The antimicrobial activity of honeys produced in the Czech Republic

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ABSTRACT: The aim of this project was to determine the antimicrobial activity of honeys produced in the Czech Republic to some bacteria (*Listeria monocytogenes, Staphylococcus aureus, Salmonella typhimurium* and *Escherichia coli*). Another aim was to find out whether there are correlations between the antimicrobial activity of honeys and their physicochemical parameters. All honeys included in the study were directly obtained from beekeepers in Southern and Northern Moravia from the summer load of 2001. The project contains 20 honeys which were determined according to their conductivity as blossom (6), blends (10) and honeydew (4). The determination of physical and chemical parameters such as content of water, conductivity, pH, water activity, invertase and diastase activities was carried out in accordance with the methods described in Harmonised methods of the European Honey Commission. The determination of the antimicrobial activity of honey was done by the MIC (minimal inhibitory concentration) method. Statistically significant relations between the antimicrobial activity and the conductivity of honey ($P < 0.05$), the diastase ($P < 0.05$) and invertase ($P < 0.001$) activities were found out in the observed physicochemical parameters. The study proved that honey produced in the Czech Republic is antimicrobially effective with the highest effect in honeydew honeys ($P < 0.01$).

Keywords: physicochemical parameters; *Listeria monocytogenes*; *Staphylococcus aureus*; *Salmonella typhimurium*; *Escherichia coli*; honey; antimicrobial activity

The antibacterial activity of honey was first published by Ketel in 1982 (Molan, 1992a) and since then a number of other scientists have dealt with this issue, trying to find the principle of the mechanism of its effect (Molan, 1992a, 1992b; Armstrong and Otis, 1995; Weston, 2000). These studies proved that all types of honey did not have the same antimicrobial activity (Molan, 1992a). There are several factors participating in the antimicrobial activity: osmolarity, acidity, hydrogen peroxide activity, botanical origin of honey and other so far not described factors.

Honey is a supersaturated solution of saccharides and the mean value of water activity for honey ranges from 0.562 to 0.620 (Tysset et al., 1980). With the exception of osmotic kinds of yeasts, this environment is unsuitable for the survival of microorganisms (Molan, 1992a). Some authors claim that the

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cause of the antimicrobial effect of honey solution is its high osmolarity, thus it is not different from a normal solution of saccharides and the antimicrobial effect results from the physical not chemical character of honey (Condon, 1993; Grobler and Basson, 1996). However, the results of a number of studies show that more factors contribute to the antimicrobial character of honey (Molan, 1992a). Sugar solutions and pastes have a high osmolarity but they are therapeutically inefficient. The low pH of honey, ranging from 3.2 to 4.5, further explains its antimicrobial activity. Acidity is primarily determined by the content of gluconic acid, which results from the enzyme reaction while nectar is ripening (Molan, 1992a). The influence of hydrogen peroxide on the antibacterial activity of honey was first described by Adcock (1962), and then White et al. (1963) proved a direct relation between the produced hydrogen peroxide and the antimicrobial activity of various types of honey. He also found out that glucose oxidase produces hydrogen peroxide efficiently only after dissolution of honey, which explains the paradoxical finding that the antibacterial effect of honey is produced when honey is dissolved. The antibacterial activity does not fully correlate with the amount of hydrogen peroxide in the samples of honey, which indirectly proves the presence of other non-peroxide antimicrobial components (Molan, 1992b). The antibacterial activity with very high stability to heating was found in some New Zealand honeys, in the honey from vipers bugloss (*Echium vulgare*) and in Jamaican honeys where the botanical source and the composition of accessory antimicrobial components were not specified (Molan, 1992a). The taste, colour and aroma of honey are also directly influenced by the botanical source (Yaniv and Rudich, 1996), some kinds of honey contain accessory antimicrobial components such as flavonoids and aromatic acids (Floris and Prota, 1989; Ferrese et al., 1993) of botanical origin. The origin and the composition of honey thus significantly influence the antimicrobial activity of honeys.

The aim of this project was to determine the antimicrobial activity of honeys produced in the Czech Republic to some Gram-positive and Gram-negative bacteria. Another aim was to find out how important is the role of osmolarity caused by a high content of saccharides in honey and whether there is a correlation between the antimicrobial activity of honeys and their physicochemical parameters.

MATERIAL AND METHODS

Material

Samples of honey. The honeys were obtained directly from beekeepers in Southern and Northern Moravia from the summer load of 2001. The project contains 20 honeys which were determined according to their conductivity as blossom (6), blends (10) and honeydew (4) ones. Our division was based on the valid legislation of that time. The samples were kept in well-sealed glass containers, in shade at a room temperature and they were analysed within 5 months from extracting.

Methods

Physical and chemical parameters. The determination of physical and chemical parameters such as content of water, conductivity, pH and invertase and diastase (according to Schade) activities was carried out in accordance with the methods described in Harmonised methods of the European Honey Commission (Bogdanov et al., 1997).

pH determination. The pH value was determined in a solution containing 10 g of honey in 75 ml of distilled water free of CO_2. Determination of pH was done with a ROSS combination spear-tip pH electrode and pH-meter model 250A (Orion Research Inc., USA). Every sample was analysed in three parallel determinations.

Water activity (a_w). Water activity was determined with the help of a_w-meter Thermoconstanter TH 200 (Novasina, Switzerland) instrument. Every sample was analysed in three parallel determinations.

Antimicrobial activity. The determination of antimicrobial activity of honey was done using the MIC (minimal inhibitory concentration) modified method according to Cooper et al. (1999).

Just before the analysis honey was dissolved to such a concentration that after being mixed with agar (Nutrient Agar HI-MEDIA, India) at a 1 : 1 ratio its concentration was 10% and 20%. On the surface of the prepared Petri dishes, the suspensions of the tested bacterial strains (15 µl) with different cell density were applied by an applicator and after drying they were incubated at 37°C for 18–20 hours. The results were visually read off in harmony with the bacterial growth on the media and were written as the number of daggers: 3 daggers indicated maximum growth, 2 daggers me-

dium growth and 1 dagger small growth of colonies. If any colony did not grow, we marked it as 0. To check the influence of the osmotic effects we used a saccharide solution containing 38.2% of fructose, 31.3% of glucose, 1.3% of sucrose and 7.3% of maltose (Belitz and Grosch, 1992).

Tested bacterial strains. We used the strains *Listeria monocytogenes* CCM 4699 (LM), *Staphylococcus aureus* CCM 3953 (SA), *Escherichia coli* CCM 4787 (EC) and *Salmonella typhimurium* F 8332 (STM) to demonstrate the antibacterial activity of honey.

The prepared suspensions contained about 10^3 and 10^8 CFU per 1 ml.

Statistical evaluation of results. Statistical evaluation of the physicochemical parameters was done using the basic statistical characteristics of the Microsoft Excel programme. The difference in the strength of antimicrobial effect of individual groups of honey was analysed by STAT Plus statistical programme using the Mann-Whitney non-parametrical test for unpaired selections (Matoušková et al., 1992). The relations between the physicochemical parameters and the antimicrobial activity were tested by UNISTAT programme, with Spearman's rank correlation coefficient.

RESULTS AND DISCUSSION

Physicochemical parameters of honeys

The results of the determination of physicochemical parameters of honeys for individual samples are shown in Table 1. Table 2 summarises basic statistical evaluation of honeys divided into three groups.

The observation of the water activity did not show any significant differences between the types of honeys. The found values ranged between 0.497 (13.8%

Table 1. Physicochemical parameters of honeys

Samples of honeys	Origin of honeys	Water content (%)	Conductivity (mS/m)	Diastase DN	Invertase (U/kg)	pH	a_w
1	Blossom	14.80	43.45	10.85	106.01	4.04	0.508
2	Blends	18.47	53.50	27.28	147.81	4.19	0.565
3	Blends	15.40	86.45	22.22	137.64	4.88	0.564
4	Honeydew	15.13	96.35	13.62	144.16	4.88	0.531
5	Blends	14.13	68.70	25.33	125.56	4.48	0.528
6	Blends	15.60	62.20	30.65	92.50	4.46	0.535
7	Blends	15.60	70.90	27.42	157.35	4.61	0.547
8	Honeydew	13.80	95.55	37.65	178.65	4.86	0.497
9	Blossom	14.47	14.45	25.71	35.92	4.25	0.528
10	Honeydew	14.20	92.80	38.71	154.01	4.91	0.517
11	Blossom	17.53	35.55	24.86	113.48	4.13	0.549
12	Blossom	14.87	30.40	24.00	114.44	4.35	0.527
13	Blends	15.27	73.50	24.83	143.68	4.88	0.505
14	Honeydew	23.27	99.60	17.32	120.00	5.46	0.659
15	Blends	17.60	65.25	24.39	115.87	5.01	0.58
16	Blends	18.47	51.50	49.11	255.26	3.95	0.599
17	Blends	19.27	64.85	22.88	196.45	4.47	0.619
18	Blossom	18.80	13.45	15.27	107.76	3.74	0.597
19	Blossom	19.07	32.40	42.37	235.87	3.66	0.599
20	Blends	16.53	79.95	37.26	143.84	4.56	0.522

DN = Diastatic Number

Table 2. Statistical evaluation of physicochemical parameters of honeys ($\bar{x}$ = average, SD = standard deviation, n = sample size)

Group of honey		Water content (%)	Conductivity (mS/m)	Diastase (DN)	Invertase (U/kg)	pH	a_w
Blossom $n = 6$	$\bar{x}$	16.59	28.28	23.84	118.91	4.03	0.551
	max	19.07	43.45	42.37	235.87	4.35	0.599
	min	14.47	13.45	10.85	35.92	3.66	0.508
	SD	2.12	11.96	10.86	64.66	0.28	0.038
Blends $n = 10$	$\bar{x}$	16.63	67.68	29.14	151.60	4.55	0.556
	max	19.27	86.45	49.11	255.26	5.01	0.619
	min	14.13	51.50	22.22	92.50	3.66	0.505
	SD	1.71	10.83	8.27	45.42	0.32	0.036
Honeydew $n = 4$	$\bar{x}$	16.60	96.08	26.83	149.21	5.03	0.551
	max	23.27	99.60	38.71	178.65	5.46	0.659
	min	13.80	92.80	13.62	120.00	4.86	0.497
	SD	4.48	2.80	13.21	24.28	0.29	0.073

of water) and 0.659 (19.3% of water), (0.545 ± 0.032). The increased water activity (from the value 0.73) influences the shelf life of honey and supports the growth of undesirable microflora, especially of osmotolerant yeasts. The highest value a_w (0.659) of samples analysed in this study was established in honeydew honey. However, this value is safe from the aspect of possible growth of microorganisms.

The statistical significance of measured pH values was variable in the groups of honey. The highest values were found in the group of honeydew honeys (4.88 ± 0.02) and the lowest in blossom honeys (4.03 ± 0.25). It is in accordance with bibliographic references which also state that the pH value is influenced by organic acids and by the concentration of mineral substances (Crane, 1990).

The values of the activity of the two most important enzymes diastase and invertase, which are used in some countries as the legislative criteria of honey quality (Bogdanov et al., 1997), proved differences between blossom and honeydew honeys, which is in accordance with bibliographic references (Krauze and Zalewski, 1991; Vorlová and Přidal, 2002). No differences were proved between blends and blossom honeys.

Antimicrobial activity of honey

The highest antimicrobial activity was recorded in honeydew honeys both at 10% and 20% concentrations of honeys as well as both at higher and lower density of bacterial strains (Figures 1–4). However, one sample of honeydew honey was an exception that showed an extremely high content of water, high water activity and deviations in organoleptic tests. The honey was probably immature or spoiled. The difference between the intensity of antimicrobial activity of honeydew honeys and that of the other groups of honeys was statistically highly significant ($P < 0.01$). The antimicrobial activity of the honey solutions was always higher than that of the saccharide solution used as a control (Table 3, Figures 1–4). The study proved that the antimicrobial activity of honey is also caused by other factors, not just by a mere osmotic effect of the high concentration of saccharides in honey. The results of particular honeys are listed in Table 3. When comparing antibacterial effects on the tested microorganisms with antimicrobial effects of honey, we could observe higher sensitivity of Gram-negative bacteria (*E. coli* and *S. typhimurium*). This trend was mostly kept in honeydew honeys, but it was not statistically significant. The study proved that honey produced in the Czech Republic is antimicrobially effective with the highest effect in honeydew honeys ($P < 0.01$). The activity of honey was also recorded in the diluted solution of honey to compare it with a saccharide solution. It signalizes that there are also other factors, not just the osmotic effect caused by a high content of saccharides,

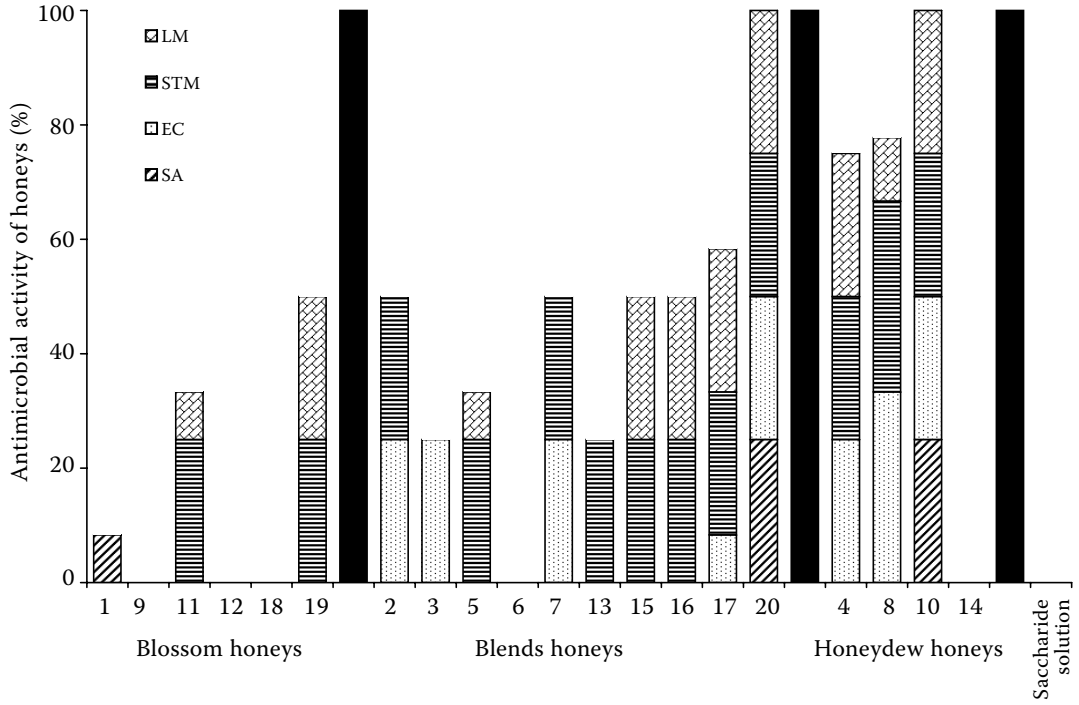

Figure 1. Antimicrobial activity of 20% honey solution at 10^8 cfu density of strains

participating in the antimicrobial activity of honey. The finding is also supported by foreign studies from many parts of the world. Scientists have found out that secondary metabolites from nectar are carried to honey by a bee and in this way honey with its specific chemical composition is produced. In Brazil Cortopasii-Laurino and Gelli (1991) revealed the strongest antimicrobial characteristics in pollen from the mimosa *(Melipona subnitida)* and eucalyptus *(Eucalyptus globulus)*. In Poland honeys from the blackberry and the lime tree were found out the therapeutically most effective (Leszcynska, 1993). In Egypt they also studied a direct influence of feeding extracts from medical herbs to bees on the anti-

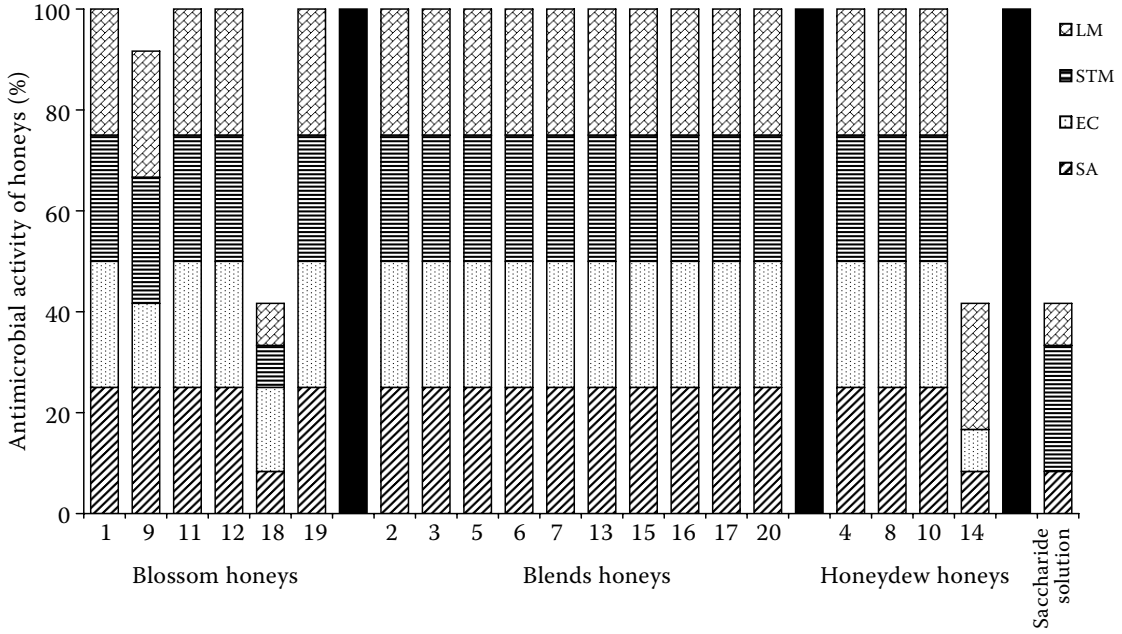

Figure 2. Antimicrobial activity of 20% honey solution at 10^3 cfu density of strains

Figure 3. Antimicrobial activity of 10% honey solution at 10^8 cfu density of strains

bacterial character of honey. Camomile, marjoram and geranium extracts were given to a bee colony once during a period of 8 weeks. At the end of the period the antimicrobial activity of honey was analysed. Honey from colonies fed medical herb extracts had a higher antimicrobial activity than honey from control colonies. Camomile showed the highest antimicrobial activity (Mishref et al., 1989). The presence of the flavonoid hesperidin was found in citrus honeys, particularly in honey from an orange extract (Ferreres et al., 1993). Honeydew honey from coniferous forests of the mountains of central Europe has a relatively high peroxide antimicrobial activity. Manuka honey from the plant *Leptospermum scoparium* grown in New Zealand also has a high antimicrobial activity being of the phytochemical origin. This kind of honey has a high non-peroxide activity (50%) (Molan, 1992a). Thus

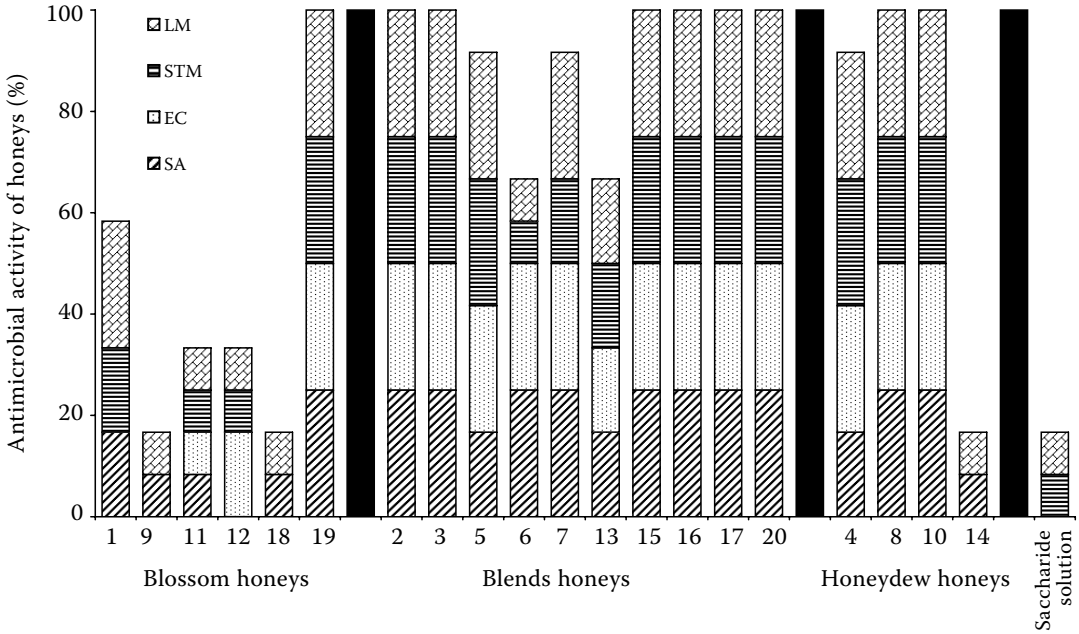

Figure 4. Antimicrobial activity of 10% honey solution at 10^3 cfu density of strains

Table 3. Growth of bacterial strains on culture medium [*Staphylococcustaureus* CCM3953 (SA), *Escherichia coli* CCM4787 (EC) and *Salmonella typhimurium* F8332 (STM), *Listeria monocytogenes* CCM4699 (LM)]

Concentrations of honeys (%)	Density of strains	Samples of honeys	SA	EC	STM	LM	Samples of honeys	SA	EC	STM	LM
10	10^8	1 blossom	+++	+++	+++	+++	11 blossom	+++	+++	+++	+++
	10^3		+	+++	+	0		++	++	++	++
20	10^8		++	+++	+++	+++		+++	+++	0	++
	10^3		0	0	0	0		0	0	0	0
10	10^8	2 blends	+++	+++	+++	+++	12 blossom	+++	+++	+++	+++
	10^3		0	0	0	0		+++	+	++	++
20	10^8		+++	0	0	+++		+++	+++	+++	+++
	10^3		0	0	0	0		0	0	0	0
10	10^8	3 blends	+++	+++	+++	+++	13 blends	+++	+++	+++	+++
	10^3		0	0	0	0		+	+	+	+
20	10^8		+++	0	+++	+++		+++	+++	0	+++
	10^3		0	0	0	0		0	0	0	0
10	10^8	4 honeydew	+++	+++	+++	+++	14 honeydew	+++	+++	+++	+++
	10^3		+	0	0	0		++	+++	+++	++
20	10^8		+++	0	0	0		+++	+++	+++	+++
	10^3		0	0	0	0		++	++	+++	0
10	10^8	5 blends	+++	+++	+++	+++	15 blends	+++	+++	+++	+++
	10^3		+	0	0	0		0	0	0	0
20	10^8		+++	+++	0	++		+++	+++	0	0
	10^3		0	0	0	0		0	0	0	0
10	10^8	6 blends	+++	+++	+++	+++	16 blends	+++	+++	+++	+++
	10^3		0	0	++	++		0	0	0	0
20	10^8		+++	+++	+++	+++		+++	+++	0	0
	10^3		0	0	0	0		0	0	0	0
10	10^8	7 blends	+++	+++	+++	+++	17 blends	+++	+++	+++	+++
	10^3		0	0	+	0		0	0	0	0
20	10^8		+++	0	0	+++		+++	++	0	0
	10^3		0	0	0	0		0	0	0	0
10	10^8	8 honeydew	+++	+++	0	+++	18 blossom	+++	+++	+++	+++
	10^3		0	0	0	0		++	+++	+++	++
20	10^8		+++	0	0	++		+++	+++	+++	+++
	10^3		0	0	0	0		++	+	++	++
10	10^8	9 blossom	+++	+++	+++	+++	19 blossom	+++	+++	+++	+++
	10^3		++	+++	+++	++		0	0	0	0
20	10^8		+++	+++	+++	+++		+++	+++	0	0
	10^3		0	+	0	0		0	0	0	0
10	10^8	10 honeydew	0	+++	+++	+++	20 blends	+++	+++	+++	+++
	10^3		0	0	0	0		0	0	0	0
20	10^8		0	0	0	0		0	0	0	0
	10^3		0	0	0	0		0	0	0	0
10	10^8	saccharide solution	+++	+++	+++	+++					
	10^3		+++	+++	++	++					
20	10^8		+++	+++	+++	+++					
	10^3		++	+++	0	++					

Table 4. The correlation between antimicrobial activity and physicochemical parameters of honeys

Concentrations of honeys (%)	Density of strains	Water content (%)	Conductivity (mS/m)	Diastase DN	Invertase (U/Kg)	pH	a_w
10	10^8	–0.4917*	0.4047*	0.4047*	0.3179	0.3184	–0.4628*
	10^3	0.0606	0.3507	0.5026*	0.7567***	0.1696	0.0565
20	10^8	–0.0436	0.4733*	0.4114*	0.7532***	0.2512	–0.1045
	10^3	–0.2653	0.1852	0.3232	0.4358*	0.0509	–0.3162

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

it appears that honeys of some botanical origins have a higher antimicrobial character than others. Various kinds of honey significantly differ in their antimicrobial strength. As we tested honey chosen at random it could happen that its antimicrobial character would be just a bit higher than in a saccharide solution. There could even be a great difference in the antimicrobial activity of honeys of the same botanical origin.

Statistical analysis

The statistical results of the relation between the antimicrobial activity and physicochemical parameters of honey are summarised in Table 4.

The content of water does not influence the antimicrobial activity statistically significantly, but it holds good the lower the content of water in honey, the higher the antimicrobial activity.

Water activity does not have a statistically significant influence on antimicrobial activity. There seems to be a trend that the lower the a_w value, the higher the antimicrobial activity.

The pH value (active acidity of honey) does not have a statistically significant influence on antimicrobial activity. It is to state here the higher the pH, the higher the antimicrobial activity. It does not correlate with data from literature which consider the low pH of honey solution as one of the factors contributing to the antimicrobial effect of honey (Molan, 1992a).

Conductivity of honey is a common criterion of honey classification into groups. It was found out that the higher the conductivity of honey (honeydew and blends honeys), the higher the antimicrobial activity, which was shown in two cases by the statistically significant coefficient of correlation ($P < 0.05$).

The correlation between the enzyme activity and the antimicrobial activity of honey was statistically most indicative. The relation between the invertase activity and the antimicrobial activity was statistically highly significant ($P < 0.001$) in the observed parameters; the significance with the diastase activity was lower ($P < 0.05$). It has not been clear so far what relation is between the invertase activity and the bacterial growth. However, to be an active invertase, it needs the action of one of the co-enzymes which are necessary for bacterial growth, or otherwise it influences the bacterial cell activity.

The statistically significant relations between the antimicrobial activity and the conductivity of honey ($P < 0.05$), the diastase ($P < 0.05$) and invertase ($P < 0.001$) activities were found out in the observed physicochemical parameters.

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