Research Article

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Antibacterial potential of Swiss honeys and characterisation of their bee-derived bioactive compounds

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Abstract

BACKGROUND: Antibacterial activity of honey is not only crucial characteristic in selection of honey for medical usage but also an important honey quality marker. The aim of the study was to characterise the antibacterial potential of 29 honey samples representing the main types of multi-floral blossom and honeydew honeys produced in Switzerland. Antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* was expressed as a minimum inhibitory and bactericidal concentrations (MIC and MBC). Furthermore, the content of bee-derived glucose oxidase (GOX) and its enzymatic product, H_2O_2, were also evaluated.

RESULTS: All honey samples successfully met basic defined criteria (moisture and hydroxymethylfurfural (HMF)) tested in this study. Honeydew honeys were the most effective honey samples and generated the highest levels of H_2O_2. A strong significant correlation was found between the overall antibacterial activity and the level of H_2O_2 among all honey samples. Interestingly, the content of GOX in honey samples did not correlate with their antibacterial activity as well as H_2O_2 production capacity. A weak antibacterial activity was determined in five floral honeys, most likely due to increased enzymatic activity of pollen-derived catalase.

CONCLUSION: This study showed that antibacterial effect of Swiss honey samples is associated mainly with H_2O_2.

Keywords: antibacterial activity; hydrogen peroxide; glucose oxidase; catalase

INTRODUCTION

Honey is considered to be a functional food and currently has gained much attention because of its positive biological and health properties. Numerous randomised controlled clinical studies have provided compelling evidence that honey possesses antibacterial/antibiofilm,[1] antiviral[2] and anti-inflammatory[3] properties when applied topically. One of the important and the most studied effects of honey is its antibacterial activity. Those honey samples including manuka honey exhibiting high and well-described antibacterial properties are commercially available as medical-grade honeys.

Antibacterial activity of honey is mainly mediated by the presence of hydrogen peroxide (H_2O_2), which is generated during the nectar processing into honey matrix or during honey dilution. Nectar already contains high levels of H_2O_2 (up to 4 mmol L^{-1}), which are produced by specific plant enzymes.[4] H_2O_2 is unstable and in the presence of metal ions breaks down to highly reactive free radicals (hydroxyl radical) that are able to kill the microorganisms. On the other hand, some plant enzymes detoxify formed free radicals and this whole mechanisms is termed the 'nectar redox cycle'.

The compounds responsible for H_2O_2 formation are of bee or plant origin. Glucose oxidase (GOX) is an enzyme that mediates conversion of glucose to H_2O_2 and gluconic acid under aerobic conditions in diluted honey.[5] It is secreted from bee hypopharyngeal glands into the processed nectar and it is a regular but quantitatively variable compound of each honey sample.[6]

Another plausible antibacterial compound found in every type of honey is defensin-1.[7,8] The concentration of defensin-1 in honey is low and it seems that its contribution to the overall antibacterial activity of honey is negligible.[6,9] Defensin-1 may rather act as an immunomodulator in wound healing, where it stimulates the re-epithelisation process.[10] Besides defensin-1, another bee antibacterial peptide – hymenoptaecin – has recently been found in honey.[11] Its role in honey antibacterial action is questioned since its concentration is negligible and it was found only in a few honey samples, and its irregular presence might be

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linked with the overall health status of particular bee colonies. Taken together, H_2O_2 is a major antibacterial compound in honey, excluding manuka honey. Nevertheless, specific phytochemicals (e.g. polyphenols, ascorbic acid) can strengthen honey antibacterial activity either by direct antibacterial action (methylglyoxal) or indirectly through additional H_2O_2 production.

According to the 2018 annual report of the National Honey Board (USA), honey consumption increased significantly over the other sweeteners (refined sugar and corn syrup) and thus there is high demand for honey. There is a similar situation is in Europe, where honey consumption increased annually and large quantities of honey of unknown quality and origin are being imported to Europe. Therefore, it is important to characterise the local natural honeys and also to determine their bioactive compounds together with evaluation of their antibacterial potential. These bioactive molecules and honey antibacterial potential could serve as a new tool for selecting honeys with higher biological functionality and commercial value.

In this context, we aimed to characterise the antibacterial potential of polyfloral and honeydew honeys or a mixture of both ($n = 29$), typical for Swiss honey production. Furthermore, the GOX content as well as the level of total H_2O_2 were determined in analysed samples. In addition, we evaluated the relationship between the H_2O_2 content and overall antibacterial activity of honey samples.

MATERIALS AND METHODS

Honey samples

Honey samples ($n = 29$) collected in 2017/2018 were received from beekeepers from different parts of Switzerland (Fig. 1). The honeys included honeydew honeys (electrical conductivity $> 0.8\ mS\ cm^{-1}$) and blossom honeys or a mixture of both origins (electrical conductivity (EC) $< 0.8\ mS\ cm^{-1}$) (Table 1). Upon receipt, they were immediately stored in glass containers at 4 °C in the dark. The basic physicochemical and microbiological characteristics are shown in Table 1.

Microorganisms

The antibacterial activity of honey samples was assessed against the isolates *Pseudomonas aeruginosa* CCM1960 and *Staphylococcus aureus* CCM4223, obtained from the Department of Medical Microbiology, Slovak Medical University (Bratislava, Slovakia).

Moisture and hydroxymethylfurfural (HMF) content

Moisture content was measured using a refractometer (model RE40, Mettler Toledo, Greifensee, Switzerland) according to the harmonised methods of the European Honey Commission.[12] The content of HMF was determined using the method of White based on the UV absorbance of HMF at 284 nm, as previously described.[12]

Microbiological analysis of honey

Ten grams of each honey sample was homogenised into 90 mL Mueller–Hinton broth (MHB) medium (pH 7.3 ± 0.1). Decimal dilutions were made into the same solution buffer. Aerobic bacteria were counted on to an MHB agar plate and incubated at 37 °C for 48 h. Microbial counts were expressed as colony-forming units per gram of honey (CFU g^{-1}). All microbial tests were performed in duplicate.

Determination of EC

EC of honey samples was measured on a 20% (w/v) honey solution[12] using an EDGE conductometer (Hanna Instruments, Vöhringen, Germany) with an EC/TDS electrode (Hanna Instruments). The conductometer was calibrated with a $1413\ \mu S\ cm^{-1}$ standard solution (Hanna Instruments) at 20 °C.

Determination of GOX content

GOX content was semi-quantitative, determined according to Bucekova *et al.*[6] Briefly, aliquots (15 µL) of 50% (w/w) honey solution were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and the proteins were transferred to a 0.22 µm nitrocellulose Advantec membrane (Sigma-Aldrich, Darmstadt, Germany) using the wet blotting procedure. The membrane was blocked for 1 h in a Tris-buffered saline–Tween (TBST) buffer (50 mmol L^{-1} Tris–HCl, pH 7.5, 200 mmol L^{-1} NaCl and 0.05% Tween 20) containing 5% non-fat dried milk and incubated overnight with a rabbit polyclonal antibody against honeybee GOX (1:2000 in TBST) which was prepared by GenCust Europe

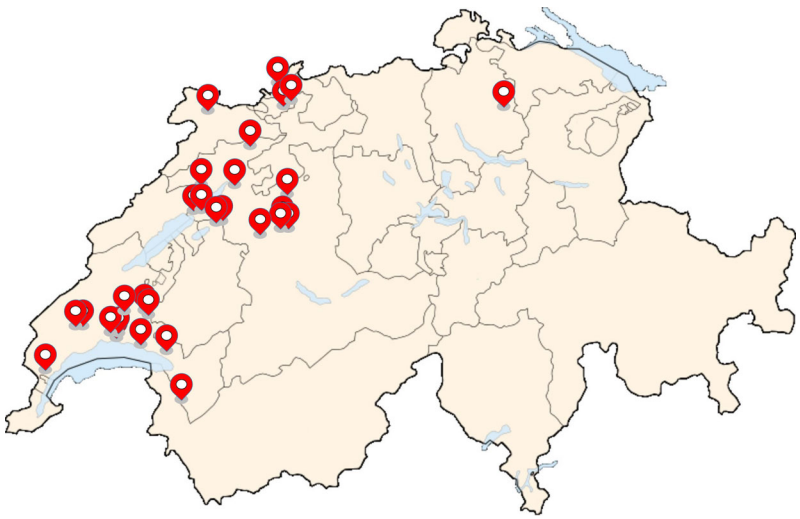

Figure 1. Map indicating the honey sampling locations in Switzerland. Honey samples ($n = 29$) were collected in years 2017 and 2018.

Table 1. Swiss honey samples analysed in this study

Honey sample	Geographic origin in Switzerland	GPS coordinates	Time of harvesting	Moisture (%)	Electric conductivity (mS cm^{-1})	HMF (mg kg^{-1})	Microorganisms (CFU g^{-1})
Blossom honeys							
1	Möringen	47.0846 N 7.2138 E	Spring 2017	17.5 ± 0.1	0.254 ± 0.003	ND	26 ± 22
2	Courgenay	47.4044 N 7.12615 E	Spring 2017	18.2 ± 0.1	0.244 ± 0.002	ND	64 ± 16
3	Cheseaux	46.5854 N 6.60614 E	Spring 2017	18.1 ± 0.0	0.232 ± 0.003	ND	81 ± 16
4	Lutry	46.5033 N 6.68679 E	Summer 2017	17.4 ± 0.1	0.417 ± 0.005	11.70 ± 0.08	89 ± 51
5	Witzwil	46.988114 N 7.058982 E	Spring 2018	17.1 ± 0.1	0.288 ± 0.003	ND	119 ± 31
6	Bellechasse	46.9738 N 7.13427 E	Spring 2018	17.4 ± 0.1	0.565 ± 0.007	ND	905 ± 1005
7	Chessiboden	46.9129 N 7.3602 E	Spring 2018	18.5 ± 0.2	0.280 ± 0.008	ND	391 ± 433
8	Krauchtal	47.008 N 7.56699 E	Spring 2018	17.2 ± 0.0	0.436 ± 0.008	ND	43 ± 22
9	Liebefeld	46.93224 N 7.420469 E	Spring 2018	17.7 ± 0.0	0.359 ± 0.005	ND	68 ± 20
10	Nyon	46.382059 N 6.240279 E	Spring 2018	17.3 ± 0.0	0.376 ± 0.004	ND	72 ± 21
11	Nods	47.113 N 7.08183 E	Spring 2018	19.3 ± 0.0	0.474 ± 0.004	ND	17 ± 19
12	Wyla	47.42 N 8.84537 E	Spring 2018	15.8 ± 0.1	0.483 ± 0.000	ND	89 ± 38
13	Schönenbuch	47.5372 N 7.50204 E	Spring 2018	18.0 ± 0.1	0.339 ± 0.028	2.08 ± 0.03	136 ± 31
14	Duggingen	47.4521 N 7.60651 E	Spring 2018	16.6 ± 0.0	0.616 ± 0.008	ND	89 ± 21
15	Nyon	46.3821 N 6.24028 E	Spring 2018	17.6 ± 0.1	0.481 ± 0.001	ND	795 ± 357
16	Apples	46.5517 N 6.42677 E	Spring 2018	17.2 ± 0.0	0.182 ± 0.001	ND	204 ± 24
17	Montblesson	46.5403 N 6.68012 E	Spring 2018	18.1 ± 0.0	0.294 ± 0.008	ND	208 ± 274
18	Mollie-Margot	46.5566 N 6.75064 E	Spring 2018	18.4 ± 0.1	0.248 ± 0.005	ND	26 ± 22
19	Bellechasse	46.9738 N 7.13427 E	Summer 2018	16.1 ± 0.1	0.680 ± 0.005	3.07 ± 0.48	74 ± 26
20	Witzwil	46.988114 N 7.058982 E	Summer 2018	16.8 ± 0.1	0.691 ± 0.004	3.42 ± 0.44	74 ± 26
21	Liebefeld	46.93224 N 7.420469 E	Summer 2018	15.3 ± 0.1	0.740 ± 0.001	ND	85 ± 46
Honeydew honeys							
22	Vérossaz	46.2129 N 6.98412 E	Summer 2017	15.9 ± 0.1	1.170 ± 0.014	ND	72 ± 47
23	Court	47.2399 N 7.33708 E	Summer 2017	16.2 ± 0.1	0.835 ± 0.003	ND	468 ± 352
24	Blonay	46.465 N 6.8952 E	Summer 2017	17.8 ± 0.1	0.862 ± 0.007	2.72 ± 0.44	30 ± 16
25	Apples	46.5517 N 6.42677 E	Summer 2017	17.3 ± 0.1	1.151 ± 0.003	ND	633 ± 167
26	Montblesson	46.5403 N 6.68012 E	Summer 2018	17.3 ± 0.1	0.953 ± 0.004	ND	55 ± 26
27	Montblesson	46.5403 N 6.68012 E	Summer 2018	17.7 ± 0.0	1.256 ± 0.008	ND	17 ± 14
28	Mollie-Margot	46.5566 N 6.75064 E	Summer 2018	16.6 ± 0.1	0.894 ± 0.002	2.37 ± 0.69	9 ± 10
29	Liebefeld	46.93224 N 7.420469 E	Summer 2018	15.5 ± 0.1	1.283 ± 0.004	ND	272 ± 80

ND, not detectable (value below 2.0 mg kg^{-1}). Data are expressed as the mean values with standard deviation.

(Dudelnag, Luxembourg). After washing with TBST, the membranes were incubated for 2 h in blocking buffer containing goat anti-rabbit horseradish peroxidase-linked antibodies (1:2500 in TBST; Promega, Madison, WI, USA). Immunoreactive bands were detected in a solution containing dissolved SigmaFast 3,3-diaminobenzidine tablets (Sigma-Aldrich), and specific bands were quantified by densitometry using ImageJ software (NIH, Bethesda, MD, USA).

Determination of H_2O_2 concentration

H_2O_2 concentration in honey samples was determined with a Megazyme GOX assay kit (Megazyme International Ireland Ltd), which is based on H_2O_2 release. For a standard, H_2O_2 diluted to 9.8–312.5 µmol L^{-1} was used. Briefly, 40% (w/w) honey solutions in 0.1 mol L^{-1} potassium phosphate buffer (pH 7.0) were prepared and either immediately used for H_2O_2 measurement or measured after 24 h incubation of prepared solutions at 37 °C. Each honey sample and standard was tested in duplicate in a 96-well microplate and the absorbance was measured at 510 nm using a Synergy HT microplate reader (BioTek Instruments, Winooski, VT, USA).

Determination of honey antibacterial activity

The antibacterial efficacy of the honey samples was evaluated with a minimum inhibitory concentration (MIC) assay as described by Bucekova *et al.*[13] Briefly, overnight bacterial culture was suspended in phosphate-buffered saline (PBS), pH 7.2, and the turbidity of the suspension was adjusted to 10^8 colony-forming units (CFU) mL^{-1} and diluted with MHB medium to a final concentration of 10^6 CFU mL^{-1}. Then, 10 µL aliquots of suspension were inoculated into each well of sterile 96-well polystyrene U-shape plates (Sarstedt, Nümbrecht, Germany). The final volume in each well was 100 µL, consisting of 90 µL sterile medium or diluted honey and 10 µL bacterial suspension. After 18 h incubation at 37 °C, bacterial growth inhibition was determined by monitoring the optical density at 490 nm. The MIC was defined as the lowest concentration of honey inhibiting bacterial growth. All tests were performed in triplicate and repeated three times.

Serial dilutions of each honey sample were prepared from a 50% (w/w) honey solution, resulting in final concentrations of 40%, 35%, 30%, 25%, 20%, 18%, 16%, 14%, 12%, 10%, 8%, 6% and 4%.

Minimum bactericidal concentration (MBC) values of honey samples were also evaluated.[14] The viability of bacteria in wells with no

turbidity was determined by spreading 100 µL on to an MHB agar plate and incubating at 37 °C for 24 h. The lowest concentration of honey that resulted in no survival of viable bacteria was recorded as MBC.

Statistical analysis

The Pearson correlation test was used for correlation analysis between antibacterial activity/relative content and H_2O_2 production in honeys. The data are expressed as mean values with standard deviation (SD). Data with *P*-values smaller than 0.05 were considered statistically significant. All statistical analyses were performed using GraphPad Prism (GraphPad Software Inc., La Jolla, CA, USA).

RESULTS

Honey samples and their physicochemical and microbiological parameters

All honey samples successfully met defined criteria (moisture and HMF) tested in this study (Table 1). The moisture content of all honey samples is within the range of 15.8–19.3% and it did not exceed the limit of 20%. Most of the honey samples showed undetectable levels of HMF (below 2 mg kg^{-1}) and could be considered fresh/non-heated. None of the samples exceeded the limit of HMF (40 mg kg^{-1}) for non-tropical regions.[15]

Based on EC values > 0.8 mS cm^{-1}, eight of the total honey samples were classified as honeydew honeys. Honeydew honeys are characterised by higher production of H_2O_2.[9] The average H_2O_2 concentration of eight honeydew honeys was 2758 µmol L^{-1} compared to 21 blossom honeys, giving an average value of 1395 µmol L^{-1}.

Microbiological analysis of honey samples was carried out by a standard total plate count. The plate counts from honey samples varied from 0 to 1000 CFU g^{-1} honey (Table 1).

Content of GOX and H_2O_2 in honey samples

GOX is proposed to be a regular compound found in every type of honey and is responsible for production of H_2O_2 in honey. Indeed, GOX was detected in all 29 honey samples but its content significantly varied from 4.5 to 61.2 µg g^{-1} honey (Fig. 2A).

In this study, concentration of H_2O_2 was determined in non-incubated undiluted honey samples ($t = 0$ h) and in 40% (w/v) honey solution incubated for 24 h ($t = 24$ h) (Fig. 2B,C). As expected, the average concentration of H_2O_2 in incubated honey samples was seven times higher (1771.0 µmol L^{-1}) in comparison to non-incubated ones (254.8 µmol L^{-1}). However, no correlation was found between the initial levels of H_2O_2 ($t = 0$ h) and the levels of accumulated H_2O_2 after incubation ($t = 24$ h) in honey samples ($r = 0.20$, $P = 0.30$). This observation suggested that additional factors influence the accumulation of H_2O_2. The final H_2O_2 content in diluted honeys may depend on ratio between the GOX (producing H_2O_2) and catalase (degrading H_2O_2). This theory is supported by the fact that no correlation was found between GOX content and the levels of H_2O_2 ($t = 0$ h, $t = 24$ h) in honey samples.

Antibacterial activity of honey samples

Antibacterial activity of tested honey samples was expressed as an MIC and MBC value (Fig. 3). The profile of MBC values was similar to that of MIC values (Fig. 3B); in particular, MBC values were either identical to, or slightly higher (one dilution above), than MIC values.

Honey samples showed different antibacterial efficacy against *S. aureus* and *P. aeruginosa*. *Staphylococcus aureus* was often more sensitive to honey bioactive compounds than *P. aeruginosa*. Differences in antibacterial efficacy was also documented among individual honey samples. The lowest average MIC value of honey samples against *S. aureus* and *P. aeruginosa* was 4% and 7%, respectively. Out of a total of 29 honey samples, 13 samples (Nos. 6, 11, 15, 19–23 and 25–29) were most effective, having MIC values against both bacteria below 10%. On the other hand, five blossom honey samples (Nos. 1, 4, 8, 13 and 18) showed the weakest antibacterial efficacy and in the case of sample Nos. 13 and 18 the antibacterial activity was even similar to the activity of artificial honey.

As mentioned above, eight of the total Swiss honey samples were classified as honeydew honeys (Table 1; electrical conductivity > 0.8 mS cm^{-1}). These honeydew honeys exhibited significantly stronger antibacterial activity in comparison to blossom honey samples ($n = 21$), as shown in Fig. 4(A,B). On the other hand, no statistically significant differences in GOX content and production of H_2O_2 were found between blossom multi-floral and honeydew honey samples (Fig. 4C,D).

Since H_2O_2 is considered to be a major antibacterial factor of blossom and honeydew honeys, we investigated the relationship between the level of initial/accumulated H_2O_2 and overall antibacterial activity of honey samples. A strong statistically significant correlation was found between the total level of H_2O_2 measured after 24 h and antibacterial activity against *S. aureus* ($r = -0.63$, $P < 0.001$) and *P. aeruginosa* ($r = -0.64$, $P < 0.001$). Similarly, a strong correlation was found between the initial content of H_2O_2 and antibacterial activity against *S. aureus* ($r = -0.59$, $P < 0.001$) and *P. aeruginosa* ($r = -0.72$, $P < 0.001$). The importance of H_2O_2 for honey antibacterial activity was well demonstrated in sample Nos. 1, 4, 8, 13, 17 and 18, which were characterised by the lowest antibacterial activity, accompanied by the lowest production of H_2O_2.

DISCUSSION

Honey, as a functional food, possesses a broad spectrum of biological activities, including antioxidant, anti-inflammatory and antibacterial activity. Most of these activities, in particular antibacterial activity, tend to decrease rapidly during storage or thermal treatment. Since the antibacterial activity reflects the capacity of a honey to combat bacterial pathogens, analysing honeys for this parameter is especially important for therapeutic applications. In accordance with EU regulations,[15] quality of honey samples is mainly examined by analysing several physicochemical parameters, including parameters for honey freshness. However, antibacterial activity represents a more sensitive parameter of honey freshness than the currently used markers and may serve in the future as an additional parameter for honey quality.

In this study, we characterised selected physicochemical parameters and the antibacterial potential of 29 Swiss blossom, mixture of blossom/honeydew and honeydew honey samples. Furthermore, we determined the GOX content as well as concentration of accumulated H_2O_2 in both non-incubated and 24 h incubated honey samples. Honeydew honey samples showed higher antibacterial efficacy as compared to blossom honey samples that was caused by twofold increased concentration of H_2O_2. This observation is supported by our previous studies, where Slovak honeydew honeys produced high levels of H_2O_2 and exhibited pronounced antibacterial activities.[9,13] Indeed, the antibacterial effect of honey is mainly mediated by the presence of

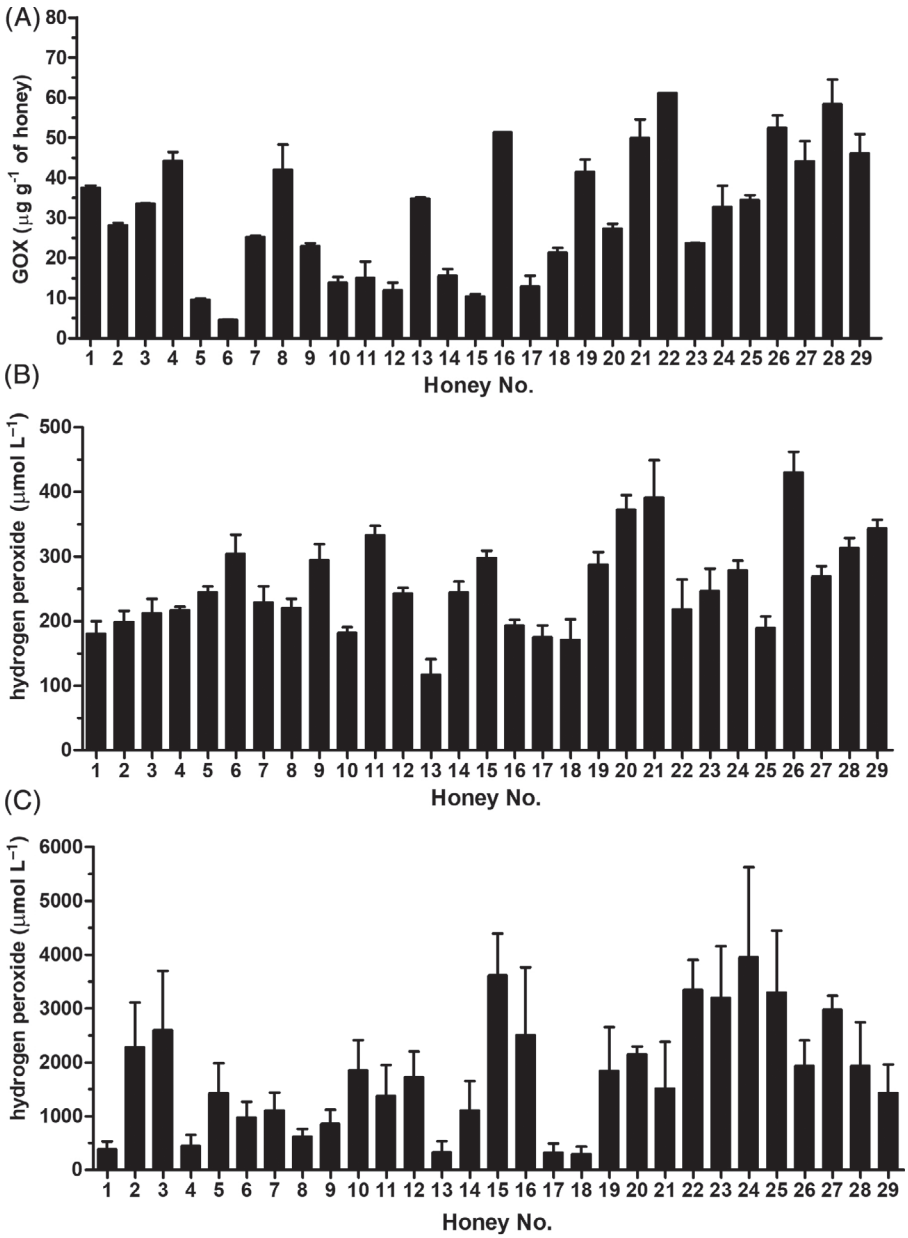

Figure 2. Glucose oxidase (GOX) content and hydrogen peroxide (H_2O_2) production in Swiss honey samples ($n = 29$). (A) GOX content was determined using a semi-quantitative assay based on polyclonal antibody against GOX; results are expressed as micrograms of GOX per gram of honey. (B) H_2O_2 content was measured in non-incubated 40% (w/v) honey solutions with a modified GOX assay kit. (C) H_2O_2 production was measured in 40% (w/v) honey solutions with a modified GOX assay kit after 24 h incubation. The data are expressed as the mean values with standard deviation (SD).

H_2O_2, which was also documented in Swiss honeys. Furthermore, honey samples generating low levels of H_2O_2 after 24 h incubation of their 40% (w/w) solutions showed the weakest antibacterial activities.

Weak honey antibacterial activity together with low concentration of H_2O_2 may indicate that enzymes and other bioactive proteinous compounds are inactivated by prolonged storage or thermal processing. In the current study, we verified honey freshness by analysing HMF content, an indicator of honey freshness and overheating. Twenty-three honeys contained no HMF above the detection limit, while six honey samples contained HMF concentrations up to a maximum of 11.7 mg kg^{-1} (honey sample No. 4). Since the HMF content did not exceed the limit of the *Codex Alimentarius* of 40 mg kg^{-1},[15] we can assume appropriate honey storage and no excessive heating for all the honey analysed. However, the enzymes responsible for H_2O_2-related antibacterial activity might be especially heat and storage sensitive, and hence not well reflected by HMF measurements. Indeed, a previous study has shown that floral honeys are especially sensitive to heating and storage, while honeydew honeys were much less affected.[16] The H_2O_2 production capacity in floral honeys decreased 38% during 3 months' storage in the light and at room temperature, and 92% of the initial capacity was lost by heating the floral honeys to 70 °C for 15 min.[16]

Our study confirmed that the antibacterial activity is linked to the botanical origin of the honeys, since the antibacterial activity is lower in blossom honeys as compared to honeydew honeys. The five honey samples in our study with especially weak

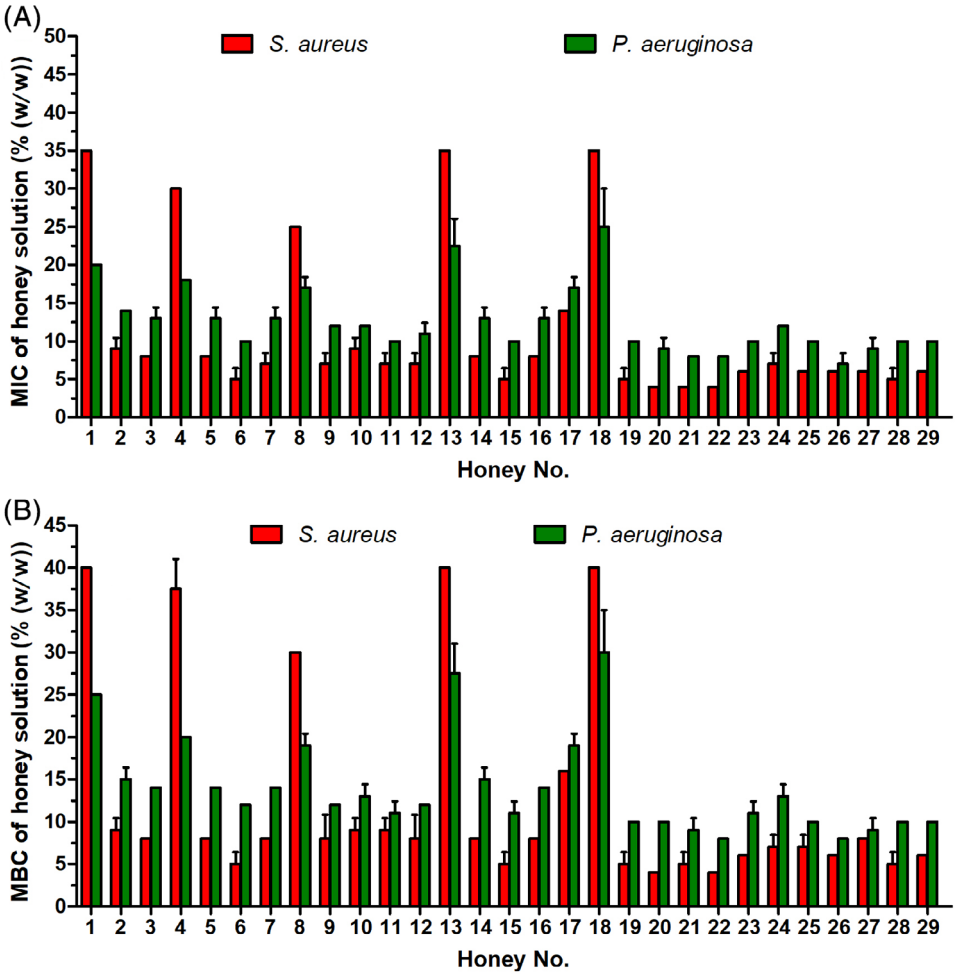

Figure 3. Antibacterial activity of Swiss honey samples ($n = 29$) against *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolates. Activity was determined with a minimum inhibitory concentration (MIC) (A) and minimum bactericidal concentration (MBC) assay (B). MIC and MBC were defined as the lowest concentration of honey solution (%) inhibiting bacterial growth and killing the bacteria, respectively. The data are expressed as the mean values with standard deviation (SD).

antibacterial activity were all blossom honeys (maximum electrical conductivity 0.436 mS cm^{-1}). Other reasons for low antibacterial activity of these five samples could be related to enhanced catalase enzymatic activity or natural GOX inhibitors present in these honeys.

GOX enzyme and polyphenol compounds are major compounds responsible for the generation of H_2O_2 in honey. H_2O_2 can also be generated via an alternative method by polyphenolic substances.[17] In other words, honeys with a high content of polyphenols (e.g. honeydew, buckwheat) accumulated substantially higher amounts of H_2O_2 and thus exhibit elevated antibacterial activity. Furthermore, our very recent study, although on blossom honeys, showed that overall antibacterial activity of honeys correlated with the level of total H_2O_2 as well as with total polyphenol content.[6] Dark honeys such as honeydew, buckwheat, chestnut or eucalyptus honeys appeared to be the richest in polyphenols and consequently showed higher biological activities.[18,19]

According to our recent study,[6] a complete honey protein content digestion did not affect the overall honey antibacterial activity, suggesting that either initial content of H_2O_2 in honey or polyphenol-mediated production of H_2O_2 is crucial for antibacterial effect of honey. A statistical analysis of data from determination of H_2O_2 in non-incubated and 24 h incubated honey samples revealed no correlation between the two groups of samples. A critical factor responsible for this observation was the ratio between the enzymatic activity of GOX and catalase. Catalase is a pollen/nectar-derived enzyme that is able to decompose H_2O_2 in a very effective and rapid way. Unfortunately, the content of catalase and its enzymatic activity compared to GOX activity in different honey samples have not been determined yet. Surprisingly, identification of catalase in honey via proteomic approaches often failed.[20–23]

High antibacterial activity is an important characteristic for those honeys that are used in wound care management as medical devices for treating infected or non-healing wounds and for treating various eye disorders such as dry eye syndrome and corneal ulcer. Natural antibacterial activity of some medical-grade honeys can be enhanced by adding biologically active molecules including fungal GOX. This bioengineered antibacterial honey product, Surgihoney RO, produces higher levels of H_2O_2 and is more effective against bacteria.[24,25] In this study, the content of GOX in Swiss honey samples did not correlate with their overall antibacterial activities. This suggests that the content of GOX is not a suitable marker of honey antibacterial potential owing to catalase enzymatic activity or the presence of natural GOX inhibitors in honey.

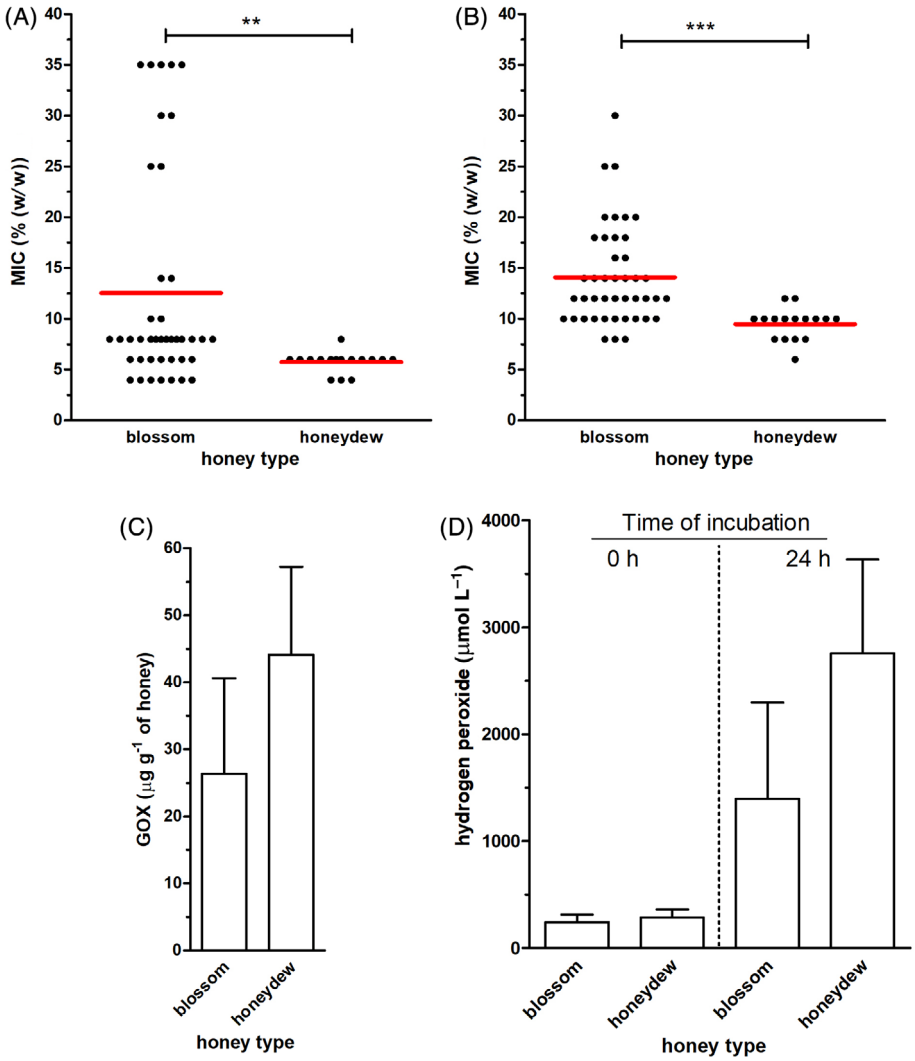

Figure 4. Comparison of antibacterial efficacy, glucose oxidase (GOX) content and hydrogen peroxide (H_2O_2) production of Swiss blossom and honeydew honey samples. Antibacterial activity of blossom ($n = 21$) and honeydew ($n = 8$) honeys was determined against (A) *Staphylococcus aureus* and (B) *Pseudomonas aeruginosa*. Activity was determined with a minimum inhibitory concentration (MIC). The red line represents the mean of all measured data. (C) GOX content was determined using a semi-quantitative assay based on polyclonal antibody against GOX; results are expressed as micrograms per gram of GOX per gram of honey. (D) H_2O_2 content was measured in non-incubated 40% (w/v) honey solutions with a modified GOX assay kit. H_2O_2 production was measured in 40% (w/v) honey solutions after 24 h incubation. The data are expressed as the mean values with standard deviation (SD). Differences between two honey groups were analysed by *t*-test. Asterisks indicate a significant difference between honey types: $^{**}P < 0.01$, $^{***}P < 0.001$.

CONCLUSIONS

We characterised the antibacterial potential of 29 honey samples representing the most prevalent types of honey in Switzerland. Honeydew honeys were the most effective honey samples and generated the highest levels of H_2O_2. A strong, significant correlation was found between the overall antibacterial activity and the level of H_2O_2 among all honey samples. The content of GOX in honey samples did not correlate with their antibacterial activity as well as H_2O_2 production capacity. A weak antibacterial activity was determined in five honey samples, most likely due to increased enzymatic activity of pollen (nectar)-derived catalase or GOX inhibitors.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to report.

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