Summary

As defined in

Art. 16 section 16 of Law no. 2 (in the English language)

Dr. Izabela Jasicka-Misiak

Department of Analytical and Ecological Chemistry

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1. Diplomas and degrees

1995	Master of Science in chemistry, major agrobiochemistry, the University of Opole. Master's thesis entitled *Wpływ różnego sposobu uprawy marchwi na występowanie szkodliwej i pożytecznej entomofauny* [The influence of various methods of carrot farming on the occurrence of beneficial and harmful entomofauna], Supervisor: dr hab. Kazimierz Wiech
2005	PhD in chemical science, the University of Opole. PhD thesis entitled *Allelochemia marchwi jadalnej Daucus carota L.* [Allelochemistry of carrot *Daucus carota* L.], Supervisor: prof. dr hab. inż. Paweł Kafarski

2. Employment in research institutions

1995-2005	assistant lecturer, Institute of Chemistry, Faculty of Mathematics, Physics and Chemistry, the University of Opole
2005-2008	assistant professor, Institute of Chemistry, Faculty of Mathematics, Physics and Chemistry, the University of Opole
2008-present	assistant professor, Faculty of Chemistry, the University of Opole

3. Selected achievements pursuant to article 16 paragraph 2 of the Act of 14 March 2003 on Academic Degrees and Title as well as on Degrees and Title in the Field of Arts (Dz. U. [Journal of Laws] of 2016 item 882, as amended in Dz. U. of 2016 item 1311):

a. Title of scientific achievement:

Authentication of nectar honeys

List of publications (author/authors, title/titles of the publication, year of publication, name of publisher)

H1 **I. Jasicka-Misiak**[*], P. Kafarski,
Chemiczne markery miodów odmianowych, **2011,**
Wiadomości Chemiczne, *65*, 821.
Overview paper. My contribution to the paper consisted of writing a part of the manuscript. I estimate my contribution as 70%.

IF = 0; MNiSW = 7

H2 **I. Jasicka-Misiak**[*], A. Poliwoda, M. Dereń, P. Kafarski, **2012,**
Phenolic compounds and abscisic acid as potential markers for the floral origin of two Polish unifloral honeys, *Food Chemistry*, 31, 1149-1156; Elsevier.

My contribution to the paper consisted of planning experiments, participation in conducting analyses and writing the manuscript. I estimate my contribution as 70%.

IF = 3,334; $IF_{5\ years}$ = 4,072; MNiSW = 40

H3 Ł. Zieliński*, S. Deja, **I. Jasicka-Misiak,** P. Kafarski, **2014,**
Chemometrics as a tool of origin determination of Polish monofloral and multifloral honeys, *Journal of Agricultural and Food Chemistry,* 62, 2973-2981; ACS**.**
My contribution to the paper consisted of planning of part of the work and participation in interpretation of NMR spectra prepared by Łukasz Zieliński as a part of his PhD thesis, of which I was an additional supervisor. I estimate my contribution as 30%.

IF = 2,912; $IF_{5\ years}$ = 3,154; MNiSW = 45

H4 **I. Jasicka-Misiak*,** E. Makowicz, N. Stanek, **2017**,
Polish yellow sweet clover (*Melilotus officinalis* L.) honey, chromatographic fingerprints, and chemical markers, *Molecules*, 22 (1), 138; MDPI.
My contribution to the paper consisted of conceptualising the work, participating in conducting chromatography analyses, interpreting test results and writing the manuscript. I estimate my contribution as 60%.

IF = 2,465; $IF_{5\ years}$ = 2,988; MNiSW = 30

H5 **I. Jasicka-Misiak***, S. Gruyaert, A. Poliwoda, P. Kafarski, **2017**,
Chemical profiling of polyfloral Belgian honey: ellagic acid and pinocembrin as antioxidants and chemical markers, *Journal of Chemistry*, 2017, Article ID 5393158, https://doi.org/10.1155/2017/5393158; Hindawi.
My contribution to the paper consisted of planning experiments, choosing appropriate methodology, supervising and participating in chromatography analyses conducted by Steven Gruyaert as his Master's thesis, of which I was the supervisor. Moreover, I gave advice on the interpretation of the research and wrote the manuscript. I estimate my contribution as 70%.

IF = 1,3; MNiSW = 20

H6 **I. Jasicka-Misiak***, E. Makowicz, N. Stanek, **2018**,
Chromatographic fingerprint, antioxidant activity and colour characteristic of Polish goldenrod (*Solidago virgaurea* L.) honey and flower. *European Food Research and Technology*, 244(7), 1169–1184; Springer.
My contribution to the paper consisted of planning experiments, supervising and participating in chromatography analyses conducted by doctoral students Ewa Makowicz and Natalia Stanek, of whom I am a scientific/technical supervisor; interpretation of the research results and writing the manuscript. Moreover, I adjusted the paper after its reviews. I estimate my contribution as 60%.

IF = 1,664; $IF_{5\ years}$ = 1,854; MNiSW = 25

H7 N. Stanek, **I. Jasicka-Misiak***, **2018**,
HPTLC phenolic profiles as useful tools for the authentication of honey, *Food Analytical Methods*, doi: 10.1007/s12161-018-1281-3, Springer.
My contribution to the paper consisted of conceptualising the research, advising on and participating in chromatography analyses conducted by doctoral student Natalia Stanek, of whom I am a scientific/technical supervisor; interpretation of the research results and writing the manuscript. Moreover, I adjusted the paper after its reviews. I estimate my contribution as 70%.

IF = 2,038; $IF_{5\ years}$ = 1,982; MNiSW = 30

H8 E. Makowicz, **I. Jasicka-Misiak***, D. Teper, P. Kafarski, **2018**,
HPTLC fingerprinting as an efficient method for the differentiation of honeys of different botanical origin based on the composition of the volatile fractions, *Molecules*, 23, 1811; DOI 10.3390/molecules23071811, MDPI.
My contribution to the paper consisted of planning experiments, participating in chromatography analyses conducted by doctoral student Ewa Makowicz as her doctoral thesis, of which I was an additional supervisor; writing the manuscript and adjusting the paper after its reviews. I estimate my contribution as 50%.

IF = 3,098; $IF_{5\ years}$ = 3,268; MNiSW = 30

H9 E. Makowicz, P. Kafarski, **I. Jasicka-Misiak***, **2018**,
Chromatographic fingerprint of volatile fraction of rare *Hedera helix* honey and biomarkers identification, *European Food Research and Technology*, DOI 10.1007/s00217-018-3127-z, Springer.
My contribution to the paper consisted of planning experiments, supervising and participating in chromatography analyses conducted by doctoral student Ewa Makowicz, of whom I am a scientific/technical supervisor; interpretation of the research results and writing a part of the manuscript. I estimate my contribution as 60%.

IF = 1,919; $IF_{5\ years}$ = 1,854; MNiSW = 25

*- correspondence author

Impact Factor (IF) of the pieces of research making up the achievement, calculated based on the database of Journal Citation Reports as for the year of publishing amounts to: **18.73** considering their 58 citations

Total points awarded by the **Polish Ministry of Science and Higher Education** (MNiSW) for the series of publications – **252**

c. Overview of the scientific objective of the above-mentioned research papers and their possible use

INTRODUCTION

Honey is a natural sweet substance produced by honey bees (*Apis mellifera* L.) from floral nectar or honeydew, which occurs on various plant species. Bees collect forage with their tongue and to it they add secretions of their salivary glands. Upon their return to the hive, they pass it to the younger bees, which suck it into their honey stomach and subsequently move the liquid back onto their tongues. They repeat this action several times. They place the nectar mixed with saliva in one of the lower cells of the honeycomb, where the water partially evaporates. After some time, honey, already thickened to a certain degree, is transported to the upper part of the honeycomb. When the cells of the honeycomb are filled, there occurs honey ripening, during which the water evaporates further. The process of evaporation takes place due to the air current generated by bees' wings. The evaporation of water prevents the sweet product from fermenting. Once a cell is filled with fully ripe honey, bees seal it with wax capping [1, 2]. The resulting honey is a product of variable chemical composition shaped by many factors, for example, by its botanical and geographic origin, abiotic conditions, extraction techniques (spinning, filtering), packaging and storage conditions.

Currently, natural products are popular due to their richness in biologically active compounds. For centuries, natural food products have been known for their health benefits, such as anti-bacterial, anti-fungal and anti-cancer properties. Today, they are gaining popularity again, their beneficial effects having been scientifically proven.

For thousands of years, natural bee honey has been valued worldwide as a nutritional component, an ingredient of natural cosmetics and a therapeutic product [3-5]. Honey is commonly used in folk and alternative medicine; however, the market also offers new preparations and bandages containing for example New Zealand manuka honey, used during hospital treatment as well.

The development of apitherapy and growing consumer awareness contribute – also in Poland – to an increase of popularity of honey as a part of everyday diet, mostly in the case of varietal honeys (nectar and unifloral). Polish honey keepers are known for producing good quality honey, hence the popularity of Polish varietal honeys is growing not only in Poland, but also in other European Union countries. The mean yield of honey of the years 2009-2016 amounted in Poland to 19 thousands of tonnes, and in 2017, it was the highest so far, amounting to 24.3 thousands of tonnes (according to Institute of Agricultural and Food Economics and Bee Research Department of Institute of Gardening in Puławy). Despite the high national production, on the Polish market, there can still be found cheap honeys of inferior quality. Polish trade companies import honey from China, Latin America and Eastern Europe. Such honey can be offered for competitive prices, but fails to fulfil the requirements of good quality honey when it comes to ingredients and properties. Selling the imported

honey of inferior quality for lower prices (often disguising it as domestic honey), the companies mislead the consumer and practice unfair competition. Undoubtedly then, bee honey should meet strictly defined requirements when it comes to its organoleptic as well as physical and chemical properties, just like certain other food products. Before the accession of Poland into the European Union structures, the requirements for bee honey were addressed by Polish Standard PN-88/A-77626 *Miód pszczeli* [Bee honey] [6]. Afterwards, the requirements were specified in Polish and international standards, of which the most important is the Global Standard drawn up and validated in 2001 by the *Codex Alimentarius* Commission (*Draft revised standard for honey 2001*) [7], and Directive 2001/110 EU as amended [8-10]. EU Member States are required to accept the Directive and bring into force any essential laws, regulations and administration provisions necessary to comply with it. The documents are periodically updated, taking into account new research methods and, what follows, new quality requirements. There are still considerable differences between European legislation and the changed standards of *Codex Alimentarius*, when it comes to declaring geographic origin of the product, assessment of its quality expressed by an indicator of diastase activity and the definition of industrial honey.

The Polish standard recognises three types of honey: floral honey, which is produced from plant nectar secreted by flower or extra-flower nectars; honeydew honey, produced from honeydew collected from plant shoots, and floral/honeydew honey produced by the bees partially from nectar and partially from honeydew. The Polish Standard distinguishes, moreover, the following varieties of honey: floral rapeseed, floral acacia, floral linden, floral buckwheat, floral heather, multifloral, floral/honeydew, coniferous honeydew honey and deciduous honeydew honey. Each honey includes evidence of its botanical and geographic origin in the form of hundreds of thousands of pollen grains suspended in it – the remains of plants of whose nectar the honey was made. Varietal honey is one whose level of dominant pollen is equal or larger than its minimal proportion specified by the Polish Standard for the particular varietal honey. The classification of honey as varietal honey is based on pollen analysis, which is a common test employed in order to determine botanical origin of honeys, i.e., the species of plants of whose nectar the honey was made. The test method consists of qualitative and quantitative microscopic assessment of the pollen grains contained in a honey sample (Photo. 1).

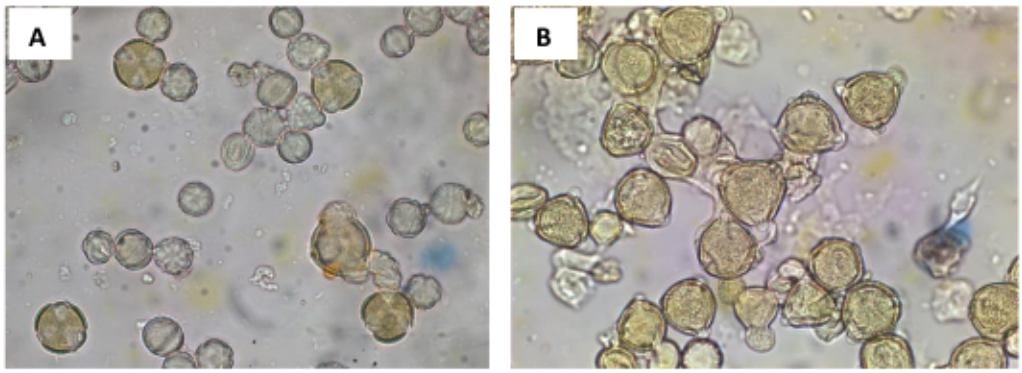

Photo. 1. Pollen image of Phacelia honey (A) and Ivy honey (B); (author: D. Teper).

The minimal percentages of the dominant pollen for five varieties of the most popular Polish honeys are specified in the Polish Standard *Miód pszczeli* [Bee honey] (PN–88/A–77626) (Table 1). Table 1 also includes relevant data concerning several other European countries. Taking into consideration that up till now about 100 (floral) varietal honeys have been described in Europe, the legislative data is not optimistic [11].

Table 1. Regulations concerning dominant pollen content in Polish honeys (PN–88/A–77626) and in honeys produced in selected European countries [11].

Pollen grains	**Country**					
	Poland	Croatia	Greece	Germany	Italy	Serbia
Fagopyrum esculentum (buckwheat)	**45**	-	-	-	-	-
Citrus spp. (citrus)	**-**	10	3	20	10	-
Brassica napus (rape)	**45**	60	-	80	-	-
Calluna vulgaris (heather)	**45**	20	-	-	-	20
Erica spp. (heath)	**-**	-	45	45	-	-
Castanea sativa (chestnut)	**-**	85	87	90	-	85
Robinia pseudoacacia (black locust)	**30**	20	-	-	-	20
Lavandula spp. (lavender)	**-**	10	-	-	-	-
Tilia *spp.* (linden)	**20**	25	-	20	-	25
Phacelia tanacetifolia (phacelia)	**-**	60	-	-	-	-

Botanical and geographical origin of honeys can be identified with the use of physical, chemical and biological methods, consisting of measuring, e.g. moisture level, ash content,

electrical conductivity, acidity and enzymatic activity [12, 13]. Specific conductivity of floral and floral/honeydew honeys ranges from 0.45-0.8 mS/cm. Honeydew honeys are characterised by a higher level of conductivity, which amounts to over 0.8 mS/cm and in the case of coniferous honeydew – over 1.2 mS/cm. In the case of honeys produced from strawberry tree (*Arbutus unedo* L.), Erica, linden (Tilia spp.), common heather (*Calluna vulgaris* L. *Hull*) or tea tree (Melaleuca spp.) specific conductivity is not indicated [12, 14].

The aforementioned methods are subject to limits concerning the extraction technique employed by the bee keepers as well as the procedures of sample preparation for particular analyses, and are unfortunately insufficient for the aim of unequivocally identifying botanical and geographic origin of honey. Doubtlessly, physical and chemical properties of honey and other apiculture products need to be precisely specified. Considering honey's valuable nutritional and therapeutic properties, the procedure of authentication of honey is critical. The research I have carried out contributes to the search of a precise system of determining the origin and assessing the quality of honey, which would be capable of specifying the quality and content of substances characteristic to the particular honey variety (markers).

PHYSIO-CHEMICAL PROPERTIES AND CHEMICAL COMPOSITION OF HONEY

Ripe honey is a thick, hygroscopic liquid of the specific weight between 1.38 and 1.45 g/cm^3. Honeys are liquid in consistency, sticky and can be partially or fully crystallised (set honey). Individual varieties of honey can differ in colour, taste, smell and texture. The colour of floral honeys varies from white (linden or rapeseed) to dark amber (heather or buckwheat). They are usually very fragrant, its aroma being similar to that of nectar. Honeydew honeys are dark with a possible green or grey tone, and have an intense smell which brings to mind resin or conifers. The shade of honey is largely dependent on plant pigments contained in the nectar, mainly carotenoids and flavonoids, which contribute to the antioxidant properties of honey.

Pursuant to applicable European Union Directives [7-10], water content in ripe honey should not exceed 20%, with the exception of heather honey where the maximum water content is specified as 23%. The water content of most honeys is between 17 and 18%. It depends on many factors, such as the level of ripeness, season and time of harvesting, as well as climatic conditions [17]. Sugars are the most numerous group of chemical compounds contained in honey (77% on average). Carbohydrate content in Polish floral and honeydew honeys ranges between 68 and 78%, of which monosaccharides constitute the main part. The average content of glucose amounts to 30%, and fructose to 38%. Among disaccharides the most pervasive one is sucrose. The content of sucrose in varietal honeys ranges from 0.8% (heather or buckwheat honey) to 7.7% (acacia honey). The content of another saccharide, melitose, constitutes around 5.4% of honey. Honeydew honeys additionally consist of melezitose, a trisaccharide whose content in this type of honey can be as high as 28%. In different types and varieties of honey there have been found 22 other sugars, such as melibiose, trehalose, isomaltose or gentiobiose [18, 19]. Another compound

particular to honey is 5-(hydroxymethyl)furfural which is formed by the breakdown of monosaccharides (mainly fructose) into acids. The level of this compound increases as the honey ages as well as during heating. An important factor, which influences the value and taste of honey and contributes to its ripeness, is the content of organic acids. The most pervading are the acids: gluconic, malic, citric, lactic, succinic, tartaric, oxalic, butyric, propionic, formic and acetic. There can be found over 15 more acids in honey, such as benzoic and pyruvic acids. Thus, regardless of its geographic origin, honey has an acidic reaction. For example, the pH reaction of Polish and Spanish honeys ranges from 3.6 to 5.0, Indian from 3.7 to 4.4, while the acidity of Algerian honeys ranges from 3.4 to 4.5 [22]. The most acidic are buckwheat honeys, the least acidic goldenrod, acacia and rapeseed honeys.

The content of nitrogenous compounds in honey is low, about 0.6%, whereas the protein content is not higher than 0.5%. Albumins and globulins are of the highest concentration. Although they occur in very slight concentrations, enzymes constitute an important group of proteins. They are mostly generated in bees' salivary glands, but some may also originate from flower pollen grains or from the honeydew. In honey, there have been observed enzymes such as α- and β-amylase, catalase, invertase, maltase, glucose oxidase, phosphatase and lysozyme [12]. α-Amylase (diastase) is an enzyme responsible for the process of complex sugar hydrolysis. Schade scale, which specifies the activity of diastase, has been devised in order to detect overheating or adulteration of honeys or other unlawful procedures [21]. Honey also consists of small amounts of amino acids (up to 0.03%). In different varieties of honey have been identified from 11 to 21 (heather honey) amino acids. The content of minerals in honeys is low and depends on its variety. In floral honeys the mineral content is from 0.05 to 0.5%, whereas honeydew honeys the mineral content is about 1%. Surprisingly low is the content of vitamins in honey, which is variable and depends on the content of floral pollen and royal jelly. They are mostly B-group vitamins: thiamine (B_1), riboflavin (B_2), nicotinic acid (B_3), pantothenic acid (B_5), pyridoxine (B_6), biotin (B_8 or H) folate (B_9), and vitamin C. These vitamins occurring in honey remain stable due to honey's low pH level [15].

In different types and varieties of honey have been identified over 600 components belonging to about a dozen chemical groups. Most of them occurs in low concentrations [15]. One of the most interesting chemical compound groups that can be found in honey are those which make up the volatile fraction and are responsible for the flavour and aroma of honey. These compounds might originate directly from the honey plant's nectar or may be a product of enzymatic transformations taking place in bees' organisms. Up till now, over 50 substances contributing to honey's aroma have been marked, the most important of them being monoterpenes, sesquiterpenes, higher aliphatic alcohols, aldehydes, ketones, esters and polyphenol compounds. Chemical content of honey changes during the ripening process. There are many factors which influence the changes in volatile substances content, such as processing method (spinning, decrystallisation, i.e. liquefying honey in high temperature) and storage conditions (Fig. 1.) [15, 24, 25].

A following group of compounds occurring in honey are phenolic compounds. They are responsible for the shade of honey as well as for its antioxidant properties. This numerous heterogeneous group consist of about 10 000 of compounds, which are synthetised in plant tissues. They are divided into two classes: phenolic acids and flavonoids (flavones, flavonols, flavanones, flavanols, anthocyanidins, isoflavones and chalcones) [26]. Phenolic acids, mostly derivatives of hydroxybenzoic and hydroxycinnamic acids, occur in nearly all plant organs: seeds, roots, leaves, bark and flowers. These compounds protect the plants from microorganisms and insects and in connection with polysaccharides, stiffen the cell walls [27]. When honeybees collect nectar, they may transfer these bioactive compounds occurring in nectar, into the honey [28]. The phenolic acids and flavonoids occur in honeys in the proportion of 0.1 to several dozen mg for 100 g of honey. The proportion of phenolic compounds depends on the variety of honey. Results of research on the composition of the phenolic fraction of honey show that the compounds occurring in the fraction most often are phenolic acids, such as vanillic acid, caffeic acid, 3-hydroxybenzoic acid, chlorogenic acid, 4-hydroxybenzoic acid, rosmarinic acid, gallic acid, syringic acid, p-coumaric acid, ferulic acid and ellagic acid, as well as flavonoids: quercetin, kemferol, myricetin, pinobanksin, pinocembrin, chrysin, galangine and hesperetin. Flavonoids significantly contribute to honey's antioxidant activity and are beneficial for human health [29, 30]. Antioxidant activity of flavonoids in most cases depends on the number and position of hydroxyl groups and other substituents, as well as on molecular glycosylation. The occurrence of hydroxyl groups at specific positions of the ring in flavonoids enhances their antioxidant activity, whereas the glycosylation of flavonoids lowers the antioxidant activity in comparison to the corresponding aglycones [31].

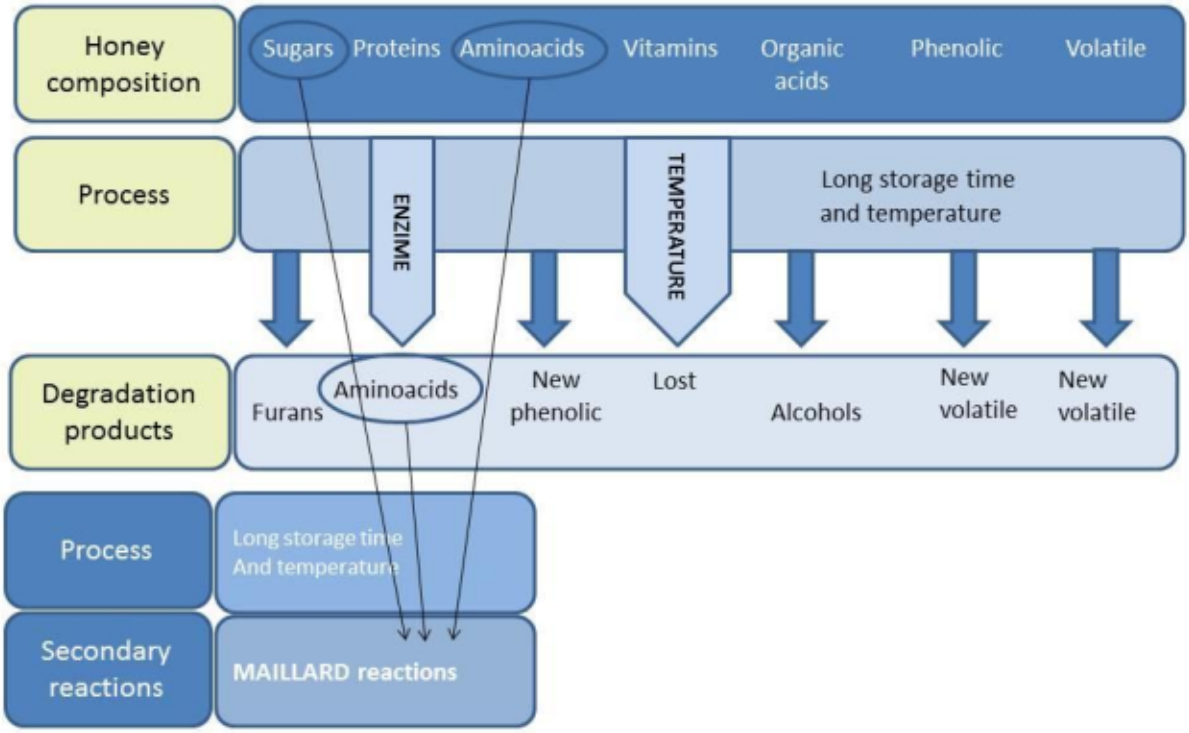

Fig. 1. Compounds occurring in honey and processes influencing the stability of honey [15].

AUTHENTIFICATION OF HONEY – RESEARCH OBJECTIVE

Today, authentication of food products constitutes a significant challenge for global food production, it ensures, however, increased quality. When choosing food products, consumers consider the information on its quality. The choice of food products can depend on one's health, food habits, lifestyle and religion. The labelling of food products should, hence be fair and precise [15, 25, 32].

Natural honey is a product that has been commonly adulterated worldwide. The most common method is adding sucrose, which can take place already before the honey is produced by the bees – by feeding them with sugar. The excess sucrose is not processed by bees; therefore sucrose content above 5% can be considered adulteration [33]. Another common method of adulterating honey is adding to it an amount of highly sweet glucose-fructose syrup, produced from starch, cane or beet sugar [34]. Moreover, it should be considered fraud to give the product a misleading name, suggesting that the honey is produced from a specific plant, when in fact it is for example a mixture of honeys [34].

In recent years, there has been an increase in search for such analysis methods of specifying geographic and botanical origin of honey, which would constitute an alternative or an addition to the microscopic pollen analysis [12, 35, 36]. Research is conducted in three

directions: (i) searching for characteristic compounds (markers) for specific varieties and geographical origins of honey, (ii) constructing chemical profiles based on specific classes of compounds (most often flavonoids or phenolic acids), which constitute a "fingerprint" of individual varietal honeys, and (iii) the use of metabolomic techniques to distinguish and authenticate different varieties and define the place of origin of honeys [**H1**].

The purpose of my research was to isolate the volatile and phenolic fractions from honeys of various botanical origins with the use of selected extraction techniques, as well as the qualitative and quantitative evaluation of compounds contained in these fractions and the construction of chemical profiles that create a characteristic "fingerprint" of these honeys. The honeys selected for the research consisting in the search for compounds, which could be useful as chemical markers of botanical origin, were uncommon honeys with possible biological activity: goldenrod (*Solidago virgaurea* L.), yellow sweet clover (*Melilotus officinalis* L.), phacelia (*Phacelia tanacetifolia* Benth.) and ivy (*Hedera helix* L.). The research also examined selected physicochemical parameters, including colour and antioxidant activity, for both the previously mentioned and popular buckwheat (*Fagopyrum esculentum* Moench), linden (*Tilia* spp.), acacia (*Robinia pseudoacacia* L.), rapeseed (*Brassica napus* L.) and heather (*Calluna vulgaris* L.) honeys. In addition, the research on goldenrod honey has been extended to include the analysis of goldenrod flower nectar composition, in order to determine which substances contained in it are transferred into honeys. The identification of botanical (flower) markers complemented the data confirming the botanical origin of honey.

The methods of analysing the honeys consisted of high-performance thin-layer chromatography (HPTLC), high-performance liquid chromatography with diode array detection (HPLC-DAD), gas chromatography mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR). The analysis of the phenolic fractions obtained from the tested honeys by solid phase extraction with the use of adsorbents of the Amberlite type, was carried out using HPTLC and HPLC. In order to obtain a more complete profile of volatile compounds, the samples were prepared with the use of the following complementary methods: steam distillation, Soxhlet extractions and ultrasonic liquid extraction (USE), as well as with the use of organic solvents of varying polarity, and by headspace solid phase microextraction (HS-SPME) onto fibres coated with different types of adsorbents, and then analysed by the GC-MS method. Additional marked properties of the selected honeys were: shade, total phenolic content (using the modified Folin-Ciocalteu method), content of flavonoid compounds (formation of colour complexes with $AlCl_3$) and antioxidant activity in DPPH tests (1,1-diphenyl-2-hydrazilic picryl radical), ABTS (diammonium sulfate 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonate) and FRAP (ferric reducing antioxidant potential test).

Chemical markers of varietal honeys and their identification methods

The development of instrumental analysis methods characterised by high sensitivity and low limit of determination of individual compounds influenced the growth of research

aimed at authentication of food products, including apiculture products. These studies focus on attempts to identify markers, substances characteristic of a given honey type, and to construct chemical profiles of varietal honeys. Current knowledge indicates that phytomarkers, i.e. compounds transferred directly from nectar or together with pollen by bees, have a very high potential for the authentication of honey and other apiculture products. The markers of varietal honeys may include: volatile substances, phenylalanine degradation products, aromatic carboxylic acids and their esters, carotenoid degradation products, aromatic aldehydes, amino acids, heterocyclic compounds, phenolic compounds, atypical sugars, minerals and trace elements. Those compounds are often found in honey in very low concentrations (Fig. 2.) [**H1,** 15, 37].

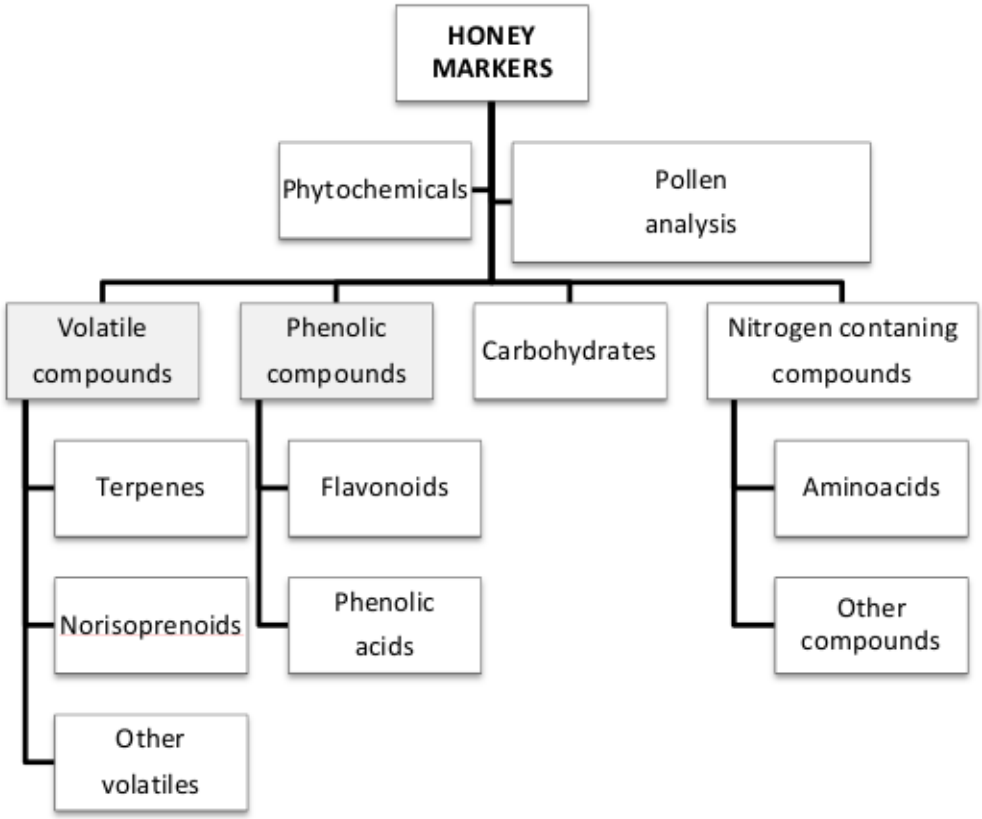

Fig. 2. The breakdown of substances which are potential honey markers.

The most promising classes of compounds in the context of the search for markers seem to be the substances contained in the volatile honey fraction and phenolic compounds. Substances that belong to those groups are most responsible for the organoleptic properties of honey, such as its aroma and taste, as well as its therapeutic properties, mainly its antioxidant activity.

Mass spectrometry and chromatographic techniques (paper chromatography, HPTLC, GC) are used as methods of identification of characteristic components of varietal honeys from the group of volatile substances [38-41]. The results of GM-MS analysis of samples of selected honey varieties indicate that the products contain various mono-sesquiterpenes,

benzaldehyde, furfural or isovaleraldehyde, forming a proper honey aroma [15]. The analysis of these derivatives, carried out using relatively simple analytical techniques, may therefore constitute a "fingerprint" determining the origin and quality of the honey. As the template – honey – is very complex in composition and as the concentrations of the compounds studied are low, it is of the utmost importance that the samples are properly prepared.

The classic technique of releasing the mixtures of compounds from the honeys is the extraction, which is carried out in the following compositions: aqueous solution of honey/organic solvent (most often: dichloromethane, ethyl acetate, *n*-pentane, hexane), often supported by ultrasounds (USE) [42]. Most often, the Solid Phase Extraction (SPE) is used in honey analysis, which consists in transferring analytes contained in the liquid sample to solid phase [43, 44]. The separation of compounds is based on the coefficient of division of organic compounds between water and solid sorbent. The analytes are released using a solvent of a suitable character. In the case of honey samples, polymer resins (copolymer polystyrene/divinylbenzene) are used as sorbents and volatile compounds are eluted with dichloromethane or ethyl acetate [44].

Occasionally, honey is extracted at elevated temperatures (e.g. extraction in a Soxhlet extractor, steam distillation). Under the influence of thermal processes, a number of successive reactions between reducing sugars and amino acids, peptides or proteins containing a free amine group take place, leading to the formation of a number of new chemical compounds (Maillard reaction products) [45, 46].

Using the described extraction methods, for the first time the composition of volatile compounds from nine honeys from yellow melilot (or melilotus) was analysed (collected in the 2014–2016 season in the Podkarpackie Province) [**H4**]. The results of the research showed that all the honeys tested contain a compound of lumichrome, which can be taken as an indicator of the botanical origin of the honeys. What is more, by steam distillation and extraction in the Soxhlet apparatus, coumarin was identified in melilot honey and its level in the samples was dependent on honey age [**H4**]. This compound was previously identified as a component of mahaleb cherry honey [47] and lavender [48]. It should be emphasised that the extracts from the melilot herb also contain coumarins (e.g. 7-hydroxycoumarin and 6,7-dihydroxycoumarin) and are used in the treatment of phlebitides, preventions of thrombosis and blood vessels fragility [49] and in the treatment of varicose veins and haemorrhoids [50]. Moreover, melilot extracts have a calming and anti-spasm effect [50].

Similar extraction procedures were used to analyse the composition of volatile compounds in European goldenrod honeys from the growing season 2014–2016. These honeys were collected at the small home apiaries in the Opolskie and Dolnośląskie Provinces. On the basis of chromatographic analyses of the examined samples, it was found that this honey is characterised by the presence of numerous shikimic acid pathways derivatives, terpenes and norisoprenoids. Compounds characteristic for the goldenrod honey turned out to be: hotrienol, nerol oxide and benzyl cinnamate [**H6**].

In the search for marker compounds from the group of volatile substances, common ivy honey from Ireland was tested. To isolate the mixture of substances, the USE and SPE

extraction methods described earlier were used, as well as solid phase microextraction (SPME) [**H9**]. Microextraction to the solid phase is a fast, solvent-free and inexpensive technique allowing for the analysis of volatile compounds contained in honeys [51-53]. This technique involves stabilising a sample of honey placed in a vial, in which the balance between the solid and gaseous phases is determined. The volatile compounds are then adsorbed on fibreglass. After thermal desorption of the fibre, the mixture of these substances is analysed with GC-MS.

The use of polydimethylsiloxane-poly(divinylbenzene) (PDMS/DVB) and polydimethylsolixane-carboxen-poly(divinylbenzene) (PDMS/CAR/DVB) fibres allowed selective isolation of volatile compounds from ivy honey. However, a comparison of the results showed that only few compounds were identified in all the extracts obtained by different methods. The comparative analysis made it possible to identify the marker compounds in ivy honey, which were found to be 4(1H)-quinolone, myrtenal, phenylacetonitrile [**H9**].

The examples described clearly show that the use of one specific method of isolation never allows to separate all the substances contained in the honeys. Moreover, the type and composition of the mixture is strongly dependent on the method used. Therefore, in various works devoted to the search for varietal honey markers, there are found different substances which perform this function for the same variety of honey. A substance shall be considered a marker if it is present in all honeys of the given variety within the defined area. They should be at a significant concentration compared to other compounds obtained after the samples were prepared for measurement. In very few cases, it can be said that the role of the marker is played by one substance which occurs exclusively in a specific variety of honey.

Phenolic derivatives are another numerous class of compounds present in honey and capable of being an identification marker of individual varieties of honey. High-performance liquid chromatography (HPLC) with different detectors is one of the most frequently used methods of quantitative and qualitative analysis of this class of compounds in samples of various types of honey by researchers. Using this method, attempts shall be made to correlate the content of non-volatile ingredients contained in the honey with their botanical or geographical origin [15]. These studies cover mainly phenolic acids and flavonoids. Some of the phenolic compounds that make up the honey have been proposed as markers of the origin of the honey. An example is the homogeneous acid specific to strawberry tree (*Arbutus unedo L*) honey [54] or the kaempferol glycosides identified as black locust (*Robinia pseudoacacia L.*) markers [55]. Myricetin, tricetin, luteolin, quercetin, kaempferol are flavonoids characteristic for eucalyptus honeys [56]. The aromatic carboxylic acids: caffeinated, *p*-coumaric and ferulic, which are compounds characteristic for chestnut tree honeys, may also be used as honey authenticity markers [57]. These acids were also identified as compounds characteristic for Australian honeys made by bees from nectar collected from eucalyptus flowers [58].

Preparation of the sample for analysis of phenolic compounds consists mainly in removal of sugars and other polar compounds, i.e. separation of analytes from the matrix, and then concentration of the tested compounds. For this purpose, the technique of solid phase extraction is most often used [30, 31]. It consists in passing the honey sample through a column filled with appropriate adsorbent and washing it with water in order to remove sugars and polar compounds. The tested compound mixtures are adsorbed on a carrier and washed out with another solvent, e.g. methanol aqueous solution [59, 60]. In these studies, the most common adsorbents are Amberlite resins [34, 61]. Of course, the composition of the obtained compound mixture is strongly dependant on both the used adsorbent and the solvents used in the process of sample elution and recovery from the solid phase [62].

Using an optimised analytical procedure, fractions of phenolic compounds from 14 Polish heather honeys (*Calluna vulgaris* L.) from Lower Silesian Wilderness, one German heather honey and 9 buckwheat honeys (*Fagopyrum esculentum* L.) from Southern Poland. On the basis of the obtained results of HPLC chromatographic analyses, a marker of heather honeys, which turned out to be abscisic acid, was indicated [**H2**]. It should be explained that this compound belongs to the isoprenoid class, but similarly to phenolic acids it has a strong UV absorption at λ=290nm. The phenolic profile of buckwheat honey was dominated by 3-hydroxybenzoic, ferric and rosmarinic acids and myricetin, which belongs to the flavonols. These compounds were indicated as non-specific markers of Polish buckwheat honey [**H2**].

Markers from the phenol class were also sought in melilot honey, goldenrod honey, and multifloral honeys from Belgium. On the basis of the results of the studies, it was found that the melilot honey is characterised by the presence of (+)catechin and gallic acid [**H4**], whereas goldenrod honey can be identified on the basis of the content of gallic, 4-hydroxybenzoic and *p*-coumaric acids [**H6**]. Interesting results were obtained by analysing the phenolic profile of multifloral honeys. On the basis of HPLC analyses, it was established that all multifloral honeys from three apiaries near Antwerp, from three consecutive seasons (2007-2009), contain significant quantities of ellagic acid and pinocembrin with interesting biological properties. It was established that these compounds could determine the geographical origin of the honey [**H5**].

Chemical compounds profiles as indicators of honey varieties

The creation of chemical profiles for food products is a relatively new way of assessing their quality. The sources of information in this case are not individual chemical compounds, but quantitative interactions in an isolated by a chosen technique mixture of compounds. Classical tests are based on the fact that as many substances as possible contained in a given mixture are identified and their content compared in specific varieties of honey. The profiles are constructed by combining the composition of the isolated chemical fractions with the botanical and geographical origin of the honey. For example, chemical profiles constructed for phenolic substances identified with HPLC-DAD methods, contained in four Polish honeys: heather honey and buckwheat honey [**H2**], melilot honey [**H4**] and goldenrod honey [**H6**]. Relative concentrations of identified phenolic compounds were compared on the example

profiles (Fig. 3). Studies conducted on heather honeys from the Lower Silesian Wilderness show that by determining level of phenolic compounds, it is easy to distinguish botanical origins of the honeys. For the first time attempts were made to construct phenolic compound profiles for Belgian multifloral honeys from three apiaries in the Antwerp region. It turned out the profiles built can determine the geographic origin of honey [**H5**].

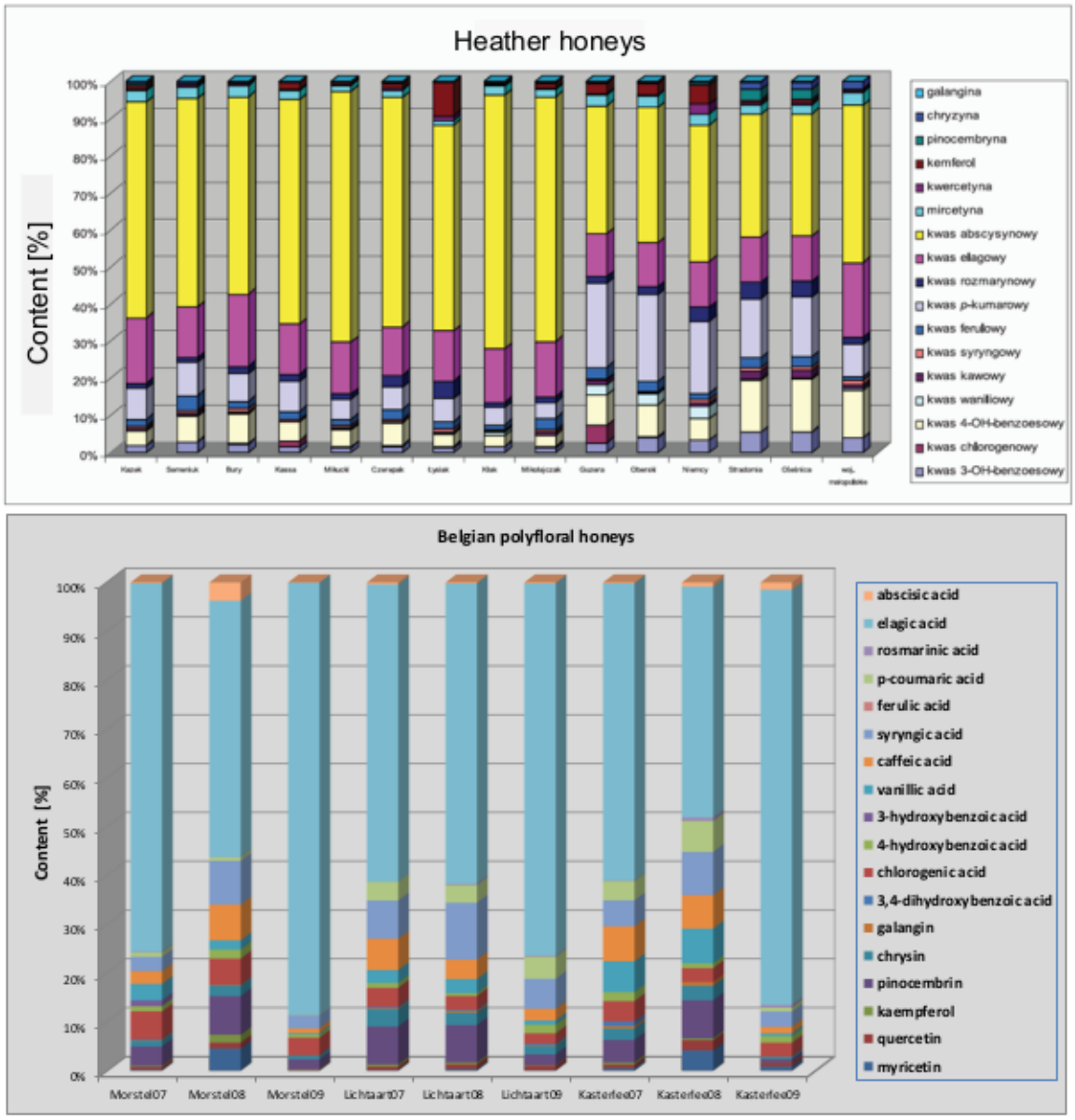

Fig. 3. Examples of profiles of phenolic compounds in the studied honeys.

One of the techniques that has recently been used to create chemical profiles of substances identified in both phenolic fractions and volatile honey extracts is the HPTLC technique.

Chromatographic data obtained on varietal honeys: goldenrod [**H6**], willow, heather, buckwheat, pine honeydew and manuka [**H7**], raspberry, dandelion, melilot, rapeseed, clover, milk thistle [**H8**], and common ivy honey [**H9**] indicate that HPTLC analysis is a very

precise visual method, which can be successfully used for fast and precise honey differentiation. Based on the R_f value obtained and colour of the stripes of the individual compounds, images resembling a barcode are generated for each of the examined honey samples (Fig. 4). This is a characteristic graphic "fingerprint" containing information on the chemical composition of the honey.

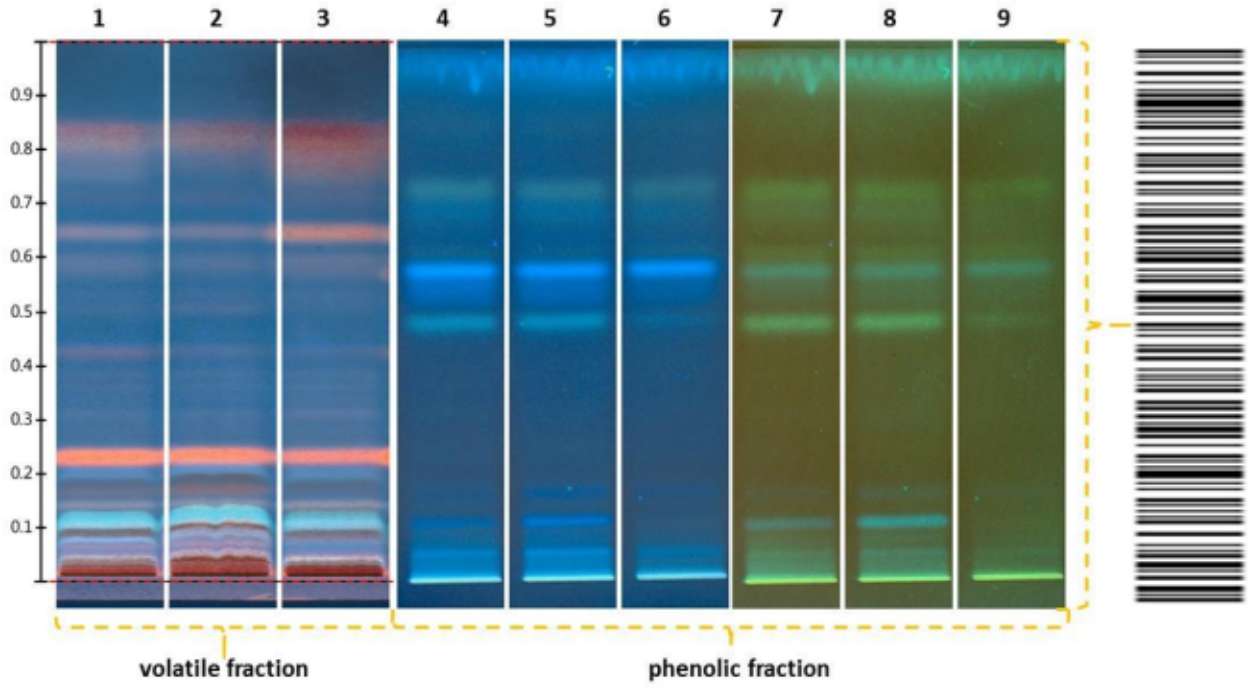

Fig. 4. HPTLC chromatograms of phenolic and volatile goldenrod honey fractions.

The technique used for direct examination of the honey is ^{1}H NMR. However, due to their high glucose and fructose contest, NMR spectra are difficult to interpret. This is possible in the spectra where are no peaks from sugars. However, the final identification of the marker still requires its separation and definition of its chemical structure, or the use of an individual compound as an internal standard [63-66].

The ^{1}H NMR analysis of heather, buckwheat, tilia (Tilia L.), rapeseed (*Brassica napus* L. var. napus), acacia (Acacia Mill.) and multifloral honeys metabolomes conducted during the research, allowed to identify characteristic markers (1-9) and to construct a heat map correlating the type of metabolite with its concentration in a specific honey sample (Fig. 5). Such maps are similar to the system of marking goods with a QR code and could serve the same function in defining the origin of honey [**H3**].

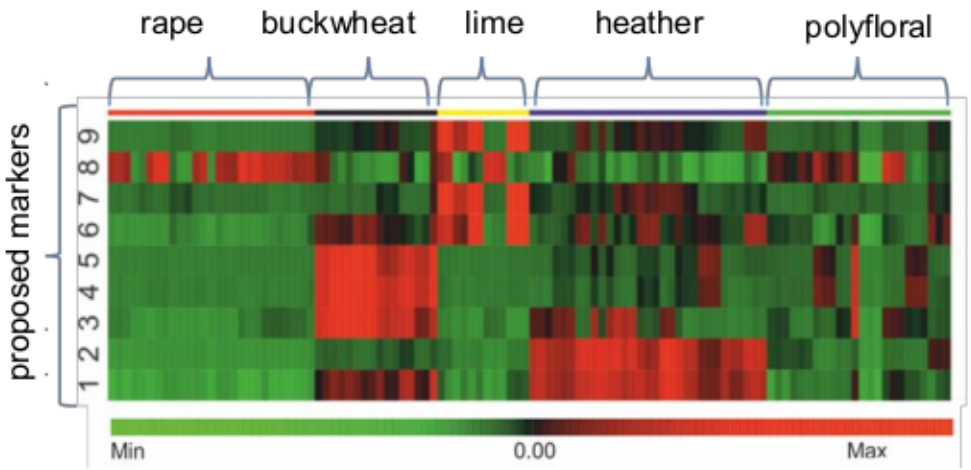

Fig. 5. NMR results based heat map.

Use of metabolomics methods

Analyses of specific substances which are "fingerprints" of specific honeys result in metabolomic studies. This technique involves systematic identifying and determining the levels of all metabolites and xenobiotics present in the analysed samples by NMR, MS or chromatographic techniques (HPLC, HPTLC). The primary goal of metabolomic studies is to identify specific chemical compounds that are formed in the studied system as its response to a specific factor [67]. In the case of honey, it is a dynamic collection of chemical compounds which illustrate the impact of the living conditions of bees and plants and the state of the environment on the quality of honey. Metabolomic studies based on chemometric and statistical tools for analysing the data collected and for extracting useful information allow to define the botanical and geographical origin of the honeys [68-70].

In order to check the authenticity of the botanical and geographical origin of honey, a comprehensive and congeneric chemometric evaluation is carried out using several highly advanced techniques (PCA, CA, LDA, KNN, SIMCA). The obtained results of the analyses allow to evaluate at a high level of statistical significance the degree of differentiation between samples of high quality varietal honeys and cheaper honeys of lower quality [46, 71-73].

The usefulness of chemometric techniques was also demonstrated by research of Polish varietal honeys and manuka honey from New Zealand and Australia. In these studies, chromatographic methods (HPTLC, HPLC) were used to identify the components of phenolic fractions. The obtained chromatographic data were subjected to a computer multi-variant analysis (PCA, HCA), which helped to show differences between samples representing honeys of different botanical origins (Fig. 6) [**H7**].

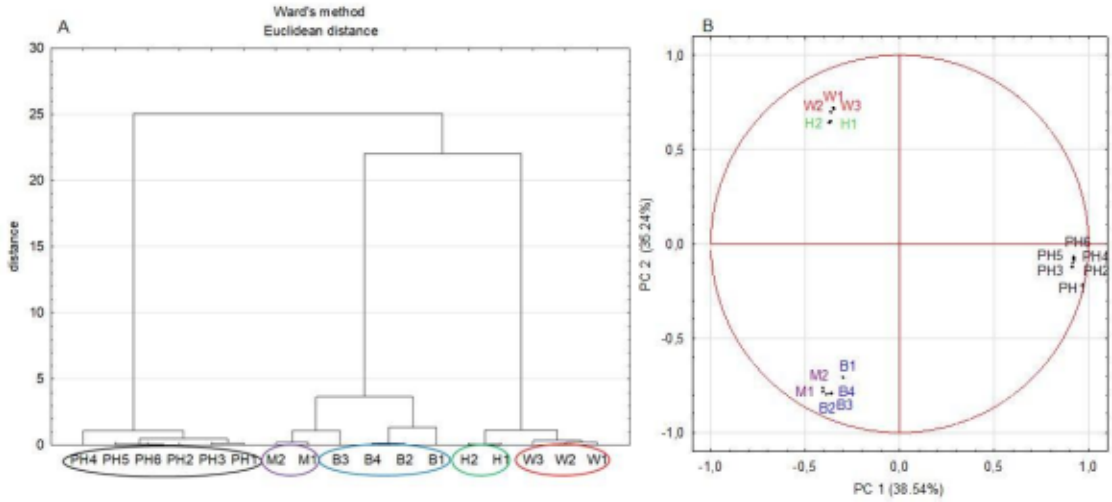

Fig. 6. Hierarchical dendrogram (a) and Principal Components Analysis (PCA) (b) of the tested honey samples. W1-W3 willow honeys, H1-H2 heather honeys, B1-B4 buckwheat honeys, Ph1-Ph6 pine honeydew honeys, M1-M2 manuka honeys.

Thanks to the application of appropriate chemometric techniques, it was also possible to classify the analysed seven nectar honeys on the basis of their content of lipophilic fraction components [**H8**]. The aim of the study was to demonstrate the usefulness of the HPTLC technique in quickly differentiating between Polish varietal honeys (rapeseed, buckwheat, clover, willow, milk thistle, dandelion, raspberry and yellow melilot) based on the content of lipophilic compounds. The results analysed using chemometric techniques clearly show that, on the basis of R_f values obtained, studied honeys were grouped into separate sections according to their botanical origin (Fig. 7).

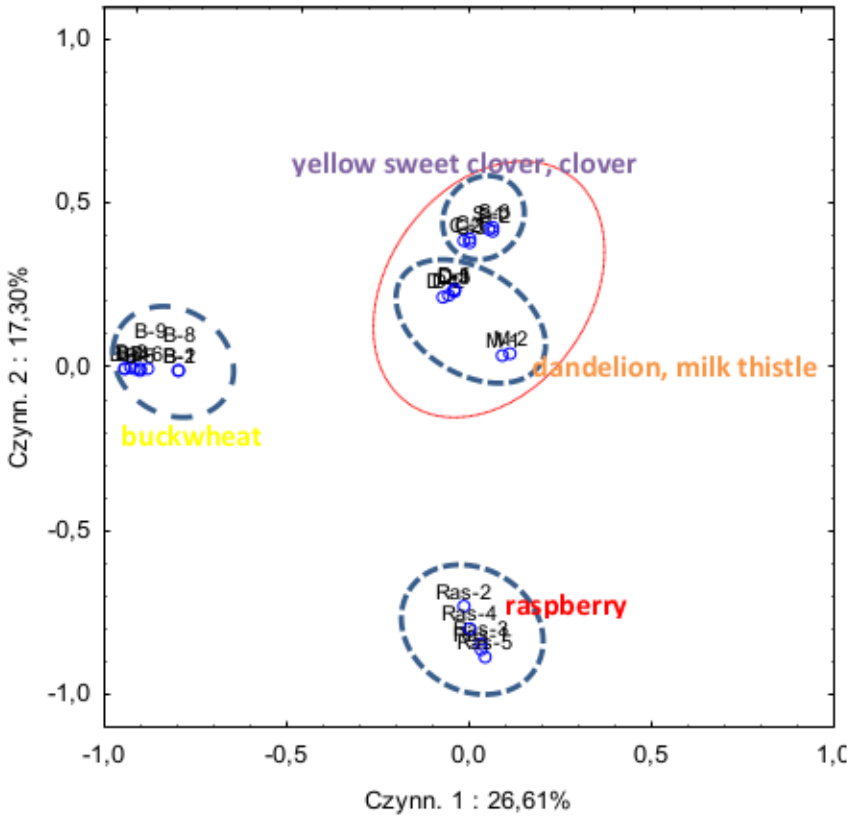

Fig. 7. Principal Components Analysis (PCA).

Among the modern techniques, NNR spectroscopy is an important tool for analysing mixtures. In combination with chemometric tools, the use of NMR significantly improved the ability to classify honey samples [69, 74-76]. However, in honey identification, methods using NMR, are still perceived as complementary tests.

For the purpose of authentication of five nectar and multifloral honeys, a multidimensional analysis of chemometric data using the Principal Components Analysis (PCA) (Fig. 8) and the Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA). The chemometric analysis supported by the pollen analysis revealed an incorrect classification of acacia honeys by the producers [**H3**].

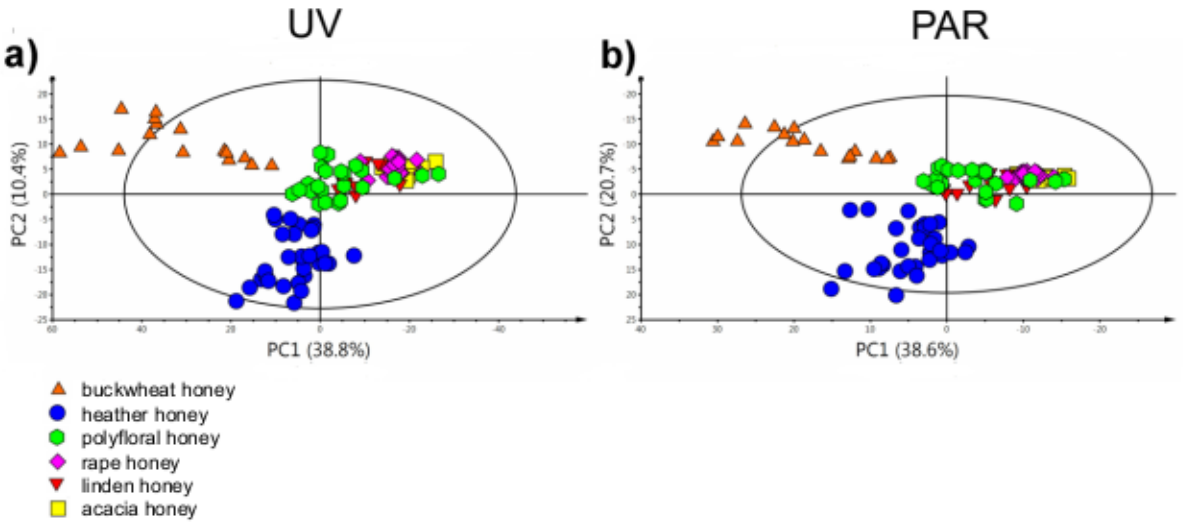

Fig. 8. PCA analysis of ^{1}H NMR spectra of tested varietal honeys for two methods of data standardisation.

SUMMARY

The results of the research, described in series 9 of the publication, provided new knowledge on phytochemistry, selected physicochemical parameters and antioxidant activity of popular and less common honeys.

For the first time, chromatographic profiles of phenolic compounds of popular Polish heather and buckwheat honeys were described, indicating the compounds which may play the role of specific markers of these varieties. Chromatographic profiles of volatile and phenolic fractions obtained from less popular goldenrod and yellow melilot honeys were constructed and volatile fraction of Irish common ivy honey was characterised, describing specific markers of those honeys.

Additionally, the total content of phenolic compounds (modified by the Folin-Ciocalteu method) and antioxidant activity of all tested honey varieties were determined. The content of phenols for different varieties was diversified and ranged from 121.6 to 1173.8 mg GAE/kg on average. These values correlate with the colour and antioxidant activity of the honeys (DDPH, FRAP, ABTS tests). Dark honeys, the richest in phenolic compounds, showed the strongest antioxidant activity.

High-performance thin layer chromatography has been shown to be useful as a fast and precise technique for creating chemical profiles, and thus for unambiguously determining the botanical origin of honey. The profiles constructed for samples of several honeys of the same variety show striking similarities and marked differences with those of other varieties.

It has been confirmed that the use of chemometric tools for the exploration of large data sets is very useful as it allows for easy and quick visualisation of similarities between the samples and the measured parameters.

The given examples also indicate that the creation of chemical profiles based on markers characteristic for varietal honeys may be an interesting alternative to the existing methods of identifying the origin of the honeys and defining their quality and utility values.

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4. Discussion of other academic and research achievements

My scientific interests and activities so far have focused on the isolation and identification of substances of natural origin, mainly of plant origin, on the assessment of their biological activity and potential applications in various branches of industry, with particular emphasis on medical devices, dietary supplements and cosmetic preparations.

I developed my interest in natural sciences while studying chemistry with the specialisation of agricultural biochemistry. The choice of such an interdisciplinary speciality enabled me to use the methodology of a group of natural sciences in analysing and understanding the phenomena occurring in nature. In 1995, under the supervision of dr hab. Kazimierz Wiech, I defended my Master's thesis entitled, *Wpływ różnego sposobu uprawy marchwi na występowanie szkodliwej i pożytecznej entomofauny* [The influence of various methods of carrot farming on the occurrence of beneficial and harmful entomofauna]. After obtaining my Master's degree, I was employed in the Department of Plant Protection at the Institute of Chemistry, now the Department of Analytical and Ecological Chemistry at Faculty of Chemistry in Opole, as an assistant professor. Initially, I was involved in research on the herbicide properties of compounds belonging to the group of bisphosphonates. At the same time, under the supervision of dr hab. Paweł Kafarski, I developed my knowledge and laboratory skills related to the isolation, purification and identification of plant substances. The objects of my interest were substances that are part of the plant defence systems, especially allelochemicals synthesised in the tissues of carrot seeds. I used this experience to conduct my research as the main researcher in the project: New agrochemicals and their safe use for health and environment, research task No. 37, *Allelochemicals as potential biopesticides in agricultural systems*, grant ordered PBZ-KBN–060/T09/2001/37. The research carried out on this project concerned plant extracts with allelopathic effects on other plant species. Impacts of this nature are associated with the presence of specific chemical compounds in plant tissues, the so called allelochemicals. Isolation and identification of these substances is useful for the development of new, natural and safe plant protection products. The final result of this research project was three works in journals from the JCR database and several chapters in monographs, of which I am a co-author.

I have developed research on the search for substances responsible for allelopathic interactions as part of the Ministry of Science and Higher Education's research grant; *Allelochemistry of carrot (Daucus carota* L.) No. 4T09B 052 24 (03.2003-09.2005). In this paper, I showed that carrot seed oil and its sesquiterpene components (carotol, daucol) significantly reduce the germination and growth of other plant species and limit the development of pathogenic fungi of *Alternaria* and *Fusarium* species. The effect of the obtained results was a PhD thesis of the same title, which I defended in 2005 and 2 articles in JCR journals (with a total IF of 3.495), as well as several chapters in monographs.

After obtaining the PhD degree in chemical sciences and being employed as an assistant professor, I continued my research related to the isolation and identification of

plant substances and the evaluation of their biological activity. In this area I cooperated with the Faculty of Biological Science at Univesity of Wrocław, Department of Technology of Biologically Active Substances, Pharmacy and Biotechnology at Lviv Polytechnic National University and Department of Chemistry at Constantine University, Algieria. This cooperation resulted in four publications in JCR journals. At the same time, I also started cooperation with the Polish Beekeeping Association and Miody Polskie Sp. z o.o., and started research on the chemical nectar honeys markers. At this stage of my scientific work, I worked on the development and optimisation of analytical procedures that would enable the identification of compounds belonging to the volatile fraction and the phenolic fraction in extracts obtained from nectar honeys.

At the same time, as one of the main researchers, in the period from December 2009 to June 2014, I conducted research in a project financed from EU funds under the Operational Programme Innovative Economy (POIG 01.01.02-02-003/08), Biotechnologies and advanced medical technologies, Research tasks 1 and 2, *Detection of hallucinogenic substances.*

The main result of the project was an atlas entitled "Grzyby neurotropowe" [Neurotropic mushrooms], a kind of guide enabling the control services to identify the species of mushrooms producing hallucinogenic substances, of which I am a co-author. The detailed mycological analysis of the fungus material described there and the analysis of the composition of the extracts by instrumental methods, including the use of metabolic tests and the use of genetic techniques, enable the species of fungi containing substances with hallucinogenic properties to be unambiguously defined. The description of structures of newly identified compounds with psychoactive properties and therefore changing the metabolism of the organism contained in the atlas, should contribute to the development of new, effective standards of treatment of poisoning with these substances. The developed analytical solutions, also being the results of the project, enable standard determination of the content of components responsible for drug effects, both in the fungus material and in physiological fluids (urine, blood). A significant part of the results obtained during the project implementation consisted of 14 scientific publications in journals included in the JCR database, and I am a co-author of seven of these works.

Following the completion of the project related to the identification of hallucinogenic substances in mushrooms, I intensified my research related to the authentication of nectar honeys. The research was continued in cooperation with Miody Polskie Sp. z o.o. and Polish Beekeeping Association, which provided samples of Polish varietal honeys. Part of the research was financed by the National Science Centre [Narodowe Centrum Nauki] (OPUS 8, No. 2014/15/B/NZ9/02182), of which I was the executor.

The results of these studies form the basis for the evaluated achievement and are discussed in part one.

5. Summary of scientific achievements

	Pre-doctoral				Post-doctoral				Total			
	Amount	IF	IF_5 years	MNiSW Points	Amount	IF	IF_5 years	MNiSW Points	Amount	IF	$IF_{5\ years}$	MNiSW Points
The orginal creative work published in journals from the database Journal Citation Reports	4	4,425	5,643	45	21	33,712	35,118	470	25	38,137	40,761	515
The orginal creative work published in journals outside the base Journal Citation Reports	5	-	-	-	7	-	-	-	12	-	-	-
Chapters in books	5	-	-	-	6	-	-	-	11	-	-	-
Total publications	**14**	**4,425**		**45**	**34**	**33,712**		**470**	**48**	**38,137**	**40,761**	**515**
Conference lectures	11	-	-	-	7	-	-	-	18	-	-	-
Conference announcements and posters	21	-	-	-	61	-	-	-	83	-	-	-

6. Future research plans

I would like to further develop my interests in natural substances in the next stages of my scientific work. Because of the knowledge I gained during the hallucinogenic substances project and preliminary results of the secondary fungal metabolites, I intend to explore the subject of the metabolism of these organisms.

Large-fruited fungi are living "bio-reactors" capable of synthesising a vast number of secondary metabolites with strong and varied biological activity, including pharmacological ones. Studies on the chemical composition conducted on these organism so far, have led to the identification of many chemical compounds responsible for particular directions of pharmacological activity. These compounds, belonging mainly to the polysaccharides, terpenoids, phenols, and lectins, were assigned a number of therapeutic effects, including immunotherapeutical, anticancer, antimicrobial, antiviral, and antioxidant. Among many species of fungi, mushrooms considered to be poisonous or hallucinogenic seem to be particularly interesting as a source of the so-called leading structures, starting compounds in search of new drugs by chemical synthesis. A good example of such a species is the fly agaric (*Amanita muscaria*).

At present, only some species of Basidiomycetes are used to obtain medical preparations, especially in the European Union, including Poland. However, the dynamic development of both biotechnology and chemometrically assisted analytical techniques suggests that the importance of these organism for the pharmaceutical industry will increase.

In order to study these research problems, it is necessary to establish cooperation with scientific groups both at home university and beyond. I intend to undertake such cooperation with, among others, Wroclaw Medical University.