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CHANGES IN MICROFLORA OF BEE POLLEN TREATED WITH UV LIGHT AND FREEZING DURING STORAGE

ZMIANY FLORY BAKTERYJNEJ PYŁKU PSZCZELEGO ZAMRAŻANEGO I EKSPONOWANEGO NA PROMIENIOWANIE UV W CZASIE PRZECHOWYWANIA

Abstract: The aim of this work was observation of microbial community of bee pollen of poppy (*Papaver somniferum*), rape (*Brassica napus*) and sunflower (*Helianthus annus*) which were treated under freezing, UV light and then stored for 6 weeks. From among microbiological parameters were tested counts and representation of microscopic fungi, total counts of microorganisms, counts of mesophilic aerobic and anaerobic sporulating microorganisms, count of coliforms bacteria and count of cells of *Escherichia coli.* Counts of microscopic fungi in the pollen treated with UV light ranged from 1.86 log cfu · g^{-1} in the rape pollen after the 5th week of storage to 3.94 log cfu · g^{-1} in the sunflower pollen after the 1st week of storage. Counts of mesophilic anaerobic sporulating microorganisms ranged from 2.54 log cfu · g^{-1} after the 6th week of storage in the poppy pollen to 4.27 log cfu · g^{-1} in the sunflower pollen after the first week. Mesophilic aerobic sporulating microorganisms varied from 2.43 log cfu · g^{-1} in poppy pollen after the 6th week of UV light treatment to 3.60 log cfu · g^{-1} in rape pollen after the first week. Counts of coliforms bacteria ranged from 0 log cfu · g^{-1} since the 4th week in sunflower pollen and since 4th week in rape pollen to 3.33 log cfu · g^{-1} in sunflower pollen after the 1st week of UV light treatment. Counts of microscopic fungi in the pollen treated by freezing ranged from 2.13 log cfu · g^{-1} after the 5th week of storage in the pollen of rape to 4.05 log cfu · g^{-1} in

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the pollen of sunflower after the first week. Counts of mesophilic anaerobic sporulating microorganisms varied from 2.13 log cfu · g^{-1} after the 5th week in the pollen of rape to 4.65 log cfu · g^{-1} after the first week in the pollen of sunflower. Mesophilic aerobic sporulating microorganisms were found from 2.60 log cfu · g^{-1} after the 6th week of freezing of poppy pollen to 4.02 log cfu · g^{-1} after the first week of freezing in the pollen of sunflower. Counts of coliforms bacteria ranged from 0 log cfu · g^{-1} after the 3rd week in the rape pollen to 3.21 log cfu · g^{-1} after first week of freezing in the poppy pollen. *Alternaria* sp., *Cladosporium* sp. and *Penicillium* sp. were the most frequent species among microbiological fungi during whole time of observation. We can conclude that counts of microorganisms during 6 weeks storage treated with UV light and freezing decreased in all kinds of pollen.

Keywords: bee pollen, poppy, rape, sunflower, microorganisms, storage

The quantity and quality of pollen collected by honeybees affect reproduction, brood rearing and longevity, thus ultimately the productivity of the colony [1]. Apart from small quantities in nectar, honeybees obtain all the proteins, lipids, minerals and vitamins they need for brood rearing and adult growth and development from pollen [2]. The proportions of these nutrients can vary widely among pollens of different plant species [3], but few complete analyses are available for the chemical composition of pollens. Pollen analyses are generally carried out on bee-collected pollens because of the ease of collection. Bees do not consume fresh pollen. During collection and storage the pollen composition is changed through the addition of mainly nectar, but also glandular secretions [4]. Together with a specific bacterial flora associated with stored pollen, this increases the digestibility and nutritive value of pollen for honeybees.

Honeybee collected pollen, a traditional product of beekeeping, is used as an ingredient in diet cooking and is thought to be a source of physiologically active elements. Commercial production of biologically active food supplements based on honeybee pollen has recently been growing. With physiological value of pollen having been studied well enough, scientists have limited access to publications on hygienic aspects of pollen which would involve microbiological analysis. This article describes microbiological quality of pollen as a food substance in respect of the set sanitary standards.

Materials and methods

Bee-collected pollen of common poppy (*Papaver somniferum* L.), rape (*Brassica napus*) and sunflower (*Helianthus annuus*) was studied. Eighteen samples of bee-collected pollen were gathered from beekeepers, during a last spring season (year 2007). There were respected qualitative criteria for gathering, drying and storage of bee pollen according to criteria proposed by Bogdanov [5]:

a) Bee pollen was obtained from selected beekeepers. Health and hygiene conditions of bee families were controlled before a season starts.

b) The pollen was collected by special pollen traps.

c) Pollen was harvested daily and in the shortest time placed to a freezer (–18 to –20 ^{o}C) for prevention of spoilage and for preservation of a maximum quality.

d) Purification of frozen bee pollen pellets from different impurities was done most efficiently by air with special constructed purifier.

e) The frozen and purified bee pollen was dried as the gentlest way as possible to keep high nutritional value of pollen. Firstly, pollen was defrosted 2–3 hours in room conditions. Time of drying in a drying-oven was 6–8 hours. The maximum temperature was 35–40 ^{o}C. The pollen was dried until humidity was 10–11 %.

f) Dried pollen was stored under cool conditions (around 8 ^{o}C), in sterilized containers.

g) During all stages of manipulation with bee pollen were kept as sterilized conditions as possible to avoid contamination.

Determination of colony forming units (cfu) counts in pollen samples

Plate diluting method was applied for quantitative cfu counts determination of respective groups of microorganisms in 1 g of pollen sample. Gelatinous nutritive substrate in Petri dishes was inoculated with 1 cm^3 of pollen samples by flushing on surface, in three replications. Basic dilution (10^{-1}) was prepared as follows: 5 g of pollen content was added to the test tube containing 45 cm^3 of distilled water.

Media and culture conditions

The composition of nutritive substrates, for total mesophilic sporulating anaerobic and aerobic microorganisms, coliform bacteria, and *Escherichia coli*, was according to the directions for use declared by the producer (Biomark laboratories). Total mesophilic sporulating anaerobic microorganisms were grown in Meat Peptone agar (anaerobiosis), at 37 ^{o}C during 72 hours. Total mesophilic sporulating aerobic microorganisms were grown in Meat Peptone agar (aerobiosis), at 37 ^{o}C during 72 hours. Coliform bacteria were grown in Mac Conkey agar (aerobiosis), at 37 ^{o}C during 24 hours. *Escherichia coli* were grown in Violet red bile agar (aerobiosis), at 37 ^{o}C during 24 hours. The composition of these nutritive substrates was according to the directions for use declared by the producer (Biomark laboratories). Bacteria were determined according to Holt et al [6].

Isolation and morphological characterization of fungi

For determination of fungi colony-forming units (cfu) 5 g of sample was soaked in 45 cm^3 sterile tap-water containing 0.02 % Tween 80 and then 30 min shaken. Dilutions (from 10^{-1} to 10^{-5}) in sterile tap-water with 0.02 % Tween 80 were prepared and 1 cm^3 aliquots were inoculated on each of three plates of Czapek-Dox agar with streptomycin (to inhibit the bacterial growth). Petri dishes were inoculated using the spread-plate technique and incubated at 25 ^{o}C. Total fungi cfu $\cdot$ g^{-1} counts in samples were determined after 5 days of incubation.

Malt agar and Czapek-Dox agar were used to isolate and identify individual genera and species. After isolation, or in some cases monosporic isolation, individual species were identified on the basis of their macro- and micromorphology in accordance with other scientific reports [7–9].

Results and discussion

Changes in microbiological properties of bee pollen by application of gamma irradiation and ozone treatment were tested by York et al [10]. Gamma irradiation at 7.5 kGy reduced the total microbial loads below detection levels ($>10^2$ cfu · g^{-1}), but after ozone treatment of up to 18 ppm for 8 h the total aerobic bacteria were found in concentrations of more than 10^3 cfu · g^{-1}.

Our results in Table 1 show microbial community of bee pollen of poppy (*Papaver somniferum*), rape (*Brassica napus*) and sunflower (*Helianthus annus)* treated under freezing, UV light and submitted to storage for 6 weeks. The results of microbiological quality of bee pollen during storage after six weeks and UV light treatment show, growing number of all microbial groups in the second week. This numbers after next weeks are gradually reduced. The same results of microbiological quality of bee pollen after six weeks with freezing treatment were achieved.

Table 1

The number of microorganisms in bee pollen [log cfu · g^{-1}] during its storage

Sample	UV light				Freezing			
	MF	MAESM	MASM	CB	MF	MAESM	MASM	CB
1st week								
Poppy	3.56	3.32	3.43	3.28	3.67	3.93	3.56	3.23
Rape	2.76	2.87	3.60	3.27	2.69	3.01	3.37	0.57
Sunflower	3.94	4.27	3.58	3.33	4.65	4.56	4.62	3.21
2nd week								
Poppy	3.38	3.83	3.74	2.10	3.61	3.47	3.67	0.90
Rape	3.10	2.93	3.25	0.57	2.53	2.82	3.18	0.67
Sunflower	3.44	3.90	0.56	1.23	3.41	4.13	3.40	2.61
3rd week								
Poppy	2.90	3.44	3.52	2.77	3.48	3.09	3.49	0.77
Rape	2.41	2.58	3.30	0.57	2.39	2.68	3.12	0.00
Sunflower	3.23	3.81	3.48	0.49	3.28	4.01	3.35	1.61
4th week								
Poppy	3.07	3.21	3.24	2.57	3.35	3.25	3.13	0.67
Rape	2.12	2.37	3.16	0.00	2.16	2.47	3.01	0.00
Sunflower	3.09	3.61	3.39	0.00	3.16	3.996	3.28	1.39
5th week								
Poppy	2.90	3.10	2.54	1.79	3.29	3.03	3.28	0.57
Rape	1.86	2.33	2.99	0.00	2.13	2.13	2.86	0.00
Sunflower	2.90	3.54	3.25	0.00	3.05	3.63	3.33	1.33
6th week								
Poppy	2.25	2.54	2.43	1.97	2.52	2.41	2.60	1.00
Rape	2.82	2.60	3.16	0.00	2.45	2.63	3.01	0.00
Sunflower	3.06	3.63	3.29	0.00	3.10	3.68	3.28	1.39

MF – microscopic fungi, MAESM – mesophilic anaerobic sporulating microorganisms, MASM – mesophilic aerobic sporulating microorganisms, CB – coliform bacteria.

Kačániová et al [11] published similar results in pollen microbiology. It is very important to continue similar research of pollen, because standards in this area are deficient.

These findings match the data obtained by the researches in China in the number of aerobic bacteria identified in pollen, which were 10^3–10^7 cfu · g^{-1} [12]. The level of pollen contamination with moulds generally exceeded the values determined by researchers in fresh pollen in Spain [13] and in French pollen [14].

Nowadays huge attention is focused on microscopic fungi, because it was discovered that some of them are able to produce toxic metabolites, called mycotoxins, which can threat health of consumer of contaminated food [15]. In our research microscopic fungi found in bee pollen samples correspond to the genus *Alternaria* sp., *Cladosporium* sp. and *Penicillium* sp. as the most abundant (100 %, 1000 % and 78 %, respectively). The rest was represented by *Fusarium* sp. (56 %), *Mucor* sp. (56 %) and *Trichoderma* sp. (33 %).

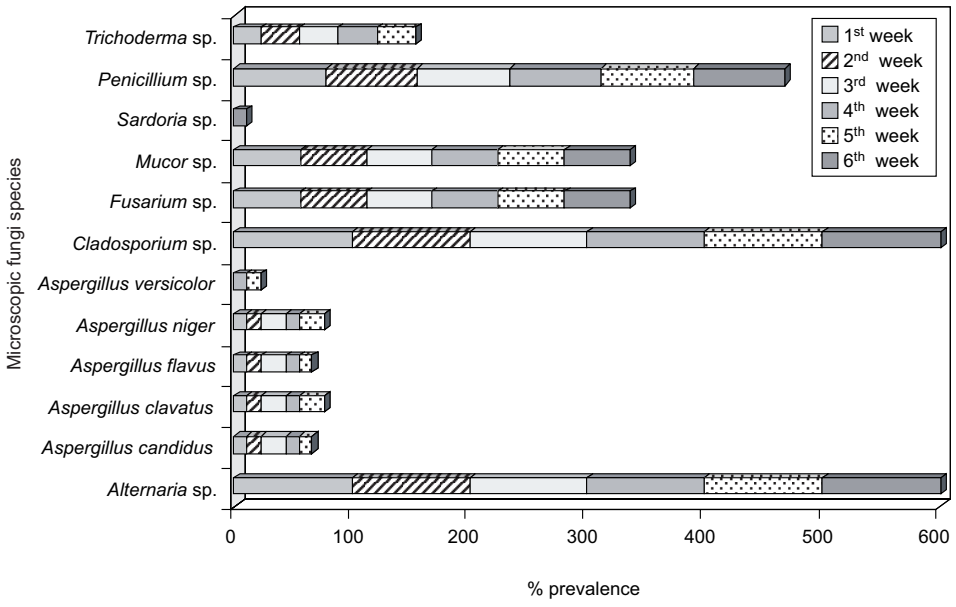

Fig. 1. Prevalence of microscopic fungi in bee pollen samples during its storage (%)

If microorganisms are responsible for fermentation and the accompanying chemical changes of pollen stored in comb cells by honey bees, the moulds may be a component of the required microbial complement. They could contribute antibiotics, organic acids and enzymes, products for which they are utilized industrially. These compounds may limit the growth of deleterious microorganisms and provide enzymes for utilization of nutrients [16].

The high water content in bee pollen is an ideal cultural medium for microorganisms as bacteria and yeast [5]. The results confirmed that the bee pollen samples contain

higher diversity of different genus of microscopic fungi than the samples of flower pollen.

Conclusion

Based on our findings, presence of moulds of up to 10^4 cfu · g^{-1} seems to be normal, which is a permissible level in Europe. In this connection studies of microflora content in relatively good quality pollen are required for the estimation of its risks for human health. From the hygienic point of view the microbiological safety is the main quality criterium. It is important to control the microbiological quality of pollen, especially the absence of pathogenic germs and yeasts. In our studies of microbiological quality we documented positive influence of UV radiation and freezing to bee pollen during its storage.

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ZMIANY FLORY BAKTERYJNEJ PYŁKU PSZCZELEGO ZAMRAŻANEGO I EKSPONOWANEGO NA PROMIENIOWANIE UV W CZASIE PRZECHOWYWANIA

Abstrakt: Celem pracy była obserwacja mikroorganizmów pyłku pszczelego pochodzącego z *Papaver somniferum*, *Brassica napus* i *Helianthus annus*, który naświetlono UV, zamrożono i przechowywano przez 6 tygodni. Spośród parametrów mikrobiologicznych oznaczono liczebność i skład gatunkowy grzybów, całkowitą liczebność mikroorganizmów, liczebność mezofilnych aerobowych i anaerobowych sporulujących mikroorganizmów, liczebność bakterii *coli* oraz liczebność *Escherichia coli*. Liczebność grzybów w pyłku *Brassica napus* naświetlanym UV wynosiła od 1.86 log cfu · g^{-1} po 5 tygodniach przechowywania do 3.94 log cfu · g^{-1} w pyłku *Helianthus annus* po 1 tygodniu przechowywania. Liczebność mezofilowych anaerobowych sporulujących mikroorganizmów wynosiła od 2.54 log cfu · g^{-1} w pyłku z *Papaver somniferum* po 6 tygodniach przechowywania do 4.27 log cfu · g^{-1} w pyłku *Helianthus annus* przechowywanym przez tydzień. Liczebność aerobowych sporulujących mikroorganizmów wynosiła od 2.43 log cfu · g^{-1} w pyłku *Papaver somniferum* przechowywanym przez 6 tygodni w obecności UV do 3.60 log cfu · g^{-1} w pyłku *Brassica napus* po pierwszym tygodniu przechowywania. Liczebność bakterii coli w pyłku *Helianthus annus* i *Brassica napus* wynosiła od 0 w 4 tygodniu przechowywania do 3.33 log cfu · g^{-1} w pyłku *Helianthus annus* po 1 tygodniu naświetlania UV. Liczebność grzybów wynosiła od 2.13 log cfu · g^{-1} w zamrażanym przez 5 tygodni pyłku *Brassica napus* do 4.05 log cfu · g^{-1} w pyłku *Helianthus annus* zamrożonym przez tydzień. Liczebność mezofilnych anaerobowych sporulujących mikroorganizmów wynosiła od 2.13 log cfu · g^{-1} w zamrażanym przez 5 tygodni pyłku *Brassica napus* do 4.65 log cfu · g^{-1} w pyłku *Helianthus annus* zamrożonym przez tydzień. Liczebność mezofilowych aerobowych sporulujących mikroorganizmów wynosiła od 2.60 log cfu · g^{-1} w pyłku *Papaver somniferum* zamrożonym przez 6 tygodni do 4.02 log cfu · g^{-1} w pyłku *Helianthus annus* zamrożonym przez tydzień. Liczebność bakterii coli wynosiła od 0 w pyłku *Brassica napus* zamrożonym przez 3 tygodnie do 3.21 log cfu · g^{-1} w pyłku *Papaver somniferum* zamrożonym przez tydzień. Spośród grzybów najczęściej występowały *Alternaria* sp., *Cladosporium* sp. i *Penicillium* sp. Zamrażanie połączone z ekspozycją na promieniowanie UV powodowało spadek liczebności mikroorganizmów we wszystkich rodzajach pyłku po 6 tygodniach przechowywania.

Słowa kluczowe: pyłek pszczeli, mak polny, rzepak, słonecznik, mikroorganizmy, przechowywanie