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THE ANTIMICROBIAL ACTIVITY OF POLYFLORAL HONEY AND ITS AWARENESS AMONG URBAN CONSUMERS IN SLOVAKIA

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ABSTRACT

The current interdisciplinary research studies the antimicrobial activity of selected polyfloral kinds of honey (n = 30) against three microorganisms (gram-positive bacteria *Enterococcus faecalis*, gram-negative bacteria *Salmonella enterica,* and one yeast *Candida krusei*) as well as investigates consumer behavior and awareness towards honey healing properties. Consumer research involved 617 honey consumers living in urban areas. T-test for Equality of means, non-parametric tests, and descriptive statistics were applied. Results showed that antimicrobial activity was found in all honey samples with a concentration of 50%. Nevertheless, better activity was obtained in honey samples from urban beekeepers compared to samples from retail stores. Results of consumer research showed that honey is mostly used as food (sweetener in beverages, ingredient in the recipe, or direct consumption) and as medicine mainly during the winter period. The consumer awareness towards honey healing effects was very high (97%), however, 1/3 of respondents were not able to list any specific examples, and only 11 % mentioned antibacterial activity. Furthermore, more than 70% of respondents did not know to explain the term "medical honey" and more than 50% of respondents are not aware of the maximum temperature used for heating honey without decreasing its biologically active compounds.

Keywords: consumer research; consumer perception; honey consumer; antimicrobial activity; Slovak honey

INTRODUCTION

In modern nutrition, honey has an irreplaceable place, as it is considered a valuable dietary food, sweetener, or medicine **(Ahluwalia et al., 2020; Meo et al., 2017; Samarghandian, Farkhondeh and Samini, 2017; Kumar et al., 2010)**. **Selmi, Irnad and Sistanto (2020)** emphasize, that honey is used for nutritional and medicinal purposes, as well as honey is required by industries, especially by pharmaceutical and cosmetic companies. In recent years, honey has been considered an important commodity in the international market **(Buba, Gidado, and Shugaba, 2013)**. In the context of the above mentioned it could be stated that the popularity of honey is increasing, and natural honey is becoming a sought-after product among consumers. The increased demand can be justified by the awareness of consumers towards the unique properties of honey, which are attributed to the influence of the various groups of substances it contains **(Puścion-Jakubik, Borawska and Socha, 2020; Yeow et al., 2013)**.

Escuredo et al. (2013) focus on the fact that honey is a food that contains about 200 substances. Honey contains a mixture of two monosaccharides (glucose and fructose) and is a source of proteins, minerals, vitamins, organic acids, flavonoids, phenolic acids, and enzymes **(Keskin and Keskin, 2021; Gündoğdu, Cakmakci and Şat, 2019)**.

The composition of honey is not uniformed and it has a very complex composition, which depends on the different factors. The most important determinants influencing the composition of honey are, in particular, the botanical and geographical origin, climatic conditions, and weather during harvest. Another important determinant is also beekeeping management, which is associated with the collection and storage of honey, as well as its conditions **(Escuredo and Seijo, 2019; Da Silva et al., 2016; Escuredo et al., 2014; Karabagias et al., 2018; Otero and Bernolo, 2020)**. **Ranneh et al. (2021)** add that honey contains macro and micronutrients which depend on bee type, floral source, but also on environmental and processing factors.

Honey can be classified as a superfood thanks to its unique natural composition, and its consumption has a positive effect on the health of consumers. Honey can boost the immune system to fight infection **(Scepankova, Saraiva and Estevinho, 2017)**, honey has proven antiviral effects **(Kala et al., 2020)**, honey is also beneficial for sore throats, coughs, and colds **(Kumar et al., 2010)** and honey is a valuable cure against pathogenic respiratory agents, including viruses that cause cough **(Al-Hatamleh et al., 2020)**. **Abbas et al. (2019)** showed that honey in a combination with other substances has also a relatively high

efficiency in patients with asthma. According to **Idrus et al. (2020)**, honey can even act as a protective agent in cardiovascular disease. **Khalil and Sulaiman (2010)** add that honey has anxiolytic, antidepressant, anticonvulsant, and antinociceptive effects and ameliorates the oxidative content of the central nervous system. Moreover, **Güneş and Rn (2007)** indicate that honey has beneficial effects in the treatment of diabetes. In addition, **Samarghandian, Farkhondeh and Samini (2017)** stated that honey could be able to act preventively against cancer, for example, breast cancer, carcinoma, melanoma, colon carcinoma, hepatic cancer, and bladder cancer. However, they also add that there are necessary more studies to improve understanding of the positive effect of honey and cancer.

In the context of the above, it could be concluded that honey is one of the most complete foods for humans, due to its therapeutic, antioxidant, antimicrobial, antitumoral, anti-inflammatory, antiviral, and activities **(Bueno-Costa et al., 2015)**. Nowadays, however, the antibacterial effects of honey are also highlighted. Antibacterial activity is considered the most investigated biological property of honey **(Bucekova et al., 2019)**. Natural unheated honey has broad-spectrum antibacterial activity honey is specific for its antibacterial activity, which it also shows by tests against pathogenic bacteria, oral bacteria as well as food spoilage bacteria **(Lusby, Coombes and Wilkinson, 2005; Mundo, Padilla-Zakour and Worobo, 2004)**. The antibacterial activity of honey is not derived from one mechanism, resp. the action of one chemical, but is based on multifactorial action. The factors responsible for the antibacterial activity of honey are the high sugar concentration, which participates at osmotic pressure, low pH value, and water activity, as well as the 1,2-dicarbonyl compound methylglyoxal (MGO) and the cationic antimicrobial peptide bee defensin-1 **(Kwakman et al., 2010; Bucekova et al., 2020)**. **Mandal and Mandal (2011)** emphasize that identification of the antibacterial purpose of honey can be beneficial and can provide relevant information related to honey quality as well as therapeutic potentials against health diseases of people.

Scientific Hypothesis

Assumption No. 1: We assume that there exist significant differences in antimicrobial activity between honey samples from urban beekeepers and those purchase from retail stores.

Assumption No 2: We assume that there exists a statistically significant dependence between annual honey consumption and respondent´s age.

Assumption No. 3: We assume that there exists statistically significant dependence between honey usage and respondent´s age.

Assumption No. 4: We assume that urban consumers evaluate the factors affecting the purchase of honey differently.

Assumption No. 5: We assume that more than 40% of respondents are not aware of the max. temperature for heating honey without decreasing biological active compounds.

MATERIAL AND METHODOLOGY

Samples

30 samples of polyfloral honey (15 samples were directly from urban beekeepers and 15 samples were purchased from retail stores).

Chemical

Muller Hinton broth, Mueller Hinton agar, Sabouraud dextrose broth, Sabouraud dextrose agar, blanc discs, antibiotics: tigecycline (30 µg per disc), chloramphenicol (30 µg per disc), fluconazole (30 µg per disc) (Oxoid, Basingstoke, UK).

Animals and Biological Material

One species of Gram-positive bacteria *Enterococcus faecalis* CCM 4224, one subspecies of Gram-negative bacteria *Salmonella enterica* subsp. *enterica* CCM 4420, and one species of yeast *Candida krusei* CCM 8271. All tested microorganisms were collected from the Czech Collection of microorganisms (Brno, Czech Republic).

Instrument

Densitometer (Biosan DEN-1, Riga, Latvia).

Laboratory Methods

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method according to CLSI criteria. The inoculums of bacteria were prepared in sterile Muller Hinton broth and yeast in Sabouraud dextrose broth. The optical density of microorganisms to 0.5 McFarland turbidity with a densitometer (Biosan DEN-1, Riga, Latvia) was used. The test microorganisms were uniformly seeded over the Mueller Hinton agar (Oxoid, Basingstoke, UK) resp. Sabouraud dextrose agar (Oxoid, Basingstoke, UK). Diameters of the zone of inhibition around the discs were measured using a ruler in mm. The antibiotics tested were for Gram-positive bacteria tigecycline (30 µg per disc), for Gram-negative bacteria chloramphenicol (30 µg per disc), and yeasts fluconazole (30 µg per disc) as a positive control.

Antimicrobial activity of honey

Susceptibility testing was performed by Kirby–Bauer disk diffusion method according to criteria by CLSI, 2016. The inoculums of each microorganism were prepared with a sterile wire loop and suspended in sterile Mueller-Hinton broth resp. Sabouraud dextrose broth. The optical density of microbial suspension ware determined with densitometer on McFarland turbidity 0.5. The test microorganism was uniformly seeded over the Mueller–Hinton agar resp. Sabouraud dextrose agar on the surface. Using a sterile cork borer (6 mm diameter, 4 mm deep, and about 2 cm apart), wells were made in the agar medium. Using a micropipette, 50 µL of honey with a concentration of 50%, 25%, 12.50%, and 6.25% was added to the wells in the plate. The plates with bacterial strains were incubated at 37°C for 24 h and with yeasts at 25 °C for 24 h. The mean diameters of inhibition zones were measured in mm, and the results were recorded. Sterile distilled water is used as negative control and antibiotics as a positive control. The experiment was repeated triplicate for each strain.

Description of the Experiment

Sample preparation:

Thirty honey samples were used for antimicrobial testing. Hundred percent pure honey (100% v/v) was obtained after filtered using sterile gauze. To get 50% honey solutions (v/v), 0.50 mL of honey was diluted in 0.50 mL sterilized Muller Hinton broth (Oxoid, Basingstoke, UK) and yeast in

Sabouraud dextrose broth (Oxoid, Basingstoke, UK). Further serial dilutions of 0.25 mL of each, 0.125 mL and 0.0625 mL of honey, and 0.50 mL of sterile Muller Hinton broth and yeast in Sabouraud dextrose broth were added to obtain 50%, 25%, 12.5%, and 6.25% honey solutions (v/v), respectively.

Number of samples analyzed: 30
Number of repeated analyses: 3
Number of experiment replication: 3

Consumer research

The second part of the research was based on a questionnaire survey conducted in 2020. The survey was carried out online in Google forms and was disseminated via emails and social media (mostly Facebook groups) by applying a snowball sampling strategy. The research sample comprised 617 honey consumers living in urban areas. The socio-demographic profile is described in Table 1. The questionnaire involved both close-ended questions and open-ended questions regarding consumption patterns, purchasing behavior, and consumer´s awareness of the healing properties of honey.

Table 1 Socio-demographic profile of research sample.

Demographic variable		(%)
Gender	male	36.63
	female	63.37
Age	18 – 30 years	47.81
	31 – 50 years	36.95
	>50 years	15.24
Education	secondary	51.70
	university	48.30
Economic status	employed	66.29
	unemployed	1.13
	student	22.37
	pensioner	5.51
	maternity leave	4.70
Individual income per month (Netto)	up to 400 €	21.39
	401 – 600 €	13.13
	601 – 800 €	20.42
	801 – 1,000 €	19.12
	>1,000 €	25.93

Statistical Analysis

The antimicrobial activity of honey samples was conducted in triplicate and recorded with standard deviations. T-test for Equality of means was used for testing differences between honey samples from stores and urban beekeepers. In consumer research, there were applied non-parametric tests such as the Chi-square test of Independence, Friedman test, and multiple pairwise comparisons using Nemenyi's procedure. The significance level was set to 0.05. All statistical analysis was carried out in statistical software SPSS Statistics v.25, IBM.

RESULTS AND DISCUSSION

Antimicrobial activity of honey samples

Results showed that antimicrobial activity was found in all tested kinds of honey (50% concentration). The highest antimicrobial activity in commercial kinds of honey was found against *E. faecalis* followed by *S. enteritidis* and *C. krusei*, while in kinds of honey from urban beekeepers was the order as follows: *E. faecalis* > *C. krusei* > *S. enteritidis* (see Table. 2). In addition, we formulated the first hypothesis, which assumes that there exist significant differences in antimicrobial activity between honey samples from urban beekeepers and those purchase from retail stores. T-test for Equality of Means confirmed statistically significant differences in all three types of microorganisms ($p \leq 0.001$).

Table 2 Antimicrobial activity of analyzed honey samples at concentration 50% (mm).

Microorganisms	*S. enteritidis*	*E. faecalis*	*C. krusei*
Beekeeper´s kinds of honey 50%	5.13 ±0.81	9.78 ±0.72	5.49 ±0.89
Commercial kinds of honey 50%	3.89 ±0.73	4.62 ±0.78	3.69 ±0.78

Note: mean (n = 15) ±standard deviation.

Moreover, the antibacterial activity of kinds of honey with a concentration of 25% was found only in one type of examined microorganism and only of few honey samples. Eight honey samples from urban beekeepers inhibited *E. faecalis* (10.75 ±0.43) while only two honey samples from stores inhibited *S. enteritidis* (4.5 ±0.23). Antibacterial activity of honey with a concentration of 12.5% was found only in the case of 5 samples from urban beekeepers and it was against *E. faecalis* (6.9 ±0.97). Antibacterial activity is the most evaluated and investigated biological activity of honey and its presence has been proven in several tested samples of honey from the different botanical and geographical origin that were the object of examination of numerous studies which were orientated on the antibacterial activity **(Al-Jabri et al., 2003; Al-Waili, 2004; Bucekova et al., 2019; Bucekova et al., 2018; Cilia et al., 2020)**. The mentioned statement confirms our achieved results related to antimicrobial activity. The similar results with samples of Slovak honey were obtained by **Šedík et al. (2018)** and by **Kačániová et al. (2012)**.

Results of consumer research

The questionnaire survey showed that Slovak consumers living in urban areas have the following annual consumption patterns: 39.7% consume only up to 1 kg and honey is mostly consumed occasionally or only during illness; 32.4% consume 1 or 2 kg in certain frequencies (few times per month or week). The rest 27.8 % consume ≥3 kg mostly every day or few times per week. Based on the realized survey in Romania **Pocol (2011)** identify that 11.0% of consumers do not consume honey, while consumption of honey at the level of a maximum of 750 g per year is recorded in a group of approximately 35% of the population. **Pocol (2011)** also added that the average consumption, between 750 g and 2 kg per year, has a rate of approximately 27%, while 20% of the population consumes over 2 kg of honey per year. Furthermore, by applying the Chi-square test of independence we confirmed (p-value = <0.0001) the statistically significant differences in honey consumption per year among different age segments (H2). Lower honey consumption (only up to 1 kg) is more

frequent for younger consumers (18 – 30 years). The highest annual consumption has consumers older than 50 years (see Figure 1). **Kopała, Balcerak and Kuźnicka (2019)** also found that elder respondents declared honey consumption more often than young people. Moreover, **Żak (2017)** realized the survey in Poland and reports that 83% of Poles over 65 years consume 500 g of honey in a month. In the context of the above **Pocol and Moldovan-Teselios (2012)** stated that young people up to 30 years consume rather small quantities of honey, consumers in the age category 32 – 45 years present a "normal" consumption behavior which is similar that the entire population, consumers between 46 – 60 years prefer consumption of average and large quantities of honey, being under-represented among non-consumers and the last category of consumers, consumers older than 61 years, consume medium quantities of honey.

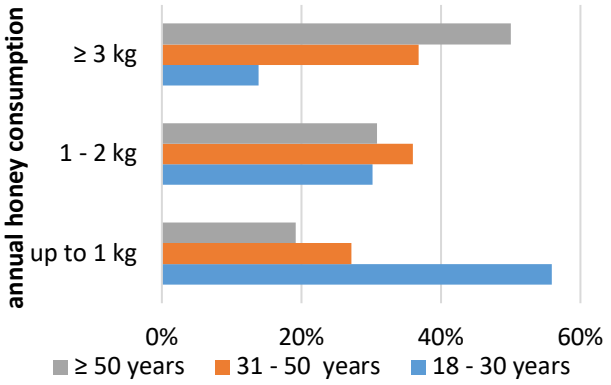

Figure 1 Annual honey consumption based on respondent´s age.

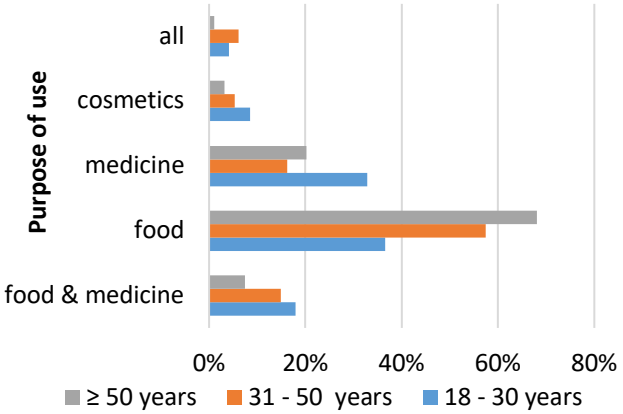

Figure 2 Honey usage based on respondent´s age.

Approximately 78% of respondents answered that honey is consumed by the whole family. The household consumption of honey is on average 6,5 kg per year. The results of the study of **Roman, Popiela-Pleban and Kozak (2013)** showed that 7.2% of respondents declared that honey is not consumed in their households and emphasized that only 20.6% of respondents stated a daily consumption of this product while almost 39% of respondents stated that they consumed it only occasionally. Regarding the purpose of honey, it can be stated that honey as food is used by 68% and as medicine by 44% of consumers. Only 10% indicated cosmetics purpose. The purpose of honey also confirmed the survey realized by **Ismaiel et al. (2014)** and stated that the major motives of consumers for purchasing and consuming honey were to use it as medicine, food, a sweetener, or for other uses. In addition, the Chi-square test of independence confirmed (H3) significant dependence between respondent's age and purpose of use (p-value = <0.0001). Young consumers use honey more as medicine while the older generation uses it more as food (see Figure. 2).

Honey is mostly consumed as follows: sweetener in beverages > ingredient in the recipe > directly from a jar. Around 1/2 of respondents consume this product all over the year and around 43% only during the winter period to support their health. Moreover, 31% use honey regularly as a healthier alternative to sugar, and approximately 60% only sometimes.

Distribution of honey takes place primarily through producer-consumer or manufacturer-retailer-consumer channels **(Borowska, 2011; Kumar, Sharma and Singh, 2012)**. Results of our study showed that the honey is mostly purchased as follows: directly from beekeeper > shops and retail stores > farmer markets > specialty shops. The least frequent place of purchase was e-shops. These results are also confirmed by **Roman, Popiela-Pleban and Kozak (2013)** who found that more than 60.0% of respondents prefer to buy honey from beekeepers, and by **Ciric, Ignjatijević and Cvijanovic (2015)** who identified more than 40% of consumers has a habit of purchasing honey directly from the manufacturer of honey. **Pocol and Bolboaca (2013)** concluded, that local producers were preferred to purchase honey, but on the other hand, respondents also purchase honey and bee products in regional markets, hypermarkets or supermarkets, fairs and exhibitions, specialized shops for organic products, and other places. **Marzec (2003)** in her research showed that 84% of consumers purchase honey at the store and only 21% directly from the beekeeper. **Krystallis, Petrovici and Arvanitoyannis (2007)** state that the most usual channel for frequent food purchases for more than half of the sample is small local stores and open markets, while supermarkets are preferred for occasional food purchases. The optimal price per 1 kg of honey was considered 7 € (22%), 8 € (20%), or 6 € (17%). In comparison with other studies, we could state that consumers from other countries also most prefer honey up to 10 € per 900 g **(Kos Skubic, Erjavec and Klopčič, 2018)**. In addition, respondents evaluated the importance of selected factors during honey purchase using 7 points scale, where 1 = the most important and 7 = the least important. Based on the Friedman test (p-value = <0.0001) it can be stated that there exist statistically significant differences in the evaluation of selected factors (H4). These differences were identified by Nemenyi´s procedure and are illustrated in Table 3. The most important factors are the following: honey quality > honey taste > honey origin > hone type > consistency. The least important were honey packaging and price.

In the context of quality as the most important factor during honey purchase is important to emphasize that honey quality is connected with food safety, credence dimension, quality mark, honey taste, as well as nutritional value **(Borodin, Arion and Muresan, 2013; Röhr et al., 2005)**. According to **Ványi, Csapo and Karpati (2010)**, the most important properties in the process of honey purchasing are the taste, quality, and color of honey.

Table 3 Results of Nemenyi's procedure applied to selected purchasing factors.

Sample	Mean of ranks	Groups				
quality	2.74	A				
taste	3.07	A	B			
origin	3.26		B			
type	4.22			C		
consistency	4.29			C		
price	4.78				D	
packaging	5.64					E

Note: questionnaire research, 2021.

Oravecz et al. (2020) found that consumers consider honey as a trusted product, which is confirmed by the fact that the source and the quality are the most important factors that influence the purchase of honey. The results of a study conducted by **Wu et al. (2015)** emphasizes that the origin of honey is a very important attribute when choosing purchased honey and that even consumers are willing to pay a higher price for local honey, which also confirms our result that price is not a decisive factor in the process of honey purchase. On the one hand, **Gyau et al. (2014)** realized the descriptive analysis of the main attributes of honey and they showed that price, packaging, and color are the three key attributes that strongly influence a consumer's choice of honey. On the other hand, they emphasized that quantity, taste, and the origin of the honey have a moderate influence on the choice, whereas the production process does not influence consumer preferences.

In addition, the survey focused also on consumer awareness and perception towards honey and its biological properties. Approximately 97% of respondents think that honey has healing properties, however, 1/3 of them were not able to answer the specific ones. The interesting result is that more than 40 % of them were younger than 30 years. The rest of the respondents listed various properties and effects. The most frequent were the following ones: immunity booster (22.7%), healing effects in case of cold, flu, or sore throat (12.5%), antibacterial activity (11.2%), anti-inflammatory (8.4%), and others. Based on the results, it can be concluded that the consumer´s awareness about the antibacterial activity is very low. A similar situation is with medical honey which is unknown for 72% of urban consumers. The rest of them perceived it as honeydew honey, honey used in hospitals, in medicine, or as clean, pure honey used for healing wounds.

In general, consumers are interested in the liquid consistency of honey, which was proved by **Cosmina et al. (2016)**. Due to this fact, crystallized honey is usually liquified by applying thermal treatment. Increasing temperature of honey can decrease its antibacterial activity **(Pimentel-González et al., 2015)**. The last hypothesis (H5) assumes that more than 50% of respondents are not aware of the maximum temperature for heating honey without decreasing biological active compounds. The survey showed that only 18% knows the correct answer – 42 degree. The rest of them either do not know (48%) or has incorrect information (34%).

CONCLUSION

Slovak multi floral kinds of honey with a concentration of 50% were able to inhibit the growth of all three microorganisms (*S. enteritidis, E. faecalis, C. krusei*). A better antimicrobial activity was found in honey samples from urban beekeepers. Consumer research showed that consumers in Slovakia use honey as food (sweetener in beverages or ingredients in the recipe) and as medicine mostly during the winter to support their health. Approximately 97% think that honey has healing effects, however, only 11% are aware of its antibacterial activity and 1/3 was not able to identify specific effects. The rest of them mostly listed immunity booster, healing properties in terms of cold or sore throat.

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