ABSTRACT
thesis for the degree of Doctor of Philosophy (PhD) on specialty
"8D05301 – Chemistry"
Ongalbek Damira
"Chemical analysis and bioactivity of bee products of Kazakhstan"

General description of the research work. The dissertation is dedicated to identifying the chemical and biological properties of honey and propolis to develop a scientific basis for their rational use and protection against adulteration. Four types of honey and three samples of propolis, which are economically important beekeeping products, were studied herein. First, microscopic pollen analyses were conducted to discriminate monofloral honey samples. Secondly, all samples were subjected to physicochemical analyses suggested by the International Honey Commission. The samples meet food codex standards, including the EU Council Directive (2002) and the Turkish Honey Communique (2020) were taken into consideration. Then, the phenolic composition and aroma compounds of all honey and propolis samples were investigated using modern analytical tools such as HPLC-DAD and GC-MS. The *in vitro* biological activities of honey and propolis samples were also determined spectrophotometrically. The data obtained were analyzed using chemometric techniques, namely, Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA), for classification and identification of biomarkers of the honey samples.

Relevance of the study. Kazakhstan has a rich flora in which various types of honey are produced. There is a growing interest in Kazakh products in many countries, including Japan, the United Arab Emirates, European countries, and the United States of America. However, the varieties of honey produced in different regions of Kazakhstan have not yet been thoroughly studied to establish their unique identity. Assigning a distinct identity to Kazakh beekeeping products will enable them to enter the global market confidently. Moreover, increasing attention is paid to using natural bioactive compounds in supportive medicine.

Analysis of scientific sources shows that research by European scientists such as F. Pasini, S. Gardini, and G. Marcazzan from the University of Bologna, as well as Chinese specialists M. Caboni and Q. Zhou, revealed the presence of *p*-coumaric and *p*-hydroxybenzoic acids in buckwheat honey. In Bulgaria, a research group led by V. Bankova, B. Trusheva, and M. Popova discovered over 300 components in beekeeping products. In Turkey, a team from Mugla University, headed by M. Ozturk, Y. Sicak, and A. Yaglioglu, found high salicylic, hydroxybenzoic, and fumaric acid levels in Turkish thyme honey.

In Spain, L. Castro-Vazquez and his colleagues found compounds such as *β*-damascenone, *cis*- and *trans*-linalool oxides, benzyl and phenylethyl alcohols, as well as *α*-, *β*-, and *γ*-eudesmol in beekeeping products. In Kazakhstan, M. Mamedova and M. Alimzhanova studied the compositions of nine polyfloral and monofloral honey varieties produced in the country and identified their aroma compounds.

Kazakhstan is rich in various types of honey and needs detailed research. There is insufficient data to differentiate monofloral honey based on its origins, such as melissopalynological analysis and thorough identification of its organic components. In this regard, the present study aims to characterize the phenolic and aroma compounds of four monofloral honey varieties - *Helianthus, Fagopyrum, Agrimonia,* and *Tamarix* using high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS), and to identify the dominant biomarkers of each monofloral honey through chemometric analysis of the data obtained.

This dissertation focuses on identifying unique biomarkers of each monofloral honey, which can be used to detect possible adulteration based on organic compounds. The fact that giving each product an identity, a better understanding of bee products' properties, and studying a comprehensive chemical composition of Kazakh beekeeping products will make this work particularly important and may enable it to be used more effectively in various fields.

The purpose of the research work: Conducting chemical analysis and evaluating the bioactivities of beekeeping products produced in Kazakhstan

Research objectives:

1. Conduct a physicochemical analysis to identify promising objects from beekeeping products (*Fagopyrum sp., Tamarix sp., Helianthus sp., Agrimonia sp.* honey, propolis) harvested in the Republic of Kazakhstan.
2. Obtain extracts and establish biomarker phenolic compounds from honey and propolis products by using HPLC.
3. Determine volatile compounds contained in beekeeping products by GC-MS.
4. Conduct a biological screening of the studied objects: buckwheat, tamarix, sunflower, agrimonia honey (*Fagopyrum sp., Tamarix sp., Helianthus sp., Agrimonia sp*.) and propolis.

Object of study: Beekeeping products collected from different regions of the Republic of Kazakhstan *Fagopyrum sp., Tamarix sp., Helianthus sp., Agrimonia sp.* samples of 4 types of honey, 3 propolis.

Research methods: refractometry, conductometry, pH-metry, titrimetry, UV spectrophotometry, chromatography, HPLC, GC-MS, headspace solid-phase microextraction, spectrophotometry.

Main provisions submitted for defense:

1. The studied samples of buckwheat honey extracts contain fumaric, *p*-hydroxybenzoic, *p*-coumaric, *trans*-2-hydroxycinnamic acids and chrysin, which exhibit antioxidant activity.
2. Among the studied samples, the highest amount of terpenes (up to 69.95%) is contain in sunflower honey samples.
3. Buckwheat honey *(Fagopyrum sp.)* shows the highest antioxidant activity (IC_{50}: from 8.65±0.50 to 17.23±0.96 mg/mL) compared to agrimonia *(Agrimonia sp.)*, tamarix *(Tamarix sp.)*, sunflower *(Helianthus sp.)* honey.

Main results of the research work:

1. All 54 samples were analyzed using physicochemical methods recommended by the International Honey Commission. The studied samples meet the standards requirements and have commercial value.

2. Phenolic compounds were identified in the composition of ethyl acetate extracts by HPLC - gallic acid (T3 in tamarix honey 1075.0 mg/kg; A7 in agrimonia honey 908.7 mg/kg), *p*-hydroxybenzoic acid (KB14 in buckwheat honey 243.0 mg/kg), trans-2-hydroxycinnamic acid (S6 in sunflower honey 47.4 mg/kg). Ethanol extract of propolis showed high results for the presence of naringenin: Pro-1 45.99 mg/g, Pro-2 22.83 mg/g, Pro-3 13.01 mg/g; hisperitin: Pro-1 9.46 mg/g, Pro-2 3.29 mg/g; cynarin: Pro-1 6.52 mg/g, Pro-2 21.12 mg/g, Pro-3 7.35 mg/g, *p*-coumaric acid Pro-3 18.65 mg/g; caffeic acid: Pro-2 9.61 mg/g, Pro-1 7.70 mg/g.

3. A statistical analysis of volatile compounds, which are the predominant components of sunflower honey - hotrienol, cosmen, α-pinene, and carvacrol, was carried out; buckwheat honey - nonanol, benzaldehyde, octanol, dehydro-p-cymene, and β-linalool; Tamarix honey - β-citral, syringaldehyde A, limonene, β-damascenone and linalool oxide; agrimonia honey - dehydro-*p*-cymene, β-linalool, 2-nonen-1-ol and linalool oxide.

4. The biological activity of the studied honey and propolis products has been proven: all samples of buckwheat honey showed high antioxidant activity. Tamarix honey T2 (*Tamarix sp*.) inhibits urease and acetylcholinesterase. Propolis samples also give high antioxidant activity results.

Novelty and principal results of scientific work:

1. The physicochemical properties of 4 types of monofloral honey from Kazakhstan were analyzed according to international standards for the first time.

2. A comprehensive analysis of phenolic components was carried out, and the taxonomic compounds were identified for honey varieties *Agrimonia sp., Fagopyrum sp., Tamarix sp.,* and *Helianthus sp.* for the first time.

3. The composition of volatile compounds in Kazakh honey types from *Tamarix* sp., *Helianthus* sp., *Agrimonia* sp., and *Fagopyrum* sp. was determined by using HS-SPME-GC/MS and followed by statistical analysis for the first time.

4. The results of the biological screening obtained of the bee product were confirmed by tests for antioxidant and enzymatic activities.

Theoretical significance of the work: The studied beekeeping products play an important role in treating human health in medicine, pharmaceuticals, apitherapy, and dentistry and arouse interest in scientific research.

Practical significance of the study: Due to the comparative analysis of different varieties of honey collected from various regions of Kazakhstan, as well as according to the chemical composition studied by HPLC, HS-SPME-GC/MS, they can be used in apitherapy and the development of effective domestic drugs with pharmaceutical activity.

Compliance with directions of scientific development or government programs (projects)

The dissertation work was carried out and continued based on the program to support invited scientists, grant TUBITAK No. 2221/2018/2 “Determination of

physicochemical properties, bioactivity and chemotaxonomic markers of the commercial potential of Kazakhstan honey" 2019-2020.

The validity and reliability of the results obtained in the study are supported using selective, accurate, and modern analysis methods as well as the scientific method. To ensure reliability and repeatability, all experiments were performed several times.

Approbation of practical results of work

The results obtained in the dissertation work were reported and discussed at the 2nd International Scientific Conference on Food Chemistry, Kemer-Antalya, Turkey, March 16-19, 2023; at the International Scientific Conference 12th Aegean Analytical Chemistry Days (AACD 2023) Istanbul, Turkey, October 19-22, 2023, at the 35th National Chemistry Congress, Diyarbakir, Turkey, September 9-12, 2024.

Publications. Based on the dissertation research results, 3 articles and 3 theses were published.

Scientific publications with a high impact factor included in the international database Scopus and Web of Science

1. Rajib Hossain, Cristina Quispe, Rasel Ahmed Khan, Abu Saim Mohammad Saikat, Pranta Ray, **Damira Ongalbek**, Balakyz Yeskaliyeva and et al. Propolis: An update on its chemistry and pharmacological applications //Chinese Medicine (2022)17:100, 1-60, IF: 4.50, (Q1 Web of Science and 78% Scopus) https://doi.org/10.1186/s13020-022-00651-2

2. **Damira Ongalbek,** Özge Tokul-Ölmez, Bihter Şahin, Selçuk Küçükaydın, Fatma Aydoğmuş-Öztürk, Yusuf Sıcak, Balakyz Yeskaliyeva, Mehmet Öztürk Classification of buckwheat honey produced in Kazakhstan according to their chemical ingredients and bioactivities by chemometric approach //Food chemistry (2024), (Q1 Web of Science and 94% Scopus) https://doi.org/10.1016/j.foodchem.2024.139409

3. **Damira Ongalbek**, Bihter Şahin, Talshyn Berdesh, Meltem Taş-Küçükaydın, Özge Tokul-Ölmez, Balakyz Yeskaliyeva, Mehmet Öztürk Biomarker Aroma Compounds of Monofloral Honey Samples from Kazakhstan by Gas Chromatography-Mass Spectrometry (GC/MS) and Chemometric Analysis //Analytical letters (2024), (Q3 Web of Science and Scopus 54%) https://doi.org/10.1080/00032719.2024.2345751

Publications in collections of abstracts of reports based on the results of International scientific conferences

1. **Ongalbek D.,** Cakir C., Çam D., Berdesh T., Karatas S., Yeskaliyeva B. and Öztürk M. The Aroma Compounds of Vitex, Helianthus, and Tamarix Honey Collected from Kazakhstan // 2nd. Food Chemistry Congress 16-19 March 2023, Kemer-Antalya, Türkiye http://doi.org/10.25135/rfac.2023.2nd.OP22.

2. **Damira Ongalbek,** Talsyn Berdesh, Meltem Taş-Küçükaydın, Özge Tokul-Ölmez, Bihter Şahin, Balakyz Yeskaliyeva, Mehmet Öztürk Chemometric studies on the aroma compounds of honey samples produced in Kazakhstan //12th Aegean Analytical Chemistry Congress, October 19-22, 2023, Istanbul, Türkiye, https://aacd2023.yildiz.edu.tr/.

3. Özge Tokul-Ölmez, **Damira Ongalbek,** Bihter Şahin, Balakyz Yeskaliyeva, Mehmet Öztürk. Kazakistan'ın Çeşitli Bölgelerinde Üretilen Tamarix ve Agrimonia Ballarının Biyoaktivite ve Kimyasal İçeriklerinin Araştırılması ve Kemometrik

Analizleri //35th National Chemistry Congress 9-12, 2024, September, Diyarbakir, Turkey, https://kimya2024.com/en/konu-basliklari/#

Individual contribution of the doctoral student to the preparation of each article. Independent planning and execution of the experimental part of the research, as well as interpretation and processing of the obtained data, while the author collaborated with scientific supervisors on problem-solving and discussing research results.

1. Propolis: An update on its chemistry and pharmacological applications //Chinese Medicine – a review article published (involved in preparing the literature analysis and gathering empirical data).

2. Classification of buckwheat honey produced in Kazakhstan according to their chemical ingredients and bioactivities by chemometric approach //Food Chemistry (participated directly in collecting experimental data, processing, and interpreting the experimental results for article preparation).

3. Biomarker Aroma Compounds of Monofloral Honey Samples from Kazakhstan by Gas Chromatography-Mass Spectrometry (GC/MS) and Chemometric Analysis // Analytical Letters (contributed to conducting experiments, processing, and interpreting the results, and preparing scientific publications).

4. Materials from reports of scientific conferences and symposia (collection and description of experimental data, discussion of analysis results, and preparation of materials).