THE STUDY OF THE PHYSICAL-CHEMICAL CHARACTERISTICS OF ACACIA HONEY WESTERN GEORGIA

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Abstract: The purpose of this study was to examine the physico-chemical characteristics, phenolic compound content, and antioxidant activity of acacia honey produced in the western regions of Georgia (Imereti, Guria, Adjara) in accordance with Codex Alimentarius standards. At the initial stage of the research, pollen analysis was conducted to confirm the botanical origin of the honey. There is no significant difference in the average characteristics of the honey across the regions. By jointly analyzing these parameters, we aim to confirm the naturalness and origin of acacia honey produced in Western Georgia.

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Keywords: Acacia honey, Pollen analysis, characteristics, HPLC.

Introduction

Honey is a natural sweetener widely consumed worldwide for its appealing taste, as well as its high nutritional and functional value. (Matkovits et al.,2023). The physical characteristics (such as color, taste, aroma, and fragrance) and chemical composition of honey vary depending on its botanical and geographical origin. In addition to its high nutritional value, honey exhibits antimicrobial, antioxidant, anti-inflammatory, and anticarcinogenic effects, primarily due to the presence of phenolic compounds, including flavonoids and phenolic acids. (Margaoan et al 2021).

production of monofloral honey is widely practiced and popular worldwide. for example in Europe, over 100 monofloral kinds of honey are produced currently (linden, acacia, sunflower, rosemary, eucalyptus, and others). In China, buckwheat honey is particularly popular due to its lower sweetness and high antioxidant activity. (Natural Honey Market 2022-2028). According to data from the National Institute of Standardisation (UNI), there are five types of monofloral honey in Italy: Black Acacia, Chestnut, Citrus, Eucalyptus, and honeydew honey. (Margaoan et al., 2021. Marcazzan et.al.,2014).

Although producing honey of uniform origin in Georgia is challenging, there are specific zones where each type of honey can be consistently produced. Notably, false acacia honey is obtained in regions such as Adigeni, Khashuri, Gurjaani, Telavi, Khoni, Van, Baghdati, and others. Acacia honey is primarily derived from the nectar of the false acacia (Robinia pseudoacacia) flower. Acacia honey is characterized by its light color. It has a sweet taste, light texture, delicate and pleasant floral aroma. Acacia honey is made special by the fact that it does not crystallize over a long period, or it may not crystallize at all. When it crystallizes, it takes on a white color. Acacia honey is considered to be one of the popular monofloral honey (Matkovits et al., 2023).

Microscopic analysis of pollen grains is primarily used to identify and confirm the botanical origin of honey, which is a very effective method (Tarapatskyy et al., 2021), But according to the researcher, this alone is insufficient to confirm the origin of honey, the method of using microscopic analysis of pollen grains has limitations, microscopic analysis of pollen grains requires a significant amount of time and a high level of professionalism (Bodóa et al., 2020). The accumulation and quantity of pollens are influenced by both the geographical origin of honey and climatic conditions, also, the type of bee, the conditions of filtering honey, and the beekeeper's honesty (Tarapatskyy et al., 2021).

Our research aimed to address that *pseudo acacia* honey in Georgia is absolutely unstudied.

Materials and Methods

Samples

The object of the study was acacia honey collected in 3 regions of Western Georgia - Imereti, Guria, and Adjara. Samples were collected from private beekeepers over two years: 2022 and 2023. (table 1). Samples were chosen by analyzing stamens under a microscope. Specifically, honey samples with an

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acacia pollen content exceeding 45% were chosen for analysis (European Commission, 2020), which proves monoflora.

Chemicals and instruments

All reagents and chemicals used were analytical grade from Sigma and Merck Chemical Company, A Mettler Toledo UV-5 model UV–VIS spectrophotometer was used for absorbance measurements, A Waters HPLC (Water 2414 Refractive Index was used for identification compounds, A UKA Technic professional light microscope for melissopalynological analysis.

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

Table 1. A sample of Acacia honey taken for analysis

Sample No.	Sampling Time	Region	Municipality
Acacia honey 1 – ACH 1	2022 – 2023	Imereti	Vani
Acacia honey 2 – ACH2	2022 – 2023	Imereti	Baghdad
Acacia honey 3 – ACH3	2022 – 2023	Imereti	Samtredia
Acacia honey 4 – ACH4	2022 – 2023	Imereti	Zestafoni
Acacia honey 5 – ACH5	2022 – 2023	Imereti	Tkibuli
Acacia honey 6 – ACH6	2022 – 2023	Imereti	Tskaltubo
Acacia honey 7 – ACH7	2022 – 2023	Imereti	Terjola
Acacia honey 8 – ACH8	2022 – 2023	Imereti	Kharagauli
Acacia honey 9 – ACH9	2022 – 2023	Imereti	Khoni
Acacia honey 10 – ACH10	2022 – 2023	Imereti	Khoni
Acacia honey 11 – ACH11	2022 – 2023	Guria	Lanchkhuti
Acacia honey 12 – ACH12	2022 – 2023	Guria	Lanchkhuti
Acacia honey 13 – ACH13	2022 – 2023	Guria	Lanchkhuti
Acacia honey 14 – ACH14	2022 – 2023	Guria	Ozurgeti
Acacia honey 15 – ACH15	2022 – 2023	Guria	Ozurgeti
Acacia honey 16 – ACH16	2022 – 2023	Guria	Ozurgeti
Acacia honey 17 – ACH17	2022 – 2023	Guria	Ozurgeti
Acacia honey 18 – ACH18	2022 – 2023	Guria	Chokhatauri
Acacia honey 19 – ACH19	2022 – 2023	Guria	Chokhatauri
Acacia honey 20 – ACH20	2022 – 2023	Guria	Chokhatauri
Acacia honey 21 – ACH21	2022 – 2023	Adjara	Khelvachauri
Acacia honey 22 – ACH22	2022 – 2023	Adjara	Khelvachauri
Acacia honey 23 – ACH23	2022 – 2023	Adjara	Keda
Acacia honey 24 – ACH24	2022 – 2023	Adjara	Keda
Acacia honey 25 – ACH25	2022 – 2023	Adjara	Keda
Acacia honey 26 – ACH26	2022 – 2023	Adjara	Keda
Acacia honey 27 – ACH27	2022 – 2023	Adjara	Keda
Acacia honey 28 – ACH28	2022 – 2023	Adjara	Keda
Acacia honey 29 – ACH29	2022 – 2023	Adjara	Khulo
Acacia honey 30 – ACH30	2022 – 2023	Adjara	Khulo

Source: Author

Methods

The refractometric method was used to determine the moisture, or conversely the soluble solids in honey. pH/Ion and free acidity meter S220 and S230 Conductivity (Seven Compact, Mettler Toledo). Determination of electrical conductivity - The electrical conductivity was measured using a Mettler Toledo conductometer. Determination of diastase activity – The conventional Schade method and proline analysis; Total content of phenolic compounds (TCPC), Analysis using the Folin-Ciocalteu method (in terms of gallic acid); Antioxidant activity assay with DPPH- 2,2-Diphenyl-1-picrylhydrazyl - DPPH 0.1 N 50% inhibition mg, with a sample. Carbohydrates: The sugar content is assessed using Waters HPLC (High-Pressure Liquid Chromatography) with RI-detection Protein: The Kjeldahl method is employed to determine nitrogen content, and applying a conversion factor yields the crude protein result

Pollen Analysis

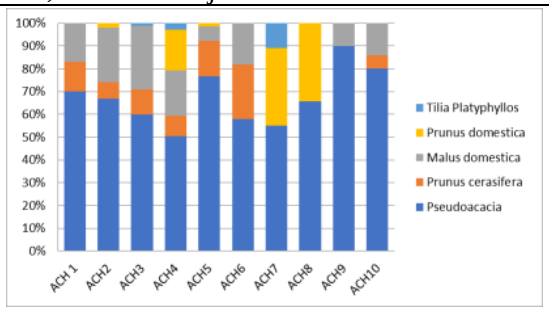
Pollen analysis followed the Louveaux et al., method, where slides were prepared from each sample and photographed under a light microscope. Identification of pollen grains relied on their size and shape. (Ma, 2019, Bouddine et al., 2022, Raweh et al., 2023, Schievano, 2019).

Microscopic study of pollens

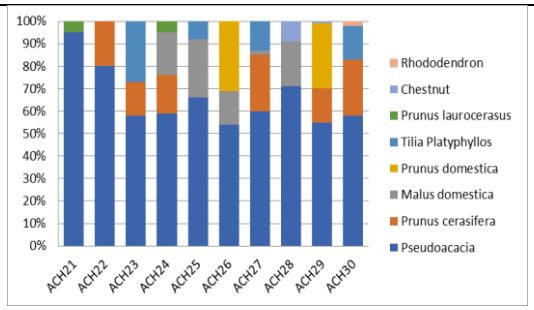
A light microscope was used for pollen analysis (UKA Technic Professional Microscope, AmScope/ Microscope Digital Camera 18MP Aptina Color CMOS/Ultra-Fine Color Engine Inside /MU1803 USB3.0DC5V.900mA), which was equipped with a video monitor (AmScope/ Microscope Digital Camera 18MP Aptina Color CMOS/Ultra-Fine Color Engine Inside /MU1803 USB3.0DC5V.900mA).

For the analysis, we took 10 g ± 0.1 g of honey sample, added 20 ml of water, and centrifuged it at 1000 rpm for 10 minutes. After the complete removal of the liquid, we transferred the remaining precipitate to a microscope slide and spread it evenly on the glycerin-gelatin surface. After maximum heating of the mixture (at 40^0C for 5 minutes), we examined it under a microscope. Reference preparations of honeydew plant pollen and pollen atlases of different plants were used for pollen identification. More than 500 pollen grains were counted in each sample. For more accuracy, pollens were counted three times and A mean was calculated for each sample. The indicator is calculated in percentages and the results are given in Figure 1 (Vanidze et al., 2018, Dhawan et al., 2018).

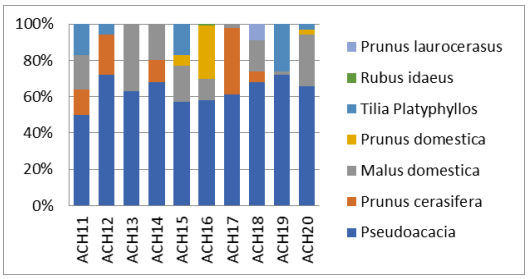
Figure 1. - Pollen types in the Acacia honey. A: Acacia honey 1-10 Imereti, B: Acacia Honey 11-20 Guria, C: 21-30 Adjara



A



B



C

Source: Authors (2024)

The water content in honey stands as an important parameter. The mass fraction of water in the honey samples taken for analysis is within the norm according to the European regulation and the technical regulation on honey in force in Georgia. (no more than 20%), in particular from 16.9 to 20% (Figure 2). It is only an exception for ACH8 (Kharagauli), ACH11 (Lanchkhuti), and ACH23 (Keda) samples, where the rate is 21.1%. A relatively low rate was observed in the sample taken in Samtredia municipality, 15.8% (Figure 2).

In the samples taken from all three regions, the average rate of water is similar, Imereti region 18.83%, Guria region 18.75%, and Ajara region 18.74%.

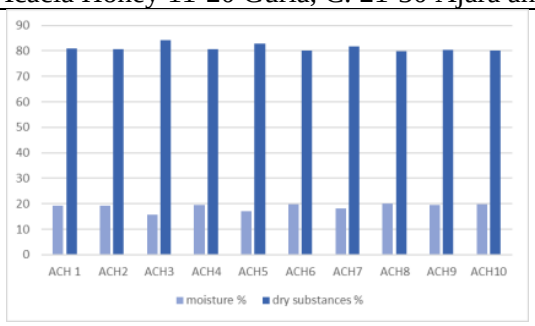
The low water content in the honey samples taken for analysis ensures the stability and resistance of honey to fermentation processes (Stefan, 2009). The total content of soluble substances in acacia honey is in the range of 78.9-84.2%.

The content of free acids is an important marker in determining the maturity of honey. Also, it reflects the changes during the storage period of honey (Ma et al., 2019, Raweh, 2022).

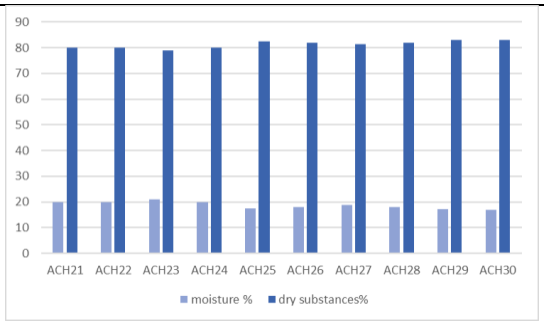
The acidity in the samples submitted for analysis is less than 50 units, In particular, the average rate in Imereti acacia honey samples is 15.45 mEq/kg, 18.22 mEq/kg in Adjara samples and It is relatively high in Guria samples at 20.2 mEq/kg.

The pH value in honey is not defined by the Codex Alimentarius Commission (Sakač, 2022), However, it is recommended to have a low level (that is, relatively high acidity), to prevent the development of microorganisms. Its rate is generally between 3.2 and 4.5. The pH value depends on many factors, including the pH of the nectar and soil (Acquarone et al., 2006). The pH value in the samples presented for analysis is almost similar. In particular, 3.64-4.14 in Imereti acacia honey, 3.58-4.25 in Guria samples, and 3.61-4.08 in Adjara samples. The electrical conductivity limit in samples of acacia honey is comparable (Stefan, B., 2009). Specifically, the average value of the minimum value for Imereti was 0.152 mls/cm, while the average value for the samples from the Adjara and Guria regions was 0.154 mls/cm. The maximum value is 0.29 mls/cm (average value) for Imereti, 0.285 mls/cm (average value) for Guria, and 0.282 mls/cm (average value) for Adjara honey.

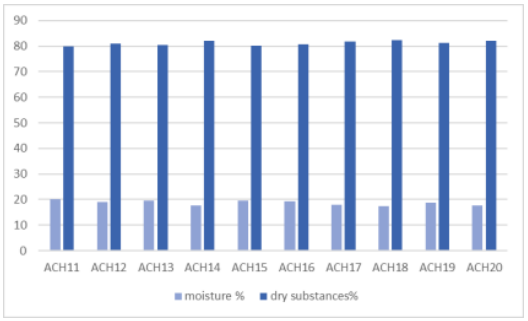
Figure 2. Acacia honey characteristics of water, dry substances – A: Acacia honey 1-10 Imereti, B: Acacia Honey 11-20 Guria, C: 21-30 Ajara and D: free acids



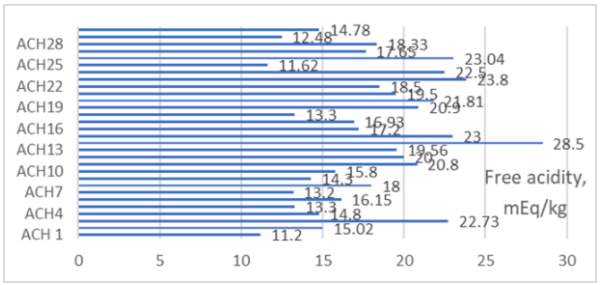
A



B



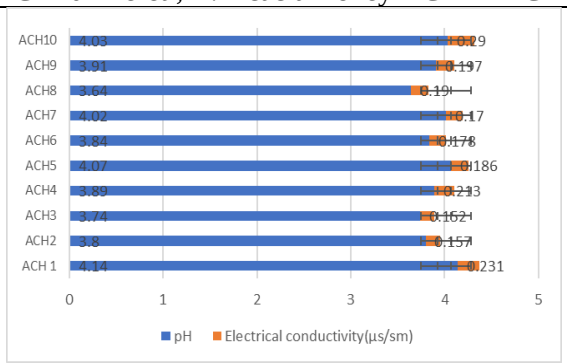
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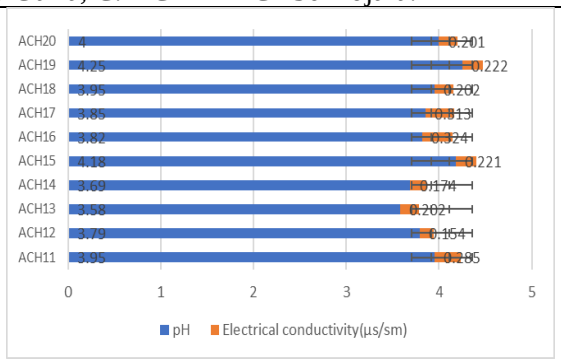
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Source: Authors (2024)

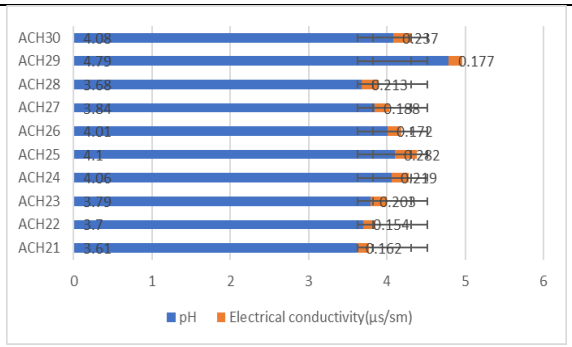
Figure 3. Acacia honey characteristics: pH and Electrical conductivity. A: Acacia honey ACH1-ACH10 Imereti, B: Acacia Honey ACH11-ACH20 Guria, C: ACH21-ACH30 Adjara.



A



B



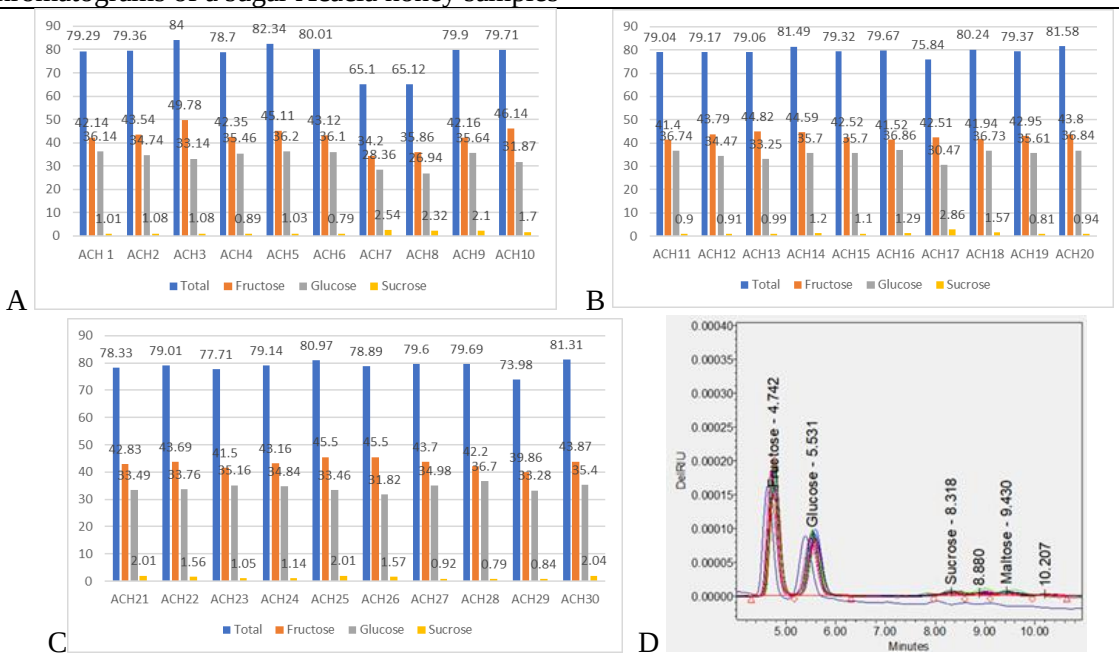
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Source: Authors (2024)

Samples of acacia honey were subjected to high-performance liquid chromatography (HPLC-Waters (RI detector Binary HPLC Pump 1525)), which detected fructose, glucose, and sucrose (Andrasi et al., 2023).

According to a comparison of the obtained results, fructose is the predominant carbohydrate in all 30 samples that were taken for analysis;

Figure 4. Quality and quantity of Acacia honey carbohydrates in Western Georgia A: Acacia honey ACH1-ACH10 Imereti, B: Acacia Honey ACH11-ACH20 Guria, C: ACH21-ACH30 Adjara and D-Chromatograms of a sugar Acacia honey samples



Source: Authors (2024)

Its amount in the samples from the Imereti region falls between 34.2-49.78% (average rate: 42.44%), in the samples from the Guria region, between 41.4–44, within 82% (average rate: 42.98%), and in the samples from the Adjara region, between 41.5–45.5% (average rate: 43.18%). Regarding the glucose content, the average value is 33.46% (ranging from 26.94 to 36.14%) in the honey from the Imereti region, 35.24% (ranging from 30.47% to 36.86%) in the samples from the Guria region, and 31.82% to 35.4% in the samples from the Adjara region.

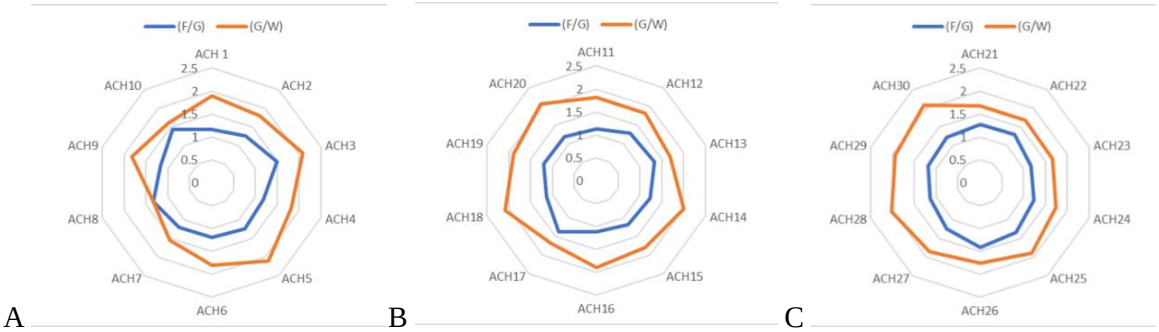
Since the technical rule on honey stipulates that the concentration of sucrose should not exceed 5%, the amount of sucrose in honey is a crucial metric in verifying the naturalness of honey. All three of the region samples have average sucrose concentration rates between 1.26 and 1.44, which is within normal ranges. The results show that the honey from the Adjara region has a relatively greater fructose content (based on the average index), whereas the samples from Guria have a higher glucose content.

The ratios of glucose to water and fructose to glucose are also significant indicators when assessing the quality of honey. When the fructose and glucose ratio is less than 1, honey crystallizes, indicating that

there is more glucose than fructose in the honey (Andrasi et al., 2023). A good characteristic of acacia honey is the average fructose/glucose ratio in the samples collected for analysis: 1.25 in Imereti honey, 1.22 in the second 10 samples (ACH11–ACH20), and 1.27 in the first 10 honey samples (Adjara region). Furthermore, the ratio of glucose and water is crucial. The ratio of glucose and water in the seven acacia honey samples (ACH3, ACH5, ACH14, ACH18, ACH20, ACH28, and ACH30) is between 2.03 and 2.11, indicating that the crystallization process is progressing. The ratio is less than 1.7 in three samples from the Imereti region (ACH7, ACH8, ACH10) and three samples from the Adjara region (ACH21, ACH22, ACH23), indicating a low or negligible possibility of crystallization. The ratios in the remaining honey samples range from 1.74 to 1.94 (Figure 5)

The most abundant amino acid in honey is proline, which is mostly found in the stamens of nectar. One crucial feature of honey is its proline level, which helps identify whether or not the honey is natural and hasn't been treated with sugar syrup. It should be mentioned that because bees are fed sugar syrup, which contains sodium chloride, contaminated honey has a higher concentration of sodium ions (Stefan, B., 2009).

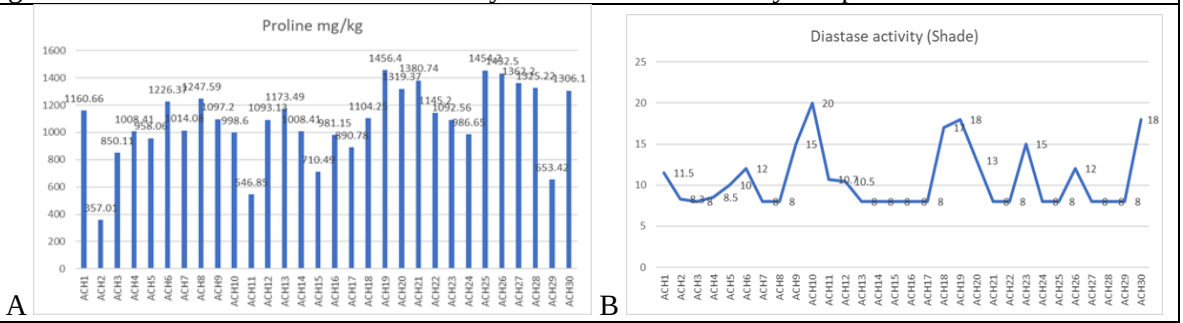
Figure 5. Glucose/Fructose and Glucose/Water ratios in Acacia honey samples A: Acacia honey 1-10 Imereti, B: Acacia Honey 11-20 Guria, C: 21-30 Adjara



Source: Authors (2024)

Both the flowery cover and the proline content differ between nations. In Imereti honey samples, proline concentration ranged from 357 to 1247 mg/kg (average value 991.8 mg/kg), in Guria honey, from 546 to 1456 mg/kg (average value 1028.43 mg/kg), and in Adjara honey, from 653 to 1380 mg/kg (average value 1213.88 mg/kg), according to a comparison of the obtained data. The Imereti region has the lowest proline content on average, followed by the regions of Guria and Adjara.

Figure 6. A: Proline and B: diastatic activity content in acacia honey samples



Source: Authors (2024)

The hydrolytic enzyme diastase (α- and β-amylases) is made by bees. It guarantees that honey and nectar mature. Diastatic activity is recognized as a crucial factor for evaluating honey quality and storage conditions by all current honey standards. High temperatures and improper storage conditions harm diastatic activity. Specifically, heating the honey during production processing is a very successful strategy for the entrepreneur as it guarantees the evaporation of excess water, which prevents the crystallization process and also kills microorganisms. Although this approach is financially advantageous, the quantity of enzymes is almost eliminated at this point, which causes the activity to drop (Stefan, B., 2009). For all varieties of honey, the diastase (amylase) activity should be at least 8 units, as determined by the shade scale. The analysis shows that the rate is less than 8 in 15 samples of

acacia honey (ACH 3, 7, 8, 13, 14, 15, 16, 17, 21, 22, 24, 25, 27, 28, 29). The Guria region's tenth sample had the highest rate of diastatic activity (20 units), followed by the twenty-third sample (15 units), the twenty-sixth sample (12 units), and the thirty-third sample (18 units). The average rate of diastatic activity in the remaining 15 samples was found to be 13.3 units, after accounting for rates higher than 8 units.

Since protein is mostly taken by bees and has a relatively low concentration in honey, the amount of protein in honey is determined by how long and how well bees process honey (Ma, 2019). The range of its concentration in the samples collected for analysis is 0.16–0.39%. Three samples from Guria (acacia honey 13 (0.37%), 17 (0.37%), and 18 (0.39%), one sample from Imereti (acacia honey 8) - 0.39%, and three samples from Adjara stand out with a relatively high rate.

The results show that the total phenol content of acacia honey ranges from 109 to 433 mg/kg. The average value of the honey samples from Imereti is 216.64 mg/kg (112 - 279 mg/kg), whereas the samples from Guria are 256.04 mg/kg (158.64-433 mg/kg) and the samples from Adjara are 176.05 mg/kg (109-259 73 mg/kg). Samples from Guria have a comparatively high phenol content. Antioxidant activity is hence high. Because only 50.55 mg of this sample was required for 50% suppression of the DPPH radical in the case of 433 mg/kg of phenols and 126.5 mg of honey sample in the case of 158.64 mg/kg of phenols, high antioxidant activity is expressed in numerically tiny amounts.

Discussion

The study's findings showed that more than 45% of the honey samples collected for examination included dominant acacia pollen, with the remaining secondary pollens being those from Liden, apple, raspberry, plum, oak, and leather. The physical and chemical properties of acacia honey do not differ by region. With the obtained results, the low water content in acacia honey samples provides honey's durability and resilience to enzymatic processes: water content - 16.90-20.47%, mass fraction of soluble components - 79.57-82.43 g/100 g. By the technical regulation on honey, the total amount of carbohydrates is 73.95-82.37%, which includes fructose (38.93-46.70%), glucose (29.74-36.13%), and sucrose (0.87-8.48%). Free acidity is limited to 50 milliequivalents (12.04-24.76 milliequivalents/kg), pH (3.61-3.16), and electrical conductivity (0.15-0.29 millisiemens/cm). The predominant sugar in samples of acacia honey is fructose. Its content is significant since the high fructose concentration of this honey contributes to its liquid structure. As a result, the ratios of glucose to water (1.57) and fructose to glucose (1.14–1.40) are high. Total phenols: 126.55-305.73 mg/kg; proline: 518.67-1361.00 mg/kg; protein: 0.207-0.397%; diastatic activity (per Shade scale): ≤8-18.67; antioxidant activity: 50% suppression of DPPH radical by mg sample: 65.24 - 179.11.

Conclusion

The research included a microscopic analysis of the pollens of smoked acacia honey in three regions of Western Georgia: Imereti, Guria, and Adjara; additionally, modern instrumental research methods were used to study the physico-chemical characteristics specified. Acacia honey's total phenolic content and antioxidant activity were also measured. Based on the findings, this study will enable us to regulate the production of acacia honey in Georgia's western region.

Additionally, the obtained results will make the physical and chemical properties of this highly popular honey known to the scientific community and consumers.

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