlation. Jara`s honey has the highest pH and conductivity values of 4.23 and 1.706 (μs/sm), respectively.

By comparing the result obtained in the determination of diastatic activity as one of the important characteristics (the activity index is higher than 8 units), it can be concluded that all samples of Jara honey are natural. Dominant sugars in the form of fructose and glucose were identified in Jara honey samples by the high-pressure liquid chromatography method. A strong correlation was found between phenolic compounds, phenolic acids, and flavonoids. Color intensity increases with the increase of phenolic compounds (0.9547), phenolic acids (0.9637), and flavonoids (0.7672) (Figure 8). A stable positive correlation was observed between color intensity and phenolic compounds (0.954745808). In addition, there is a strong correlation between antioxidant activity and biologically active compounds, so there is a linear association.

The obtained results showed that protein and proline content as well as enzyme activities were within the limit that ensures the freshness of Jara honey samples. Potassium is the most abundant element in all the samples analyzed. This mineral is the most quantitatively important in honey, accounting for around 89% of the total mineral content. Depending on the melissopalynology analysis result, we can conclude that Jara`s honey can be classified as multi-floral honey because of its botanic origin.

5. Conclusions

This study encompasses the characterization of semi-wild Jara honey, a unique product distinguished by its origin and extraction method. The results of this study show that all honey samples indicate a good level of quality, maturity, and freshness. Physicochemical and chemical analyses of Jara honey are in agreement with European and Georgian legislation. Considering the pollen profile and physicochemical characteristics, the samples were classified as multifloral honey. Because of Grayatoxin 3's consistency, it is inevitable to take precautions when using Jara Honey. The uniqueness of Jara is due to its production and harvesting methods. This study will allow us to obtain the results of the first targeted study on semi-wild jara honey.

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