# Methylglyoxal in Manuka Honey – Correlation with Antibacterial Properties

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**Abstract**: A perfect linear correlation was found for methylglyoxal levels in 61 samples of Manuka honey, ranging from 189 to 835 mg/kg, and the corresponding antibacterial activities of the samples, which were between 12.4% and 30.9% equivalent phenol concentration. This clearly underlines that methylglyoxal is the dominant bioactive compound in Manuka honey and above concentrations of around 150 mg/kg directly responsible for the characteristic antibacterial properties of Manuka honey. Methylglyoxal can be a suitable tool for labelling the unique bioactivity of Manuka honey.

Keywords: methylglyoxal; honey; bacteria; RP-HPLC; functional food

## INTRODUCTION

Manuka honey, derived from the Manuka tree (Leptospermum scoparium) in New Zealand, is well-known for a pronounced antibacterial activity which cannot be found by any other honey. Manuka honey has been reported to exhibit antimicrobial activity against pathogenic bacteria such as Staphylococcus aureus and Helicobacter pylori, making this honey to a promising functional food for the treatment of wounds or stomach ulcers (FRENCH et al. 2005). Besides hydrogen peroxide, which is produced in most "conventional" honeys by the endogenous enzyme glucoseoxidase, several other "non-peroxide" factors were discussed to be responsible for the unique antibacterial activity of Manuka honey, but the chemistry behind this phenomenon remained unclear for decades (Rus-SEL et al. 1990; HENRIQUES 2006). Nevertheless, the so-called "Unique Manuka Factor" (UMF) was introduced some years ago for marketing purposes, leading to a classification of premium products based on microbiological assays. A UMF of 10, for instance, has the same antibacterial activity to a 10% solution of phenol (Allen et al. 1991). Recently, we were able to demonstrate unambiguously that the surprisingly high amounts of the 1,2-dicarbonyl compound methylglyoxal are present in certain samples of Manuka honey (MAVRIC *et al.* 2008). Methylglyoxal, at the levels at which we found it in Manuka honeys, proved to be the long sought-after non-peroxide antibacterial constituent. This observation was confirmed by ADAMS *et al.* (2008). The purpose of the present study was to investigate to which extent methylglyoxal is responsible for the non-peroxide antibacterial activity of Manuka honey, in order to check whether it is possible to back-reference from the methylglyoxal content to the antibacterial properties of a honey sample.

### MATERIALS AND METHODS

The source of the 61 samples of Manuka honey from New Zealand came from drums of honey selected on a spread of non-peroxide contents and of varying ages. The amount of methylglyoxal was measured as the corresponding quinoxaline after pre-column derivatisation with *o*-phenylendiamine using RP-HPLC with UV detection (MAVRIC *et al.* 2008). The data for non-peroxide antibacterial activity of all samples were provided by Manuka Health Ltd., Te Awamutu, New Zealand, and were analysed by a specially appointed laboratory for testing for antibacterial activity using criteria laid down by the Honey Research Unit at Waikato University, New Zealand, following the method described by ADAMS *et al.* (2008). Antibacterial activity was expressed as equivalent phenol concentration (% w/v). This value is used commercially as so-called Unique Manuka Factor (UMF).

#### **RESULTS AND DISCUSSION**

Methylglyoxal levels in 61 samples of Manuka honey ranged from 189 to 835 mg/kg honey. Corresponding antibacterial activities were between 12.4 and 30.9% equivalent phenol concentration (Figure 1A). A good linear correlation (y = 8.388 +0.0263*x*;  $r^2 = 0.905$ ) between methylglyoxal and the antibacterial activity was found. This indicates that methylglyoxal is directly responsible for the characteristic antibacterial properties of Manuka honey. Our data are in perfect agreement with results published by ADAMS et al. (2008), who had reported data for 49 samples of Manuka honey. In their study, concentrations of methylglyoxal as measured according to MAVRIC et al. (2008) ranged from 25 to 709 mg/kg. Corresponding antibacterial activity was between "not detectable" and 27.5% equivalent phenol concentration. Among the samples analysed by ADAMS et al. (2008), 30 samples had antibacterial activities higher or equal 10% equivalent phenol concentration. Plotting these 30 samples together with data obtained in our study, a perfect match of the data sets can be obtained (Figure 1B). This remarkable agreement of results obtained in two independent studies clearly underlines the final statement that methylglyoxal is the dominant bioactive compound in Manuka honey and starting from concentrations of approximately 150 mg/kg is exclusively responsible for the pronounced antibacterial activity. Due to limited sensitivity and inaccuracy of the used test system, data obtained for antibacterial activities below 10% equivalent phenol concentration must be handled with care. This may explain the fact that the corresponding regression lines in Figure 1 doe not pass through the origin (parameter a =8.388 or 7.783, respectively). Furthermore, for such low antibacterial activities, other factors such as polyphenols, organic acids or currently unknown compounds may additionally contribute to nonperoxide antibacterial properties.

In conclusion, methylglyoxal is a unique antibacterial compound found in high concentrations in Manuka honeys from New Zealand and directly responsible for the specific antibacterial activity of these samples. Methylglyoxal can serve as a suitable tool for the labelling of the bioactivity of commercial products.

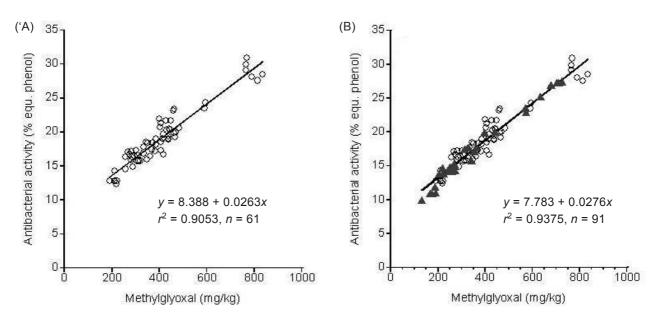


Figure 1. (A) correlation between methylglyoxal and antibacterial activity for 61 samples of Manuka honey analysed in this study plus data from ALLEN *et al.* (1991), shown as filled triangles

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