Manuka honey (*Leptospermum scoparium*) inhibits jack bean urease activity due to methylglyoxal and dihydroxyacetone

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**A B S T R A C T**

Manuka honey (*Leptospermum scoparium*) exerts a strong antibacterial effect. Bacterial enzymes are an important target for antibacterial compounds. The enzyme urease produces ammonia and enables bacteria to adapt to an acidic environment. A new enzymatic assay, based on photometric detection of ammonia with ninhydrin, was developed to study urease activity. Methylglyoxal (MGO) and its precursor dihydroxyacetone (DHA), which are naturally present in manuka honey, were identified as jack bean urease inhibitors with IC₅₀ values of 2.8 and 5.0 mM, respectively. Urease inhibition of manuka honey correlates with its MGO and DHA content. Non-manuka honeys, which lack MGO and DHA, showed significantly less urease inhibition. MGO depletion from manuka honey with glyoxalase reduced urease inhibition. Therefore, urease inhibition by manuka honey is mainly due to MGO and DHA. The results obtained with jack bean urease as a model urease, may contribute to the understanding of bacterial inhibition by manuka honey.

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**1. Introduction**

The enzyme urease catalyzes the formation of carbon dioxide and ammonia from urea. The activity of urease is essential for *Helicobacter pylori* survival. The Gram-negative bacterium colonizes the human stomach and neutralizes the acidic pH value with continuous ammonia production. *Helicobacter pylori* infections are responsible for gastric inflammation and ulcers (Marshall et al., 1987). Eradication of bacterial infection is commonly achieved by antibiotic treatments. The increasing resistance of bacteria towards antibiotics is problematic and new treatments of bacterial infections are becoming necessary. Manuka honey has an inhibitory effect on *Helicobacter pylori* (Al Somal, Coley, Molan, & Hancock, 1994). Five clinical isolates of *H. pylori* were inoculated on agar plates, which contained 2.5–10.0% manuka honey. A minimal inhibitory concentration (MIC) of 2.5% of manuka honey was determined visually. The authors concluded that such an amount could be easily taken up with a normal diet. Thus, manuka honey could be used as an alternative therapeutic treatment in *Helicobacter pylori* infections. A supportive effect of food against *Helicobacter pylori* infections is also described by other groups (Keenan, Salm, Hampton, & Wallace, 2010; Keenan, Salm, Wallace, & Hampton, 2012). The authors studied the synergistic and individual antibacterial effect of a range of foods and found a dose-dependent effect of manuka honey on *Helicobacter pylori* growth and reported MIC values of 2.5–5.0% for manuka honey. A possible target of *Helicobacter pylori* inhibition by food stuff is the enzyme urease. Several authors have studied the inhibitory effect of plant extracts on urease activity (Juszkiewicz, Zaborska, Laptas, & Olech, 2004; Olech, Zaborska, & Kot, 2014; Shaikh et al., 2016; Xie et al., 2016). The compounds responsible for urease inhibition were claimed to be alkenylthiosulfinates, glycosides, flavonoids and catechines (Adeniyi & Anyiam, 2004). Moreover, the inhibitory effect of honey on urease was described (Salin, 2015). The author assumed that the inhibitory effect of aqueous extracts of chestnut and oak honey is due to their high phenolic content. Manuka honey (*Leptospermum scoparium*) from New Zealand is known for its exceptional antibacterial activity. The antibacterial effect is caused by the compound methylglyoxal (MGO) (Mavric, Wittmann, Barth,
and acetoxyhydroxamic acid was provided by Alfa Aesar (Karlsruhe, Germany). Catalase from bovine liver and glutathione (reduced) were obtained from Merck (Darmstadt, Germany). For all experiments, ultrapure water was used and was prepared using an ELGA LabWater Purelab Plus water system (Celle, Germany).

2.3. Urease activity assay

The commercial jack bean urease was solubilized in ultrapure water (80 KU/l). To measure the enzyme activity, 25 µl of urease solution was mixed with 10 µl ninhydrin (2% (w/w) in ethanol), 90 µl phosphate buffer (200 mM, pH 6.8) and 100 µl ultrapure water in a 96-well microtiter plate. The mixture was pre-incubated at 37 °C for 2 h. The reaction was started by adding 50 µl of urea solution (550 mM in ultrapure water). For the enzyme working at its maximum velocity, the final urea concentration for the assay was set to 100 mM, which is approximately 5 times the Michaelis-Menten constant (see below). The microtiter plate was further incubated at 37 °C for 80 min and the detection of ammonia released from urea cleavage was continuously monitored by measuring the absorption at 440 nm with a multimode reader Tecan Infinite M200 (Mainz, Germany). A blank with 50 µl ultrapure water instead of the substrate urea was included in the assay to test whether ninhydrin reacts with any other compounds than ammonia to form a yellow complex which absorbs at 440 nm. Urease activity was defined as the linear slope of absorbance between 10 and 30 min of incubation.

2.4. Urease inhibition assay

To measure the inhibition of the enzyme activity caused by honeys, methylglyoxal or dihydroxyacetone, respectively, 25 µl of urease solution was mixed with 10 µl ninhydrin (2% (w/w) in ethanol), 90 µl phosphate buffer (200 mM, pH 6.8) and 100 µl of the solution of the inhibitory compound, in a 96-well microtiter plate. The mixture was pre-incubated at 37 °C for 2 h and the reaction was started by adding 50 µl of the substrate urea solution (550 mM in ultrapure water). The microtiter plate was further incubated at 37 °C for 80 min and the detection of ammonia release from urea cleavage was continuously monitored by measuring the absorption at 440 nm with a multimode reader Tecan Infinite M200 (Mainz, Germany). A blank of each inhibitory compound without urease but 25 µl of ultrapure water instead was included in the assay to test for any interfering substances which absorb at 440 nm. Urease activity was defined as the linear slope of absorbance between 10 and 30 min of incubation. The results are expressed as a percentage of the residual activity measured in the presence of an inhibitor compared to the urease activity of a control (water) sample.

2.5. Inhibitory test compounds

Lime, honeydew, rape and artificial honey, as well as four manuka honeys with MGO and DHA contents classified as low, medium, high and very high (see Table 1) were analysed for their inhibitory effect. Hydrogen peroxide, which is formed via enzymatic glucose oxidation in diluted honey, inhibits the ninhydrin-ammonia reaction, which leads to apparently decreased activities. Therefore, hydrogen peroxide was eliminated by the addition of 20 µl of catalase to each honey stock solution (75% w/v). The honey stock solutions were diluted with ultrapure water to reach a final concentration of 0.04, 0.4, 1.8, 5.5, 10.9, 16.4, 21.8 and 27.3% (w/v) in the assay. Eight concentrations ranging between 0.4–83.3 and 0.2–44.4 mM of methylglyoxal (MGO) and dihydroxyacetone (DHA), respectively, were tested for their inhibitory effect. Acetoxyhydroxamic acid (AHA) was used as a positive control according

<table>
<thead>
<tr>
<th>MGO (mg/kg)</th>
<th>DHA (mg/kg)</th>
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<tr>
<td>MH 1</td>
<td>73</td>
</tr>
<tr>
<td>MH 2</td>
<td>284</td>
</tr>
<tr>
<td>MH 3</td>
<td>595</td>
</tr>
<tr>
<td>MH 4</td>
<td>435</td>
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to Tanaka, Kawase, & Tani (2004) and tested in eight concentrations ranging between 1.9 and 400 μM. All tested substances were diluted in ultrapure water. Rape honey was artificially spiked with MGO and DHA by adding 114 μl of a MGO stock solution (39.2 g/l) and 1 ml of a DHA stock solution (12.0 g/l) to 7.5 g of honey in 10 ml of ultrapure water. The aqueous solution with MGO and DHA was prepared similarly to the spiked rape honey, but without honey addition. To eliminate the effect of MGO in manuka honey, glutathione (GSH) and glyoxalase were added in a final concentration of 0.5 mM and 141 μM/ml to the 75% (w/v) stock solution of manuka honey sample MH 3, respectively.

2.6. Determination of the Michaelis–Menten constant

To determine the affinity of urease to the substrate urea, the urease assay conditions mentioned above were used. The velocity of the reaction was measured using eight different concentrations of the substrate urea (15–100 mM) and a constant enzyme concentration in the absence of inhibitors.

2.7. Angiotensin-converting enzyme (ACE) activity assay

ACE activity assay in the absence or presence of methylglyoxal (MGO) or dihydroxyacetone (DHA) was carried out according to Lunow, Kaiser, Rückriemen, Pohl, & Henle (2015). The ACE-induced cleavage of the fluorescence resonance energy transfer (FRET) substrate Abs-FR(Dnp)P-OH was continuously monitored by measuring the fluorescence at λ_em/λ_ex = 320/420 nm for 60 min at 37°C with a multimode reader Tecan Infinite M200 (Mainz, Germany). MGO stock solution was diluted in Tris-HCl buffer to give a final concentration of 4 mM. DHA was dissolved and diluted in Tris-HCl buffer to a final concentration of 7 mM. Inhibitor solutions and ACE were pre-incubated at 37°C for 2 h. The reaction was started with the addition of the FRET-substrate and substrate cleavage control samples, which correspond to 100% activity, were prepared by adding Tris-HCl buffer instead of inhibitor solutions to the enzyme. A blank, containing no enzyme but inhibitor and substrate, was included in the assay and subtracted from the inhibitor sample and control sample. The results are expressed as a percentage of the residual activity measured in the presence of an inhibitor compared to the pepsin activity measured for a control sample.

2.8. Pepsin activity assay

Pepsin activity was measured in the presence and absence of MGO or DHA, respectively, according to Minekus et al. (2014). All reagents were solubilised in ultrapure water unless otherwise stated. Trichloroacetic acid (TCA) was diluted to 5% (w/w) and MGO stock solution was diluted to 4 mM. DHA was solubilised and diluted to a final concentration of 7 mM. 0.5 g of the substrate haemoglobin was diluted in 20 ml of ultrapure water and further diluted with 5 ml HCl (300 mM) to reach a final concentration of 2% (w/v) with a pH of 2. For the pepsin stock solution, 20 mg of pepsin was diluted in 20 ml NaCl solution (150 mM). To prevent autoysis, the pH was brought to 6.5 with 100 mM NaOH. The pepsin stock solution was diluted 1:10 in 100 mM HCl freshly for each test. Inhibitor solutions and diluted pepsin were pre-incubated at 37°C for 2 h. The reaction was started with the addition of haemoglobin substrate and the reaction mixture was incubated at exactly 10 min at 37°C. The reaction was stopped with the addition of TCA, followed by centrifugation at 10,000 rpm for 10 min. 200 μl of the supernatant was transferred in a UV-star microtiter plate (from Greiner) and UV absorbance at 280 nm was measured with a multimode reader Tecan Infinite M200 (Mainz, Germany). A blank, containing no enzyme but inhibitor and substrate, was included in the assay and subtracted from the inhibitor sample and control sample. The results are expressed as a percentage of the residual activity measured in the presence of an inhibitor compared to the pepsin activity measured for a control sample.

2.9. Chromatographic quantitation of ammonia

In order to check values obtained from photometric quantitation of ammonia, data were compared with results obtained from quantitation via an amino acid analyser based on ion-exchange chromatography and post-column detection with ninhydrin. For this, incubation of urease with or without inhibitory substances was conducted without the addition of ninhydrin. Volume correction was achieved by adding 10 μl of phosphate buffer (100 μl phosphate buffer in total) instead of ninhydrin solution. Urease was pre-incubated with the inhibitors 2 h prior to start of the reaction with substrate addition (50 μl urea, 550 mM). The samples were further incubated for 30 min and directly diluted 1:250 with lithium citrate buffer (0.12 N, pH 2.2) to stop the enzymatic reaction. The analyses were performed using a SYKAM S4300 amino acid analyser (Fuerstenfeldbruck, Germany) with conditions according to manufacturer’s instructions. Ammonia eluted at 59.6 min and was detected after post-column derivatization with ninhydrin at 570 nm. External calibration with ammonia standards was performed.

2.10. RP-HPLC-UV analysis of MGO and DHA after derivatization to quinoxalines

The determination of MGO and DHA was performed according to Atrott et al. (2012) with slight modifications. For quantitation of MGO, 1 ml aliquots of 10% (w/v) honey solutions in 0.5 M phosphate buffer (pH 6.5) were mixed with 300 μl phosphate buffer (pH 6.5) and 300 μl oPD solution (1% in phosphate buffer pH 6.5). Samples were incubated in the dark at room temperature overnight and membrane filtered (0.45 μm). For quantitation of DHA, 1 ml aliquots of 10% honey solutions in 0.5 M acetate buffer (pH 4.0) were mixed with 300 μl acetate buffer (pH 4.0) and 300 μl oPD solution (1% in acetate buffer pH 4.0), followed by incubation for 16 h at 37°C and membrane filtration (0.45 μm).

HPLC analyses were performed using a HPLC system (Knauer, Berlin, Germany) equipped with a Smartline 1000 pump, a dynamic mixing chamber, a solvent organizer K-1500, an online degasser K-5004, a Basic Marathon auto sampler and an Anzura DAD detector 2.1 L. Peak evaluation was managed using the software ClarityChrom 6.1.0. The separation of quinoxalines was realized on a stainless steel column filled with Eurospher 100 RP18 material (250 mm × 4.6 mm, 5 μm particle size with integrated pre-column; Knauer, Berlin, Germany). The mobile phase was 0.075% acetic acid in water (solvent A) and a mixture of 80% methanol and 20% solvent A (solvent B). The gradient started with 40% solvent B for 1 min and was elevated linearly to 100% B over a period of 20 min, was changed back to 40% B in 4 min and was held there for 7 min. The flow rate was 0.9 ml/min, the separation was done at 30°C, 20 μl sample solution was injected and peaks were detected by measurement of UV absorbance at 312 nm. Quantitation was achieved by external calibration with MGO standard solution or by the standard addition method for DHA.

2.11. Statistical analysis

Statistical analysis was carried out using Origin 9 Software (OriginLab Corporation, Northampton, USA). Results are expressed as mean values ± standard deviation (SD) of three separate measurements, unless otherwise indicated.
3. Results and discussion

3.1. Evaluation of the photometric method for urease activity measurement

There are several methods published in the literature to study urease activity in vitro. An earlier study used an approach based on the pH increase caused by the continuous ammonia production to analyse the inhibitory effect of α-hydroxyketones (Tanaka et al., 2004). Phenol red was used to indicate appropriate ammonia production through urease with a colour change from yellow to pink. Other authors measured the urease inhibiting effect of aqueous honey samples and detected the ammonia production by using the indophenol method (Sahin, 2015). During this reaction, known as the Berthelot reaction, a phenol solution reacts with ammonia and sodium hypochlorite to form indophenol which can be detected photometrically at 625 nm. Both methods were tested in this study for their applicability (data not shown). According to our results, the reducing sugars in honey suppress the oxidation of phenol to indophenol, thus leading to wrong positive inhibition values. Hence, the reducing nature of the sugars, especially of fructose, makes this detection method unsuitable to study urease activity in combination with honey. Also, the method used by Tanaka et al. (2004), based on the detection of ammonia via pH control with phenol red, was negatively influenced by reducing sugars. Therefore, the first aim of this study was to develop a new method for reliable quantitation of ammonia production by urease in vitro, which is not influenced by honey sugars. Ammonia reacts with ninhydrin to form a purple dye, called Ruhemann purple (Ruhemann, 1910). Based on this knowledge, urea and urease, with or without inhibitors, were incubated and the resulting ammonia was separated via ion exchange chromatography and detected via post-column derivatization with ninhydrin at 570 nm. The chromatographically quantitated amount of ammonia was dependent on the presence or absence of an inhibitor (see Fig. 1). We concluded that ammonia quantitation via ninhydrin reaction was suitable to analyse urease activity. To quantitate urease activity with high sample throughput, the assay was optimized by using a photometric approach instead of chromatographic separation. Hence, urea and urease, with or without inhibitor, was incubated in a microtiter plate and ninhydrin was directly added to the reaction. The incubation resulted in the formation of a yellow coloured product, with an absorption maximum at 440 nm (see Fig. 2, black squares). The absorption maximum at this wavelength was unexpected, since Ruhemann purple detected via post-column derivatization of ammonia with ninhydrin absorbs at 570 nm. Nevertheless, the incubation of ammonia with ninhydrin in the microtiter plate resulted in the formation of a light-brown product with an absorption spectrum similar to the spectrum of the yellow product mentioned above. There was no increase at 440 nm when urease and urea were incubated without ninhydrin, or when ninhydrin was incubated with urease without urea. Fig. 3 shows a perfect correlation between the absorbance measured using the photometric assay and the ammonia concentration as quantitated chromatographically. This proves the hypothesis that the absorbance at 440 nm directly correlates with the ammonia concentration. The increase of absorbance at 440 nm was therefore solely due to the formation of ammonia and subsequent reaction with ninhydrin. Moreover, Fig. 3 illustrates the limit of detection of the microplate assay. At a concentration of 65 mM ammonia, no absorbance at 440 nm was detected. A minimum ammonia concentration of above 65 mM is necessary to detect ammonia photometrically. To ensure that the lack of absorption was not misinterpreted because of the limit of detection, the assay was incubated for 80 min. No increase of absorption after this time was observed, which was interpreted as complete inhibition of the enzyme. The inhibitor concentration, which inhibits 50% of enzyme activity (IC₅₀), was determined by plotting the percentage of urease inhibition against the logarithm of different concentrations of the inhibitor (see 2.5.). The IC₅₀ value of acetohydroxamic acid (AHA) was determined with the newly developed method to be 10.1 μM (see supplementary material for dose-response curve). In an earlier study an IC₅₀ of 5 μM after 3 h pre-incubation time (current assay: 2 h) was determined (Tanaka et al., 2004). This proves that the assay is suitable to determine inhibition constants similar to those previously published.

![Fig. 1. Amino acid analysis via ion-exchange chromatography and post-column ninhydrin detection](image1)

![Fig. 2. UV spectra after incubation of urea with urease and ninhydrin (■), urea with ammonia and ninhydrin (●), ninhydrin and ammonia (“Ruhemann purple”) (▼) and urea with ammonia (▲).](image2)
3.2. Inhibition of urease

The next aim of this study was to analyse urease inhibition by MGO and DHA and to evaluate the inhibitory potential of the carbonyl compounds in manuka honey. Therefore, aqueous solutions of MGO and DHA were studied for their urease inhibiting effect and the percentage of urease inhibition was plotted against the logarithm of the MGO and DHA concentrations. The fitting of the sigmoid dose-response curves resulted in IC_{50} values of 2.8 ± 0.2 mM for MGO and 5.0 ± 0.1 mM for DHA, which equals 202 mg/l and 450 mg/l, respectively (see supplementary material for dose-response curves). Compared to other natural ingredients of food, which are known to inhibit urease, like alkyl thiosulfimates in garlic or onion juice (IC_{50} values between 1–27 mM) (Olech et al., 2014), MGO and DHA are moderate inhibitors. Jack bean urease contains 15 cysteine residues per subunit, whereby Cys-592 is crucial for its enzymatic activity. It was proposed that the urease contains 15 cysteine residues per subunit, whereby Cys-592 is crucial for its enzymatic activity. Moreover, urease from jack bean contains 50 lysine and 37 arginine residues, which accounts for 6% and 4% of total amino acids, respectively (according to jack bean urease amino acid sequence, UniProt database entry P07374). Both amino acids are known to react quickly with MGO and form glycation products, which could lead to a decrease of urease activity. To check whether enzyme activity is non-specifically affected by MGO or DHA, pepsin and angiotensin converting enzyme (ACE) activity was measured in the presence of MGO and DHA. Both enzymes contain lysine (pepsin: 3%, ACE: 4%) and arginine (pepsin: 1%, ACE: 6%) residues (according to pep-sin and ACE amino acid sequence, UniProt database entry P00791 and P12822, respectively). The hydrolytic activities of these two enzymes are not affected by the presence of MGO or DHA (see supplementary material for data). Similar results for pepsin and pancreatin were recently published (Daglia, Ferrari, Collina, & Curti, 2013). The authors concluded that glycation of digestive enzymes may occur, but it does not influence the three-dimensional structure and thus enzyme activity. These “protecting effects” may be a consequence of protein evolution, since MGO is present in different food, which is commonly consumed and physiologically relevant enzymes got adapted to incubation with car-bonyls. Besides naturally present MGO and DHA, manuka honey contains mainly fructose and glucose. The effect of a sugar matrix on the urease inhibition was studied by preparing an artificial honey. No influence on urease activity was observed until concentrations of 10.9% (w/v) and maximum inhibition was 32% (see Fig. 4A) at 27.3% (w/v). This effect might be due to the osmotic activity of the sugars and the limited availability of water for the enzymatic reaction. Hence, in high concentrations, the effect of MGO and DHA is a combination of inhibition caused by sugars and other honey compounds, e.g. MGO and DHA, whereas in small concentrations of honey, sugars do not influence the enzyme activity. To prove whether MGO and DHA also inhibit urease activity when present in manuka honey, four different manuka honeys with varying MGO and DHA contents were tested. Fig. 4B shows the urease inhibition of manuka honeys in concentrations ranging between 0.04 and 27.3% (w/v). The concentrations of MGO and DHA of the corresponding honeys are listed in Table 1. The results indicate that manuka honeys efficiently inhibit urease and that the effect is dose-dependent on the MGO and DHA content of the hon-ey. Fig. 4A shows the inhibition of urease by non-manuka honeys, namely rape, lime and honeydew honey. Compared to manuka honeys, rape and lime honey showed significantly lower inhibitory activity on urease. This can be explained by the absence of MGO and DHA in these honeys (concentrations below 5 mg/kg). The inhibitory effect of the non-manuka honeys is mainly due to the effect of the sugars. A 27.3% (w/v) solution of artificial honey resulted in 32% inhibition of urease. For rape honey in the same concentration, 34% and for lime honey 43% urease inhibition was observed. The honeydew honey showed a stronger effect compared to the other honeys with 62% inhibition at 27.3% honey (w/v). Honeydew honeys are known to be rich in phenolic compounds originating from the plant phloem which is absorbed and excreted by the insects. These compounds may be responsible for the increased inhibitory effect of honeydew honey. Earlier reports already showed that honeydew honeys cause significantly stronger urease inhibition than other monofloral honeys (Sahin, 2015). This was confirmed by the results of our study. In contrast, a 5.5% (w/v) solution of manuka honey MH 3 inhibited around 50% of the enzyme. This solution contained 33 mg/l MGO and 85 mg/l DHA. An aqueous solution of MGO and DHA in the same concentrations was prepared to study, if this effect found for honey MH 3 is solely due to these compounds. Fig. 5 compares the inhibition of manuka honey MH 3 and the aqueous MGO and DHA sample. Both solutions inhibited 50% of the enzyme activity, thus demonstrating that the inhibition of manuka honey is exclusively caused by the effect of MGO and DHA. To test whether this inhibition is specific for manuka honey, MGO and DHA were artificially added to rape honey in concentrations similar to MH 3. After addition of MGO and DHA, the inhibitory effect of rape honey was higher than rape honey alone, but not as strong as caused by manuka honey MH 3 (see Fig. 5). This indicates that the strong urease inhibition is exclusive for manuka honey and cannot be achieved by adding MGO or DHA to non-manuka varieties. The decreased effect of artificially added MGO and DHA in the rape honey matrix could be due to trapping reactions caused by honey polyphenols. Benzene structures with hydroxyl groups can form MGO adducts (Lo, Hsiao, & Chen, 2011). This reaction may reduce the apparent MGO concentra-tion and its urease inhibition capacity. In manuka honey, MGO is naturally formed during honey maturation, thus an equilibrium between trapped and free MGO should exist. To elucidate whether MGO depletion from manuka honey results in decreased urease inhibition, glyoxalase I and glutathione (GSH) were added to MH 3. GSH forms a hemithioacetal adduct with MGO which is isomerized to S-2-hydroxyacylglutathione by glyoxalase I (Racker, 1951).
Thus, MGO should no longer be available for urease inhibition. After addition of glyoxalase I and GSH, the inhibitory effect of manuka honey MH 3 decreased by 23%, but no complete decrease down to the level of a non-manuka honey was observed (see Fig. 5). The remaining inhibitory effect is due to DHA, which is not affected by glyoxalase or GSH. This unambiguously demonstrates that MGO and DHA are essentially responsible for urease inhibition with manuka honey.

4. Conclusion

In this study, MGO and DHA, which are naturally present in manuka honey, are described as urease inhibitors for the first time. Since other honey ingredients, like polyphenols, melanoidins or, to a lesser extent sugar, can act as urease inhibitors, the inhibitory nature of a honey is difficult to predict. Nevertheless, we demonstrated that honeys which do not contain MGO or DHA show significantly less urease inhibition. In manuka honey mainly MGO and DHA are responsible for the inhibition of urease. This effect is unique to manuka honey and cannot be achieved by adding MGO and DHA to non-manuka honey. A manuka honey concentration of 0.4% (w/v) is sufficient to achieve 20% urease inhibition. That equals a dose of 4 g manuka honey containing 595 mg MGO per kg and 1549 mg DHA per kg, solubilized in 1 l of stomach fluid. Thus, an amount of manuka honey which causes significant urease inhibition can easily be achieved within the diet. Further studies are needed to clarify if the inhibition of jack bean urease can be transferred to urease present in *Helicobacter pylori*. There are several studies published in the literature, which use jack bean urease as a model to study potential urease inhibition in *Helicobacter pylori* (Krajewska, 2011; Wu et al., 2013). Moreover, studies, which compared the inhibition of certain plant extracts on jack bean urease and urease from *Helicobacter pylori* did not find significant differences (Matongo & Nwodo, 2014; Matsubara et al., 2003). The results of this study are a contribution towards the explanation of the mechanism of the antibacterial activity of manuka honey against *Helicobacter pylori*. According to our results, manuka honey could be a nutritional support for a treatment against *Helicobacter pylori* and may help to decrease the use of antibiotics.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.03.075.
References


