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PRODUCTION OF POLLEN CANS BY FERMENTATION OF BEE POLLEN IN MODEL CONDITIONS WITH REGARD TO FILAMENTOUS MICROMYCETES OCCURRENCE

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ABSTRACT

Pollen is produced by flowering plants and gathered by bees and it is rich in nutrients and important phytochemicals. Bee collected pollen is used in human nutrition, but the digestibility is limited. The possible solution is the production of fermented pollen cans according to the pattern of bee bread production by bees. The study was devoted to the testing of 3 variants of pollen cans production, from the point of view of filamentous microscopic fungi (FMF) occurrence. Totally 3 types of bee pollen and 3 types of honeys of Slovak origin, water and yoghurt as a starter (in one variant) were used for the production of pollen cans. Raw materials and fermented pollen cans were subjected to mycological analyzes (plate dilution method). Samples of honey and yoghurt were free of FMF. Pollen represents the main potential source of pollen cans contamination. The average count of FMF in pollens was 4.70 log CFU.g^{-1}. The highest number of fungi was found in the late spring bee pollen and *Cladosporium* spp. occurred with the highest relative density. The counts of FMF were reduced by fermentation and botanical origin of pollen had the greatest impact on their expansion. The greatest diversity of genera was recorded in bee pollen samples, subsequently in pollen cans has declined and after total fermentation was the lowest. *Aspergillus* spp. were isolated at a low frequency, *Alternaria* spp. appeared mainly in cans of late spring bee pollen, the highest counts of *Penicillium* spp. were recorded in cans of early spring bee pollen, in variant with added yoghurt.

Keywords: honey, bee pollen, pollen can, beebread, filamentous microscopic fungi

INTRODUCTION

Bee pollen as one of bee product is considered as the most complete food in the nature **(Linskens *et* Jorde, 1997)**. Honeybees (*Apis mellifera*) forage pollen grains of specific floral species, resulting in highly agglutinated pollen loads attached to their hind legs. The bees initiate the preparation of food for the beehive by adding nectar and salivary substances to the pollen. Part of these pollen loads is collected by the beekeeper before the bees enter the beehive and is treated as bee pollen **(Deveza *et al.*, 2015)**.
The old Egyptians describe pollen as "a life-giving dust" **(Bogdanov, 2017)** and pollen as a food and medicine was traditionally used in The Far East region, especially in China **(Brindza *et al.*, 2010)**. Bee collected pollen began to be used for human nutrition only after the Second World War, when pollen traps were developed **(Bogdanov, 2017)** and the consumption is constantly increased due to grooving consumers demand for the healthier and nutritious diet **(Linskens *et* Jorde, 1997)**.
Bee pollen consists of proteins, lipids, sugars, dietary fibers, trace elements, enzymes, fatty acids, vitamins and minerals **(Bonvehí *et* Jordà, 1997; Block *et al.*, 1994)**. As a functional food, one of the main health enhancing properties of pollen is the strong antioxidant activity **(Bogdanov, 2017)**. It has high contents of biological active substances such as polyphenols, mainly flavonoids, which possess this high antioxidant capacity **(Carpes *et al.*, 2009; Leja *et al.*, 2007)**.
Doubts have been raised, whether the tough shell of pollen can be cracked and digested by humans. It has been found out, that in animal experiments pollen does longer contain their content when they have left the digestion tract **(Roulston *et* Cane, 2000)**. In *in vitro* simulation of human digestion the pollen was partly digested **(Franchi *et al.*, 1997)**, and there were differences in the degree of digestion of various types of pollen, with an average degree of digestibility of 15% for carbohydrates and 53% for proteins. In this case has been hypothesized that pollen is insufficiently digested and that cracking will improve the digestibility and bioavailability **(Rimpler, 2013)**.
Bees naturally induce bioprocesses in the interior of their hive to make pollen nutrients more bioavailable **(Salazar-González *et* Díaz-Moreno, 2016)**. They store pollen in the hive as beebread **(Bogdanov, 2017)**, which is a primary protein source necessary for the proper development and growth of adult bees and bee larvae **(Yoder et al., 2012)**. Pollen is mixed with honey and bee secretions and stored in the combs. Bee bread undergoes lactic acid fermentation and can be thus preserved. This type of fermentation is similar to that in yoghurts (and other fermented milk products) and renders the end product more digestible and enriched with new nutrients. One advantage is almost unlimited storability of beebread in comparison with dried or frozen pollen in which nutritional values are rapidly lost. The natural process carried out by the bees can more or less be repeated artificially with dry or fresh bee-collected pollen. It is important however, to provide the correct conditions during the fermentation process. From hygienic point of view, the microbiological safety is the main quality criterion, especially the absence of pathogenic germs and fungi in the pollen **(Bogdanov, 2017)**. Pollen is prone to fungal contamination that could potentially generate toxins, which are harmful to human health **(Deveza *et al.*, 2015)**.
Considering that the consumption of bee pollen and fermented pollen cans could be a potential risk for the human health if it is contaminated with the mycotoxigenic fungi, the main objective of this study was to determine the occurrence of natural mycobiota in bee pollen originated from Slovakia and in fermented pollen cans.

MATERIAL AND METHODS

Material

In the present study, we have tested the production of several variants (I., II., III.) of fermented pollen cans. For their production we used fresh bee pollen, honey, boiled water and yoghurt (in case of variant III.). There were three different samples of bee pollen, three different samples of honey and one sample of yoghurt. All honey and pollen samples were obtained directly from beekeepers. Fresh pollen was stored in the freezer until processing (about 1 week). Yoghurt came from the commercial production of small Slovak producer. Used raw materials are characterized in Table 1.

Table 1 Raw materials used for pollen cans production

Sample	Type	Geographical origin	Year
BEE POLLEN			
A	early spring (from plants before *Brassica napus* blooming)	Male Krstenany (Slovakia)	2017
B	rape (*Brassica napus*)	Male Krstenany (Slovakia)	2017
C	late spring (from plants after *Brassica napus* blooming)	Dolny Pajer* (Slovakia)	2017
HONEY			
a	blossom	Male Krstenany (Slovakia)	2017
b	rape (*Brassica napus*)	Male Krstenany (Slovakia)	2017
c	blended (*Tilia cordata* + honeydew)	Dolny Pajer* (Slovakia)	2017
YOGHURT			
	cow whole milk	eastern part of Slovakia	2018

* unpolluted area surrounded by forest with cottages and only a few inhabitants

Production of pollen cans

The production of pollen cans was carried out according to **Dany (1988)**, as described by **Krell (1996)**. Water (75 ml) was boiled and cooled to 30 ± 2 °C. Honey (45 g) was added to the water and the solution was carefully mixed. Finally, the pollen (300 g) was added and mixed to obtain homogenous material. Then the products were bottled and bottles were filled up to 70 – 75%.

In the study, we tested three methods of preparing pollen cans - we worked with three variants of the experiment. Totally, 21 pollen cans were made – each variant of 9 cans (Table 2). In the first two variants we made nine combinations of the honeys and pollens and in the third variant we made 3 combinations, each with 3 different amounts of added yoghurt (1, 2 or 3 g).

Table 2 Raw materials used in the production of individual variants of pollen cans

Variant	Raw material	Pollen can number								
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
I.	Honey sample	a	b	c	a	a	b	b	c	c
	Pollen sample	A	B	C	B	C	A	C	B	A
	Yoghurt amount	-	-	-	-	-	-	-	-	-
II.	Honey sample	a	b	c	a	a	b	b	c	c
	Pollen sample	A	B	C	B	C	A	C	B	A
	Yoghurt amount	-	-	-	-	-	-	-	-	-
III.	Honey sample	a	a	a	b	b	b	c	c	c
	Pollen sample	A	A	A	B	B	B	C	C	C
	Yoghurt amount	1 g	2 g	3 g	1 g	2 g	3 g	1 g	2 g	3 g

a – blossom honey, b – rape honey, c – blended honey, A – early spring bee pollen, B – rape bee pollen, C – late spring bee pollen

The fermentation was carried out under the conditions shown in the Table 3. Differences between variants consisted of oxygen access in the first stage of the cans production, in the length of the next fermentation and in addition of the yoghurt as an additional easily-available starter of fermentation. In the first two variants, the fermentation was carried out only through the microorganisms naturally occurring in the raw materials (in the pollen and partly in the honey). Variant with oxygen access during the first stage of the production was traditional, according to **Hajdušková (2006)**, where bottles were covered by linen cloth, consequently oxygen was present during this stage.

Table 3 Fermentation conditions in the individual stages of the production of pollen cans

Variant	Initial fermentation			Next fermentation			Total time
	Temperature	**Time**	**O_2 access**	**Temperature**	**Time**	**O_2 access**	
I.	30 ± 1 °C	2 days	no	RT (23 ± 2 °C)	16 days	no	18 days
II.	RT (23 ± 2 °C)	5 days	yes	RT (23 ± 2 °C)	13 days	no	18 days
III.	30 ± 1 °C	2 days	no	RT (23 ± 2 °C)	6 days	no	8 days

RT – room temperature

Water activity

Samples of pollen, honey and yoghurt, used for can production, were analyzed for water activity (a_w). Analyses were performed using the reference device LabMaster-aw from Novasina.

Microbiological analysis

Raw materials (pollen, honey and yoghurt) as well as examined pollen cans (after initial and after next fermentation) were tested for the presence of filamentous microscopic fungi. In all cases, we used plate dilution method. A total of 5 g of tested material was added to 45 ml of sterile 0.1% peptone water with 0.85% NaCl. Prepared suspensions were shaken on horizontal shaker for 20 minutes. Dilutions from 10^{-1} to 10^{-5} (as needed) were inoculated in amount of 1 ml to the sterile Petri dishes, poured with molten cooled DG18 medium (agar with dichloran and 18% of glycerol), which is recommended as a selective medium for the isolation and cultivation of xerophilic fungi, and mixed well. After the solidification of the agar, the plates were incubated for 5 to 7 days in darkness at 25 ± 1 °C. Grown micromycetes were counted and classified into the genera.

Calculations and expressions of the results

Data from microbiological analyses were calculated as log CFU.g^{-1}. Results for experimental variants were processed in MS Excel 2007 as mean ± sd (standard deviation). Significant difference was assessed if it was at least 1.00 log CFU.g^{-1}.

The obtained data on occurring genera were evaluated and expressed in relative density (RD) at the genus level. The relative density (%) is defined as the percentage of isolates of the genus, occurring in the analysed sample **(Guatam *et al.*, 2009)**. These values were calculated according to **González *et al.* (1996)** as follows:

$$RD\ (\%) = (ni / Ni) \times 100$$

where ni = number of isolates of a genus, Ni = total number of isolated fungi.

RESULTS AND DISCUSSION

Analyses of raw materials

The results of microbiological analyses of raw materials used for the production of pollen cans are given in the Table 4. The water activity (a_w) is one of the factors that affect the ability of microorganisms to grow in different materials. In many practical situations it is the dominant environmental factor governing food stability or spoilage. Like all other organisms, fungi are profoundly affected by the availability of water **(Pitt *et* Hocking, 1999)**. Micromycetes (especially those that most often cause food spoilage) can grow even at a_w 0.80 **(Tančinová *et al.*, 2017)**. Honey is generally considered as a safe commodity. In addition, has proven antimicrobial properties. In our honey samples, the presence of filamentous microscopic fungi was not detected. Also, the presence of microscopic fungi was not detected in the yoghurt sample. Spores of microscopic fungi can survive in bee pollen and thus the pollen represents the main potential

source of pollen cans contamination. The average count of filamentous microscopic fungi in tested pollens was 4.70 log CFU.g^{-1}.

For comparison, in the study of **Kačániová *et al.* (2011)**, the total number of microscopic fungi ranged from 2.98 ± 0.02 in frozen sunflower bee pollen to 4.06 ± 0.10 log CFU.g^{-1} in sunflower bee pollen after UV radiation. According to **Bogdanov (2017)**, fresh, bee collected pollen contains about 20-30 g water per 100 g. This high humidity is an ideal culture medium for microorganisms. For prevention of spoilage and for preservation of a maximum quality the pollen has to be harvested daily and immediately placed in a freezer. After thawing, pollen can be kept only for a few hours and should be further processed as soon as possible. After drying the water content should be 6 g water per 100 g pollen. Authors **Kačániová *et al.* (2011)** reported, that the collection, ways of treatment and storage of bee-collected pollen influence the food safety of this special product of hive.

Table 4 Water activity (a_w) and quantitative detection of microscopic fungi in raw materials used in production of pollen cans

Tested material	Water activity	Quantity of filamentous microscopic fungi [log CFU.g^{-1}]
pollen A	0.572	4.28
pollen B	0.708	4.49
pollen C	0.547	5.35
mean ± sd	**0.609 ± 0.087**	**4.70 ± 0.57**
honey a	0.523	< 1.00
honey b	0.498	< 1.00
honey c	0.540	< 1.00
mean ± sd	**0.520 ± 0.021**	**< 1.00**
yoghurt	0.881	< 1.00

Isolated fungi have been subjected to genus identification. Totally 8 genera were detected (Table 5). Microscopic fungi that did not produce typical identification structures were classified as *Mycelium sterilium*. The highest number of fungi was found in the late spring bee pollen (C). In general, *Cladosporium* spp. occurred with the highest relative density. In the early spring bee pollen (A) we noticed higher relative density of *Penicillium* spp. and in late spring bee pollen *Alternaria* spp. (potentially toxigenic genera).

In the similar study of **Kačániová *et al.* (2011)** the most frequent isolates of microscopic fungi found in Slovak bee pollen samples of all prevalent species were *Mucor mucedo*, *Alternaria alternata*, *Mucor hiemalis*, *Aspergillus fumigatus* and *Cladosporium cladosporioides*. Situation in Serbia has been documented in papers of **Petrovic *et al.* (2014)**. Authors report that the predominant fungi were from the genera *Alternaria* (48.48%) and *Aspergillus* (39.39%), followed by *Penicillium* spp. (24.24%) and *Mucor* spp. (21.21%), while the most common isolated species was *A. flavus* with an incidence of 27.27%. Analysis of hygienic quality of Brazilian pollen based on fungal load showed that *Aspergillus*, *Cladosporium* and *Penicillium* were the most common genera and even the toxigenic evaluation showed that 25% of the *A. flavus* strains produced aflatoxins **(Deveza *et al.*, 2015)**.

Table 5 Numbers of isolates and relative density [%] of isolated filamentous microscopic fungi from the samples of raw bee pollen used in production of pollen cans

Genus	Number of isolates (relative density [%])		
	A	B	C
Alternaria spp.	1 (1.59)	5 (4.63)	23 (3.38)
Arthrinium sp.	-	-	1 (0.15)
Aspergillus sp.	-	-	1 (0.15)
Aureobasidium spp.	-	2 (1.85)	4 (0.59)
Botrytis cinerea	1 (1.59)	1 (0.93)	3 (0.44)
Cladosporium spp.	28 (44.44)	91 (84.25)	630 (92.65)
Epicoccum spp.	-	1 (0.93)	6 (0.88)
Mycelium sterilium	1 (1.59)	3 (2.78)	2 (0.29)
Penicillium spp.	32 (50.79)	5 (4.63)	10 (1.47)

A - early spring bee pollen, B – rape bee pollen, C - late spring bee pollen

Analyses of pollen cans

Detected counts of filamentous micromycetes of tested pollen cans are listed in Table 6. In the most cases we noticed, that the pollen cans in which the late spring pollen was used, were the most contaminated with microscopic fungi. Botanical origin of pollen is obviously an important factor in term of microscopic fungi propagation. On the other hand, the lowest counts of filamentous microscopic fungi were recorded in cans with rape bee pollen, in all tested variants. The amount of the used starter (1, 2 or 3 g of yoghurt) did not have a significant effect on the counts of micromycetes in cans. Even lower numbers of micromycetes after total fermentation were recorded in variants I. and II. (without starter).

Table 6 Occurrence of filamentous microscopic fungi [log CFU.g^{-1}] after initial and next fermentation of the pollen cans

Var.	Bee pollen	A			B			C		
	pollen can	**1**	**6**	**9**	**2**	**4**	**8**	**3**	**5**	**7**
	initial fermentation	3.85	3.95	4.16	3.48	<3.00	3.00	4.87	4.78	4.80
I.	**mean ± sd (n = 3)**		**3.99 ± 0.16**			**3.24 ± 0.34**			**4.82 ± 0.05**	
	next fermentation	3.00	1.70	3.30	<1.00	<1.00	<1.00	1.48	3.00	1.48
	mean ± sd (n = 3)		**2.67 ± 0.85**			**ND**			**1.99 ± 0.88**	
	pollen can	**1**	**6**	**9**	**2**	**4**	**8**	**3**	**5**	**7**
	initial fermentation	3.95	3.48	3.48	<3.00	3.30	3.00	4.42	4.45	4.32
II.	**mean ± sd (n = 3)**		**3.64 ± 0.27**			**3.15 ± 0.21**			**4.40 ± 0.07**	
	next fermentation	1.30	1.48	1.70	<1.00	1.30	1.00	1.70	3.00	3.30
	mean ± sd (n = 3)		**1.49 ± 0.20**			**1.15 ± 0.21**			**2.67 ± 0.85**	
	pollen can	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
	initial fermentation	4.10	4.16	4.19	3.00	1.78	1.78	5.03	4.87	4.95
III.	**mean ± sd (n = 3)**		**4.15 ± 0.05**			**2.19 ± 0.70**			**4.95 ± 0.08**	
	next fermentation	3.70	3.30	3.30	1.60	1.60	3.00	3.48	3.00	3.30
	mean ± sd (n = 3)		**3.43 ± 0.23**			**2.07 ± 0.81**			**3.26 ± 0.24**	

A – early spring bee pollen, B – rape bee pollen, C – late spring bee pollen, ND – not detected

In general, fungal contamination of food involves the presence of mycotoxins, which are products of fungal secondary metabolism **(FAO, 1990)**. Studies have demonstrated their toxigenic, nephrotoxic, hepatotoxic, carcinogenic, immunosuppressive and mutagenic characteristics **(Bezerra da Rocha *et al.*, 2014)**. The main genera of potentially toxigenic microscopic fungi are *Alternaria*, *Aspergillus*, *Fusarium* and *Penicillium*, where the *Aspergillus* and *Penicillium* genera are able to grow under conditions of lower water activity.

Isolated micromycetes, their counts and relative density are listed in Tables 7, 8 and 9 (according to individual variants). The greatest diversity of genera was recorded in bee pollen samples, subsequently in pollen cans the diversity has declined. After total fermentation, the genera diversity was the lowest. No micromycetes were recorded after the total fermentation in the case of pollen can originating from early spring bee pollen (A) - in variant II. and in the case of pollen cans originating from rape bee pollen (B) -in variants I. and II. From the perspective of used pollens, the widest genera range was recorded in pollen cans originating from late spring bee pollen (C), in all tested variants.

The highest numbers of isolates were represented by the members of the genus *Cladosporium*. It is very common fungi and can grow under refrigerated storage conditions. The main consequence of *Cladosporium* species in foods is spoilage and discoloration **(Bullerman, 2003)**.

From the perspective of toxigenic micromycetes occurrence, the potential risk may arise from isolates of *Alternaria*, *Aspergillus* and *Penicillium* genera. Out of these genera, isolates of the genus *Aspergillus* were isolated at a low frequency. Representatives of the genus *Alternaria* appeared mainly in cans of late spring bee pollen (C), but after total fermentation were not isolated. *Alternaria* spp. were not detected in samples with rape bee pollen (B). The highest counts of *Penicillium* spp. were recorded in cans of early spring bee pollen (A), especially in variant III.

Table 7 Numbers of isolates and relative density [%] of isolated filamentous microscopic fungi from the samples of pollen cans - variant I. after initial and next fermentation

Genus	Number of isolates (relative density [%])[a, b]								
	A			B			C		
	1	6	9	2	4	8	3	5	7
Alternaria spp.	1 (14.29)[a]	-	2 (12.50)[a]	-	-	-	5 (6.17)[a]	10 (14.29)[a]	5 (7.04)[a]
Arthrinium spp.	-	-	-	-	-	-	1 (1.23)[a]	2 (2.86)[a]	1 (1.41)[a]
Aspergillus spp.	-	-	2 (12.50)[a] 2 (100.00)[b]	-	-	-	-	-	-
Aureobasidium spp.	-	-	-	-	-	-	2 (2.47)[a]	-	2 (2.82)[a]
Botrytis cinerea	-	-	-	-	-	-	-	-	1 (1.41)[a]
Cladosporium spp.	2 (28.57)[a]	3(30.00)[a]	7 (43.75)[a]	-	-	-	70 (86.42)[a]	54 (77.14)[a]	57 (80.28)[a]
Epicoccum spp.	-	-	-	-	-	-	-	2 (2.86)1[a]	2 (2.82)[a]
Mucor sp.	-	-	-	-	-	-	-	1 (100.00)[b]	
Mycelium sterilium	-	-	-	1 (33.33)[a]	-	-	3 (3.70)[a]	2 (2.86)[a]	-
Penicillium spp.	4 (57.14)[a] 1 (100.00)[b]	7 (70.00)[a]	5 (31.25)[a]	2 (66.67)[a]	1 (100.00)[a]	1 (100.00)[a]	-	-	3 (4.23)[a]

a – initial fermentation, b – next fermentation

Table 8 Numbers of isolates and relative density [%] of isolated filamentous microscopic fungi from the samples of pollen cans - variant II. after initial and next fermentation

Genus	Number of isolates (relative density [%])[a, b]								
	A			B			C		
	1	6	9	2	4	8	3	5	7
Absidia sp.	-	-	-	-	-	-	-	1 (3.13)[a]	-
Acremonium sp.	-	-	-	-	-	-	-	-	1 (4.35)[a]
Alternaria spp.	-	-	-	-	-	-	7 (23.33)[a]	5 (15.63)[a]	5 (21.74)[a]
Arthrinium spp.	-	-	-	-	-	-	1 (3.33)[a]	-	1 (4.35)[a]
Aureobasidium spp.	-	-	-	-	-	-	-	2 (6.25)[a]	-
Cladosporium spp.	2 (22.22)[a]	-	-	-	-	-	17 (56.67)[a]	23 (71.88)[a]	14 (60.87)[a] 1 (50.00)[b]
Epicoccum spp.	-	-	-	-	-	-	2 (6.67)[a]	-	1 (4.35)[a]
Mucor spp.								1 (100.00)[b]	1 (50.00)[b]
Mycelium sterilium	-	-	-	-	-	-	2 (6.67)[a]	1 (3.13)[a]	-
Penicillium spp.	7 (77.78)[a]	3 (100.00)[a]	3 (100.00)[a]	-	2 (100.00)[a]	1 (100.00)[a]	1 (3.33)[a]	-	1 (4.35)[a]

a – initial fermentation, b – next fermentation

Table 9 Numbers of isolates and relative density [%] of isolated filamentous microscopic fungi from the samples of pollen cans - variant III. after initial and next fermentation

Genus	Number of isolates (relative density [%])[a, b]								
	A			B			C		
	1	2	3	4	5	6	7	8	9
Absidia sp.	-	-	-	-	-	1 (16.67)[a]	-	-	-
Alternaria spp.	-	-	1 (5.88)[a]	-	-	-	12 (10.17)[a]	3 (3.53)[a]	7 (7.07)[a]
Arthrinium spp.	-	-	-	-	-	-	2 (66.67)[b]	1 (1.18)[a] 1 (100.00)[b]	1 (1.01)[a] 3 (100.00)[b]
Aspergillus spp.	-	-	-	-	-	4 (66.67)[a]	-	-	1 (1.01)[a]
Aureobasidium spp.	-	1 (33.33)[b]	1 (5.88)[a]	-	-	-	-	-	-
Cladosporium spp.	7 (50.00)[a]	6 (37.50)[a]	2 (11.76)[a]	-	-	-	100 (84.75)[a] 1 (33.33)[b]	78 (91.76)[a]	87 (87.88)[a]
Epicoccum sp.	-	-	1 (50.00)[b]	-	-	-	-	-	-
Mucor spp.	-	-	-	-	-	1 (16.67)[a]	1 (0.85)[a]	-	-
Mycelium sterilium	-	1 (33.33)[b]	1 (5.88)[a]	1 (100.00)[a]	-	-	4 (3.39)[a]	-	2 (2.02)[a]
Penicilium spp.	7 (50.00)[a] 5 (100.00)[b]	10 (62.50)[a] 1 (33.33)[b]	12 (70.59)[a] 1 (50.00)[b]	-	-	1 (100.00)[b]	1 (0.85)[a]	3 (3.53)[a]	1 (1.01)[a]

a – initial fermentation, b – next fermentation

CONCLUSION

Pollen is commodity with relatively high counts of microorganisms, which occur naturally and probably most of them are positive for human health, however it is necessary to eliminate the activities of pathogenic bacteria and microscopic filamentous fungi. According to our mycological analyses, the bee pollen is a very good matrix for microscopic fungi. Microscopic filamentous fungi belong to risk microbial groups, mainly for children, older people and people, who have problems with immune system. In Slovakia, no qualitative and quantitative microbiological regulations for pollen as a food supplement have been established and the research studies could help to set some limits in the future.

The study showed that the counts of filamentous microscopic fungi were reduced by fermentation, as in bee pollen samples we recorded higher numbers and in fermented products (in all tested variants) they have been reduced. Differences in mycocenosis were mainly observed depending on the used pollen. Botanical origin of pollen is obviously an important factor in term of microscopic fungi propagation. It is questionable how the next storage of cans will affect the mycocenosis. Therefore, another study will focus on monitoring the mycological quality of stored pollen cans.

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