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Research Article

Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka (*Leptospermum scoparium)* honeys from New Zealand

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The 1,2-dicarbonyl compounds 3-deoxyglucosulose (3-DG), glyoxal (GO), and methylglyoxal (MGO) were measured as the corresponding quinoxalines after derivatization with orthophenylendiamine using RP-HPLC and UV-detection in commercially available honey samples. Whereas for most of the samples values for 3-DG, MGO, and GO were comparable to previously published data, for six samples of New Zealand Manuka (*Leptospermum scoparium*) honey very high amounts of MGO were found, ranging from 38 to 761 mg/kg, which is up to 100-fold higher compared to conventional honeys. MGO was unambigously identified as the corresponding quinoxaline *via* photodiodearry detection as well as by means of mass spectroscopy. Antibacterial activity of honey and solutions of 1,2-dicarbonyl towards *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) were analyzed using an agar well diffusion assay. Minimum concentrations needed for inhibition of bacterial growth (minimum inhibitory concentration, MIC) of MGO were 1.1 mM for both types of bacteria. MIC for GO was 6.9 mM (*E. coli*) or 4.3 mM (*S. aureus*), respectively. 3-DG showed no inhibition in concentrations up to 60 mM. Whereas most of the honey samples investigated showed no inhibition in dilutions of 80% (v/v with water) or below, the samples of Manuka honey exhibited antibacterial activity when diluted to 15 – 30%, which corresponded to MGO concentrations of 1.1 – 1.8 mM. This clearly demonstrates that the pronounced antibacterial activity of New Zealand Manuka honey directly originates from MGO.

Keywords: 1,2-Dicarbonyl compounds / 3-Deoxyglucosulose / Bacteria / Glyoxal / Methylglyoxal

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1 Introduction

The use of honey as a traditional remedy for bacterial infections is known since ancient times. According to [1], scientific research in that field aiming at the identification of antibacterial compounds started with the pioneering reports of van Ketel in 1892. Dold *et al.* [2] established the term "inhibine" for the light and temperature sensitive antibacterial substances in honey without further chemical characterization. Since then, numerous investigations have been undertaken in order to explain the antibacterial activity of individual honey samples by osmotic effects, or the low pH-value caused by several organic acids [3 – 6]. The most known inhibine is hydrogen peroxide [7, 8], which is formed in the honey by glucose oxidase. Several studies, however, have shown that certain honey samples possess an antibacterial activity which persists after removal of hydrogen peroxide by catalase [8]. It has been observed that Manuka honey, derived from the Manuka tree (*Leptospermum scoparium*) in New Zealand, has a very high level of "non-peroxide" antibacterial activity [9 – 10]. The pronounced antibacterial activity of Manuka honey is an important commercial property, which is referred to in marketing purposes as the so-called "Unique Manuka Factor" (UMF), leading to a classification of premium products based on microbiological assays [11].

Although several studies have been undertaken in order to characterize the components responsible for the "nonper-

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Abbreviations: 3-DG, 3-deoxyglucosulose; **GO,** glyoxal; **MGO,** methylglyoxal; **OPD,** orthophenylendiamine; **UMF,** Unique Manuka Factor

oxide" antibacterial activity of Manuka honey [12], the chemistry behind this phenomenon still remains unclear. In a recent report, Weigel *et al*. [13] showed that honey contains varying amounts of 1,2-dicarbonyl compounds such as glyoxal (GO), methylglyoxal (MGO), and 3-deoxyglucosulose (3-DG) besides 5-hydroxymethylfurfural, a well-known indicator for heat-treatment. 1,2-Dicarbonyl compounds are formed in the course of the Maillard reaction or caramelization reactions as degradation products from reducing carbohydrates [14]. Motivated by a report from Suortti and Mälkki [15], who found that heated solutions of glucose or fructose, respectively, exhibit a pronounced antibacterial activity against *Escherichia coli* (*E. coli*), we investigated the hypothesis whether 1,2-dicarbonyl compounds present in Manuka honey may be related to the non-peroxide activity of these food items. 1,2-Dicarbonyl compounds were quantified after derivatization with orthophenylenediamine as the corresponding chinoxalines using RP-HPLC with UV-detection. Antibacterial properties of individual 1,2-dicarbonyls as well as of honey samples were evaluated using an agar well diffusion assay.

2 Materials and methods

2.1 Chemicals

GO and MGO, each 40% in water and MGO, were from Sigma (Taufkirchen, Germany). MGO was purified according to [16] with additional redistillation [17]. 3-DG was synthetized according to [18] with some modifications [19]. Acetic acid p.a. was obtained from BioChemica (Darmstadt, Germany). Phosphoric acid (85%, p.a.) was from Merck (Darmstadt, Germany). Orthophenylendiamine (OPD) was obtained from Fluka (Munich, Germany). HPLC grade methanol was from Riedel de Haen (Seelze, Germany). Water used for preparation of buffers and solutions was obtained with a Purelab plus purification system (USFilter, Ransbach-Baumbach, Germany). Agar agar, yeast extract, peptone, tryptone, and sodiumchloride were applied from AppliChem (Darmstadt, Germany).

2.2 Honey samples

A total of 50 honey samples of various origin were obtained from local retail stores or were received as a gift from Beekeeper's association Dresden (Imkerverein Dresden, e.V.), respectively. The six samples of Manuka honey were ordered *via* the internet from suppliers of New Zealand honey.

2.3 Analytical RP-HPLC

1,2-Dicarbonyl compounds were analyzed as the corresponding quinoxalines after derivatization with OPD according to [13] with some slight modifications. 1.0 mL of 15% w/v solutions of honey in 0.5 M sodium phosphate, pH 6.5, or standard solutions of the 1,2-dicarbonyl compounds were mixed with 0.6 mL of a 1.0% w/v solution of OPD in 0.5 M sodium phosphate buffer, pH 6.5. The mixture was kept in the dark for 12 h at room temperature. After membrane filtration (0.45 µm), 20 µL of this samples were subjected to chromatography analysis. This was performed using an Äkta Basic System with a pump P-900, UV monitor UV-900 and an auto sampler A-900 (all from Pharmacia, Freiburg, Germany). A stainless steel column, 250 mm × 4.6 mm, filled with Eurospher 100 RP18-material of 5 µm particle size (Knauer, Berlin, Germany), was used. The flow rate was 0.8 mL/min. The column temperature was set at 30°C. The mobile phases were 0.15% acetic acid (solvent A) and 80% methanol containing 20% solvent A (solvent B). The gradient started with 20% solvent B over a period of 2 min, then it was changed linearly to 40% solvent B over a period of 20 min and to 100% solvent B within 15 min, followed by an elution with 100% solvent B over a period of 5 min and then it was changed to 20% solvent B in 7 min with subsequently equlibration with 20% solvent B for 5 min. Peaks were detected by measurement of UV-absorbance at 312 nm. External calibration using reference compounds in the range from 10 to 500 mg/L for 3-DG, from 0.1 to 20 mg/L for GO and from 0.1 to 300 mg/L for MGO was performed. All calibration curves showed linearity within these concentration ranges. Detection limits were 0.3 mg/kg for 3-DG and 0.2 mg/kg for GO or MGO, respectively.

2.4 LC/mass spectroscopy

LC-MS measurement was performed with a LC system 1100 Series (Agilent Technologies, PaloAlto, USA) and a Mariner ESI-TOF mass spectrometer (PerSeptive Biosystem, Framingham, USA). Chromatographic conditions were as above. Electrospray ionization was performed in the positive ionization mode. Nitrogen was used as curtain gas (1.5 L/min) and nebulizer gas (0.8 L/min). The mass spectrometer operating conditions were as follows: spray tip potential 5190 V, SCIEX heater 290°C, nozzle potential 90 V, skimmer 1 potential 11.50 V, quadrupole DC potential 7.50 V, deflection voltage −1.00 V, einzel lens potential −34 V, quadrupole RF voltage 1000 V, quadrupole temperature 140°C, nozzle temperature 140°C, push pulse potential 545 V, pull pulse potential 210 V, pull bias potential 3 V, acceleration potential 4000 V, reflector potential 1550 V, and detector voltage 2250 V. Full scan mass spectra were measured in mass range 100–3000 *m/z* in the tic-mode. The instrument was calibrated using a protein mixture containing bradykinin, angiotensin I, and neurotensin (Sigma–Aldrich, Steinheim, Germany). Data acquisition and handling was preformed using the software Data Explorer Version 4.0.0.1 (Applied Biosystems, Foster City, USA).

Table 1. 1,2-Dicarbonyl compounds and HMF in honey samples and one pharmaceutical preparation (data given in mg/kg as median, minimum and maximum value; for samples of Manuka honey data are mean ± SD resulting from triplicate analysis; abbreviations are as follows: 3-DG, 3-deoxyglucosulose; GO, glyoxal; MGO, methylglyoxal; HMF, 5-hydroxymethylfurfural; n.d., not detectable, below 0.2 mg/kg; n.a., not analyzed)

Sample	3-DG	GO	MGO	HMF
Commercial honey samples (n = 50)	342 (119 – 1451)	1.7 (n.d. -4.6)	3.1 (n.d. -5.7)	3.9 (1.0 – 75)
Manuka 1 "active 5"	1060 ± 54	0.7 ± 0.2	38.4 ± 5.0	3.0 ± 0.2
Manuka 2 "active"	668 ± 30	3.0 ± 1.0	347 ± 20	22.6 ± 0.5
Manuka 3 "active"	563 ± 26	3.9 ± 1.0	411 ± 24	17.6 ± 0.6
Manuka 4 "UMF 10"	747 ± 40	1.2 ± 0.5	416 ± 35	21.3 ± 1.1
Manuka 5 "UMF 20"	807 ± 39	4.2 ± 1.1	743 ± 40	43.9 ± 2.0
Manuka 6 "UMF 25"	697 ± 44	7.0 ± 1.0	761 ± 25	n.a.
Antibacterial wound gel	n.a.	n.a.	312 ± 20	n.a.

2.5 Assessment of antibacterial activity

Antibacterial activity of honey and solutions of 1,2-dicarbonyl were analyzed using an agar well diffusion assay according to Patton *et al*. (2005) [20]. *E. coli* or *Staphylococcus aureus* (*S. aureus*) were precultivated overnight at 37°C in 50 mL flasks containing 10 mL of nutrient broth according to [21]. Afterwards 0.1 mL of the cultures were spreaded on plates containing solidified nutrient medium. Wells 10 mm in diameter (0.2 mL capacity) were bored into the surface of the agar medium. 0.15 mL of solutions of the 1,2-dicarbonyl compounds GO, MGO, and 3-DG in 0.5 M sodium phosphate buffer, pH 6.5, or honey diluted to concentrations ranging from 15 to 80% in the same buffer were placed into the wells. Plates were incubated at 37°C for 20 h. The zones of inhibition were measured. MIC values (minimum inhibitory concentration) were determined. MIC corresponds to the lowest concentration, for which an inhibition zone was visually detectable.

3 Results

The trapping of 1,2-dicarbonyl compounds with orthophenylenediamine and subsequent chromatographic analysis of the corresponding chinoxalines using RP-HPLC with UV detection at 312 nm is a generally accepted method for the quantification the degradation compounds formed from carbohydrates during Maillard reactions or caramelization. Recently, we were able to quantify 1,2-dicarbonyls in honey [13] for the first time. In the present study, a large number of commercially available samples were investigated and it could be shown, that for most of the conventional honeys, the amount of GO and MGO was low when compared to 3-DG (Table 1). GO and MGO did not exceed maximum levels of 5 mg/kg and were not affected by storage conditions, whereas up to 1451 mg/kg of 3-DG were measured. The observation by Weigel *et al*. [13] that no correlation exists between the amount of HMF and 3-DG, the latter representing the direct precursor for HMF, could be confirmed.

Table 2. MIC (minimally inhibitory concentration) of solutions of 1,2-dicarbonyls or diluted honey samples, respectively, and concentrations of MGO in honey samples diluted to corresponding MIC

Sample	MIC for *E. coli*	MIC for *S. aureus*
3-DG	No inhibition observed up to 60 mM	
GO	6.9 mM	4.3 mM
MGO	1.1 mM	
Honey samples	>80%	
Manuka honeys 2 – 6	15 – 30%	
MGO at MIC dilution	1.1 – 1.8 mM	

While analyzing further honey samples, we noticed for samples of commercially available Manuka honey from New Zealand (Fig. 1A) surprisingly high peaks of a chinoxaline eluting with identical retention time as the chinoxaline formed from MGO and orthophenylenediamine (Fig. 1B). Using a photodiodearry detector, it could be shown that the UV-spectra of the peaks detected in the honey samples were identical to that in the reference sample of MGO. For unambiguous identification of the chinoxaline derived from MGO, LC-TOF-MS was performed. Identical mass spectra with a dominant signal at an m/z of 145.1 when measured as $[MH]^+$ were found for the peak eluting at 39 min in the chromatograms of Manuka honey samples as well as the reference sample of MGO (Fig. 2). This clearly proves the unambiguous identification of the chinoxaline formed from MGO and orthophenylendediamine. In six samples of Manuka honey from New Zealand, concentrations for MGO ranged from 38 to 761 mg/kg (Table 1), which is up to 1000-fold higher than corresponding data for the conventional honey samples. Interestingly, there was an indication that the "UMF-value", which is a commercially used parameter to rate the antibacterial activity of Manuka honey, is directly related to the content of MGO (Table 1).

Based on that surprising result, we hypothesized that the antibacterial activity of Manuka honey may at least in part be due to MGO. In order to prove this hypothesis, first the antibacterial properties of the 1,2-dicarbonyl compounds

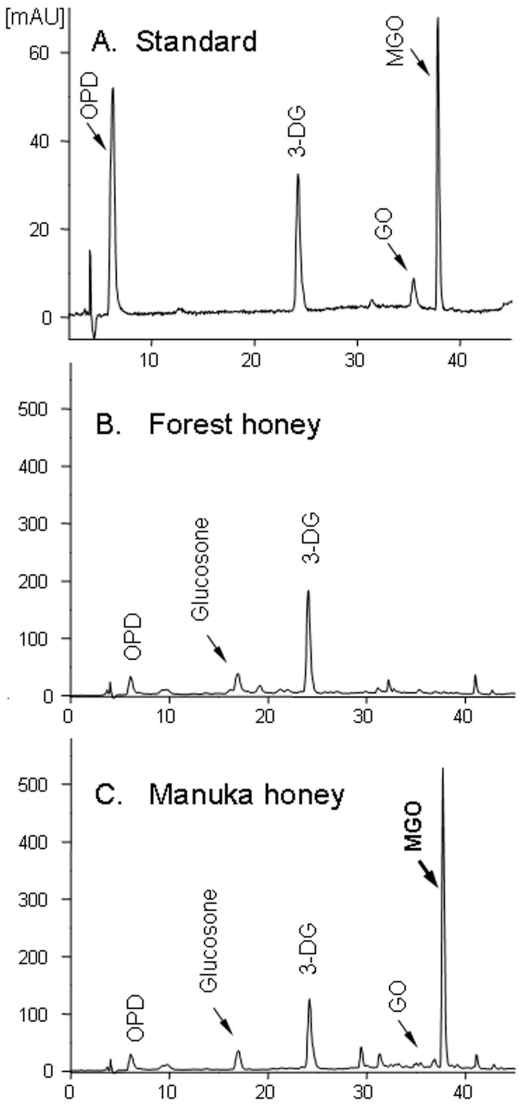

Figure 1. RP-HPLC with UV-detection at 312 nm. (A) Standard mixture consisting of 3-DG (155 mg/L), GO (12 mg/L), and MGO (45 mg/L); (B) forest honey; (C) Manuka honey.

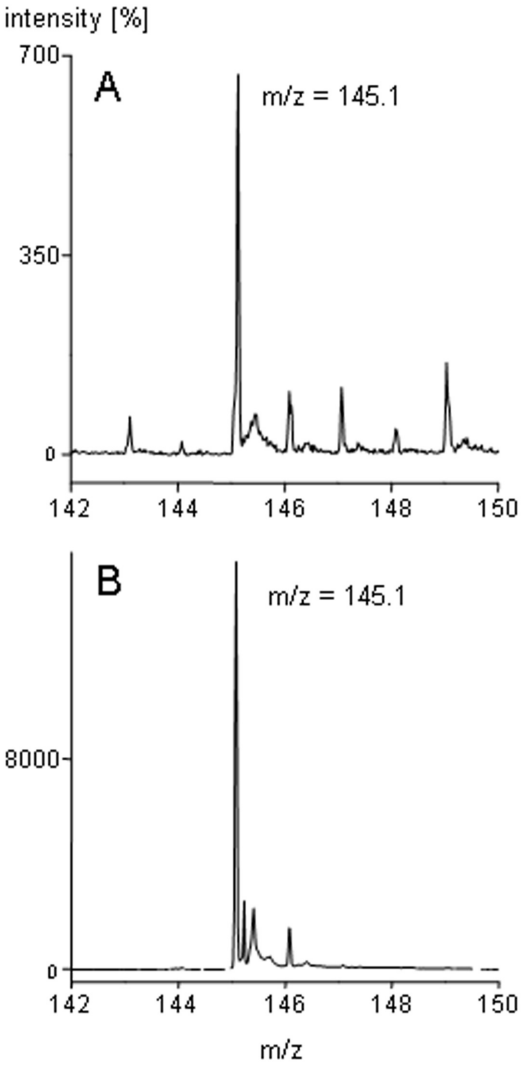

Figure 2. ESI-TOF-MS spectra for the peak eluting with a retention time of 39 min in the RP-HPLC chromatograms shown in Fig. 1. (A) Manuka honey sample; (B) reference sample of MGO.

were evaluated using an agar well diffusion assay. As can be seen from Table 2, all compounds studied exhibited an inhibiting effect against *E. coli* and *S. aureus*. The pronounced antibacterial effect was found for MGO, which is expressed by a MIC value of 1.1 mM for both bacteria strains. The values for MIC represent the minimum concentration of a compound for which an inhibiting effect was detectable. GO, for which a MIC of 6.9 mM for *E. coli* and 4.3 mM for *S. aureus* were measured, and 3-DG, which showed no inhibition at concentrations up to 60 mM, were significantly less effective inhibitors for bacterial growth when compared to MGO (Table 2). Next, it was evaluated whether honey samples exhibit an antibacterial effect under the conditions used in our assay. A pronounced antibacterial activity was only found for the samples designated as "active Manuka honey". For this samples, MIC values, expressed as concentrations after dilution in 0.5 M phosphate buffer, ranging from 15 to 30% were measured. All other honeys did not show any antibacterial effect in dilutions below 80%. Based on the quantitative data measured for MGO *via* RP-HPLC, it could be calculated that the amount of MGO "active" Manuka honeys diluted to the corresponding MIC, were similar to the determined MIC values of the standard solutions of the 1,2-dicarbonyl compound. In other words, diluting of Manuka honeys to concentrations between 15 and 30% resulted in concentrations of MGO from 1.1 to 1.8 mM, which are high enough to exhibit antibacterial effects.

This assumption was finally verified by adding the amount of pure MGO, which is present in a 20% solution of

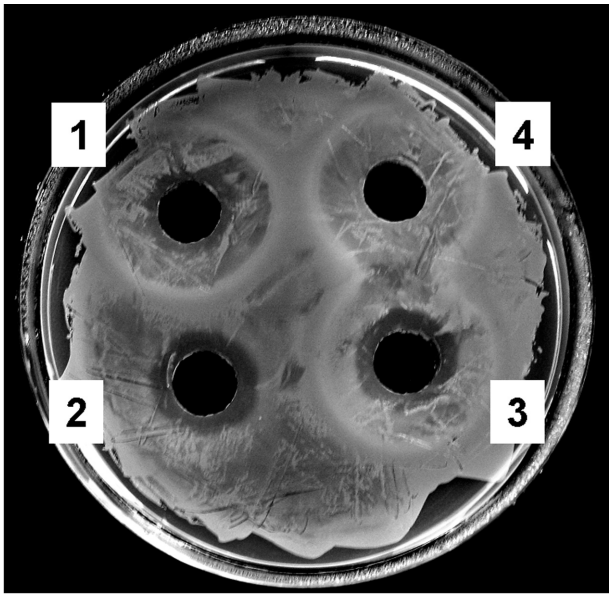

Figure 3. Agar well diffusion assay *(S. aureus).* Sample 1, active Manuka honey (dilution 20%); sample 2, MGO (1.9 mM); sample 3, inactive forest honey (dilution 20%) supplemented with 1.9 mM MGO; sample 4, inactive forest honey (dilution 20%).

an active Manuka honey, to a 20% dilution of an "inactive" forest honey. This "inactive" honey did not show an antibacterial activity at a concentration of 20% (Fig. 3, sample 4), whereas for the sample of Manuka honey, an inhibition zone was clearly visible (Fig. 3, sample 1). This Manuka honey dilution contained 1.9 mM MGO. After adding 1.9 mM MGO to the 20% solution of the forest honey, an inhibition zone comparable to that of an active Manuka honey was visible (Fig. 3, sample 3). Based on this observation, it can be concluded the MGO present in Manuka honey is directly responsible for the pronounced antibacterial activity of Manuka honey.

4 Discussion

The fact that honey originating from the Manuka tree (*L. scoparium*) has a significantly higher level of antibacterial activity when compared to other honeys has been reported by several authors [9 – 11]. This antibacterial activity could not be explained solely by the enzyme glucose oxidase, which is present in honey originating from the bee, inducing the formation of hydrogen peroxide when honey is diluted [7, 12]. This additional contribution in antibacterial activity was referred to a "nonperoxide activity" or "UMF", and several attempts were made in order to identify the compounds responsible for this effect. Preliminary phenomenological studies showed that the compounds are heat and light stable, and are not affected when the pH value is shifted to values above 11 during fractionation procedures [5, 22]. Several antibacterial phenolic acids such as caffeic and ferulic acid as well as syringic and methylsiringic acid and flavonoids like quercetin, isorhamnetin, and luteolin [23 – 25] were identified. The concentrations of these compounds in honey, however, were far too low to cause antibacterial effects. Nevertheless, a patent published recently describes methods for the preparation of UMF fortified honey and methods for the preparation of UMF-containing fractions of honey without insight into chemical details [26].

With our findings, we unambiguously demonstrate for the first time that MGO is directly responsible for the antibacterial activity of Manuka honey. It is noteworthy that such high amounts of MGO as present in Manuka honey (Table 1) have not yet been found for any other food item. Low amounts of enzymatically formed GO and MGO were reported for fermented foods such as milk products as well as beer and wine, with concentrations ranging from 3 to 11 mg/kg [27, 28]. Furthermore, MGO is known to form during coffee roasting in amounts of 23 – 47 mg/mg [29]. Quantitative data for 3-DG in food are not available. At present, only speculations can be made concerning the origin of MGO in Manuka honey. A nonenzymatic formation *via* retro-aldolization in the course of heat- or storage-induced Maillard or caramelization reactions [14] can be excluded, as relatively low amounts of HMF were measured. HMF is a sensitive indicator for heat-treatment. MGO is known as a by-product of increased glycolysis in bacteria [30], therefore a microbiological origin of the 1,2-dicarboynl compound should be taken into account. Furthermore, a recent report [31] for the first time demonstrated the estimation of MGO level in the range of 30 – 75 μM in various plant species and its increase in response to salinity, drought, and cold stress conditions. Whether this may be of importance for Manuka from a botanical point of view remains to be elucidated. Further studies must also clarify whether the promising experience in wound care issues reported for Medihoney, which is a pharmaceutical preparation of *Leptospermum* honeys from Australia certified for wound care, may directly be due to MGO [32] (www.medihoney.com). One commercially available pharmaceutical sample of an antibacterial wound dressing contained 312 mg/kg MGO (Table 1). This concentration of MGO should be high enough to cause antibacterial effects when applied to wounds.

Finally, from the nutritional standpoint, the physiological significance resulting from the uptake of MGO and other 1,2-dicarbonyl compounds must be a topic of further investigations. MGO and glycation compounds resulting from the reaction of MGO with amino acid side chains of lysine or arginine, respectively, have been identified *in vivo* and are associated with complications of diabetes and some neurodegenerative diseases, although the role of these compounds in the pathogenesis of different diseases have not

yet been fully understood [33 – 35]. Information concerning a potential toxicity of dietary MGO are rare and ambivalent, as the intake of MGO has also exerted an anticancer effect [35 – 37]. The physiological implications resulting from an uptake of bioactive carbohydrate degradation products and a risk-benefit analysis resulting there from must be topic of further investigations.

In conclusion, with the present investigation the occurrence of high amounts of MGO in New Zealand Manuka (*L. scoparium*) honey was demonstrated. MGO was identified as a bioactive compound which is responsible for the antibacterial activity of these honey samples. Studies in order to clarify the pathways for the biochemical formation of MGO in Manuka plants and honey are underway in our laboratory.

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The authors have declared no conflict of interest.

5 References

[1] Dustmann, J. H., Antibacterial effect of honey. *Apiacta* 1979, *14*, 7 – 11.

[2] Dold, D., Du, D. H., Dziao, S. T., Nachweis antibakterieller, hitze- und lichtempfindlicher Hemmstoffe (Inhibine) im Naturhonig. *Z. Hyg. Infekt.* 1937, *120*, 155 – 167.

[3] Dustmann, J. H., Über den Einfluss des Lichtes auf den Peroxidwert (Inhibin) des Honigs. *Z. Lebensm. Unters. Forsch*. 1972, *148*, 263 – 268.

[4] Bogdanov, S., Characterization of antibacterial substances in honey. *Lebensm. Wiss. Technol*. 1984, *17*, 74 – 76.

[5] Bogdanov, S., Nature and origin of the antibacterial substances in honey. *Lebensm. Wiss. Technol*. 1997, *30*, 748 – 753.

[6] Molan, P. C., The antibacterial activity of honey. 1. The nature of the antibacterial activity. *Bee World* 1992, *73*, 5 – 28.

[7] White, J. W., Subers, M. H., Schepartz, A., The identification of inhibine. *Am. Bee J.* 1962, *102*, 430 – 431.

[8] Adcock, D., The effect of catalase on the on the inhibine and peroxide values of various honeys. *J. Apic. Res.* 1962, *1*, 38 – 40.

[9] Allen, K. L., Molan, P. C., Reid, G. M., A survey of the antibacterial activity of some New Zealand honeys. *J. Pharm. Pharmacol*. 1991, *43*, 817 – 822.

[10] Russell, K. M., Molan, P. C., Wilkins, A. L., Holland, P. T., Identification of some antibacterial constituents of New Zealand manuka honey. *J. Agric. Food Chem*. 1990, *38*, 10 – 13.

[11] Molan, P. C., Why honey is effective as a medicine. 2. The scientific explanation of its effects. *Bee World* 2001, *82*, 22 – 40.

[12] Weston, R. J., The contribution of catalase and other natural products to the antibacterial activity of honey: A review. *Food Chem*. 2000, *71*, 235 – 239.

[13] Weigel, K., Opitz, T., Henle, T., Studies on occurence and formation of 1,2-dicarbonyls in honey. *Eur. Food Res. Technol*. 2004, *218*, 147 – 151.

[14] Mavric, E., Kumpf, Y., Schuster, K., Kappenstein, O. *et al*., A new imidazolinone resulting from the resulting from the reaction of peptide-bound arginine and oligosaccharides with 1,4-glycosidic linkages. *Eur. Food Res.* 2004, *218*, 213 – 218.

[15] Suortti, T., Mälkki, Y., Antimicrobial activities of heated glucose and fructose solutions and their elucidation by high performance liquid chromatography. *Food Chem.* 1984, *15*, 165 – 173.

[16] Kellum, M. W., Oray, B., Norton, S. J., A convenient quantitative synthesis of methylglyoxal for glyoxalase I assays. *Anal. Biochem*. 1978, *85*, 586 – 590.

[17] McLellan, A. C., Phillips, S. A., Thornalley, P. J., The assay of methylglyoxal in biological systems by derivatization with 1,2-diamino-4,5-dimethoxybenzene. *Anal. Biochem.* 1992, *206*, 17 – 23.

[18] Madson, M. A., Feather, M., An improved preparation of 3-deoxy-erythro-hexos-2-ulose via the bis(benzylhydrazone) and some related constitutional studies. *Carbohydr. Res*. 1981, *94*, 183 – 191.

[19] Henle, T., Bachmann, A., Synthesis of pyrraline reference material. *Z. Lebensm. Unters. Forsch.* 1996, *202*, 72 – 75.

[20] Patton, T., Barrett, J., Brennan, J., Moran, N., Use of a spectrophotometric bioassay for determination of microbial sensitivity to manuka honey. *J. Microbiol. Meth.* 2006, *64*, 84 – 95.

[21] Zimbro, M. J., Power, D. A. (Eds.), *Difco and BBL Manual: Manual of Microbiological Culture Media. Becton*, Dickinson and Co. Sparks, MD 2003.

[22] Molan, P. C., Russell, K. M., Non-peroxide antibacterial activity in some New Zealand honeys. *J. Apic. Res.* 1988, *27*, 62 – 67.

[23] Tan, S. T., Wilkins, A. L., Molan, P. C., Holland, P. T., Reid, M., A chemical approach to the determination of floral sources of New Zealand honeys. *J. Apic. Res*. 1989, *28*, 212 – 222.

[24] Weston, R. J., Brocklebank, L. K., Lu, Y., Identification and quantitative levels of antibacterial components of some New Zealand Manuka honeys. *Food Chem.* 2000, *70*, 427 – 435.

[25] Russell, K. M., Molan, P. C., Wilkins, A. L., Holland, P. T., Identification of some antibacterial constituents of New Zealand Manuka honey. *J. Agric. Food Chem*. 1990, *38*, 10 – 13.

[26] Snow, M.J., Manlye-Harris, M., Farr, J.M., Unique manuka factor fortified honey. *PCT Internat. Patent Appl.* 2005, Patent Number WO2005120250-A1.

[27] Bednarski, W., Jedrychowski, L., Hammond, E. G., Nikolov, Z. L., A method for the determination of alpha-dicarbonyl compounds. *J. Dairy Sci.* 1989, *72*, 2474 – 2477.

[28] De Revel, G., Bertrand, A., A method for the detection of carbonyl compounds in wine: Glyoxal and methylglyoxal. *J. Sci. Food Agric.* 1993, *61*, 267 – 272.

[29] Hayashi, T., Shibamoto, T., Analysis of methylglyoxal in foods and beverages. *J. Agric. Food Chem.* 1985, *33*, 1090 – 1093.

[30] Ferguson, G. P., Tötemeyer, S., MacLean, M. J., Booth, I. R., Methylglyoxal production in bacteria: Suicide or survival? *Arch. Microbiol.* 1998, *170*, 209 – 218.

[31] Yadav, S. K., Singla-Pareek, S. L., Ray, M., Reddy, M. K., Sopory, S. K., Methylglyoxal levels in plants under salinity stress are dependent on glyoxalase I and glutathione. *Biochem. Biophys. Res. Commun.* 2005, *337*, 61 – 67.

[32] Simon, A., Sofka, K., Wiszniewsky, G., Blaser, G.*et al*., Wound care with antibacterial honey (Medihoney) in pediatric hematology-oncology. *Support. Care Cancer* 2006, *14,* 91 – 97.

[33] Henle, T., Protein-bound advanced glycation endproducts (AGEs) as bioactive amino acid derivatives in foods. *Amino Acids* 2005, *29*, 313 – 322.

[34] Henle, T., Miyata, T., Advanced glycation end products in uremia. A*dv. Ren. Replace. Ther.* 2003, *10*, 321 – 331.

[35] Nemet, I., Varga-Defterdarovic, L., Turk, Z., Methylglyoxal in food and living organism. *Mol. Nutr. Food Res.* 2006, *50*, 1105 – 1117.

[36] Kalapos, M. P., Methylglyoxal in living organisms: Chemistry, biochemistry, toxicology and biological implications. *Toxicol. Lett.* 1999, *110*, 145 – 175.

[37] Ghosh, M., Talukdar, D., Ghosh, S., Bhattacharyya, N., *et al*., In vivo assessment of toxicity and pharmacokinetics of methylglyoxal. Augmentation of the curative effect of methylglyoxal on cancer-bearing mice by ascorbic acid and creatine. *Toxicol. Appl. Pharmacol.* 2006, *212*, 45 – 58.