VERSITA

Bull Vet Inst Pulawy 56, 161-163, 2012
DOI: 10.2478/v10213-012-0029-x

EFFECT OF STORAGE ON MICROBIOLOGICAL QUALITY OF HONEY

HANNA RÓŻAŃSKA AND JACEK OSEK

Department of Hygiene of Food of Animal Origin,
National Veterinary Research Institute, 24-100 Pulawy, Poland
bruna@piwet.pulawy.pl

Received: February 17, 2012 *Accepted: May 8, 2012*

Abstract

One hundred and nine samples of honey representing different botanical types were microbiologically retested for the total number of aerobic bacteria per 1 g, the presence of anaerobic bacteria in 0.1 g, and number of yeasts and moulds per 1 g after one year of storage. The samples displayed different levels of microbiological contamination. The mean of total number of aerobic bacteria varied from 1.9 x 10^1 CFU/g to 4.6 x 10^3 CFU/g depending on the type of honey. This value, in comparison with year 2010 was lower in the case of 75 samples (68.8%), higher in 14 samples (12.8%), and stable in the remaining 20 samples (18.4%). The mean number of moulds and yeasts was 9.8 x 10^1 CFU/g and it was lower in 46 samples (42.2%). In 46 samples no changes were noted. The presence of anaerobic spore forming bacteria was noted in 18 samples. The presence of these microorganisms in 73 honey samples (67.0%) did not change since 2010.

Key words: honey, storage, microbological quality.

Honey may be contaminated with different microorganisms. In general, the spore forming anaerobes and aerobes are reported as predominant microflora of honey, but several others genera of microorganisms were also detected (1, 3, 4, 8, 13, 19, 20). The primary sources of microorganisms in honey may be pollen, digestive tract of honey bees, soil, water, air, and nectar. These natural sources are very difficult to control. Secondary sources are closely connected with hygiene of processing, handling, and storage of honey. These sources may be effectively controlled by Good Manufacturing Practice (GMP) (5, 8, 10, 15, 22). Theoretically, physicochemical properties of honey, such a low water activity, low pH, high sugars concentration, and the activity of different enzymes prevent the growth or even survival of different bacteria in honey (2-5, 8, 10). In practice, this process depends on the level of microbiological contamination as well as the time and conditions of storage (9, 15). The aim of the presented study was to evaluate the effect of storage on microbiological quality of different types of honey from Polish apiaries.

Material and Methods

One hundred and nine samples of unifloral (lime, buckwheat, acacia, rape, forest) and multifloral honey purchased from Polish apiaries were retested after one year of storage in dark, at room temperature, in hermetic containers. Total viable count of aerobic microorganisms per 1 g, presence of anaerobic spore forming bacteria in 0.1 g, and number of yeasts and moulds in 1 g were investigated and compared with the results obtained in 2010. The investigation was performed in accordance to the PN EN ISO standards (16-18).

Results

The results are summarised in Tables 1 and 2. The mean total number of aerobic bacteria ranged from 1.9 x 10^2 CFU/g to 4.6 x 10^3 CFU/g in 2011, depending on the type on honey. In comparison with 2010, it was lower in the case of 75 samples (68.8%), higher in 14 samples (12.8%), and stable in 20 (18.4%). The mean number of moulds and yeasts was 9.8 x 10^1 and was lower in 46 samples (42.2%). In 46 samples, no changes were noted. The presence of anaerobic sporulating bacteria decreased in 18 samples (16.5%) and increased in 18 samples (16.5%). The quality of the remaining 73 samples (67.0%) has not changed in this matter.

Table 1
Comparison of the results of microbiological analysis of honey before and after one year storage at room temperature

Type of honey	Total number of aerobes (CFU/g)		Presence of spore forming anaerobes (number of samples/percentage)		Total number of yeasts and moulds (CFU/g)	
	2010	2011	2010	2011	2010	2011
Multifloral (n = 59)	4.2×10^3	2.5×10^3	11/18.6	12/20.3	2.1×10^2	1.2×10^2
Lime (n = 13)	3.7×10^3	2.4×10^3	2/15.3	4/30.8	1.5×10^2	6.3×10^1
Buckwheat (n = 11)	6.8×10^3	4.5×10^3	1/9.1	1/9.1	2.9×10^2	5.5×10^1
Acacia (n = 11)	6.8×10^3	4.6×10^3	2/18.2	3/27.3	8.2×10^1	5.9×10^1
Forest (n = 8)	3.6×10^3	1.9×10^3	6/75.0	0/0	1.2×10^2	1.5×10^2
Rape (n = 7)	3.4×10^3	8.4×10^2	3/42.9	4/37.1	8.6×10^1	9.3×10^1
Mean	4.6×10^3	2.9×10^3	25/22.9	25/22.9	1.9×10^2	9.8×10^1

Table 2
Detailed comparative analysis of the results obtained in 2010–2011

Type of honey	Changes	Total number of aerobes – number of samples/%	Presence of spore forming anaerobes – number of samples/%	Total number of yeasts and moulds – number of samples/%
Multifloral	↓*	40/67.8	6/10.2	23/39.0
	↑**	5/8.5	7/11.9	6/10.2
	=***	14/23.7	46/78.0	30/50.9
Lime	↓	9/69.2	2/15.4	5/38.5
	↑	3/23.1	4/30.8	2/15.4
	=	1/7.7	7/53.8	6/46.2
Buckwheat	↓	8/72.7	1/9.1	7/63.6
	↑	0/0	1/9.1	0/0
	=	3/27.3	9/81.8	4/36.4
Acacia	↓	6/54.3	2/18.2	5/45.5
	↑	3/27.3	3/27/3	2/18.2
	=	2/18.2	6/54.6	4/36.4
Forest	↓	8/100.0	5/62.5	2/25.0
	↑	0/0	0/0	4/50.0
	=	0/0	3/37.5	2/25.0
Rape	↓	4/57.1	2/28.6	4/57.1
	↑	3/42.9	3/42.9	3/42.9
	=	0/0	2/28.6	0/0
Total	↓	75/68.8	18/16.5	46/42.2
	↑	14/12.8	18/16.5	17/15.6
	=	20/18.4	73/67.0	46/42.2

*- decreasing; ** - increasing; = - constant

Discussion

Many authors indicated physicochemical properties of honey, which should inhibit or even destroy the microorganisms (1, 2, 8, 10, 15, 22). Several experiments showed a very strong antibacterial activity of honey against different microorganisms, including human and animal pathogens (3-5). On the other hand, under certain conditions, such as leaky containers, accessibility of oxygen and water, or high temperature of storage, some microorganisms may survive or even multiply (7, 9, 15). From the point of view of consumer health security, occurrence of anaerobe sporulating bacteria, including *Clostridium botulinum*, may have some significance (2, 5, 6, 14). According to Küplülü *et al*. (12) *Cl. botulinum* was isolated from 12.5% of honey

samples from retail market in Ankara, Turkey. Rall *et al*. (20) isolated these bacteria from 3% of samples. Schocken-Hurrino *et al*. (21) detected *Cl. botulinum* in 7% of Brasilian honey samples. These bacteria are responsible for the cases of infant botulism. The spores of *Cl. botulinum* can survive in honey, but they are not able to produce toxins (2, 6). In some cases, infant botulism may be caused by honey ingestion, therefore, it should not be given to infants under one year of age (5, 6, 11). In fresh honey, the number of yeasts and moulds is usually low but under some conditions these organisms are able to multiply in honey during its storage (3, 8, 10, 13). Jimenez *et al*. (9) noted a significant increase in the number of yeasts and moulds after storage of honey during two years, especially at 28^0C. Carvalho *et al*. (3) and Iurlina *et al*. (8) also indicated the ability of yeasts and moulds to grow in honey. These organisms may cause fermentation of honey during storage, which may be a significant economic problem (3). In normal fresh honey, aerobic bacteria are found in a small number (1, 7, 8). Their high number indicates a contamination of honey from secondary sources (10, 22). These organisms theoretically should not grow in honey but some of them may persist in it (7, 13, 15). Our results confirmed the opinions that microorganisms are not able to multiply in honey. Only in a low number of samples, the increasing number of aerobes, moulds, and yeasts, and growth of anaerobe spore forming bacteria was noted.

References

1. Adenekan M.O., Amusa N.A., Lawal A.O., Okpeze V.E.: Physico-chemical and microbiological properties of honey samples obtained from Ibadan. J Microbiol Antimicrob 2010, **2**, 100-104.
2. Bogdanov S.: Contaminants of bee products. Apidologie 2006, **17**, 1-18.
3. Carvalho C.M., Meirinho S., Estevinho M.L.F., Choupina A.: Yeast species associated with honey: different identification methods. Arch Zootec 2010, **59**, 103-113.
4. Ceausi C., Togoe I., Tudor L., Furnaris F.: The evaluation of microbiological quality of some bee honey sorts. Bull UASVM Vet Med 2009, **66**, 273-277.
5. European Commission. Health & Consumer Protection Directorate–General. Opinion of the Scientific Committee on Veterinary Measures Relating to Public Health on Honey and Microbiological Hazards. 2002.
6. Gilbert S., Lake R., Hudson A., Cressey P.: Risk profile: *Clostridium botulinum* in honey. New Zealand Food Safety Authority, 2006.
7. Hosny I.M., Abdel El-Ghani S., Nadir A.S.: Nutrient composition and microbiological quality of three unifloral honeys with emphasis on processing of honey probiotic yoghurt. Glob Vet 2009, **3**, 107-112.
8. Iurlina M.O., Fritz R.: Characterization of microorganisms in Argentinian honeys from different sources. Int J Food Microbiol 2005, **105**, 297-304.
9. Jimenez M., Mateo J.J., Huerta T., Mateo R.: Influence of the storage conditions on some physicochemical and mycological parameters of honey. J Sci Food Agric 1994, **64**, 67-74.
10. Kačaniová M., Melich M., Kňazovická V., Haščik P., Sudzinova J., Pavličova S., Čuboň J.: The indicator microorganisms value in relations to primary contamination of honey. Lucrări Sintifice Zootehnie Biotehnol 2009, **42**, 159-163.
11. Koepke R., Sobel J., Arnon S.S.: Global occurrence of infant botulism, 1976–2006. Pediatrics 2008, **122**, e73–e82.
12. Küplülü Ö., Göncüoğlu M., Özdemir H., Koluman A.: Incidence of *Clostridium botulinum* spores in honey in Turkey. Food Control 2006, **17**, 222-224.
13. Martins H.M., Martins M.L., Bernardo F.M.A.: *Bacillaceace* spores, fungi and aflatoxins determination in honey. Rev Port Cien Vet 2003, **98**, 85-88.
14. Migdał W., Owczarczyk H.B., Kędzia B., Hołderna-Kędzia E., Madajczyk D.: Microbiological decontamination of natural honey by irradiation. Radial Phys Chem 2000, **57**, 285-288.
15. Olaitan P.B., Adeleke D.E., Ola I.O.: Honey: a reservoir for microorganisms and an inhibitory agent for microbes. Afr Health Sci 2007, **7**, 159-162.
16. PN-A-82055-12:1997. Obecność beztlenowych bakterii przetrwalnikujących i redukujących siarczany (IV) w badanej masie próbki. Polish Committee for Standardisation, Warszawa.
17. PN-EN ISO 4833:2004 + Ap. 1:2005. Microbiology of food and animal feeding stuffs – horizontal method for the enumeration of microorganisms – colony count technique at 30°C. Polish Committee for Standardisation, Warszawa.
18. PN-ISO 21527-2:2009. Microbiology of food and animal feeding stuffs – horizontal method for the enumeration of yeasts and moulds. – Part 2. Colony count technique in products with water activity less than or equal to 0.95. Polish Committee for Standardisation, Warszawa.
19. Popa M., Vica M., Axinte R., Glevitzky M., Varvara S.: Study concerning the honey qualities in Transylvania region. Ann Univ Apul S Oecon 2009, **11**, 1034-1040.
20. Rall V.M.L., Bomba A.J., Lopes T.F., Carvalho L.R., Silva M.G.: Honey consumption in the state São Paulo: a risk to human health? Anaerobe 2003, **9**, 299-303.
21. Schocken-Hurrino R.P., Carneiro M.C., Kato E., Sorbara J.O.B., Rossi O.D., Gerbasi L.E.R.: Study of the presence of the spores of *Clostridium botulinum* in honey in Brazil. FEMS Immunol Med Microbiol 1999, **24**, 379-382.
22. Snowdon J.A., Cliver D.O.: Microorganisms in honey. Int J Food Microbiol 1996, **31**, 1-26.