JAP&T
TPA-Timişoara

Available on-line at www.japt.tpa.usab-tm.ro

Journal of Agroalimentary Processes and Technologies 2009, *15*(3), 353-360

Journal of Agroalimentary Processes and Technologies

Microbiological Role in Hazards Analysis of Natural Honey Processing

Mihaela Vica[a], M. Glevitzky[a*], Gabriela-Alina Dumitrel[b], Maria Popa[c], Simona Varvara[c]

[a] *Alba Sanitary-Veterinary and Food Safety Directorate, 510217 – str. Lalelelor Street 7A, Alba Iulia, Romania*
[b] *"Politehnica" University of Timisoara, Faculty of Industrial Chemistry and Environmental Engineering, 300006 – P-ta Victoriei 2, Timisoara, Romania*
[c] *University "1 Decembrie 1918" of Alba Iulia, Faculty of Sciences, 510009 – str. Nicolae Iorga 11-13, Alba Iulia, Romania*

Abstract

The microbiological characteristics of honey collected from beekeepers located in Alba County were determined. The presence of moulds: Penicillium spp., Aspergillus spp., Absidia spp., Rhizpus spp., Fusarium spp. were found in normal limits in investigated samples. The microbiological contamination indicates inadequate hygiene condition in handling and storage of raw honey. An efficient management system for food safety can ensure optimum monitoring and control conditions in all stages of honey processing. An HACCP generic model was developed. In this case study the analysis and assessment of hazards that may occur during processing was implemented for honey processing stages.

Keywords: honey bee, microbiology, honey processing, HACCP

1. Introduction

Honey is used in food industry due to its nutritive, therapeutic and dietetic quality. Honey is the sweetest natural product, obtained by processing flowers nectar or plants manna [1].

Honey's chemical composition consist of water, glucose, fructose, saccharose, dextrin, vitamins, minerals and small quantities of microelements and proteins [2].

Honey is a unique natural product and does not require major processing [3]. Quality control is considered as a mark of the food industry. This is a prerequisite for the product competitiveness. Regarding the taste, color, flavor, or consistency, the smallest exception will lower the standard.

The Romanian apiculture has had to suffer after 1989 and until the present. The production of honey has been around the value of 19000 tons/year, except for the years 2007 and 2008 when the worst productions were recorded because of the unfavourable weather conditions (draught, rains and floods). The quantity of honey produced in the country did not exceed 11000 tons for a number of a million families and around 40000 apiarists registered at the national level [4].

To establish the standards that ensure honey quality, microbiological proprieties must take into consideration. A large interest was shown in the last few years for the composition of Romanian honey and its prospective value on the market regarding the U.E. standards [5]. The honey exports, especially in U.E., meet with difficulties concerning the alignment to U.E. quality standards.

The presence of micro-organisms in the honey can influence, sometimes, the stability of the product and its hygienic quality. The micro-organisms from the honey come from the nectar and pollen, from the working halls, from the machines which are poorly washed or from the recipients. The spores' micro-organisms

* Corresponding author: *e-mail address*: glevitzky.mirel-ab@ansvsa.ro

met more frequently are part of the *Bacillus* type. The bacteria without spores (*Micrococcus, Pseudomonas, Flavobacterium*) are less numerous, while the filamentous fungi, presenting thermo-resistant spores, with a high ability to survive, can be introduced into the honey even by people, through the dust, through the water used to wash the installations or the recipients or even by the bees through the pollen [6].

The presence of the yeast is due to its introduction in the hive by the bees, case when the quality of the honey is not affected, or it comes from the extraction and manipulation spaces, from the machines etc. and it is dangerous because of its quantity and acidophilia. Due to the chemical composition, the honey favours the development of osmophilic yeast that produces the fermentation, the decrepit aspect and the crystallisation of the honey [7].

The honey has been very often incriminated as a source of spores of *Clostridium botulinum* responsible for causing the infant botulism. A low percentage of the contamination with B. Cereus and fungi has been proved: yeast, *Mucor spp., Penicillium* spp and other species from the *Aspergillus* type, in particular *Asp. Flavus, Asp. Candidus*, *Asp. Fumigatus and Asp. Niger*. In another study, spores of Clostridium *perfringens* have not been detected in any of the samples. B. Cereus has been identified in 13.7% of the samples. Yeast and moulds have been detected in 88.8% of the samples. Three kinds of moulds (*Aspergillus, Penicillium* and *Mucor)* and two types of yeast (*Saccharomices* and *Candida*) have been identified. No sample has presented contamination with aflatoxins [9].

The microbiological contamination during and after the processing of the honey has been demonstrated through the absence of micro-organisms from the collected samples from the primary sources and the presence of a type of bacterium (*Bacillus spp*) and eight types of fungi (more frequently *Candida, Aspergillus, Geotrichum and Rhizopus)* in the collected samples from the local markets. This indicates the contamination from secondary sources while further manipulation and processing. The contamination with the fungi and bacteria indicates improper hygiene conditions during the collection, manipulation, processing and storage [10].

The study was accomplished by testing honey bee samples from Alba County from microbiological point of view and established the critical control points of natural honey processing.

2. Materials and Method

18 bulk liquid honey samples of known origin(1,2,11,12,13,14,15-Polyfloral honey, 3,4,16,17- Linden honey, 5,6,18- Acacia honey, 7,8- Forest honey, 9,10- Sun flower honey), aseptically collected from beekeepers located in different areas of Alba County during 2008, were used for analysis. Each honey sample was purchased in duplicate in sterilised sealed jars of 200g.

From a microbiological point of view, the contamination of the samples has been followed by determining the total aerobe mesophilic bacteria count (TCB) and by determining the yeast and the moulds. The liquid of dilution used has been the physiological serum with peptons (SFP): 10g from the sample have been homogenised with 90ml of SFP.

The total bacteria count, TCB [11]. The working technique from SR EN ISO 4833-2003 is complied with, but the Petrifilm Aerobic Count Plate (*3M Microbiology USA*) is used. The petrifilm is a reactive film covered by an environment of dehydrated culture which contains standard nutrients, an jellification agent which is soluble in cold water and a tetrasolium indicator which facilitates the enumeration of colonies. The petrifilms are placed on a plain surface, using two films for each sample. With a sterile dropper, 1ml of bee honey dilution is passed on each of the two films, and then it is left to solidify. These are incubated at 30˚C ±1˚C for 72 hours.

Then all the red colonies are numbered, regardless of the size and intensity.

Yeast and moulds, YM [12]. Two boxes of sterile Petri are taken. With a sterile dropper, 1ml of the bee honey dilution 10^{-1} is passed through each box. Approx. 15 ml extracted from the environment formed of yeast-glucose-cloramphenicol – agar is poured (being melted in advance and kept at 45˚C±1˚C in a water bath) in each Petri box. The mixture is stirred carefully with the environment and the mixture is left to solidify, placing the Petri boxes on a horizontal and cold surface. A witness box is prepared, with 15ml of the environment, for the sterility check-up. The boxes are placed with the lid down, in the incubator, at 25˚C±1˚C. After three, four and five days of incubation, the colonies from each Petri box are numbered.

3. Results and Discussion

In order to establish the microbiologic features of the samples of bee honey, the parameters TCB/g and YM/g have been analysed. The obtained results are presented in the figure 1, respectively the table 1.

The values of the microbial load in the analysed samples have not exceeded 100 TCB/g, which represents a reduced contamination with aerobe mesophilic germs.

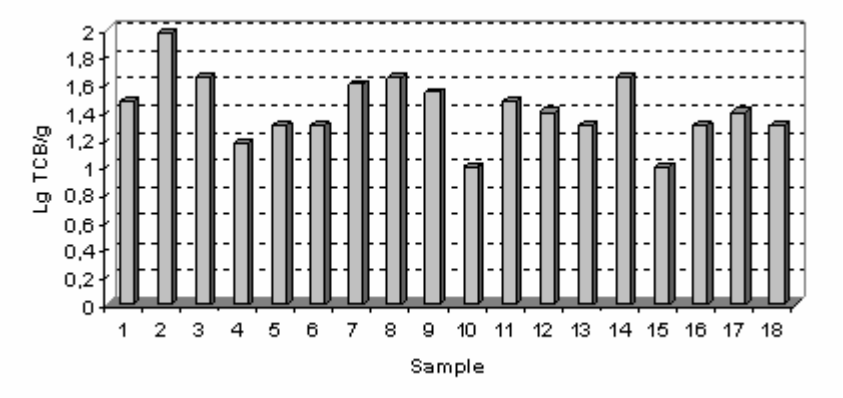


Figure 1. Microbial development (TCB/g) in the studied samples

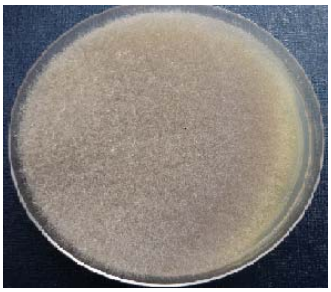
Table 1. Microbiologic control of the bee honey samples

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
YM	30	15	<10	15	<10	20	40	20	<10	10	<10	<10	10	10	<10	<10	<10	<10

The contamination with moulds in the analysed samples does not exceed 40/g. Yeast has not been developed in the 18 samples. In the pictures 2÷10 the macro and microscopic species are presented and they are identified using a Hund Wetzlar H600LL microscope connected to a PC using the Pinuacle program TV Centre for the image capture.

Sample 1 plate 1 (a) Sample 1 plate 2 (a) *Penicillium* spp. (b)

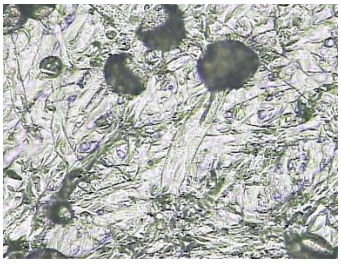
Figure 2. Macro(a) and microphotography(b) for sample 1



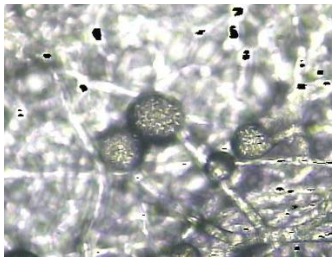
Sample 2 plate 1 (a)

Sample 2 plate 2 (a)

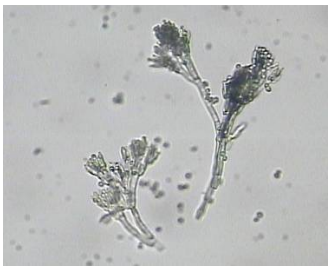
Sample 2 plate 2 (a)



Rhizopus spp. (b)

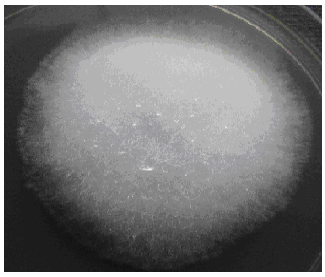


Aspergillus spp. (b)



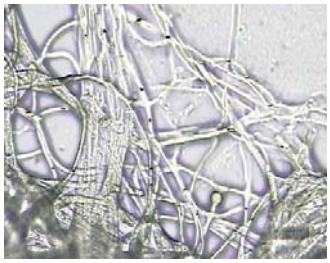
Penicillium spp. (b)

Figure 3. Macro(a) and microphotography(b) for sample 2



Sample 4 plate (a)

Sample 4 plate 2 (a)

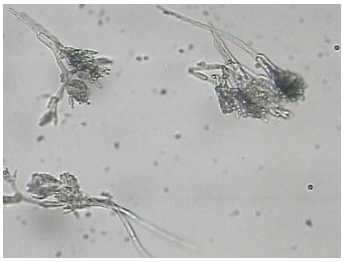


Absidia (*Mycocladus*) spp. (b)

Figure 4. Macro(a) and microphotography(b) for sample 4

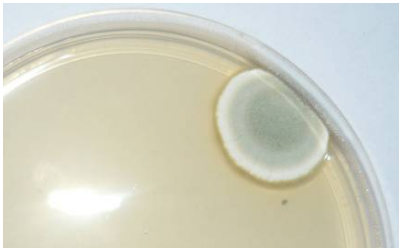
Sample 6 plate 1 (a)

Sample 6 plate 2 (a)



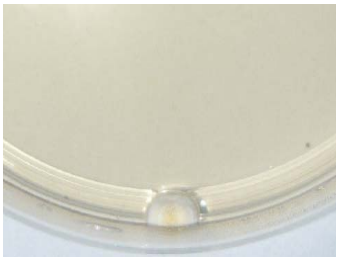
Penicillium spp. (b)

Figure 5. Macro(a) and microphotography(b) for sample 6



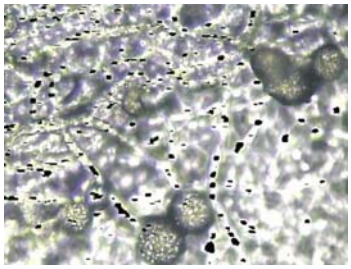
Sample 7 plate (a)

Penicillium spp. (b)



Sample 7 plate (a)

Sample 7 plate 2 (a)



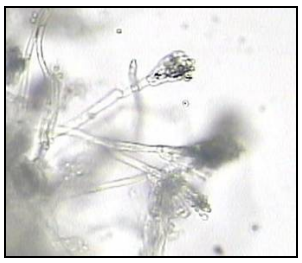
Aspergillus spp. (b)

Figure 6. Macro(a) and microphotography(b) for sample 7

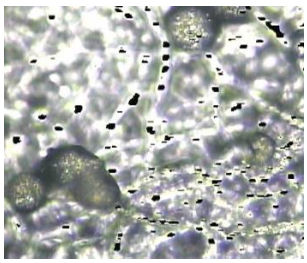
Sample 8 plate (a)

Sample 8 plate (a)

Sample 8 plate 2 (a)

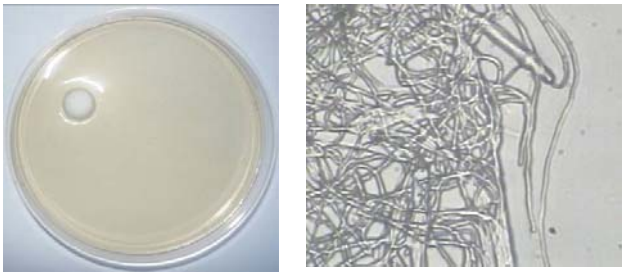


Penicillium spp. (b)



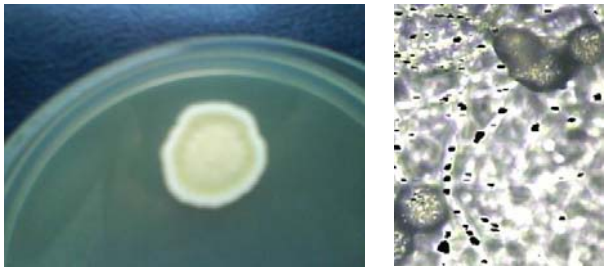
Aspergillus spp. (b)

Figure 7. Macro(a) and microphotography(b) for sample 8



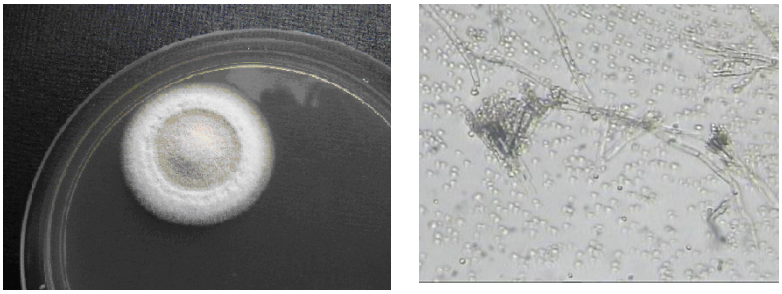
Sample 10 plate 1 (a) *Fusarium* spp. (b)

Figure 8. Macro(a) and microphotography(b) for sample 10



Sample 13 plate 1 (a) *Aspergillus* spp. (b)

Figure 9. Macro(a) and microphotography(b) for sample 13



Sample 14 plate 1 (a) *Penicillium* spp. (b)

Figure 10. Macro(a) and microphotography(b) for sample 14

Contaminated samples with yeast have not been identified, and the most frequent moulds have been the types *Penicillium* and *Aspergillus*, then *Absidia* (*Mycocladus), Rhizpus* and *Fusarium*.

Following the analysis and the evaluation of the dangers that can appear during all the technological stages of bee honey conditioning, collected by the apiarists in the collection-packaging centres (figure 11), the conclusion of the inclusion in the HACCP plan of the honey reception and jar sterilisation control (PCC) as critical points has been drawn.

The HACCP system approaches the hygienic quality of the food products, being the most efficient means of guaranteeing and controlling the security of the food products. All types of potential hazards (biological, chemical, physical) that appear in a natural way in the honey, either as a consequence of the exposure or the contact with a certain environment, or as an effect of a deficiency of the production process are taken into account.

The analysis of this plan leads to the idea that the reception of the honey and the sterilisation of the jars constitutes PCC – critical control points with a chemical, biological* and physical risk, for the security of the food product.

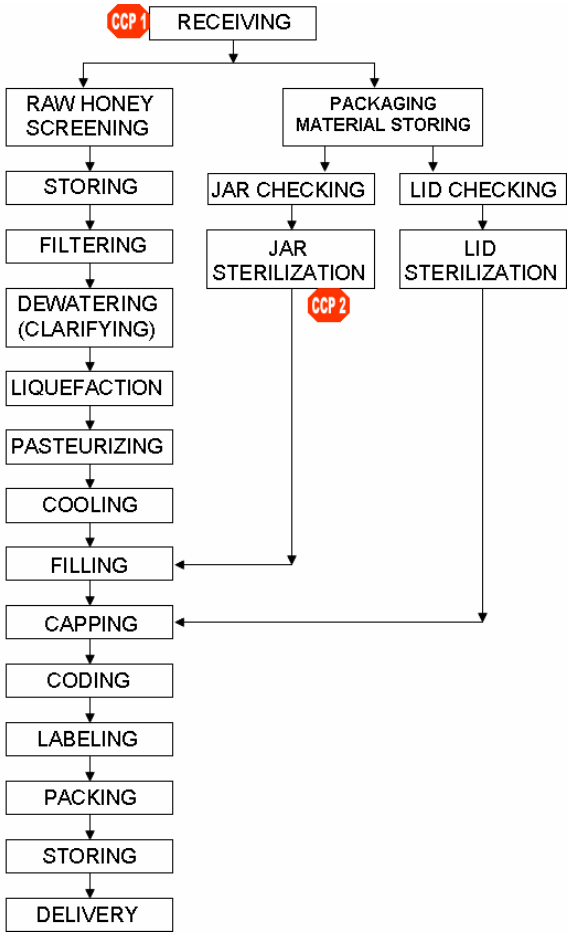


Figure 11. Process Flow Diagram of natural honey processing

Table 2. The analysis and evaluation of the dangers corresponding to the established PCCs

Stage	Type of hazard	Hazard description	G[1]	F[2]	Hazard class	Q_1	Q_2	Q_3	Q_4	PCC
Raw honey reciving	Chemical	Antibiotic residues, phenol and pesticide, chemical residues from unsafe barrels, liners or inner coatings	Medium	Low	2	yes	yes	-	-	PCC1
	Biological	*Clostridium botulinum* spores*	High	Low	3	no*	-	-	-	PCC2*
Jar sterilization	Physical	Glass fragments	High	Low	3	yes	no	yes	no	PCC3

[1] - Gravity, [2] – Frequency, Q_{1-4} – Question 1-4

*There is no conclusive evidence that the *C. botulinum* spores are a hazard in honey. The medical community must recommend to new and expectant parents against the use of honey for infants under one year of age.

4. Conclusion

The study constitutes an investigation regarding the hygienic quality of the bee honey of the apiarists from Alba County.

The anti-microbial feature of the honey is confirmed by the results regarding the TCB (the total bacteria count).

The following types of moulds have been identified: *Penicillium* spp., *Aspergillus* spp., *Absidia* spp., *Rhizpus* spp., *Fusarium* spp.

The study justifies the importance of the proper processing (conditioning) of the honey within the collection-packaging centres in order to destroy the microbial load.
The implementation of an efficient management system for the security of the food products ensures the optimal conditions of monitoring and control within the honey processing and at the same time preventing the danger of its contamination.

References

1. Mărghitaş, L.A., *Albinele şi produsele lor*, Editura Ceres, Bucureşti, **2008**, pp. 280.
2. Balan, O., *Materii prime şi materiale pentru industria alimentară*, Tipografia Universităţii Tehnice „Gh. Asachi" Iaşi, **1999**.
3. Bratu, I., Studiu asupra factorilor ce influenţează culoarea diferitelor sortimente de miere din România, *Buletin ştiinţific Sibiu*, 1998, vol.IV, 27-30.
4. *** Productiile apicole - sub media ultimilor 20 de ani, *Piaţa agricolă,* **2008**, 47(III), www.piataagricola.eu.
5. Bratu, I.; Georgescu, C., Aspecte privind compoziţia şi calitatea unor sortimente de miere din România în raport cu normele prevăzute de Codex Alimentarius, *Buletin ştiinţific Sibiu*, 1998, vol.IV, 31-36.
6. Gillian, M.; Moffett, J.O.; Kauffeld, N. M, Examination of floral nectar of citrus, cotton and Arizona desert plants for microbes, *Apidologie*, **1983**, 14, 299-302.
7. Clemansa, T., *Microbiologie alimentara*, Ed. Agir, Bucuresti, 2004, pp. 285;
8. Amon, S.S.; Damus K.; Chin, J., Infant botulism: epydemiology and relation to sudden infant death syndrome, *Epidemiol. Rev.,* **1981**, 3, 45-66.
9. Martins, H. M.; Martins, M. L;, Bernardo, F. M. A., Bacillaceae spores, fungi and aflatoxins determination in honey, *Revista Portuguesa de Ciencias Veterinarias*, **2003**, 989 (546), 85-88.
10. Tchoumboue, J.; Awah-Ndukum J.; Foneth, F.A.; Dongock, N.D.; Pinta, J.; Mvondo Ze, A., Physico-chemical and microbiological characteristics of honey from the sudano-guinean zone of West Cameroon, *African Journal of Biotechnology,* **2007**, 6(7), 908-913.
11. ***SR EN ISO 4833-2003, *Microbiologia produselor alimentare şi nutreţurilor. Metodă orizontală pentru enumerarea microorganismelor. Tehnică de numărare a coloniilor la 30^0C*, **2003**.
12. ***SR ISO 7954-2001, *Directive generale pentru numărarea drojdiilor şi mucegaiurilor. Tehnica numărării coloniilor la 25^0C*, **2001**.