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BOTANICAL ORIGIN OF WEST POMERANIAN HONEYS

Abstract

Melisopalinological studies were carried out on 24 samples of honeys from beekeepers affiliated in the Regional Association of Beekeepers in Szczecin. Honeys were obtained in 2014.

Microscopic slides were prepared by performing a smear of honey on a slide. To close the slides a glycerol-gelatine adhesive with alkaline fuchsin was used. Quantitative and qualitative analysis of pollen contained in 1 g of honey was performed.

More than 90 000 pollen grains were identified in the examined samples of honeys: 47 of entomophilous taxa and a few of anemophilous taxa. Among the nectariferous plant pollen, the highest pollen frequency (above 50%) has been estimated for Brassicaceae (with *Brassica napus*), *Calluna vulgaris*, Asteraceae (with *Centaurea sp.).* In individual honey samples, from 5 up to 22 taxa of nectariferous plants were noted. One gram of honey contained 4196 pollen grains on average. Based on the microscopic analysis results, 11 multifloral and 11 monofloral honeys were distinguished, the latter of which containing 4 honeys not covered by Polish Standard. One sample had an especially low content of pollen. One sample was not a bee honey.

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Introduction

Bees produce honey from nectar and honeydew. These raw materials are subject to different chemical processes: first in the digestive tract of the insect, where they are mixed with enzymes and other secretions, then later in the honeycomb cells. Here honey matures and thickens as a result of evaporation (Dyakowska 1959).

Honey has a rich chemical composition, which determines its nutritional and medicinal properties. Honey is also widely used in apitherapy. However, children under the age of 1 cannot eat honey due to the fact that as an unprocessed product it may contain bacteria *Clostridium botulinum*. This poses the risk of producing botulinum toxin in the body of a child (Bogdanov et al. 2008). Another factor limiting the consumption of honey is allergy. Allergic reaction to honey afflicts mainly people with allergies to pollen, foods or *Hymenoptera* venom, while occurring very rarely in healthy people (Kędzia, Hołderna-Kędzia 2006). Allergic symptoms after ingestion of honey may be caused by the following substances contained in honey:

- bee body components and their secretions (enzymes produced in the salivary glands),
- fungal spores, proteins and metabolic products of sucking insects that occur in honeydew,
- pollen.

If after ingestion of honey dermatological disorders (hives, itching, swelling), gastrointestinal disorders (nausea, vomiting, diarrhea) or disorders of the respiratory system (bronchospasm, rhinitis) occur, its administering should be discontinued (Kędzia, Hołderna-Kędzia 2006).

Each honey contains a certain amount of solids, fungal spores, algae and mainly pollen. The pollinic composition in honey allows to evaluate the plant species from which bees collect nectar and is of practical importance in the planning of cultivation of species providing nectar and pollen (Dyakowska 1959). Additionally, it enables to specify the geographical origin of honey (Pfister 1895). It is important from a commercial point of view, because mixing domestic and imported honeys is against the interests of local beekeepers.

Individual groups and varieties of honey contain different amounts of pollen. The absolute amount of pollen in honey depends on several factors.

Firstly, the construction of the flower, the pollen content in the nectar and how bees collect and process the nectar. In flowers with easily accessible and exposed nectaries pollen falls into the nectar before the insect comes. In others the bee covers its mouth instruments in pollen while inserting them into the flower. The pollen is then mixed with nectar (Dyakowska 1959). Some plants, for example Lamiaceae, produce very little pollen. The others for comparison produce more pollen yet bees collect it in small quantities. This happens for various reasons, for example insects cannot form pollen loads when the grains are too large (e.g. cucumber) or sticking in pollinia (e.g. Asclepias). Linden tree pollen is rarely collected due to the presence of calcium oxalate crystals which adversely affect the digestive system of bees (Lipiński 1979).

Secondly, the time elapsed since the visit to the flower to the donation of nectar from the crop, and, consequently, the distance from the flower to the hive. Nectar with pollen grains is carried by worker bees to the hive. However, the pollen content in the nectar at the time of collecting it from the flower is different of the contents in the nectar placed at the comb (Todd, Vansell 1942). The content of pollen in the crop decreases rapidly. This is due to the structure of the digestive system of bees. The crop is connected to the middle intestine via the prior stomach whose front part creates a so called honey valve. The valve is composed of four triangular folds to allow the accurate clipping. The final section of the prior stomach is a thin tube hanging down deep into the middle intestine. The contents of the honey crop is still mixed which enhances enzymatic processes. Moreover, prior stomach flaps have the ability to capture pollen grains contained in the crop and transfer them to the middle intestine where they are digested (Tomaszewska, Chorbiński 2008). Todd and Vansell (1942) compared the amount of pollen in the crop immediately after feeding (338 000 pollen grains) and after 15 minutes (100 000 pollen grains).

Lastly, the method of extracting honey from the combs by the beekeeper (Dyakowska 1959). Centrifugation of the honey combs with bee bread cells or leftovers from the previous bee produce causes the pollen image to be inconsistent with the actual origin of the honey. That is because the propolis may be from an earlier period, or even from the previous year.

The aim of the study has been pollen analysis and marking the botanical origin of honey offered by the West Pomeranian beekeepers.

Materials and methods

Melisopalinological studies were carried out on 24 samples of honeys from beekeepers affiliated in the Regional Association of Beekeepers in Szczecin. Honeys were obtained in 2014.

Among the many ways to prepare microscope slides with samples of honey (Crompton, Wojtas 1993), the smear method was chosen due to the method being simple, cheap and not time-consuming.

Microscope slides were prepared from each sample weighing 1 g of honey, which was subsequently separated evenly over 5 slides and smears performed. Slides were closed using an adhesive containing gelatine, glycerin, distilled water, phenol and pigment – alkaline fuchsin (used to colour pollen grains and make them identifiable). Pollen grains were assigned, if possible, to the species, genus or family, based on collected comparative preparations and available pollen atlases.

To determine the content of pollen in honey (pollen spectrum), a qualitative analysis of pollen in the sample was made and numerical relations between taxa were evaluated (Dyakowska 1959).

Pollen spectra of honeys by International Commission for Bee Botany (Louveaux 1970):

A. predominant: a species that constitutes 45% of pollen grains;
B. secondary dominant: a species that constitutes 16–45% of pollen grains;
C. important minor pollen: a species that constitutes 3–15% of pollen grains;
D. minor pollen: a species that constitutes less than 3%.

In assessing the botanical origin of honeys non-nectariferous and entomophilous pollen grains were not included. For nectariferous plants the percentage share was calculated.

Quantitative analysis of pollen in honey allows to determine the absolute content of pollen in honey, which makes it possible to distinguish natural and nutritious flower honeys from the products resulting from feeding bees with sugar. Bees fed with sugar produce honey containing particularly low amounts of pollen (Dyakowska 1959).

Results

One sample did not contain honey bee. In 1 g of this product the presence of only a few grains of pollen was showed, including those from non-native flora. This sample is not taken into account in further analyzes.

More than 90.000 pollen grains were identified in the examined samples of honeys: 47 of entomophilous taxa and a few of anemophilous taxa. Among the entomophilous plant pollen, the highest pollen frequency has been stated for Brassicaceae (with *Brassica napus*) – 20,67%, *Calluna vulgaris* – 12,89%, Asteraceae – 9,54%, *Centaurea sp.* – 8,41% (Fig. 1). On average, an individual honey sample contained 10 pollen types of nectariferous plants (range 5–22). The range of occurrence entomophilous pollen in honey samples is presented in Figure 2.

One gram of honey contained 4196 pollen grains on average; 274 were represented by anemophilous and non-nectariferous plants and 3922 by nectariferous plants. Participation of anemophilous plant pollen in individual samples ranged between 0 and 28%, average 6,5%.

In one of the samples in 1 g of the product only 332 pollen grains were found, of which 239 came from nectariferous plants. Due to the very low content of pollen in this sample, pollen spectra were not specified. The analysis of the remaining 22 samples of honeys showed that 11 do not have predominant pollen. These are multifloral honeys. The remaining 11 examined samples were variety honeys, of which 7 compatible with the Polish Standard (PN-88/A-77626): 3 samples of *Brassica napus* honeys, 2 *Robinia pseudacacia*, 2 *Fagopyrum esculentum*. The pollen contents of *Brassica napus* pollen in rape honeys were 47%, 64% and 89%. *Robinia* honeys had a 46% and 63% share of *Robinia pseudacacia* pollen. However, in buckwheat honeys pollen contents of *Fagopyrum esculentum* were 46% and 54%.

In the multifloral honeys the most common admixture were clover (Fig. 3) and phacelia (Fig. 4). Based on the microscopic analysis results, 11 multifloral honeys were determined, 2 honeys with a predominance of buckwheat and clover pollen, 2 honeys with a predominance of phacelia and clover pollen and multifloral honeys with secondary dominant pollen spectrum of: phacelia and raspberries, heather and clover, clover and raspberry, heather and buckwheat, heather and cornflowers, phacelia and thistle and also mustard family (except canola) and aster family.

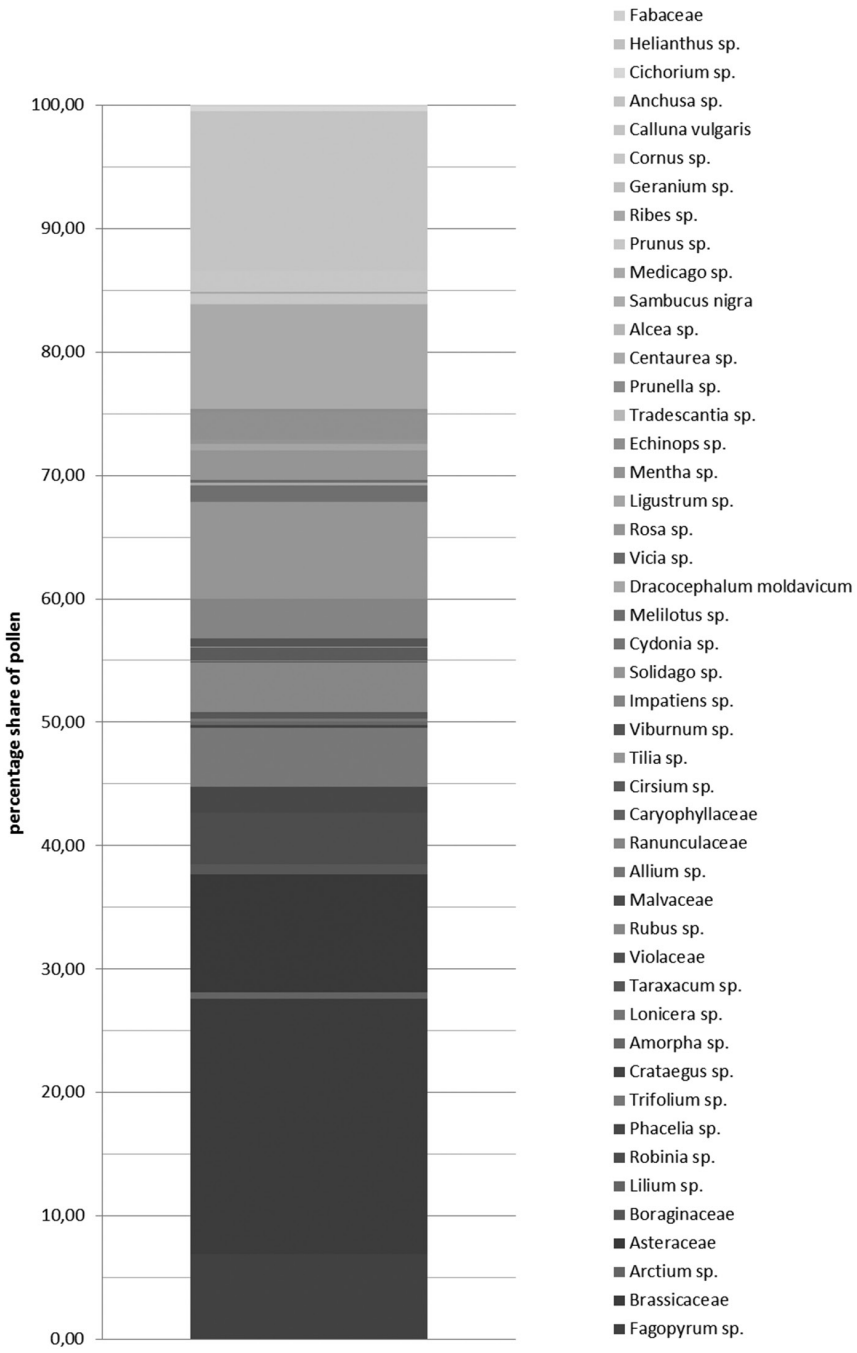

Figure 1. Percentage share of entomophilous pollen in honey samples

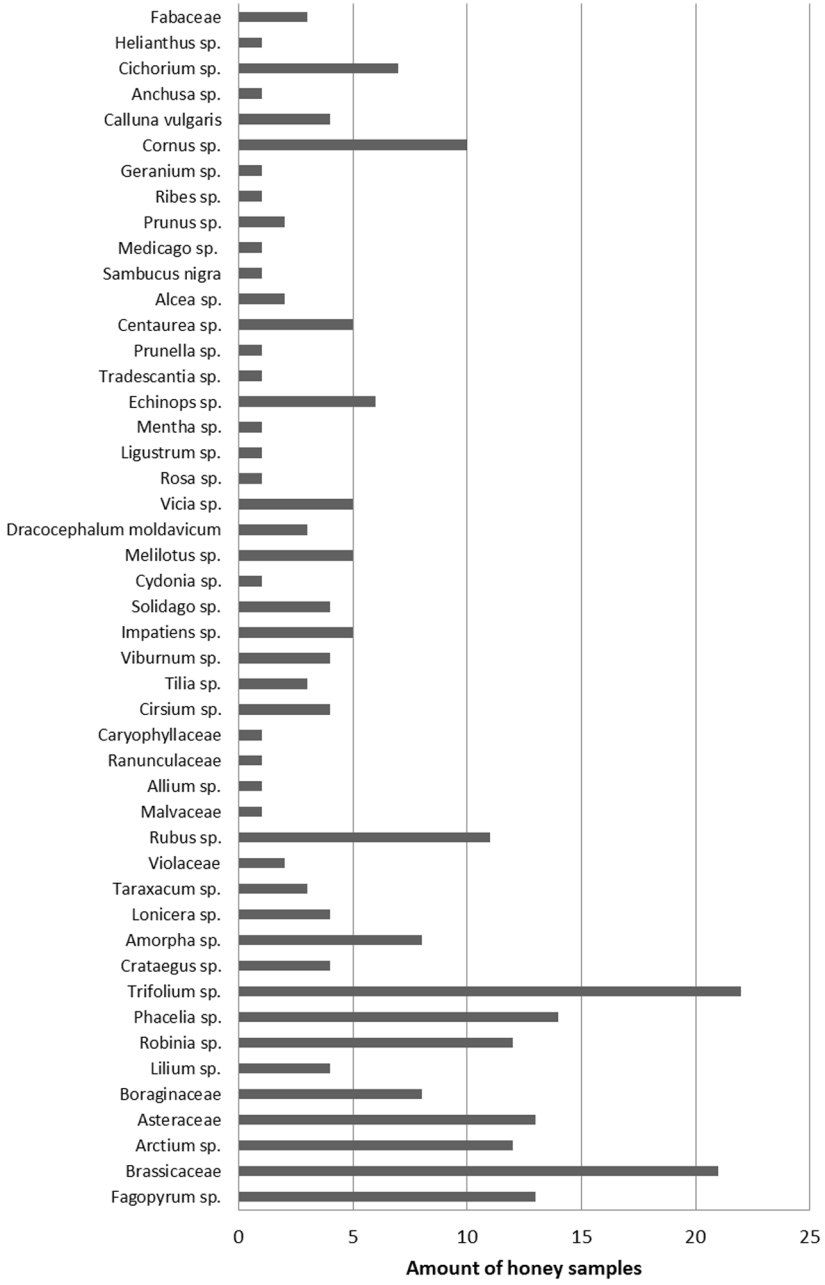

Figure 2.The range of occurrence entomophilous pollen in honey samples

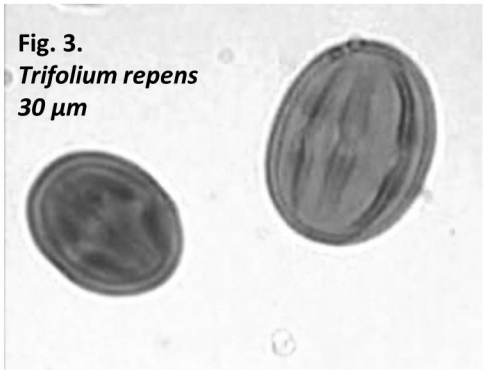

Figure 3. *Trifolium repens* (clover) pollen

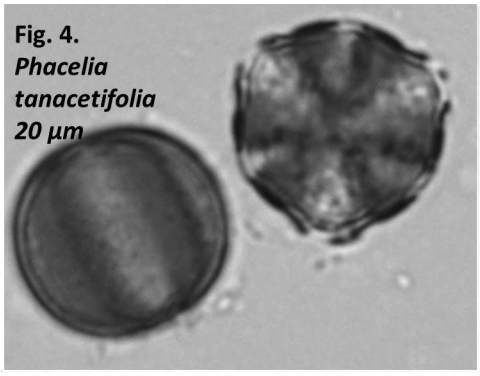

Figure 4. *Phacelia tanacetifolia* pollen

Discussion

Melisopalinological studies help distinguish real honey and identify non-wholesome products. Low percentage of pollen characterizes filtered honeys. Pollen is filtered out of them during the process of removal of foreign organic or inorganic substances. However, according to legal regulations, when such filtering results in removing significant amounts of pollen, the consumer must be informed

through an appropriate symbol on the product label. (Council Directive 2001/110/WE Dz.U. L 10 z 12.1.2002). Therefore a sample for which, due to a particularly low pollen content, a pollen spectre has not been determined should be given additional analyses, for example free acidity, sugar content, electrical conductivity, diastase activity.

Based on the number of plant fractions in 10 g of honey, 5 groups of honeys can be distinguished (Maurizio 1939):

Group I: fewer than 20 000 (low pollen variety honeys).

Group II: 20 000 – 100 000 (nectar and nectar- honeydew honeys).

Group III: 100 000 – 500 000 (pollen-rich and honeydew honeys).

Group IV: 500 000 – 1 000 000 (pollen-abundant and extruded honeys).

Group V: more than 1 000 000 (honey extruded using presses).

Group I includes *Robinia* honey which contains one of the least amounts of pollen. The structure of *Robinia* flowers reduces the amount of pollen in the nectar to the minimum. Another low-pollen honey is linden honey. Pure Linden honey in Central Europe is rare. It is more often obtained on the south east of Europe where linden form pure tree stands (Dyakowska 1959). In this study 52% of samples fall within group I, 35% within group II and 13% within group III, which indicates high quality of honeys provided for analysis.

Although *Robinia* honey is considered low pollen, the results of research show that samples contain 46% and 63% of *Robinia pseudoacacia* pollen. Two samples were marked by the beekeepers as Linden honeys, however pollen analysis showed that they were multifloral honeys. Linden pollen grains were identified in 3 samples, reaching respectively 3,33%, 2,07% and 0,5% of content. The result was similar in numerous studies conducted on honeys from German beekeepers.

Multifloral honeys are characterized by greater variety of pollen spectres (8 taxa on average) than variety honeys (12 taxa on average). A similar dependency was found in studies of Ukrainian honeys (Lokutova et al. 2005).

The significant content of phacelia pollen in the spectres of secondary pollen in 4 multifloral honeys and primary pollen in a variety honey suggests that this plant is an important source of bee nutrient during summer and autumn. This has been confirmed by the studies of Klepacz-Baniak and Czekońska (2005).

Among anemophilous plants the most often occurring types of pollen were grass, birch, willow and *Chenopodium*. The presence of birch pollen in honeys was also established by Warakomska (1997). Birch pollen, due to high content of proteins, is a valuable nutrition source for bees (Banaszak 1993) and positively

influences the physiology of bees as well as their lifespan (Maurizio 1953). *Chenopodium* pollen grains in honeys and on bee legs were identified by Warakomska (1997) and Wróblewska (2002).

In this study microscope slides were prepared using a simple method enabling identification and counting of pollen grains through making honey smears and colouring the preparations with alkaline fuchsin. The majority of studies on pollen analysis of honey base themselves on the methodology specified in Polish Standard (PN -88/A-77626), because of which detailed results are not comparable. However, the conducted research has been based on Polish Standard regarding the percent content of pollen in variety honeys.

To basic legal documents concerning honey quality belongs Polish Standard – Bee Honey (PN-88/A-77626), which described in details pollen analysis of honeys. It specified methodology of preparing slides and performing qualitative pollen analysis of honeys. Based on Polish Standard the following honey types could be distinguished: nectar-honeydew, two kinds of honeydew and nectar honeys – multifloral, rape, acacia, linden, buckwheat, heather. Pure honeys from other plants (dandelion, phacelia, borage) were treated as multifloral. Polish Standard officially ceased to apply on October 3rd 2003 (Regulation of the Minister of Agriculture and Rural Development – Dz.U. 2003, Nr 181, poz. 1773). The document (Regulation of the Minister of Agriculture and Rural Development – Dz.U. 2003, Nr 181, poz. 1773) does not precisely define the topics concerning pollen content in variety honeys. It distinguishes multifloral honey, i.e. coming from multiple various plants, and varieties of nectar honey determined by the name of plant whose pollen content is predominant. Such definition allows beekeepers to offer nectar honeys from various plants from which it can be obtained in an appropriately pure form (raspberry honey, globe thistle honey etc.). In the European Council Directive relating to honey (Council Directive 2001/110/WE Dz.U. L 10 z 12.1.2002) no detailed information about pollen content in honey and pollen analysis occurs.

The research of botanical origin of West Pomeranian honeys allowed to distinguish rape, acacia and buckwheat honey as well as interesting varieties not included in Polish Standard (PN-88/A-77626) but compatible with Polish law (Regulation of the Minister of Agriculture and Rural Development – Dz.U. 2003, Nr 181, poz. 1773). These are phacelia, impatiens, goldenrod and raspberry honeys. Moreover, 9 different types of multifloral honeys were determined. The ob-

tained results are important for honey commerce, making beekeepers sure about the plants from which the honey they sell comes.

Conclusions

1. In the investigated material, the presence of pollen grains of 47 entomophilous taxa was noted.
2. More than 50% of the participation of the nectariferous plants pollen grains showed four taxa together: Brassicaceae (with *Brassica napus*), *Calluna vulgaris*, Asteraceae (with *Centaurea sp.*).
3. 11 variety honeys, in which in the predominant pollen spectre grains of rape, robinia, buckwheat, phacelia, imaptiens, goldenrod and raspberry occurred, were determined.
4. In the eleven determined multifloral honeys in the secondary pollen spectre grains of clover, phacelia, buckwheat, heather, raspberry, Cirsium, Brassicaceae (except for *Brassica napus*) and Asteraceae were predominant.

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POCHODZENIE BOTANICZNE MIODÓW ZACHODNIOPOMORSKICH

Streszczenie

Badania melisopalinologiczne objęły 24 próbki miodów uzyskanych w 2014 roku przez pszczelarzy zrzeszonych w Wojewódzkim Związku Pszczelarzy w Szczecinie.
Preparaty mikroskopowe przygotowywano wykonując rozmaz miodu na szkiełku. Do zamknięcia preparatów użyto lepiku glicerożelatynowego z fuksyną zasadową. Wykonano analizę ilościową i jakościową pyłku zawartego w 1g miodu.
Zidentyfikowano ponad 90 000 ziaren pyłku należących do 47 taksonów roślin owadopylnych i kilku wiatropylnych. Wśród roślin nektarodajnych ponad 50% stanowią ziarna pyłku Brassicaceae (w tym *Brassica napus*), *Calluna vulgaris*, Asteraceae (w tym *Centaurea sp.).* W jednej próbie stwierdzano występowanie ziaren pyłku od 5 do 22 taksonów. Jeden gram miodu zawierał średnio 4196 ziaren pyłku. Oznaczono 11 miodów wielokwiatowych i 11 miodów odmianowych, z czego 4 poza Polską Normą. Dla jednej próbki wykazano szczególnie niską zawartość pyłku. Jedną próbkę stanowił produkt niebędący miodem pszczelim.

Słowa kluczowe: melisopalinologia, analiza pyłków, alergia

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