Methylglyoxal - a causative agent of detrimental glucose oxidase modification in manuka honey

Juraj Majtán[a], Jana Bohová[a], Emanuel Procházka[a]*, Jaroslav Klaudiny[b]

[a] Institute of Zoology, Slovak Academy of Sciences, Bratislava, Dúbravská cesta 9, 84506, Slovakia

[b] Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Dúbravská cesta 9, 84538, Slovakia

** e-mail: emanuel.prochazka@savba.sk*

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INTRODUCTION

Honey is considered to be an important therapeutic agent possessing significant antibacterial properties. Hydrogen peroxide (H_2O_2) is one of the major antibacterial components, and is produced by **glucose oxidase (GOX)-mediated conversion of glucose under aerobic conditions** in diluted honey (Fig.1).

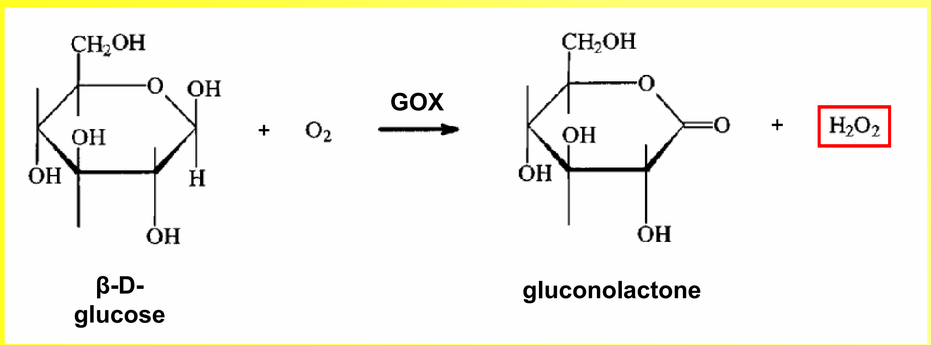


Fig.1 GOX-catalyzed production of H_2O_2 from glucose and oxygen. Taken and adapted from [1].

However, **H_2O_2 does not accumulate in medical-grade manuka honey**, and the pronounced antibacterial activity directly originates from methylglyoxal (MGO, $C_3H_4O_2$, Fig.2). Manuka honey is produced in New Zealand by domesticated European honeybees from the nectar of the manuka tree (*Leptospermum scoparium*). **MGO originates from dihydroxyacetone (present in the nectar)** upon storage of freshly produced honey.

AIMS

Recently, **destructive effects of MGO on some proteins in manuka honey have been documented** [2]. Based on this knowledge, we hypothesized that the **inability to produce high levels of H_2O_2 could be associated with high reactivity of MGO with GOX enzyme** (Fig.2). Accordingly, we investigated the effect of artificially added MGO on H_2O_2 accumulation in natural non-manuka honeys, which are capable to generate high levels of H_2O_2. Furthermore, we examined the effect of MGO on in vitro cross-linking of purified GOX and its biological activity.

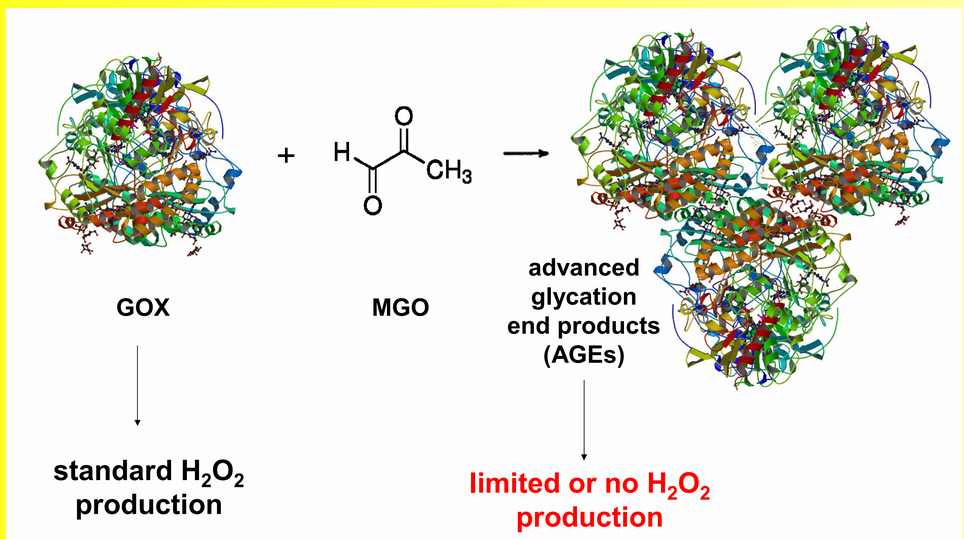


Fig.2 Proposed scenario of H_2O_2 production inhibition. Reaction between GOX and MGO presumably leads to the formation of advanced glycation end products (AGEs) with limited or completely lost ability for H_2O_2 production. 3D structure of GOX enzyme taken from [3].

RESULTS

To evaluate the production of H_2O_2 we employed fluorometric resorufin assay (OxiSelect™ Hydrogen Peroxide/Peroxidase Assay Kit; Cell Biolabs). Among several tested honeys, the most potent producers were the two honeydew honeys (Table 1). Contrary to this, the **MGO-treated honeys generated significantly lower amounts of H_2O_2** (Fig.3).

Table 1. H_2O_2 concentrations in different honey solutions (50% w/v) after 24 h incubation at 37°C.

Honey sample	Principal botanical origin	Sample age (year)	Geographic origin	H_2O_2 concentration (μM)
honeydew 1	*Abies alba* Mill	2	Bardejov (Slovakia)	495.8 ± 9.1
honeydew 2	*Abies alba* Mill	2	Banská Štiavnica (Slovakia)	306.9 ± 6.8
acacia	*Robinia pseudoacacia*	1	Šahy (Slovakia)	36.3 ± 0.7
manuka	*Leptospermum scoparium*	3	New Zealand	78.9 ± 1.9
artificial	N/A	1	N/A	16.1 ± 1.2

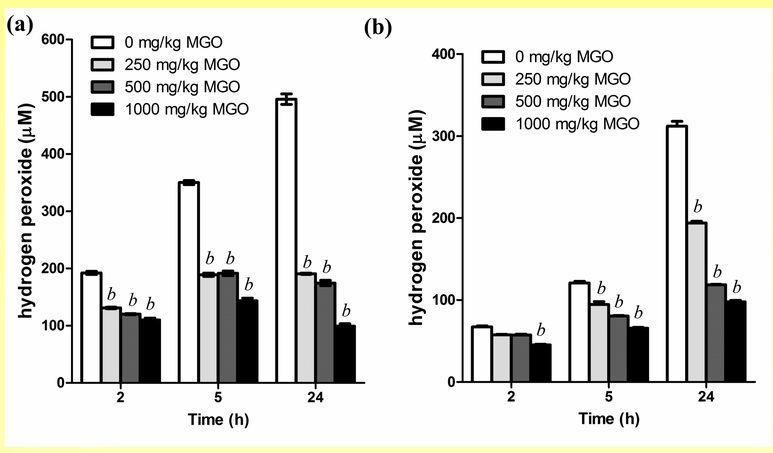


Fig.3 H_2O_2 concentrations in honeydew honey solutions (50% w/v) treated with MGO at different concentrations after 24 h incubation at 37°C.
(a) honeydew honey (Bardejov)
(b) honeydew honey (Banská Štiavnica)
b $P<0.001$ vs. control (0 mg/kg MGO).

Furthermore, we observed **formation of high molecular weight adducts** on SDS-polyacrylamide (SDS-PAGE) gels when GOX was treated with MGO (Fig.4). This observation was confirmed by ultra high-performance liquid chromatography (UHPLC) analysis (Fig.5).

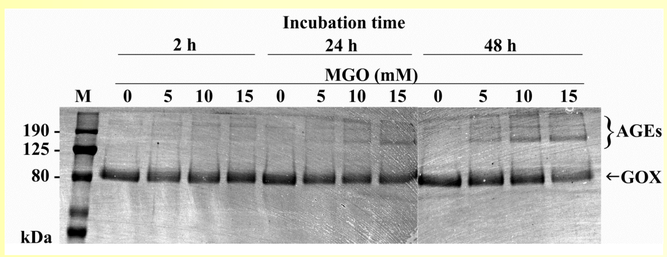


Fig.4 In vitro cross-linking of GOX by MGO – SDS-PAGE analysis. GOX at concentration of 1 mg/ml was incubated with or without MGO at concentrations of 5, 10 and 15 mM for indicated time period.
M – molecular weight marker

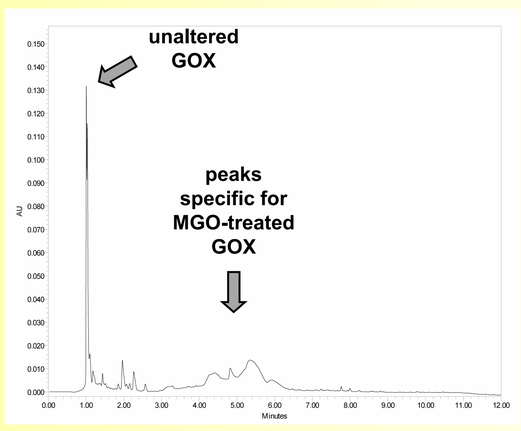
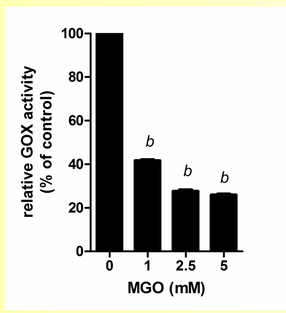


Fig.5 In vitro cross-linking of GOX by MGO – UHPLC analysis. Ten μl of GOX (1 mg/ml) after treatment with 15 mM MGO for 48 h was applied on a Waters Acquity UPLC H-Class system equipped with BEH300 C4 column. Chromatographic separation was carried out using a gradient elution of acetonitrile (ACN)-water solvent system.
AU – absorbance units at 220 nm.



In addition, formation of high molecular weight adducts was accompanied by **significant loss of its enzymatic activity** (Fig.6).

Fig.6 Effect of MGO on enzymatic activity of GOX. GOX (0.1 mg/ml) was incubated with 1, 2.5 and 5 mM MGO at 37°C for 7 days. Control and treated GOX was mixed with glucose and enzymatic activity was examined upon H_2O_2 development.
b $P<0.001$ vs. control (0 mM MGO).

CONCLUSION

Our observation suggests that high levels of MGO in manuka honey are responsible for suppressing H_2O_2 generation. These data highlight the detrimental effect of MGO on proteinaceous components, including those contributing to the antibacterial activity of honey.

However, comprehensive clinical trial study should be performed to assess the advantageousness of various selected honeys (manuka, other floral, honeydew) for specific types of wounds.

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