ISSN: 1220-3173; E-ISSN: 2457-7618

MICROBIOLOGICAL EVALUATION OF SOME HONEY ASSORTMENT PRODUCED IN ROMANIA

EVALUAREA MICROBIOLOGICĂ A UNOR SORTIMENTE DE MIERE PRODUSE ÎN ROMÂNIA

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ABSTRACT | REZUMAT

Due to its particular composition, honey has the ability to inhibit or even destroy microorganisms. However, certain types of microorganisms persist in honey, usually in a latent state, and some yeast species, due to the fact that they are pronounced osmophilic, can multiply and cause fermentation of the product, making it unsuitable for human consumption. The research was carried out between 2023 and 2024, with a total of 552 samples from 5 varieties (acacia honey, linden honey, sunflower honey, rapeseed honey and polyfloral honey) being collected and examined. The laboratory analyses aimed to determine the total number of yeasts and molds, using the colony counting technique (cfu/g). It was found that the honey samples examined had minimal contamination, with the presence of conditionally pathogenic or pathogenic bacteria being detected in some samples, and some samples showing a level of yeasts and moulds greater than 100 cfu/g, the varieties with the highest number of fungi being sunflower and rapeseed honey.

Keywords: honey, microbiota, microbiological analyses, statistics

Datorită compoziției ei particulare, mierea are capacitatea de a inhiba sau chiar distruge microorganismele. Cu toate acestea, anumite tipuri de microorganisme persistă în miere, de regulă în stare latentă, iar unele specii de levuri, datorită faptului că sunt pronunțat osmofile, pot să se multiplice și să producă fermentarea produsului, făcându-l impropriu pentru consum uman. Cercetările au fost derulate în perioada 2023 și 2024, fiind recoltate și examinate un număr total de 552 de probe din 5 sorturi (miere de salcâm, miere de tei, miere floarea soarelui, miere rapiță și miere polifloră). Analizele de laborator au vizat determinarea numărului total de levuri și mucegaiuri, folosindu-se tehnica numărării coloniilor (ufc/g). S-a constatat că probele de miere examinate aveau o contaminare minimală, în unele probe fiind detectată prezența de bacterii condiționat patogene sau patogene, iar unele probe au prezentat un nivel de levuri și mucegaiuri mai mare de 100 ufc/g, sortimentele cu cel mai mare număr de fungi fiind mierea de floarea soarelui și rapiță.

Cuvinte cheie: miere, microbiota, analize microbiologice, statistică

Honey, unlike other foods, is not a favourable substrate for the survival and multiplication of microorganisms; due to its particular composition, honey has the ability to inhibit or even destroy them. For this reason, the microbiological examination of honey has not been a concern for specialists in the field. However, rare types of microorganisms persist in honey, usually in a latent state, and some yeast species can multiply and modify the product, making it unsuitable for human consumption. This has recently led producers and specialists to pay increasing interest in the microbiological quality of honey and to impose its microbiological examination before marketing (1, 3, 4, 12, 13).

Microbial contamination of honey occurs at the hive level, by bees, during nectar collection and deposition, as well as after honey collection and primary processing. Contamination during honey collection and primary processing has the most important hygienic and sanitary significance because it often includes pathogenic or potentially pathogenic microorganisms originating from the people performing these operations. It is determined by unhygienic working conditions and the inadequate microbiological quality of the water used. Moreover, the inadequate microbiological quality of the water used by bees can also contribute to contamination during nectar collection and deposition in combs (2, 5, 6, 12, 13).

The property of honey to inhibit or even destroy microorganisms is due to a complex of factors, the most important of which are: the high sugar content and very low free water content, which causes dehydration, shriveling and death of microbial cells; acidity (pH $\leq$ 4.5); the glucose oxidation system in honey, which causes the formation of hydrogen peroxide, a toxic product for microorganisms, known long before its identification as "inhibin"; the very low protein content and high C/N ratio of honey; the lack of oxygen in honey, atmospheric oxygen not being able to penetrate honey due to its viscosity (most contaminating microorganisms need oxygen to develop); the presence in honey of some chemical substances and enzymes unfavorable to the development of microor-

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ganisms (pinocembrin, lysozyme, phenolic acids, terpenes, benzyl alcohols, various volatile substances); the unfavorable electrical charge created by the reducing sugars in honey that inhibit the multiplication of molds and aerobic bacteria. According to current data, primary and industrial processing can increase or decrease the total number of microorganisms in honey (2, 4, 7, 8, 13).

MATERIALS AND METHODS

The study was conducted in the Bucharest area (city and suburbs), aiming to include different assortment types, produced by different economic agents and sold in different profile units. For this study, 5 honey assortments were chosen: acacia honey, linden honey, sunflower honey, rapeseed honey and polyfloral honey. The samples taken were placed in sterile containers and were transported under optimal temperature conditions. Sample processing was carried out in the food microbiology laboratory according to STAS microbiological norms and ISO standardised methods (10, 11, 12, 14-21).

The study was conducted over a period of 1 year (between 2023 and 2024), analysing a total of 670 samples (132 samples of linden honey, 127 samples of acacia honey, 112 samples of sunflower honey, 124 samples of rapeseed honey, 78 samples of hay honey and 97 samples of polyfloral honey). To determine the total number of mesophilic and aerobic germs, decimal dilutions were performed in peptone water; from each dilution, 1 cm^3 was distributed with sterile pipettes into 2 Petri dishes. Melted agar was poured into each plate and cooled to 40 – 45 °C, homogenised and incubated for 24 hours at 37°C. The average number of colonies/g of product was determined (1, 3, 11, 12, 14-21). To determine the probable number of coliform bacteria, decimal dilutions were performed; from each dilution, 1 mL was introduced into 3 tubes with BBLV medium (lactose broth with bile salts and brilliant green) and Durham tube. Incubation was carried out for 24–48 hours at 37°C. The interpretation was made after gas production and calculation of the 3-digit average (depending on the grade received by each of the 3 tubes with BBLV medium), the average obtained being interpreted using the Mac Grady table. In parallel, the method of determining the number of coliform bacteria by colony counting (ISO 4832) was also used. From the decimal dilutions, 1 ml was distributed with sterile pipettes into 2 Petri dishes. VRBL medium (lactose agar, bile salts, crystal violet and indicator), melted and cooled to 45°C, was poured into each plate. The prepared plates are incubated at 35°C for 24 hours. After incubation, the red-violet colonies are counted and the average is calculated using the formula: $N = \Sigma c / (n1+0.1\ n2)\ d$ [where Σc represents the sum of the colonies counted; n1 is the number of plates from the first dilution retained for counting; n2 is the number of plates from the second dilution retained for counting; d is the dilution rate corresponding to the first dilution used] (1, 3, 11, 13, 14-21).

The determination of the number of pathogenic staphylococci was done using a technique similar to that used for the determination of NTGMA, using Chapman agar or Baird Parker agar as solid medium (3, 9, 12, 13). The determination of the number of sulphite-reducing bacteria was performed on sodium sulphite and iron citrate medium, melted and cooled to 45°C, in which 1 ml of each decimal dilution was inoculated. It was incubated in anaerobic conditions at 37°C for 24 – 48 hours, after which the black colonies were counted (1, 3, 11, 14-21). For the determination of pathogenic and conditionally pathogenic species, the following ISO standardised methods were used: for the identification of *Salmonella* bacteria – SR ISO 6597, for the identification of plasma – coagulase – positive staphylococci – STAS ISO 6888, for the identification of *Proteus bacteria* – SR 2356/1, for the identification

Table 1

The microbiological analysis results for honeybee product samples

Assortment	The bacteriological parameter investigated									
	Coliform bacteria		*E. coli*		Coagulase-positive staphylococci		*B. cereus*		Sulfite-reducing bacteria	
	No. samples	%	No. samples	%	No. samples	%	No. samples	%	No. samples	%
Acacia honey	14	11.02	7	5.5	19	14.96	2	1.57	4	3.15
Sunflower honey	5	4.45	1	0.89	5	4.45	2	1.78	-	-
Linden honey	11	8.33	4	3.03	12	9.09	4	3.03	1	0.76
Canola honey	4	3.22	-	-	2	1.61	1	0.81	2	1.61
Meadow honey	5	6.41	3	3.85	5	6.41	4	5.13	1	1.28
Polyfloral honey	17	17.52	11	11.34	11	11.34	4	38.8	6	6.18

Table 2

The microbiological analysis results for honeybee product samples

Assortment	The bacteriological parameter investigated									
	Proteus spp.		*Clostridium perfringens*		NTDM		*Aspergillus* sp.		*Mucor* sp.	
	No. samples	%	No. samples	%	No. samples	%	No. samples	%	No. samples	%
Acacia honey	1	0.79	2	1.57	6	4.72	1	0.79	-	-
Sunflower honey	2	1.78	4	3.57	-	-	3	2.68	5	4.46
Linden honey	-	-	2	1.52	2	1.52	-	-	-	-
Canola honey	1	0.81	1	0.81	5	4.3	2	1.61	-	-
Meadow honey	-	-	1	1.28	-	-	-	-	-	-
Polyfloral honey	3	3.09	2	2.06	2	2.06	2	2.06	1	1.03

NTDM – total number of yeasts and moulds

of *Escherichia coli* bacteria – STAS ISO 4832, for the determination of the probable number of *E. coli* – SR ISO 7251, for the identification of the *Bacillus cereus* species – SR ISO 7932, for the determination of the number of molds – STAS 12965 – 91, for the determination of the number of yeasts – STAS 12964 – 91 (1, 11, 12, 14-21). All isolated bacterial strains that were presumptively identified as pathogenic or conditionally pathogenic were biochemically tested for confirmatory diagnosis, the scientific data obtained being thus rigorously demonstrated.

RESULTS AND DISCUSSION

The results obtained from the complete bacteriological analysis of each collected and processed sample were statistically processed by calculating averages for each type of product.

Microbiological analysis to evaluate the presence and number of coliform bacteria, *Escherichia coli* and coagulase-positive staphylococci demonstrated that a series of samples presented such microorganisms. Microbiological analysis to evaluate the number of sulphite-reducing bacteria and *Bacillus cereus* demonstrated the presence of these bacterial species in the samples studied; although they do not represent an absolute danger for consumer safety, biochemical processes can be generated that lead to fermentation and denaturation of the respective products. The number of analysed samples in which the different species or genera of microorganisms were recorded are shown in Table 1, statistically analysing the variations for the number of analysed samples from each honey variety.

Statistical analysis of the results allowed the finding that all honey varieties recorded a number of inappropriate samples.

From all the samples analysed, no bacteria belonging to the genus *Salmonella* were isolated. Instead, bacteria belonging to the genera *Proteus* and *Clostridium* were isolated, or samples with a yeast and mould load (NTDM) higher than 100 were recorded. Also, in some cheeses, the presence of mould species was found that either produce toxic compounds (*Aspergillus* spp.) or degrade the biochemical structure of proteins and lipids and modify the organoleptic indicators (Mucor sp.). The number of positive samples and the percentage determined from the total number of samples analysed for each assortment are shown in Table 2.

Based on the results obtained from the bacteriological analyses performed, it is demonstrated that a number of products are contaminated either during transport or directly upon marketing due to storage in inappropriate conditions or due to being kept at temperatures inappropriate for the type of product.

The presence of *Aspergillus* spp. and *Mucor* spp. species was found in sunflower honey and polyfloral honey, this fact practically determining the frequent development of fermentative biochemical phenomena (4, 5, 6, 7, 12, 13).

CONCLUSIONS

All the analysed assortment types recorded microbiologically unsuitable samples. Three assortments stand out in particular: linden honey, polyfloral honey and rapeseed honey, which recorded the highest number of unsuitable samples for most of the analysed microbiological parameters. Following microbiological analysis, no product recorded samples contaminated with germs belonging to the *Salmonella* genus. The microbiological examination to identify bacteria belonging to the genus Proteus revealed the presence of these bacteria in 7 samples, of which 3 samples belonged to a single type of product (polyfloral honey).

The Clostridium perfringens species was identified in 12 samples (1.79% of the total samples examined), with the highest number of positive samples being recorded in sunflower honey samples (3.57%).

REFERENCES

1. *Bărzoi D.M,* (1985), Microbiology of food products of animal origin, (Ed.) Ceres, Bucharest, Romania
2. *Bonvehi J.S., Jorda R.E.*, (1993), The microbiological quality of honey as determined by aerobic colony counts. Journal of Food Protection, 56(4): 336-337
3. *Efem S.E.E., Udoh K.T., Iwara C.I.*, (1992), The antimicrobial spectrum of honey and its clinical significance. Infection, 20:51-53
4. *Gilliam M., Prest D.B., Lorenz B.J.*, (1989), Microbiology of pollen and bee bread: taxonomy and enzymology of molds. Apidologie, 20:53-68
5. *Gilliam M.*, (1997), Identification and roles of non pathogenic microflora associated with honey bees. FEMS Microbiol Lett, 155:1-10
6. *Jiménez M., Mateo J.J., Huerta T., Mateo R.*, (1994),Influence of the storage conditions on some physicochemical and mycological parameters of honey. Journal of the Science of Food and Agriculture, 64 (1):67-74
7. *Mundo A. M., Padilla-Zakour I.O., Worobo W.R.,* (2004), Growth inhibition of foodborne pathogens and food spoilage organisms by select raw honeys. International Journal of Food Microbiology, 97(1): 1-8
8. *Ruiz-Argueso T., Rodriguez-Navarro A.*, (1975), Microbiology of ripening honey. Applied Microbiology, 30(6):893-896
9. *Snowdon J.A., Cliver D.O.*, (1996), Microorganisms in honey. International Journal of Food Microbiology, 31(1-3):1-26
10. *Tysset C., Haas P., Durand C., Lebert F.*, (1979), Survival of some mycobacteria in honey stored at room temperature (De la survie de quelques mycobacteries dans les miels du commerce conserves - la temperature ambiante). Bull Acad Vet de France, 52:447-452
11. *Tysset C., Rousseau M., Duran C.*, (1980), Microbism and wholesomeness of commercial honey. Apiacta, 15:51-60
12. *Tudor L.*, (2009), Quality control of animal agrifood products, (Ed.) Printech, Bucharest, Romania
13. *Tudor L., Jalbă M.A.*, (2023), Considerations on the degree of yeast and mould contamination of bee honey. Rev Rom Med Vet, 33(3):81-85
14. ***, (2009), SR 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, (Ed.) ASRO, Bucharest, Romania.
15. ***, (2006), ISO 4832:2006 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms—Colony-count technique, Edition 3, (Ed.) ISO, Geneva, Switzerland
16. ***, (2023), ISO 6888-2:2021/Amd 1:2023 Microbiology of the food chain — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Part 1: Method using Baird-Parker agar medium; Part 2: Method using rabbit plasma fibrinogen agar medium, (Ed.) ISO, Geneva, Switzerland
17. ***, (2020), SR ISO 6579-1:2017/A1:2020, Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella, (Ed.) ASRO, Bucharest, Romania, 11.
18. ***, (2008), SR 2356-1:2008 Meat and meat preparations. Determination of Proteus bacteria (in Romanian), (Ed.) ASRO, Bucharest, Romania
19. ***, (2009), SR ISO 7251:2009, Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of presumptive Escherichia coli — Most probable number technique, (Ed.) ASRO, Bucharest, Romania
20. ***, (2005), SR EN ISO 7932:2005 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of presumptive Bacillus cereus — Colony-count technique at 30 degrees C, (Ed.) ASRO, Bucharest, Romania
21. ***, (2009), SR ISO 21527-1:2009 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and moulds, Part 1: Colony count technique in products with water activity greater than 0.95, (Ed.) ASRO, Bucharest, Romania.