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REGULAR ARTICLE

MICROSCOPIC FUNGI ISOLATED FROM POLISH HONEY

Soňa Felšöciová[1], Miroslava Kačániová[1], Lukáš Hleba[1], Jana Petrová[1], Adriana Pavelková[2], Molgorzata Dzugan[3], Dorota Grabek-Lejko[4]*

Address: [1]Department of Microbiology, Faculty of Biotechnology and Food Science, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic;
[2]Department of Animal Products Evaluation and Processing, Faculty of Biotechnology and Food Science, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic;
[3]Department of Food Chemistry and Toxicology, Faculty of Biology and Agriculture, University of Rzeszow, 2 Ćwiklińskiej St., 35-601 Rzeszow, Poland;
[4]Department of Biotechnology and Microbiology, Faculty of Biology and Agriculture, University of Rzeszow, 2 Ćwiklińskiej St., 35-601 Rzeszow, Poland.

*Corresponding author: sona.felsociova@uniag.sk, Phone no.: + 421 037 641 5813

ABSTRACT

The characterization of some honey samples from Poland was carried out on the basis of their microbiological (fungi and yeasts) analysis. Most of the samples contained less than 20 % water. The amount of fungi found in the honey samples was less than 1 x 10^2 CFU.g^{-1} but 19 % of the samples had more yeasts than 1 x 10^2 CFU.g^{-1} – up to 5.7 x 10^2 CFU.g^{-1}. The isolated fungi were *Alternaria* spp., *Aspergillus* spp., *Cladosporium* spp., *Fusarium* spp., *Mycelia sterilia, Rhizopus* spp. and *Penicillium* spp. The last genus was isolated very frequently. A total number of eight fungal *Penicillium* species were identified namely, *Penicillium brevicompactum*, *P. commune, P. corylophilum, P. crustosum, P. expansum, P. griseofulvum, P. chrysogenum* and *P. polonicum*. They were isolated using dilution plate method. The results showed that honeys produced in this region are of good microbiological quality.

Keywords: Honey, Mycobiota, Fungi, Yeast, *Penicillium*

INTRODUCTION

The quality of honey is mainly determined by its sensorial, chemical, physical and microbiological characteristics. Honey has two sources of contamination with microorganisms: primary sources include pollen, the digestive tracts of honey bees, dust, air, soil and nectar. Secondary sources are those arising from honey manipulation by people, they include air, food handlers, cross-contamination, equipment and buildings. Primary sources of honey contamination are very difficult to control. Conversely, secondary sources of honey contamination can be controlled by good manufacturing practices. The microbes of concern in honey are fungi, yeasts and spore-forming bacteria. The presence of micro-organisms in honey can sometimes influence the stability of the product and its hygienic quality (**Popa et al., 2009**). Fungi and yeasts are responsible for honey fermentation when the moisture content is high (above 21 %). *Penicillium* and *Mucor* are microorganisms usually found in honey. Moreover, the presence of strains of *Bettsya alvei, Acosphaera apis* and *Acosphaera major* may be indicative of bad bee-hive managment practices. On the other hand, strains of *Saccharomyces, Schizosaccharomyces* and *Torula* predominate among yeasts (**Migdal et al., 2000**).

Yeast and fungi presence in honey is unavoidable, since bees collect them together with the nectar. According to the present regulations, honey aqueous activity ranges between 0.593 and 0.637, which inhibits the development of almost all its microorganisms. Even the osmophile yeast (*Saccharomyces rouxii*) and fungi (*Aspergillus echinulatus, Monascus bisporus*) are inhibited when moisture content is lower than 171 $g.kg^{-1}$. If moisture content is comprised between 171 and 200 $g.kg^{-1}$, the product stability will depend on the microbial content, and if moisture content is higher than 200 $g.kg^{-1}$, osmophile yeast may develop. Their action upon fructose and glucose can produce carbon dioxide and ethanol. The latter, combined with oxygen, may produce acetic acid (**Snowdon and Cliver, 1996**).

Honey distinctive characteristics are not due to its stable major compounds, which can be found in any other sweet product such as sugar, molasses, syrup and marmelade, but to its multitude of minor components originated from the nectar and the bees themselves. Many of these substances, which give honey its specific aroma, flavour and some of its biological activity are unstable over time and thermolabile. Heating has a negative effect on honey due

to the loss of those substances proportionally to the temperature and duration of the thermal treatment applied. Honey high-temperature thermal treatments for inhibiting fungi and yeast development capacity and delaying honey crystallisation are not accepted as a current practise for quality standards (**Bogdanov, 1993**).

MATERIAL AND METHODS

Collection of samples

The study was carried out on 43 selected honey samples (Table 1) what make up 13 types of honey. Honey samples were provided by beekeepers and produced during the 2006 – 2010 harvest in Poland. All of them were not heated by the producers and showed no signs of fermentation or crystallisation. The samples were preserved at 0 - 4 °C until use in plastic bottles and examined for mycobiota during the storage.

Table 1 Characterization of Poland honey samples

Samples	Type of honey	Year of production
1.	Polyfloral (Mixed floral honey)	2010
2.	Polyfloral	2009
3.	Polyfloral	2010
4.	Polyfloral	2010
5.	Polyfloral	2010
6.	Polyfloral	2010
7.	Polyfloral	2010
8.	Polyfloral	2009
9.	Polyfloral	2010
10.	Polyfloral	2010
11.	Buckwheat	2010
12.	Buckwheat	2010
13.	Buckwheat	2009
14.	Buckwheat	2008
15.	Buckwheat	2007
16.	Buckwheat	2006
17.	Buckwheat	2010
18.	Heather	2010
19.	Heather	2009
20.	Heather	2010
21.	Monofloral *Solidago* L.	2010
22.	Monofloral *Solidago* L.	2010

23.	Lime (*Tilia*)	2010
24.	Lime	2010
25.	Rape	2009
26.	Rape	2010
27.	Rape	2007
28.	Coniferous honeydew	2009
29.	Coniferous honeydew	2009
30.	Coniferous honeydew	2009
31.	Coniferous honeydew	2007
32.	Coniferous honeydew	2010
33.	Deciduous honeydew	2010
34.	Deciduous honeydew	2009
35.	Deciduous honeydew	2009
36.	Monofloral (*Phacelia tanacetifolia Benth.*)	2010
37.	Monofloral (*Phacelia tanacetifolia Benth.*)	2010
38.	Monofloral (*Phaseolus coccineus*)	2010
39.	Monofloral (*Acacia*)	2010
40.	Monofloral (*Taraxacum officinale*)	2010
41.	Blossom nectar honey	2010
42.	Blossom nectar honey	2010
43.	Blossom nectar honey	2010

Isolation of fungi from the stored honey

Dilution plate method

This method was used to determine the type of fungi present in the stored honey. Five gram of the sample was mixed with 45 ml of physiological solution. This was shaken thoroughly and 1 ml of suspension was pipetted into a sterile test tube containing 9 ml of physiological solution. This was thoroughly mixed together and from previous solution was obtained dilution 10^{-2}. The sample was serially diluted and 0.1 ml each of aliquots of 10^{-1} and 10^{-2} were added to stiff Malt Extract Agar (MEA) plates. All determinations were performed in duplicate. Finally, the plates were incubated at 25 ± 1°C from 5 to 7 days in the dark.

Expression of results

The fungal and yeast colonies were counted in plates. Average number of colonies, multiplied by the dilution factor, was considered for the counting of yeast and fungi colonies. The colony forming unit number (CFU) was reported per gram of sample.

Identification of mycobiota

The fungi were identified by their cultivated and morphological features (Samson et al., 2010). The members of genera *Aspergillus* were isolated on diagnostic media of CYA (Czapek Yeast Extract agar, **Samson et al., 2010**), MEA (Malt extract agar, **Samson et al., 2010**) and the isolates of genera *Penicillium* were consequently isolated on MEA and CYA. Additional agar media were used for some species in the terverticillate *Penicillium* group. Creatine-Sucrose agar (CREA, **Samson et al., 2010**) and Yeast Extract agar (YES, **Samson et al., 2010**) are very useful for distinguishing closely related species. On CREA characteristics of colony growth, production of acids (turning the medium from purple to yellow) and base production can be used as diagnostic features of the species. The degree of sporulation, colony diameters and obverse colours are used when examining YES agar. The Petri dishes were incubated at 25 ± 1°C in darkness for 5 – 7 days. *Aspergillus* species were diagnostic by **Samson et al. (2010)** and *Penicillium* by **Pitt and Hocking (1997), Samson et al. (2002, 2010)** and for more detailed descriptions and keys of ter– and quaterverticillate Penicillia atlas from **Frisvad and Samson (2004)**.

RESULTS AND DISCUSSION

Alternaria spp., Aspergillus fumigatus, A. niger, Aspergillus sp., Cladosporium spp., Fusarium spp., Mycelium sterilium, Penicillium brevicompactum, P. commune, P. corylophilum, P. crustosum, P. expansum, P. griseofulvum, P. chrysogenum, P. polonicum, Rhizopus spp. were the fungi found in the 43 selected honey samples from Poland. The results of fungi isolated from stored honey using dilution plate method is shown on Table 2.

Table 2 Occurrence of fungi in different types of honey

Species and genera	Number of isolates in different types of honey													
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	Σ
***Alternaria* spp.**	1													1
Aspergillus sp.	1													1
A. fumigatus	1						1							2
A. niger													1	1
Aspergillus														4
***Cladosporium* spp.**	1	2												3
***Fusarium* spp.**	1													1
Mycelium sterilium	2		2				1							5
P.brevicompactum								4	2					6
P. commune	1						1							2
P. corylophilum			1											1
P. crustosum			1				1	1			1			4
P. expansum	1													1
P. griseofulvum	2			1										3
P. chrysogenum	4	3	1				1	1	1	1	1		2	15
P. polonicum													1	1
Penicillium														33
***Rhizopus* spp.**						1	2							3
Σ	15	5	5	1	-	1	7	6	3	1	2	-	4	50

Legends: **1.** – Samples no. 1-10; **2.** – Samples no. 11-17; **3.** – Samples no.18-20; **4.** – Samples no.21-22; **5.** – Samples no.23-24; **6.** – Samples no. 25-27; **7.** – Samples no. 28-32; **8.** – Samples no. 33-35; **9.** – Samples no. 36-37; **10.** – Sample no. 38; **11.** – Samples no. 39; **12.** – Sample no. 40; **13.** – Samples no. 41-42; **14.** – Sample no. 43

We recovered 50 isolates of these samples whereby 33 were represented by the genus *Penicillium*. The rest of the isolated filamentous fungi were less common. They were isolated in 10 % (*Mycelium sterilium*) and less often. Fungi belonging to the genus *Penicillium* were detected in 66 % of samples. *P. chrysogenum* (30 %) with total number of 15 isolates appeared to be the most encountered species. *P. chrysogenum* is common on dried, salted foods with sucrose and other carbohydrates. It is halotolerant and psychrotolerant (**Samson et al., 2010**). Occurence of this species is panglobal, very common (**Frisvad and Samson, 2004**). *P. brevicompactum* was found in 6 samples (12 %). *P. brevicompactum* can tolerate low pH values and rather low water activities (**Samson et al., 2010**). Species is cosmopolitan (**Frisvad and Samson, 2004**). Other species of *Penicillium* were found only in less than 10 % of the samples. Some similar study was done from 46 samples of Slovak honey (**Kačániová**

et al., 2012a, b). Also the most frequently encountered taxa from *Penicillium* genera were *P. chrysogenum* (23 %), *P. brevicompactum* (14 %), *P. crustosum* and *P. griseofulvum* (11 %). According to several studies the species of the genus *Penicillium* belong to the dominant honey mycobiota (**Migdal et al., 2000, Vica et al., 2009)**. Penicillia are also known as potential mykotoxin producers (**Samson et al., 2010**). The possible presence of mycotoxigenic fungi in foods, and rational decisions on the status of foods suspected to contain mycotoxins, are ever present problems in the food industry around the world **(Frisvad et al., 2006)**. Therefore it is important their identification.

From 13 types of honey no number of fungi was isolated from Lime (23, 24) and *Taracacum officinale* (40). From remaining types of honey at least one sample was contaminated by fungi. Except honey 23, 24 and 40 *Penicillium* spp. isolates were not detected in any of the rape samples (25, 26, 27). From 9 types of honey *P. chrysogenum* was detected and again this species was predominant. The contamination level of samples by fungi was law with a charge variable between 0.5 x 10^1 and 1 x 10^2 CFU.g^{-1} (average 1.1 x 10^1 CFU.g^{-1}). All analyzed type of samples were contaminated with yeasts and their level was generally higher ranging from 0.5 x 10^1 to 5.7 x 10^2 CFU.g^{-1} (average: 7.6 x 10^1 CFU.g^{-1}). Better results were in 23 multifloral honey samples from southern Córdoba (Argentina). **Finola et al. (2007)** were carried out these honey samples on the basis of their microbiological (*Clostridium*, fungi and yeast) analysis. The amount of yeast and fungi found in the honey samples was less than 1 x 10^2 CFU.g^{-1}. The results showed that honeys produced in this region are a good quality. On the contrary **Tosi et al. (2004)** found fungi and yeast in the 28 selected multifloral honey samples from Argentina with a charge variable between 2 x 10^3 and 33 x 10^4 CFU.g^{-1}. The most frequented isolated fungi and yeast were: *Aspergillus flavus, Penicillium viridicatum, P. vulpinum, Trichosporon cutaneum, Cladosporium cladosporoides, Alternaria alternata* and *Zygosaccharomyces mellis*.

Normal honey must lack pathogenic micro-organisms or micro-organisms that produce enteric illnesses **(Popa et al., 2009)**. In 18 honey samples collected from local markets in the six states of Southwestern Nigeria , there were a presence of seven species of heterotrophic fungi. There were isolated *Cladosporium Wernecki, Mucor mucedo, Cephalosporium sp., Rhizopus japonicus, Cladosporium herbarum, Trychophyton rubrum* and *Scopulariopsis brevicaulis*. However, out of the seven species, only one, *Trychophyton rubrum* was pathogenic, and this was the first record of the presence a pathogenic fungus in honey samples from Nigeria. The presence of pathogenic fungus necessitates an urgent need to monitor microbial status of marketed honey in South-western Nigeria (Ayansola, 2012).

Table 3 Levels of fungal a yeasts contamination in Poland honey samples (CFU/g)

Samples	Fungal contamination (CFU/g)	Yeasts contamination (CFU/g)
1.	2.5×10^1	2.5×10^1
2.	5.0×10^1	-
3.	-	-
4.	-	1.0×10^2
5.	5.0×10^1	2.0×10^1
6.	-	0.5×10^1
7.	-	1.0×10^1
8.	0.5×10^1	1.7×10^2
9.	0.5×10^1	2.5×10^1
10.	0.5×10^1	2.5×10^1
11.	0.5×10^1	4.0×10^2
12.	-	1.0×10^1
13.	1.5×10^1	2.5×10^1
14.	-	-
15.	0.5×10^1	0.5×10^1
16.	-	5.0×10^1
17.	-	-
18.	1.0×10^1	-
19.	-	3.5×10^1
20.	1.5×10^1	-
21.	-	3.5×10^1
22.	0.5×10^2	1.5×10^2
23.	-	3.3×10^2
24.	-	5.7×10^2
25.	1.5×10^1	3.5×10^1
26.	-	2.9×10^2
27.	-	4.5×10^1
28.	0.5×10^1	2.0×10^1
29.	-	8.5×10^1
30.	-	3.0×10^2
31.	0.5×10^2	1.6×10^1
32.	0.5×10^2	3.0×10^1
33.	1.0×10^1	3.5×10^1
34.	-	4.0×10^1
35.	1.0×10^1	2.0×10^1
36.	1.5×10^1	4.0×10^1
37.	-	2.5×10^1
38.	0.5×10^1	4.5×10^1
39.	1.0×10^1	6.0×10^1
40.	-	1.2×10^2
41.	1.0×10^1	3.0×10^1
42.	-	1.5×10^1
43.	-	1.0×10^1

CONCLUSION

The mycobiota of stored 13 types of honey from Poland as isolated in this study were *Alternaria* spp., *Aspergillus fumigatus, A. niger, Aspergillus* sp., *Cladosporium* spp., *Fusarium* spp., *Mycelium sterilium, Penicillium brevicompactum, P. commune, P. corylophilum, P. crustosum, P. expansum, P. griseofulvum, P. chrysogenum, P. polonicum, Rhizopus* spp. The most prevalent genera of microcopic filamentous fungi found in the honey, were: *Penicillium, Mycelium sterilium* and *Aspergillus*. The most encountered species were *P. chrysogenum* (30 %), *P. brevicompactum* (12 %) and *P. crustosum* (8 %). In spite of 50 isolates the contamination level of samples was law from 0 to 1 x 10^2 CFU.g^{-1}. The level of yeasts was higher from 0 to 5.7 x 10^2 CFU.g^{-1}. Fungal colonization and contamination of stored honey can cause depletion of its nutritive value. This is dependent on the moisture content and several environmental factors such as temperature and relative humidity of the harvested honey prior to storage. Therefore, honeys which are stored before sale or use should be kept dry. Prevention of moisture re-absorption and the general improvement of storage facilities at all levels are recommended as a safe guard against mould deterioration of honey.

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REFERENCES

AYANSOLA, A. A. 2012. Fungal Isolates from the Honey Samples Collected from Retail Outlets in Southwestern Nigeria. In *Journal of Biology and Life Science*, vol. 3, 2012, no. 1, p. 189-199.

BOGDANOV, S. 1993. Liquefaction of honey. In *Apiacta*, vol. XXVIII, 1993, p. 4-10.

FINOLA, M. S. - LASAGNO, M. C. - MARIOLI, J. M. 2007. Microbiological and chemical characterization of honeys from central Argentina. In *Food Chemistry*, vol. 100, 2007, p. 1649-1653.

FRISVAD, J. C. – THRANE, U. - SAMSON, R. A. - PITT, J. I. 2006. Important mycotoxins and the fungi which produce them. In *Advances in experimental medicine and biology*, vol. 571, 2006, p. 3-31.

KAČÁNIOVÁ, M. - HLEBA, L. - DŽUGAN, M. - PASTERNAKIEWICZ, A. - KŇAZOVICKÁ, V. - PAVELKOVÁ, A. - FELSÖCIOVÁ, S. - PETROVÁ, J. - ROVNÁ, K. - KLUZ, M. - GRABEK-LEJKO, D. 2012a. Microbiological properties and antimicrobial

effect of slovakian and polish honey having regard to the water activity and water content. In *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 2, 2012, no. 1, p. 272-281.

KAČÁNIOVÁ, M. - KŇAZOVICKÁ, V. - FELŠÖCIOVÁ, S. - ROVNÁ, K. 2012b. Microscopic fungi recovered from honey and their toxinogenity. In *Journal of Enviromental Science and Health*, vol. 47, 2012, p. 1-6.

KAČÁNIOVÁ, M. – PAVLIČOVÁ, S. – HAŠČÍK, P. – KOCIUBINSKI, K. – KŇAZOVICKÁ, V. – SUDZINA, M. – SUDZINOVÁ, J. – FIKSELOVÁ, M. 2009. Microbial communities in bees, pollen and honey from Slovakia. In *Acta Microbiol Immunol. Hung*., vol. 56, 2009, no. 3, p. 285-95.

MIGDAL, W. - OWCZARCZYK, H. B. - KEDZIA, B. - HOLDERNA-KEDZIA, E. - MADAJCZYK, D. 2000. Microbiological decontamination of natural honey by irradiation. In *Radiation Physics and Chemistry*, vol. 57, 2000, p. 285-288.

PITT, J. I. - HOCKING, A. D. 1997. Fungi and food spoilage. 2nd ed. London : Blackie Academic & Professional, 1997, 593 p. ISBN 0-8342-1306-0.

POPA, M. - VICA, M. - AXINTE, R. - GLEVITZKY, M. - VARVARA, S. 2009. Study concerning the honey qualities in Transylvania Region. In *Annales Universitatis Apulensis Series Oeconomica*, vol. 2, 2009, p. 1034-1040.

SAMSON, R. A. - FRISVAD, J. C. 2004. *Penicillium subg. Penicillium*: *new taxonomic schemes and mycotoxins and other extrolites.* Utrecht:Centraalbureau voor Schimmelcultures, 2004, 260 p. ISBN 90-70351-53-6.

SAMSON, R. A. - HOEKSTRA, E. S. - FRISVAD, J. C. - FILTENBORG, O. 2002. Introduction to food and airborne fungi. Utrecht : Centraalbureau voor Schimmelcultures, 2002. 389 p. ISBN 90-70351-42-0.

SAMSON, R. A. - HOUBRAKEN, J. - THRANE, U. - FRISVAD, J. C. - ANDERSEN , B. 2010. Food and Indoor Fungi. Utrecht : CBS – KNAW Fungal Biodiversity Centre, 2010, 390 p., ISBN 978-90-70351-82-3.

SNOWDON, J. - CLIVER, D. O. 1996 Micro-organisms in honey. In *International Journal of Food Microbiology*, vol. 31, 1996, p. 1-26.

TOSI, E.A. - RÉ, E. - LUCERO, H. - BULACIO, L. 2004. Effect of honey high-temperature short-time heating on parameters related to quality, crystallisation phenomena and fungal inhibition. In *Lebensm.-Wiss. u.-Technology*, vol. 37, 2004, p. 669-678.

VICA, M. - GLEVITZKY, M. - DUMITREL, G. A. - POPA, M. - VARVARA, S. 2009. Microbiological role in hazard analysis of natural honey processing. In *Journal of Agroalimentary Processes and Technologies*, vol. 15, 2009, no. 3, p. 353-360.