

# Assessment of the microbiological quality and bactericidal properties of flavoured honey

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## Assessment of the microbiological quality and bactericidal properties of flavoured honey

### Summary

The aim of the study was to evaluate the microbiological quality and bactericidal properties of flavoured honeys. The study evaluated 20 different flavoured honeys available in Polish retail shops. These included 17 nectar honeys enriched with flavourings, i.e. herbs, marshmallow root, seaberry, elderberry, cranberry, hops, cloves, ginger, vanilla, black cumin, lemon, blackberry, strawberry, orange, raspberry, and European blueberry, as well as three creamed honeys with ginger and lemon grass, lemon and peppermint, and strawberry. The study showed that vast majority of the flavoured honeys were microbiologically safe and exhibited high bactericidal activity. The highest level of contamination was found in honey with black cumin and elderberry. The results showed that 50% honey solutions had a bactericidal effect against *E. coli*. Its growth was inhibited most strongly (20.6 mm) by nectar honey enriched with marshmallow root and elderberry. Similar properties of flavoured honeys were observed in the case of *Salmonella* Enteritidis. The zone of inhibition for honey with orange paste and cloves was on average 18.7 mm. The study showed that the honey had no effect at all on *Pseudomonas aeruginosa*, irrespective of the added flavourings.

**Keywords:** microbiological quality, bactericidal properties, flavoured honeys

The honey market is distinguished by a great diversity of products. Honey producers are introducing modifications as well. One of these is creamed honey, which has a thick, homogenous consistency making it easier to spread (42). Flavoured honeys with added fruit, such as raspberries, or herbs and spices (e.g. cinnamon or ginger) are also becoming increasingly common. The main purpose of such additions to honey is to enrich it with biologically active substances, which enhances the product's health-promoting properties. Information provided on the labels indicates that fruit and herbs are added to honey in a freeze-dried form (maximizing preservation of their nutritional value and bioregulatory properties) or as juice. Thus the consumers are offered a wide range of products with a variety of quality characteristics. Despite the lack

of strong pharmaceutical effects, honey is believed to have medicinal properties, which is reflected in folk medicine. Owing to its antibacterial, anti-inflammatory and antioxidant properties, it is one of the oldest traditional substances used in pharmaceuticals, cosmetology and processing (14, 26, 28). It exhibits immunomodulatory and anticancer properties, as well as regulates the blood glucose level (2, 10).

Like all food products, honey is subject to mandatory inspection for purposes of consumer safety. At the international level, the quality of honey is regulated by the FAO/WHO Codex Alimentarius (11), and at the European level, by the Council Directive of 2001 (13). In Poland, the main legal act is the Regulation of the Minister of Agriculture and Rural Development of 3 October 2003 on detailed requirements for com-

mercial quality of honey (33). Unfortunately, European legislation does not specify microbiological standards for honey.

The presence of microbial contaminants in honey can pose a threat to human health as well as to the bees themselves. Some microbes naturally occurring in honey, including bacteria from the genera *Lactobacillus*, *Bifidobacterium* and *Bacillus*, produce compounds that prevent food from spoiling. The sources of microbial contamination of honey can be primary or secondary. Primary sources are related to the digestive tract of bees, the nectar and pollen they gather, atmospheric air and the hive environment. Microorganisms associated with honeybees and their food include bacteria (mainly *Bacillus* spp. and bacteria from the family *Enterobacteriaceae*), moulds (primarily *Aspergillus* spp. and *Penicillium* spp.), and yeasts (mainly *Torulopsis* spp.) (19). Wen et al. (41) showed that *Bacillus* spp. account for more than 67% of bacterial population in honey. Most identified species are considered safe, and some are able to produce antibiotics, bacteriocins and antifungal compounds. Secondary microbial contamination takes place during the collection and processing of honey. In this case, it is humans, soil and equipment that are the major sources of contamination with Gram-negative *Enterobacteriaceae* (including *E. coli*, *Salmonella* spp., and *Proteus* spp.) and *Pseudomonas* spp., as well as Gram-positive staphylococci and enterococci (35, 41).

The most important factors protecting honey from the development of microbes can be divided into three major groups: physical (low pH and high osmotic pressure), chemical (flavonoids, components of essential oils, organic acids, tannins, and other substances derived from nectar and honeydew), and enzymatic (mainly glucose oxidase and lysozyme) (20). Physical characteristics that contribute to the product's effectiveness against microorganisms include high osmotic pressure (about 500 Pa), which results from high sugar content accompanied by low moisture content (on average 17.2%), and low pH (on average 3.9) due to the presence of organic acids (mainly gluconic acid).

In undiluted honey, acidity between 3.2 and 4.5 is a significant antibacterial factor, as most pathogens do not develop in such low pH. The minimum pH for the growth of *Escherichia coli* is 4.3, for *Salmonella* spp. 4.0, for *Pseudomonas aeruginosa* 4.4, and for *Streptococcus pyogenes* 4.5 (4).

Antibiotic enzymes, which include glucose oxidase and lysozyme, enhance nonspecific immunity. Hydrogen peroxide produced by glucose oxidase exerts a strong antimicrobial effect, easily penetrating cell membranes owing to its small size. The bactericidal mechanism of action of hydrogen peroxide involves its decomposition within the cell by protective enzymes (including catalase) as well as iron and copper ions. The decomposition of H<sub>2</sub>O<sub>2</sub> generates unstable and highly reactive hydroxyl radicals, which attack micro-

bial proteins and DNA (24). In the case of anaerobic organisms, the main cytotoxic agent is the end product of hydrogen peroxide decomposition, i.e. molecular oxygen (29).

Another enzyme responsible for the antiseptic properties of honey is lysozyme, a low-molecular-weight protein sensitive to light. This enzyme causes lysis of cell walls, mainly those of Gram-negative bacteria composed of glycosaminoglycans (14).

Physical, chemical and enzymatic factors all contribute to the stability of honey, preventing the development and multiplication of microbes (18). Under these conditions, few microbes are able to develop or survive in honey (35).

The same agents that protect bees against disease and honey against spoilage are used in therapeutic procedures. Due to their non-selective mechanism of action, these compounds exhibit a very broad spectrum of action against both Gram-negative and Gram-positive bacteria, and most importantly, against multi-drug resistant microbes. Honey is an effective agent in the fight against antibiotic-resistant bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) or vancomycin-resistant *Enterococcus* (VRE), as well as multi-drug resistant Gram-negative rods, including *Pseudomonas aeruginosa*, *Acinetobacter* spp. and bacteria from the family *Enterobacteriaceae* (4, 12).

The antimicrobial properties of honey will differ depending on the kind of honey, the geographic location and plant it comes from, weather conditions, storage time and conditions, and the means of processing and enrichment (37).

The aim of the present study was to evaluate the microbiological quality and bactericidal properties of Polish flavoured honeys.

## Material and methods

**Microbial contamination of flavoured honeys.** The study evaluated 20 different flavoured honeys available in Polish retail shops. These included 17 nectar honeys enriched with flavourings, i.e. herbs, marshmallow root, seaberry, elderberry, cranberry, hops, cloves, ginger, vanilla, black cumin (*Nigella sativa*), lemon, blackberry, strawberry, orange, raspberry, and European blueberry, as well as three creamed honeys with ginger and lemon grass, lemon and peppermint, and strawberry. Until analysis, the honey samples were stored in the dark at room temperature in sterile single-use plastic containers with screw caps.

A 10 g sample of each honey was homogenized in 90 ml NaCl (initial suspension) for 15 min at 180 rpm at room temperature. Tenfold serial dilutions were prepared from the suspension and surface plated on appropriate media. Numbers of microorganisms were expressed in colony-forming units per gram of honey (cfu/g).

The microbiological analyses included determination of the total number of mesophilic bacteria, fungi, bacteria from the family *Enterobacteriaceae*, lactic acid bacteria and *Clostridium perfringens*, as well as the presence of coliforms and *Salmonella* spp.

Conditions for microbiological analyses:

- total number of mesophilic bacteria on PCA agar (BTL, Łódź, Poland), incubation at 30°C for 3 days;
- total number of fungi on Sabouraud agar with chloramphenicol (BTL, Łódź, Poland), incubation at 25°C for 5 days;
- total number of bacteria from the family *Enterobacteriaceae* on VRBG agar (BTL, Łódź, Poland), incubation at 3°C for 24-48 hr;
- total number of lactic acid bacteria on MRS agar (BTL, Łódź, Poland), incubation at 30°C for 3 days under microaerophilic conditions (GENbag microaer – BioMérieux);
- number of *Clostridium perfringens* on tryptose-sulfite cycloserine agar (TSC) (BioMérieux Polska sp. z o.o., Warsaw, Poland), incubation at 37°C for 2 days under anaerobic conditions (Genbag anaer – BioMérieux);
- number of faecal coliform bacteria on mFC agar (BTL, Łódź, Poland), incubation at 44°C for 18-24 hr;
- number of *Salmonella* spp. on SS and XLD agar (BTL, Łódź, Poland), incubation at 37°C for 24 hr after pre-enrichment on a non-selective medium (BTL, Łódź, Poland) and multiplication on a selective RV enrichment medium (BTL, Łódź, Poland). Confirmation by biochemical tests (BioMérieux).

**Bactericidal effects of flavoured honeys.** Six flavoured nectar honeys containing substances with known anti-inflammatory properties were selected, including honey with marshmallow root, elderberry, lemon, cranberry, orange, cloves, and ginger. Nectar honey without added flavourings was tested as well.

The microbicidal effects of the honey were tested by the agar well method against test strains obtained from MicroBioLogics, Inc. (St. Cloud, USA), including Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Salmonella* Enteritidis and *Pseudomonas aeruginosa*) bacteria. The inoculum suspensions were prepared in sterile saline from 24-h-old cultures with a density adjusted to 0.5 McFarland turbidity standards.

A sterile cork borer (diameter 12 mm) was used to make wells in the agar. A micropipette was used to add 0.1 ml of the 50% honey sample (v/v) to the wells. The positive control well was filled with an equal amount of chloramphenicol (31 µg/ml), and sterile distilled water was used as a negative control (7).

The plates were incubated at 37°C for 24 h. The mean diameters of the zones of inhibition were measured in mm, and the results were recorded. The diameter of the zones of growth inhibition was measured together with the diameter of the well, and the results were given in mm after subtracting the diameter of the well.

Statistical analysis of the results was performed by one-way analysis of variance (ANOVA). Differences were considered statistically significant at  $p \leq 0.05$ . All computations of statistical data were performed using the Statistica 13.1 software (StatSoft, Kraków, Poland)

## Results and discussion

### Microbial contamination of flavoured honey.

Table 1 presents the results of the analysis of microbial contamination of flavoured honey. The highest

level of contamination ( $1.3 \times 10^5$  cfu/g) was noted in the case of honey with black cumin and elderberry ( $p < 0.05$ ). A high content of mesophilic bacteria was also found in honey enriched with orange ( $3.8 \times 10^3$ ) and herbs ( $6.3 \times 10^2$ ). The statistical analysis showed that these honeys differed significantly from the others ( $p < 0.05$ ).

No bacteria from the family *Enterobacteriaceae*, coliforms, or *Salmonella* spp. were observed in the honey. Only the honey with black cumin and elderberry contained a high level of moulds ( $p < 0.05$ ).

The highest number of *C. perfringens* was noted in honeys containing blackberry, seaberry and black cumin. These values were statistically different ( $p < 0.05$ ) compared with those for the other honeys. The highest number of lactic acid bacteria were found in the honey enriched with black cumin and elderberry. The remaining honeys did not differ statistically ( $p > 0.05$ ).

**Bactericidal effects of flavoured honeys.** The results showed that the 50% honey solutions had a bactericidal effect against *E. coli* (Tab. 2). Its growth was most strongly inhibited (20.6 mm) by nectar honey enriched with marshmallow root and elderberry ( $p < 0.05$ ). The effects of the other honeys were observed within a wide range from 13.0 mm (ginger paste and lemon) to 16.6 mm (concentrated lemon juice), and the differences were not statistically significant ( $p > 0.05$ ). Similar properties of flavoured honeys were noted in the case of *Salmonella* Enteritidis. The zone of inhibition for honey with orange paste and cloves was on average 18.7 mm, which was larger than for the other honeys ( $p < 0.05$ ).

The study showed that the honey had completely no effect on *Pseudomonas aeruginosa*, irrespective of the added flavourings.

Comparison of the bactericidal activity of nectar honey with and without flavourings showed statistically significant differences in the case of *E. coli* for honeys with marshmallow root and elderberry, in the case of *S. Enteritidis* for honey with orange paste and cloves, and in the case of *S. aureus* for honey enriched with concentrated lemon juice and honey with cranberry ( $p < 0.05$ ).

According to the regulation that remained in force in Poland until 2004 (34), the total number of mesophilic bacteria in 1 g of bee product could not exceed  $5 \times 10^4$ , while the number of fungi and moulds could not exceed  $5 \times 10^2$ . The honey samples analysed in the present study did not exceed these limits. In most of the flavoured honeys the level of contamination with mesophilic bacteria ranged from  $10^1$  to  $10^2$  cfu/g of honey. Similar results have been reported by Gomes et al. (21), Estevinho et al. (16), Śliwińska and Bazylak (40), Azonwade et al. (9) and Galhardo et al. (18), who analysed nectar honey commercially available in Portugal, Poland, Benin and Brazil. Fernández et al. (17), who analysed the quality of honey in Argentinian

Tab. 1. Microbial contamination of flavoured honey (cfu/g)

Type of honey	Total number*						
	Mesophilic bacteria	Fungi	Enterobacteriaceae	Coliforms	Salmonella spp.	Clostridium perfringens	Lactic acid bacteria
Nectar honey + marshmallow root 2%, elderberry 2%	1.5 × 10 <sup>2</sup> a (1.5 × 10 <sup>2</sup> )	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>
Nectar honey + concentrated lemon juice 2%	0.0 <sup>b</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>
Nectar honey + blackberry 7%	1.3 × 10 <sup>2</sup> a (2.2 × 10 <sup>2</sup> )	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	3.5 × 10 <sup>3</sup> b (1.6 × 10 <sup>3</sup> )	0.0 <sup>a</sup>
Nectar honey + vanilla 2%	5.0 × 10 <sup>1</sup> a (5.0 × 10 <sup>1</sup> )	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	5.0 × 10 <sup>1</sup> a (5.0 × 10 <sup>1</sup> )
Nectar honey + strawberry 3%	2.8 × 10 <sup>2</sup> a (2.9 × 10 <sup>2</sup> )	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	4.0 × 10 <sup>2</sup> a (3.3 × 10 <sup>2</sup> )	2.5 × 10 <sup>1</sup> a (4.3 × 10 <sup>1</sup> )
Nectar honey + concentrated cranberry juice 2%	2.5 × 10 <sup>1</sup> a (4.3 × 10 <sup>1</sup> )	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>
Nectar honey + hops 2%	0.0 <sup>b</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	2.5 × 10 <sup>1</sup> a (4.3 × 10 <sup>1</sup> )
Nectar honey + orange paste and cloves 2%	7.5 × 10 <sup>1</sup> a (8.3 × 10 <sup>1</sup> )	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	5.0 × 10 <sup>1</sup> a (5.0 × 10 <sup>1</sup> )
Nectar honey + seaberry 2%	2.8 × 10 <sup>2</sup> a (4.2 × 10 <sup>2</sup> )	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	8.0 × 10 <sup>2</sup> b (8.2 × 10 <sup>1</sup> )	0.0 <sup>a</sup>
Nectar honey + ginger paste 1%, lemon paste 1%	5.3 × 10 <sup>2</sup> a (7.5 × 10 <sup>2</sup> )	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>
Nectar honey + orange 3%	3.8 × 10 <sup>3</sup> c (3.9 × 10 <sup>2</sup> )	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	3.3 × 10 <sup>1</sup> a (4.7 × 10 <sup>1</sup> )	5.0 × 10 <sup>1</sup> a (5.0 × 10 <sup>1</sup> )
Nectar honey + herbs 2%	6.3 × 10 <sup>2</sup> c (3.8 × 10 <sup>2</sup> )	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>
Nectar honey + concentrated raspberry juice 5%	8.5 × 10 <sup>2</sup> a (7.4 × 10 <sup>2</sup> )	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>
Nectar honey + cranberry 7%	5.3 × 10 <sup>2</sup> a (4.8 × 10 <sup>2</sup> )	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>
Nectar honey + black cumin 4%, elderberry 2%	1.3 × 10 <sup>5</sup> d (6.3 × 10 <sup>4</sup> )	7.5 × 10 <sup>2</sup> b (8.3 × 10 <sup>2</sup> )	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	4.5 × 10 <sup>3</sup> b (3.0 × 10 <sup>3</sup> )	7.3 × 10 <sup>3</sup> b (3.5 × 10 <sup>3</sup> )
Nectar honey + raspberry 2%	5.0 × 10 <sup>1</sup> a (8.7 × 10 <sup>1</sup> )	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	3.3 × 10 <sup>1</sup> a (4.7 × 10 <sup>1</sup> )	22.5 × 10 <sup>1</sup> a (4.3 × 10 <sup>1</sup> )
Nectar honey + European blueberry 7%	5.0 × 10 <sup>1</sup> a (8.7 × 10 <sup>1</sup> )	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	8.0 × 10 <sup>2</sup> a (1.4 × 10 <sup>2</sup> )	0.0 <sup>a</sup>
Creamed honey + ginger and lemon grass (2%)	7.5 × 10 <sup>1</sup> a (1.3 × 10 <sup>1</sup> )	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>
Creamed honey + lemon and peppermint (2%)	3.0 × 10 <sup>2</sup> a (4.1 × 10 <sup>2</sup> )	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>
Creamed honey + strawberry (1.5%)	6.3 × 10 <sup>2</sup> a (5.9 × 10 <sup>2</sup> )	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>

Explanations: \* – mean and standard deviation (SD). Values in columns with the same letters do not differ statistically at  $p < 0.05$

apiaries, reported much lower levels of microbial contamination, as the number of mesophilic bacteria in most samples (80%) did not exceed 30 cfu/g of honey.

In the present study, a higher level of contamination, above the established threshold, was noted in the case of honey with black cumin and elderberry. The results ( $1.3 \times 10^5$  cfu/g or 5.1 log cfu/g) indicate a secondary contamination of the honey and a failure to observe

adequate hygiene practices during its collection, extraction and handling.

The presence of bacteria from the family *Enterobacteriaceae*, such as coliforms and *Salmonella* spp., was not detected. In contrast, Fernández et al. (17) and Galhardo et al. (18) detected these groups of bacteria in Argentinian and Brazilian honey, although their number did not exceed 20 cfu/g and depended on the year in which the honey was harvested (17). Galhardo

**Tab. 2.** Effect of 50% solutions of flavoured honey on the size of the zones of inhibition of selected bacteria (mm)

Group	Stat.	Microorganism			
		<i>Escherichia coli</i>	<i>Salmonella Enteritidis</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>
Nectar honey + marshmallow root 2%, elderberry 2%	M	20.6 <sup>c</sup>	15.0 <sup>c</sup>	0.0 <sup>b</sup>	26.5 <sup>c</sup>
	SD	2.1	1.9	0.0	2.3
Nectar honey + concentrated lemon juice 2%	M	16.6 <sup>d</sup>	13.3 <sup>c</sup>	0.0 <sup>b</sup>	21.8 <sup>d</sup>
	SD	1.7	6.2	0.0	2.7
Nectar honey + cranberry 7%	M	14.4 <sup>d</sup>	16.0 <sup>c</sup>	0.0 <sup>b</sup>	31.6 <sup>e</sup>
	SD	1.2	1.1	0.0	1.5
Nectar honey + concentrated cranberry juice 2%	M	15.5 <sup>d</sup>	13.1 <sup>c</sup>	0.0 <sup>b</sup>	28.5 <sup>f</sup>
	SD	2.9	3.4	0.0	0.5
Nectar honey + orange paste and cloves 2%	M	14.0 <sup>d</sup>	18.1 <sup>d</sup>	0.0 <sup>b</sup>	28.6 <sup>f</sup>
	SD	2.6	1.7	0.0	0.7
Nectar honey + ginger paste 1%, lemon paste 1%	M	13.0 <sup>d</sup>	14.3 <sup>c</sup>	0.0 <sup>b</sup>	28.1 <sup>f</sup>
	SD	3.0	3.0	0.0	1.5
Nectar honey without additives	M	15.8 <sup>d</sup>	17.6 <sup>e</sup>	0.0 <sup>b</sup>	28.2 <sup>f</sup>
	SD	3.9	1.1	0.0	0.4
Positive control	M	31.8 <sup>a</sup>	39.0 <sup>a</sup>	28.6 <sup>a</sup>	37.8 <sup>a</sup>
	SD	5.0	1.1	3.3	4.6
Negative control	M	0.0 <sup>b</sup>	0.0 <sup>b</sup>	0.0 <sup>b</sup>	0.0 <sup>b</sup>
	SD	0.0	0.0	0.0	0.0

Explanation: values in columns with the same letters do not differ statistically at  $p < 0.05$

et al. (18) reported an average of 0.78 log cfu/g of coliform bacteria in honey, including 0.50 log cfu/g of faecal coliforms.

Our analysis of flavoured honey did not reveal the presence of either moulds or yeasts. Only the honey with black cumin and elderberry contained a significant number of moulds, but it did not contain yeasts. The absence of yeasts in honey may be linked to its low water content. This is supported by Wen et al. (41), whose analysis of microbial contamination during the first fifteen days of maturation of honey showed that the number and species diversity of microbes decreased dramatically as the honey matured and its moisture level decreased. According to Galhardo et al. (18), who detected contamination of honey with microscopic fungi at a level of 0.46 log cfu/g for yeast and at 2.95 log cfu/g for moulds, a moisture level higher than 21% in honey is conducive to the proliferation of microorganisms, which survive under acidic conditions, causing honey to ferment. Sereia et al. (36) confirm that high water content is the main factor reducing the microbiological quality of honey. In a study on samples of honey from nectar collected by bees during different flowering periods, Sinacori et al. (39) isolated various species of fungi, including *Alternaria alternata*, *Aspergillus niger*, *Aspergillus proliferans*,

*Aspergillus spelunceus*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Daldinia concentrica*, *Emericella discophora*, *Penicillium polonicum* and *Penicillium echinulatum*. The authors stress that moulds produce mycotoxins as secondary metabolites, which are compounds toxic to humans and animals even at low concentrations.

In the present study, a large number of samples (35% of honeys tested) were contaminated with *C. perfringens*. In the literature, the presence of *Clostridium* sp. is reported mainly in the context of botulism induced by *Clostridium botulinum* in infants (31). Contamination of honey with *Clostridium perfringens* due to inadequate hygiene (during harvesting and processing) is rarely assessed despite the fact that *Clostridium perfringens* is known to induce a number of diseases in humans and animals. It is considered one of the most common aetiological agents of foodborne disease (22).

The presence of lactic acid bacteria in honey should be regarded as an additional antibacterial property. Products of their metabolism include bacteriocins and organic acids produced during homo- and heterofermentation. Bacteriocins are proteins or protein complexes exhibiting inhibitory activity towards Gram-positive and Gram-negative bacteria, in particular, foodborne pathogens (i.e., *Listeria monocytogenes*, *Staphylococcus* sp. and *Escherichia* sp.) (38).

The bactericidal and bacteriostatic effect of honey depends on multiple factors (discussed above), including the degree of its dilution. Honey exhibits bacteriostatic properties even at low concentrations, and at higher concentrations it exerts a bactericidal effect. According to Olaitan et al. (30), undiluted honey inhibits pathogenic bacteria responsible for food poisoning, while even diluted honey (50%) inhibits the growth of *Escherichia coli*, *Vibrio cholerae*, *Yersinia enterocolitica*, *Plesiomonas shigelloides*, *Aeromonas hydrophila*, *Salmonella* Typhi, *Shigella boydii* and *Clostridium jejuni*.

Ayub et al. (8) achieved inhibition of bacterial growth at concentrations of just 6.25% (v/v) in the case of *E. coli* and *K. pneumoniae* and 12.5% (v/v) for *S. Typhi*, while a 25% concentration exerted bactericidal effect. The authors also showed that continuous exposure to honey did not lead to the development of any resistance in the strains. Guttentag et al. (23) emphasize that aqueous solutions of honey have much higher antibiotic activity because dilution accelerates the reaction producing bactericidal hydrogen peroxide.

Our results showed that 50% honey solutions can effectively eliminate *E. coli* and *Salmonella* Enteritidis. The highest bactericidal activity was shown for nectar honey enriched with marshmallow root and elderberry and for honey with orange paste and cloves.

The research showed the honey had completely no effect on *Pseudomonas aeruginosa*, which proved fully resistant to the honeys irrespective of their added flavourings. However, many studies emphasize a high bactericidal efficacy of honey against *Pseudomonas aeruginosa*, a bacterium considered to be a particularly virulent pathogen in wounds, capable of forming a biofilm. Most of those studies refer to manuka honey from New Zealand (3, 5, 25, 27).

ElBorai et al. (15), in an analysis of six different local Egyptian honeys, found that only half of them showed bactericidal activity against *P. aeruginosa*. In all cases, this bacterium was more resistant than *E. coli*. The results were in agreement with findings of Agbabiaka et al. (1), who showed that *P. aeruginosa* was susceptible to most of the antibiotics tested, but completely resistant to the active ingredients of honey. These discrepancies in the results suggest that not all honey is equally active against *P. aeruginosa*, and its effectiveness depends largely on the botanical origin and geographic location of the honey.

In the present study, the inhibitory activity of each of the honeys was much higher against Gram-negative bacteria than against Gram-positive *Staphylococcus aureus*. Nectar honey with 2% cranberry showed strong bactericidal activity against staphylococcus, similar to that of chloramphenicol.

Some of the honeys enriched with flavourings showed higher activity against the test strains of microorganisms. The bactericidal properties of honey were enhanced by the addition of marshmallow root and elderberry, orange paste with cloves, lemon juice and cranberry.

Rajeswari et al. (32) observed lower bactericidal properties in Nigerian honey. The zone of inhibition for *S. aureus* did not exceed 21 mm, and the zone for *E. coli* did not exceed 14 mm. Unlike in our study, the authors noted inhibitory activity against *P. aeruginosa*. Godlewska and Świsłocka (20) evaluated the antimicrobial activity of 11 honeys and reported that dark honeys such as buckwheat, heather, forest, and coniferous honeydew honey showed the highest activity against *Staphylococcus aureus* and *Escherichia coli*, with inhibition zones ranging from 8 to 11 mm. In the case of light honey, the inhibition zone was only 4.9-7.1 mm. Anand et al. (6) determined the bacteriostatic and bactericidal effects of honeys and showed that all of them inhibited the growth of staphylococci more effectively than that of Gram-negative bacteria.

Although assessment of the level of microbial contamination in Europe is not mandatory, it can improve food safety and help to diagnose hygiene errors in the management of honeybee colonies or product

handling. The study showed that the vast majority of the flavoured honeys were microbiologically safe and exhibited high bactericidal activity. Our research confirmed that honey can be an effective antibacterial agent, especially in treatment of food poisoning, while enrichment of honey with substances that not only diversify the flavour of honey, but are also known for their bacteriostatic effects, can improve its inhibitory properties.

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