

New approaches for determination of honey quality in relation to its biological activity

Dr. Juraj MAJTAN

**Laboratory of Apidology and Apitherapy
Institute of Molecular Biology SAS
Bratislava
SLOVAKIA**



Essential composition and quality factors

European Honey Directive (2002)
Codex Alimentarius Standard for Honey (2001)

Biochemical and physical properties:

- sugar content
- moisture content (not more than 20%)
- water insoluble solids content
- electrical conductivity
- free acidity
- diastase activity (Schade units)
- hydroxymethylfurfural (HMF) content

CODEX STAN 12-1981

Page 1 of 8

CODEX STANDARD FOR HONEY CODEX STAN 12-1981¹

The Annex to this Standard is intended for voluntary application by commercial partners and not for application by Governments.

1. SCOPE

1.1 Part One of this Standard applies to all honeys produced by honey bees and covers all styles of honey presentations which are processed and ultimately intended for direct consumption. Part Two covers honey for industrial uses or as an ingredient in other foods.

1.2 Parts Two of this Standard also covers honey which is packed for sale in bulk containers, which may be repacked into retail packs.

PART ONE

2. DESCRIPTION

2.1 DEFINITION

Honey is the natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature.

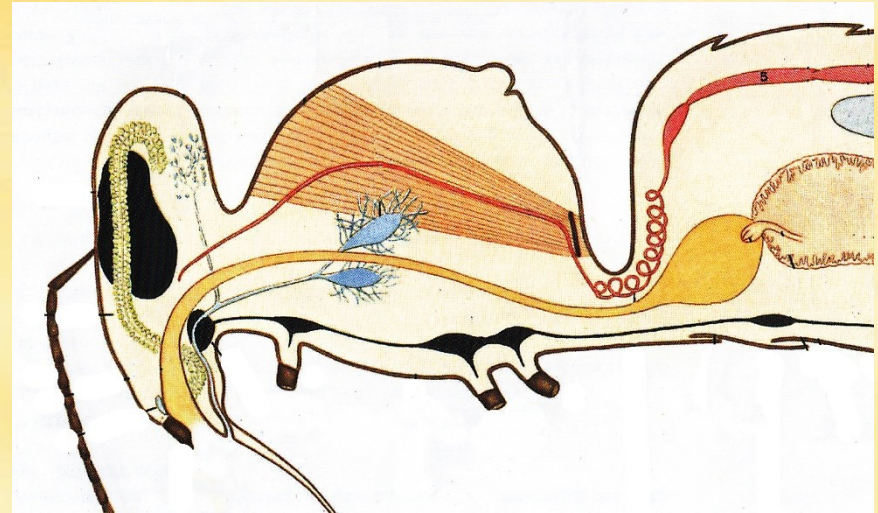
2.1.1 Blossom Honey or Nectar Honey is the honey which comes from nectars of plants.

2.1.2 Honeydew Honey is the honey which comes mainly from excretions of plant sucking insects (*Hemiptera*) on the living parts of plants or secretions of living parts of plants.

Bee proteinous content as a suitable criterion for honey authenticity and quality



Bee foragers collect the nectar into the honey crop

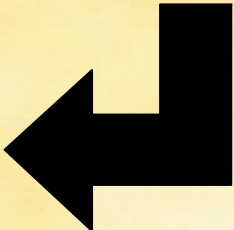


Nectar is mixing with glands secretion in honey crop (stomach)

All major proteins identified in honey are of bee origin (Girolamo *et al.* 2012)

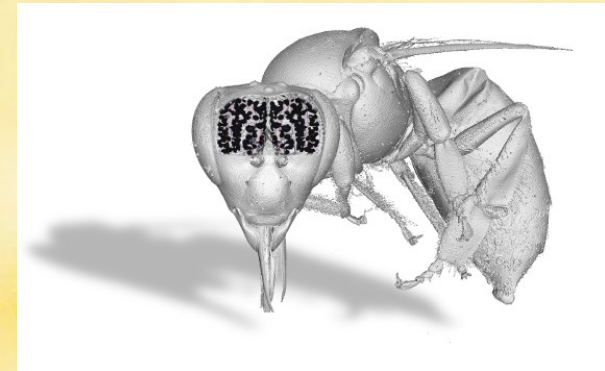


+
bee
proteins/peptides

A large black arrow pointing from the honeycomb to the text.

Bee defensin-1

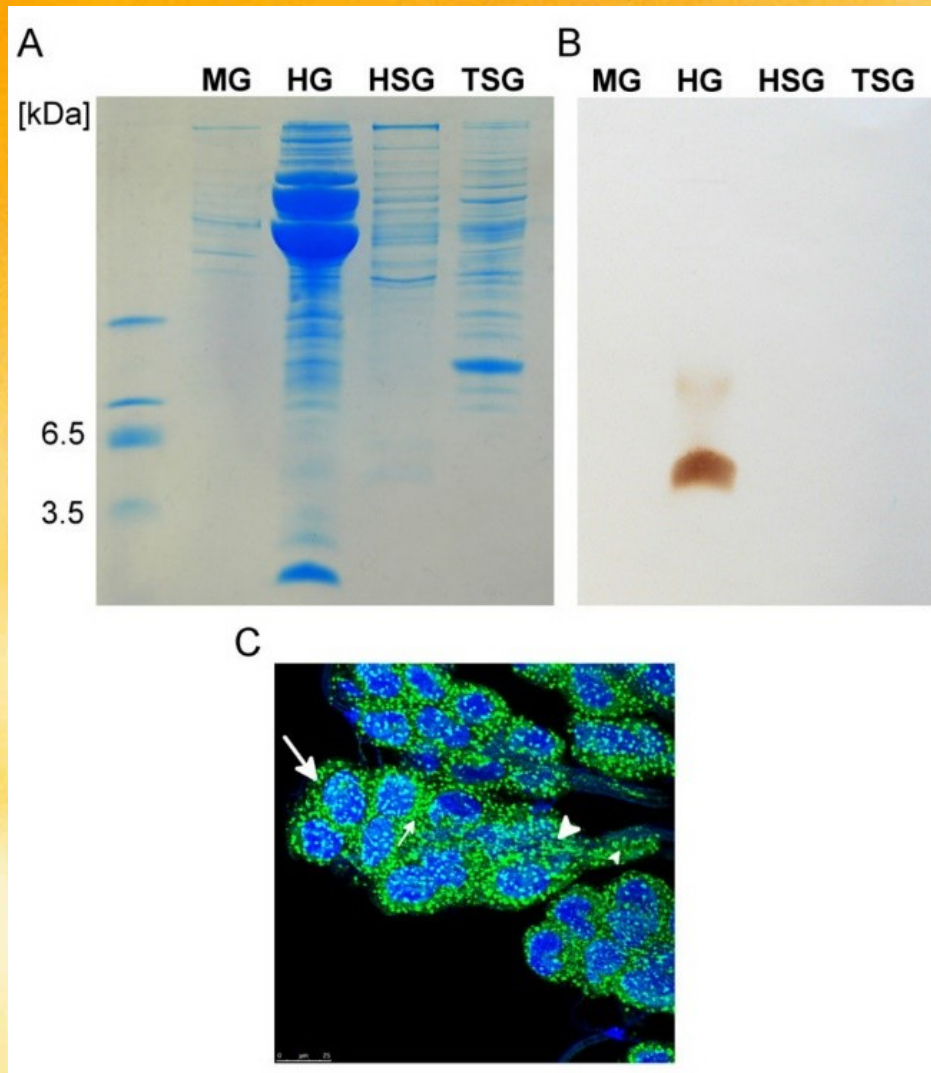
- bee defensin-1 was first isolated from royal jelly (described as royalisin) (Fujiwara et al. 1990), subsequently found in honey (Kwakman et al, 2010)
- it is regular but variable component of natural honey (Majtan et al, 2012)
- it belongs to insect defensin group, is composed of 51 AA with MW of 5.52 kDa
- it is active against Gram-positive planktonic and biofilm-embedded bacterial cells (antibacterial and antibiofilm activity)
- it occurs at low concentration in bee products
- it is heat-stable (protein-defensin-1 interaction)



Aims of the study

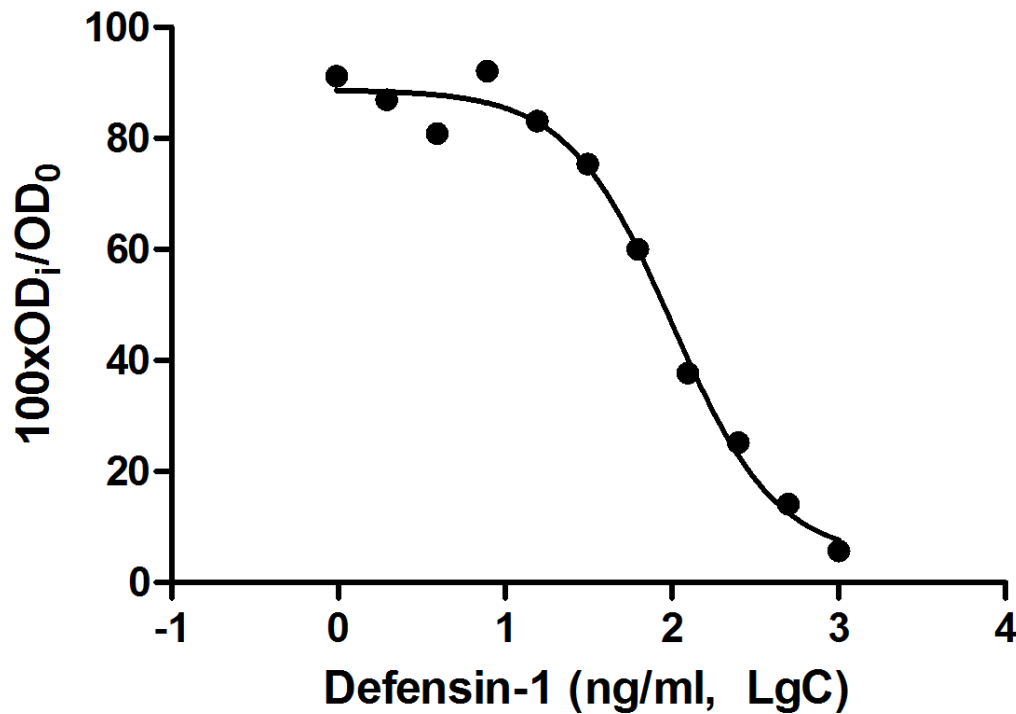
- to develop and evaluate a polyclonal antibody based competitive ELISA test for the quantification of bee-derived defensin-1 in honey
- to monitor its concentration after thermal processing (conventional heating and microwave radiation)
- to monitor its concentration after honey sterilisation using gamma radiation (medical-grade honeys)

Immunospecificity of an anti-defensin-1 polyclonal antibody



- antigen = C-terminus synthetic peptide (CRKTSFKDLWDKRFG)
- affinity purified polyclonal antibody
- **SDS-PAGE (Fig. A)**
- HG extract is rich on secreted proteins
- **immunoblot assay (Fig. B)**
- Specific immunoreaction with one band around 5 kDa in HG glands
- **Immunohistochemistry (Fig. C)**
- Defensin-1 is readily visible in the cytoplasm of secretory cells in HG glands

Quantification of defensin-1 using competitive ELISA test

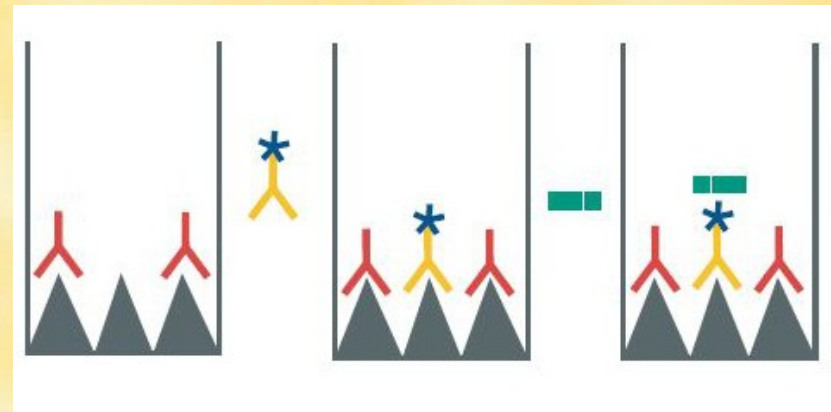


Calibration curve of ELISA test for quantification of defensin-1 in honey

Detection limit: 7.8 ng/ml (2.5% honey solution)



Higher amount of native defensin-1 = brighter colour



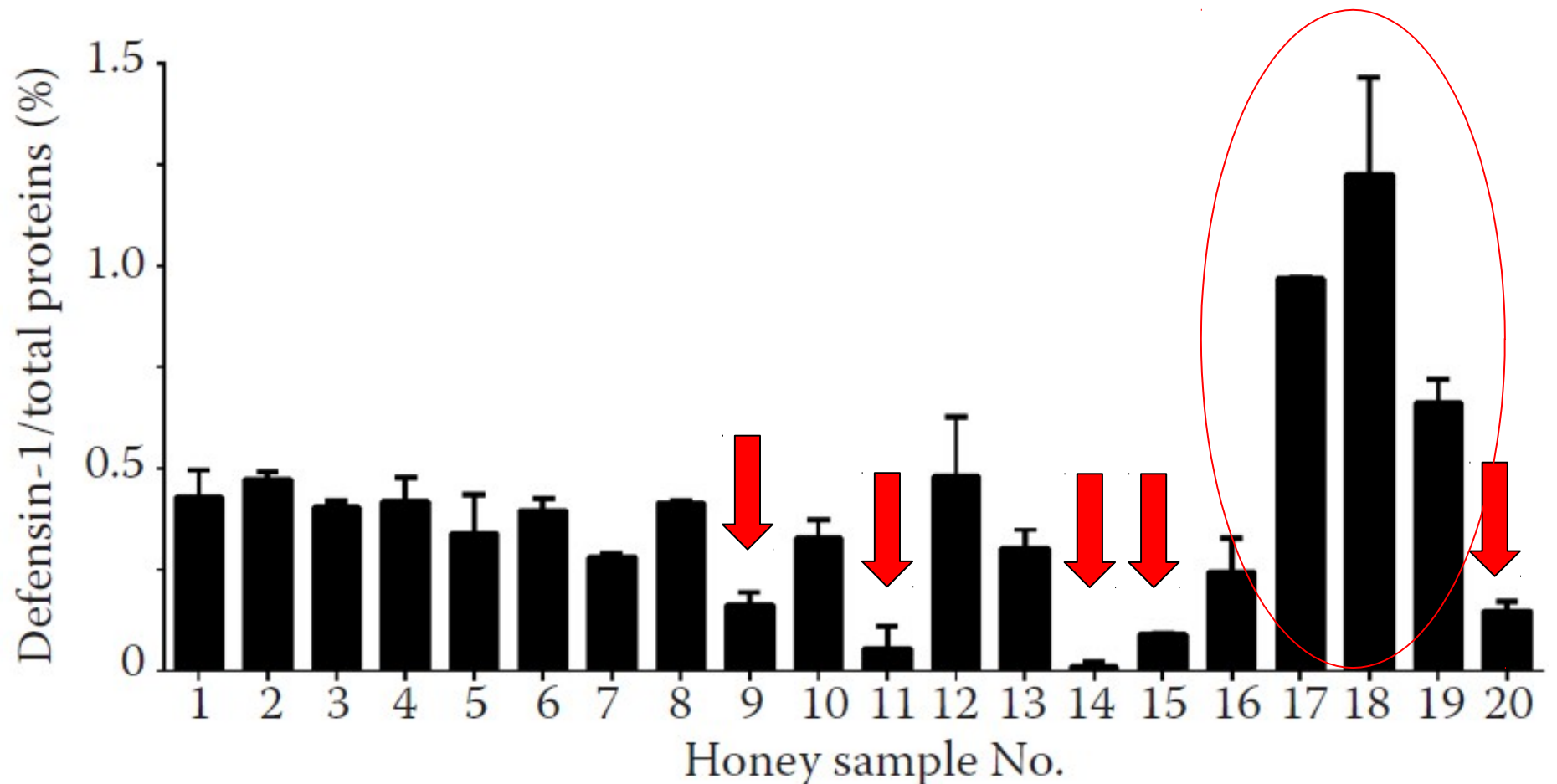
Competition between native defensin-1 in honey and synthesized HRP-conjugated defensin-1

Validation of ELISA test using natural honey samples

| Honey sample | Dominant nectar/honeydew source | Geographic origin in Slovakia | Harvesting in 2013 |
|--------------|---------------------------------|-------------------------------|--------------------|
| 1 | <i>Robinia pseudoacacia</i> | Veľký Krtíš | June |
| 2 | <i>Robinia pseudoacacia</i> | Lamač | June |
| 3 | <i>Robinia pseudoacacia</i> | Lamač | June |
| 4 | <i>Robinia pseudoacacia</i> | Borský Mikuláš | June |
| 5 | <i>Robinia pseudoacacia</i> | Borský Mikuláš | June |
| 6 | <i>Robinia pseudoacacia</i> | Borský Mikuláš | June |
| 7 | <i>Tilia platyphyllos</i> | Jarovce | June |
| 8 | <i>Tilia platyphyllos</i> | Slovenská Lupča | June |
| 9 | <i>Castanea sativa</i> | Lamač | June |
| 10 | <i>Helianthus annuus</i> | Jarovce | June |
| 11 | <i>Brassica napus</i> | Jarovce | June |
| 12 | <i>Abies alba</i> | Slovenská Lupča | August |
| 13 | <i>Abies alba</i> | Čergov | August |
| 14 | multifloral | Čergov | July |
| 15 | multifloral | Podpolanie | May |
| 16 | multifloral | Vysoká nad Uhom | June |
| 17 | multifloral | Vysoká nad Uhom | July |
| 18 | multifloral | Vysoká nad Uhom | August |
| 19 | multifloral | Inovec | May |
| 20 | multifloral | Inovec | August |

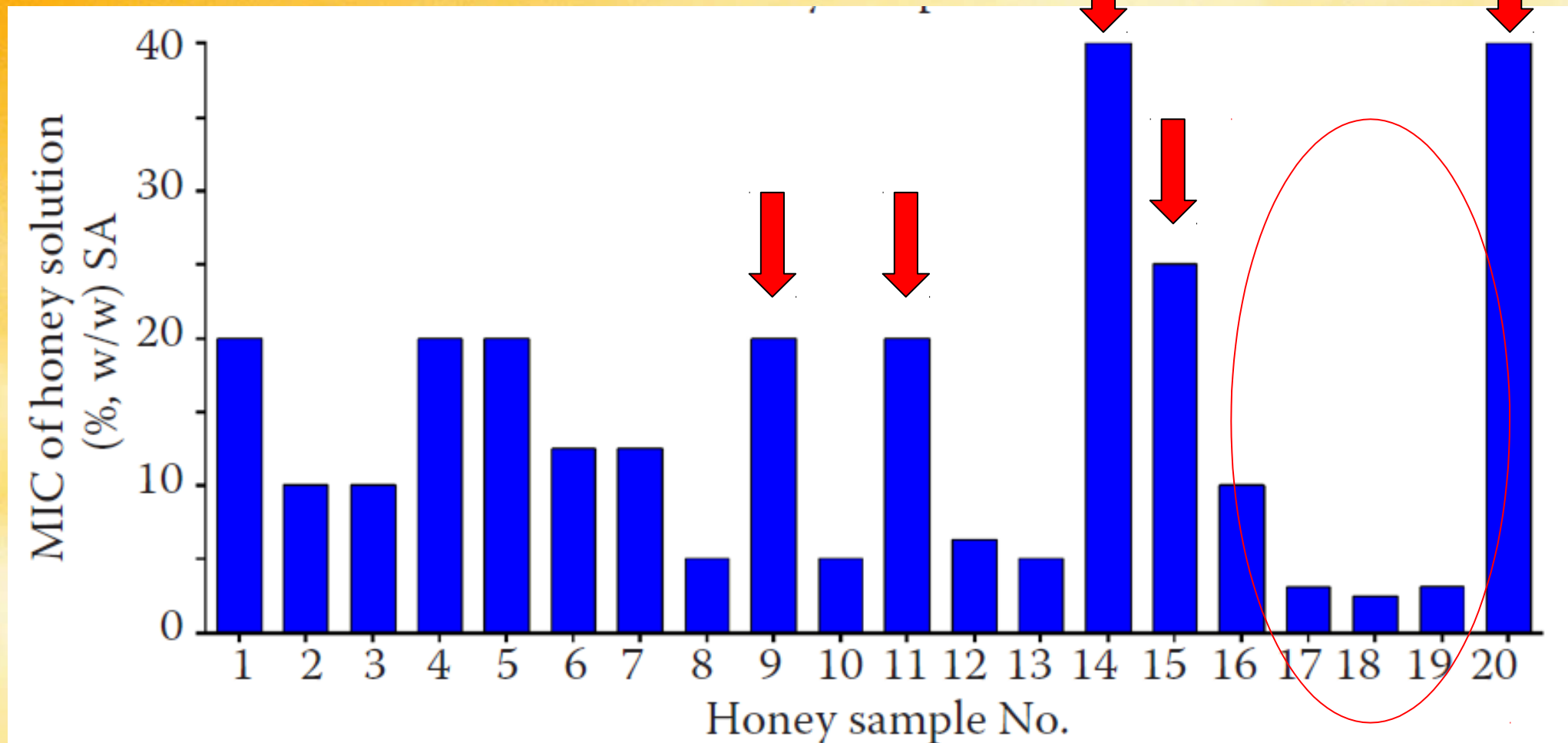
Level of defensin-1 in natural honeys

- Quantification of defensin-1 in honey solutions (2.5; 5 and 10%)
- Determination of total proteins using a Bradford assay

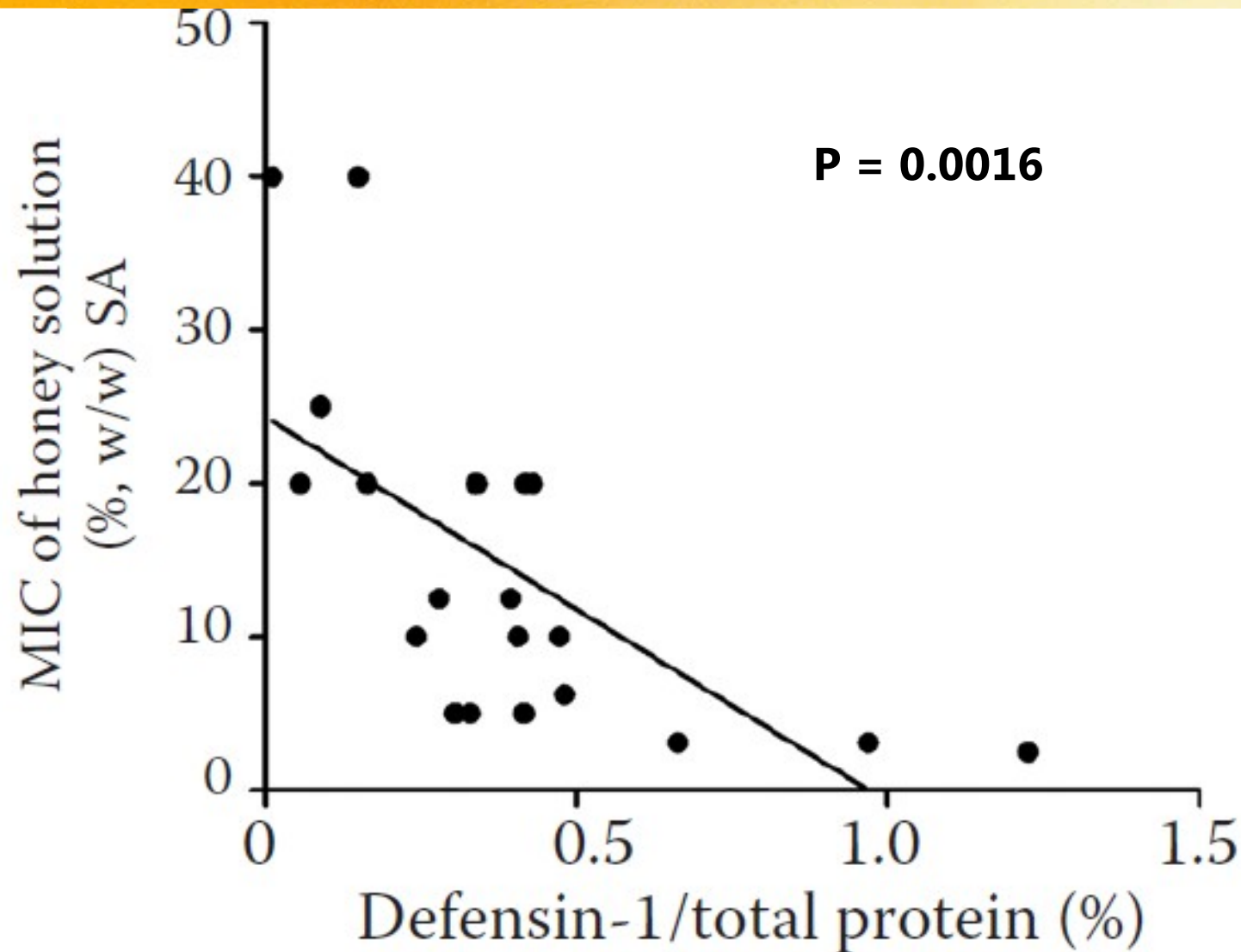


Antibacterial activity of natural honeys against *Staphylococcus aureus* (G+)

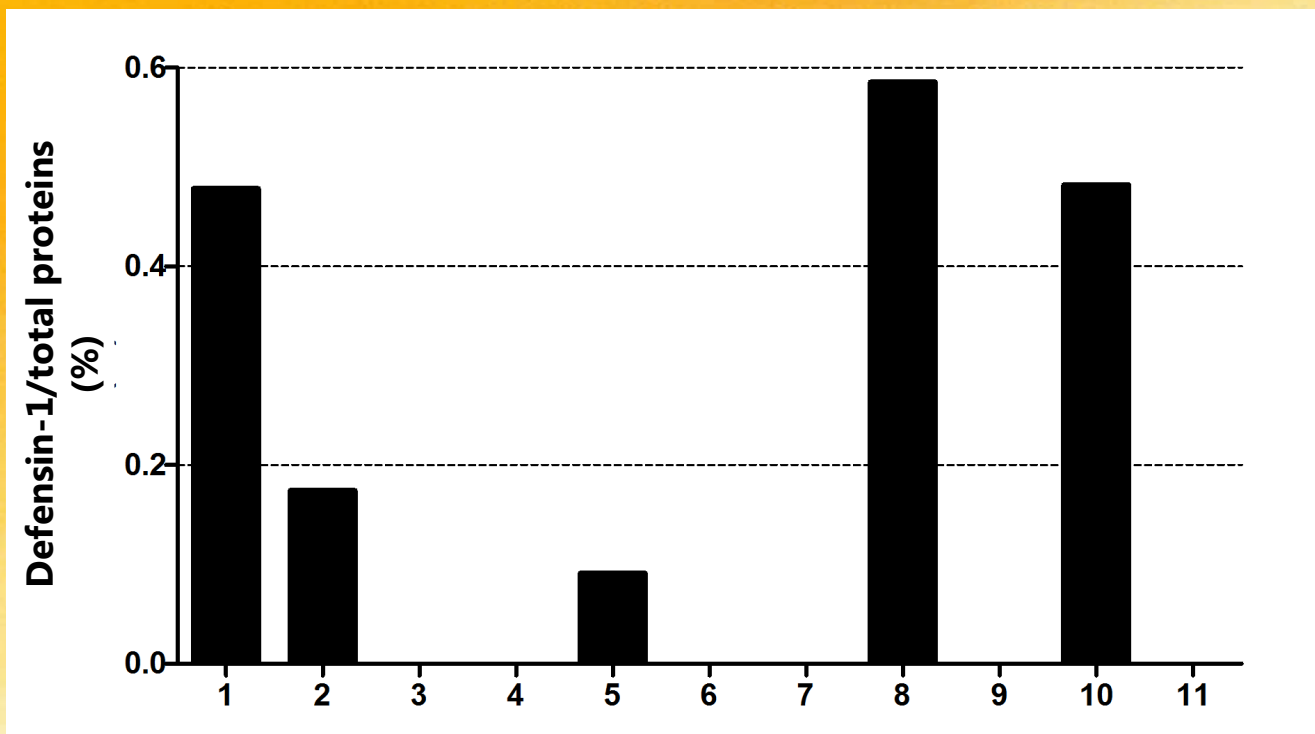
- Antibacterial activity of honey is expressed as minimal inhibitory concentration (MIC)



Correlation between the defensin-1 content and antibacterial activity



Defensin-1 in commercial honeys



1

2

3

4

5

6

7

8

9

10

11



Lidl Stiftung
& Co. KG
(Germany)

Lidl Stiftung
& Co. KG
(Germany)

Natur
Products
(Slovakia)

Natur
Products
(Slovakia)

Medokomerc
(Czechia)

JSG med a.s.
(Czechia)

JSG med a.s.
(Czechia)

Medas, s.r.o.
(Slovakia)

Medas, s.r.o.
(Slovakia)

Medas, s.r.o.
(Slovakia)

Medokomerc
(Czechia)

Effect of thermal processing on honey major antibacterial components

From scientific literature.....

- current honey quality standards are not sufficient
- heating (90-100°C) for 30 min causes changes in HMF or diastase activity but within the interval according to Codex
- microwave radiation does not significantly increase HMF
- But...honey biological activity is already completely destroyed

METHODs

3 rape honey samples (solid-creamy)
50 g / glass beaker

▶ Microwave

▶ 800W - 10s, 30s

▶ 400W - 10s, 30s, 50s

▶ 80W - 60s, 120s, 180s

▶ Conventional (incubator)

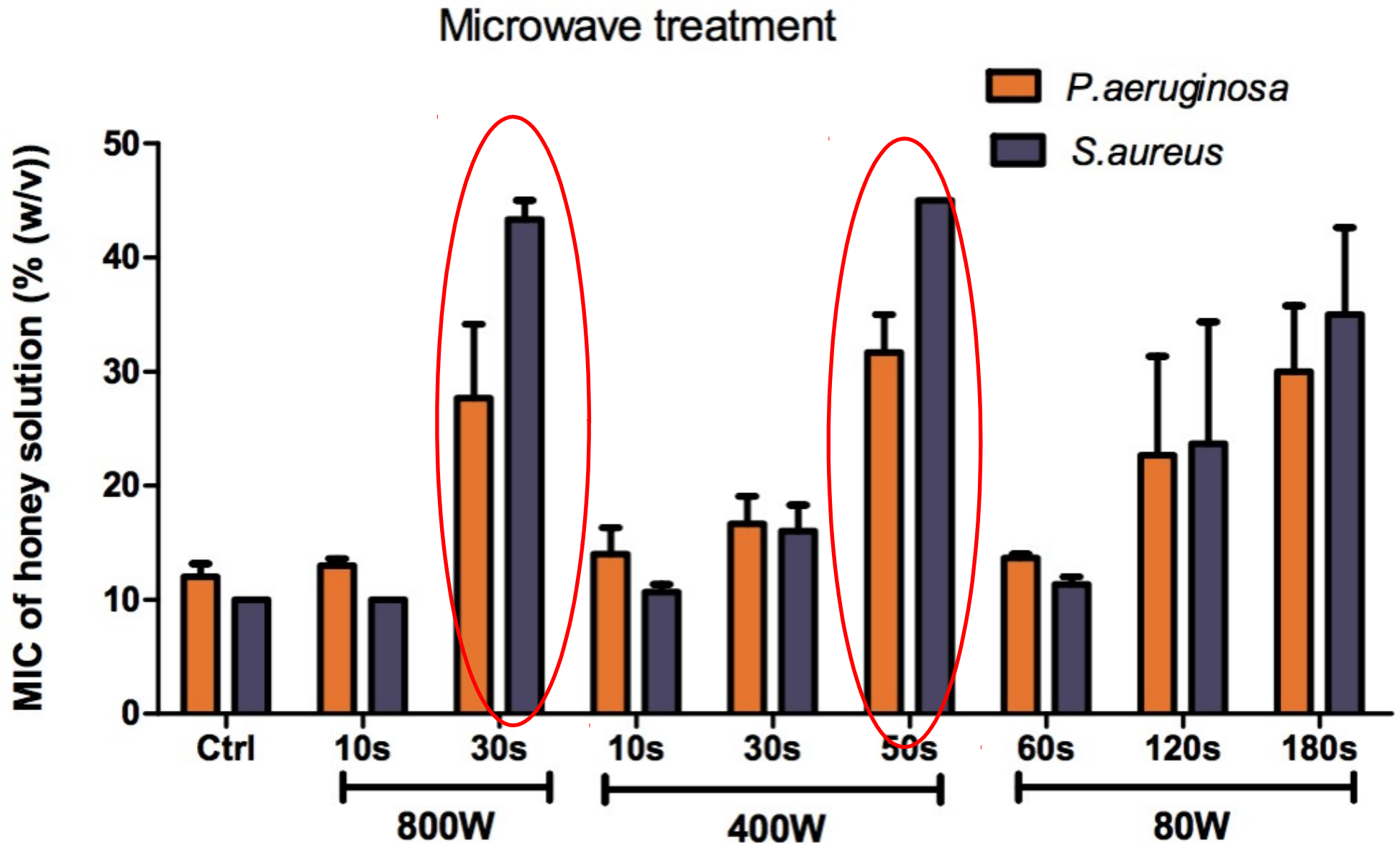
▶ 45 °C - 8h, 24h, 48h

▶ 55 °C - 8h, 24h, 48h

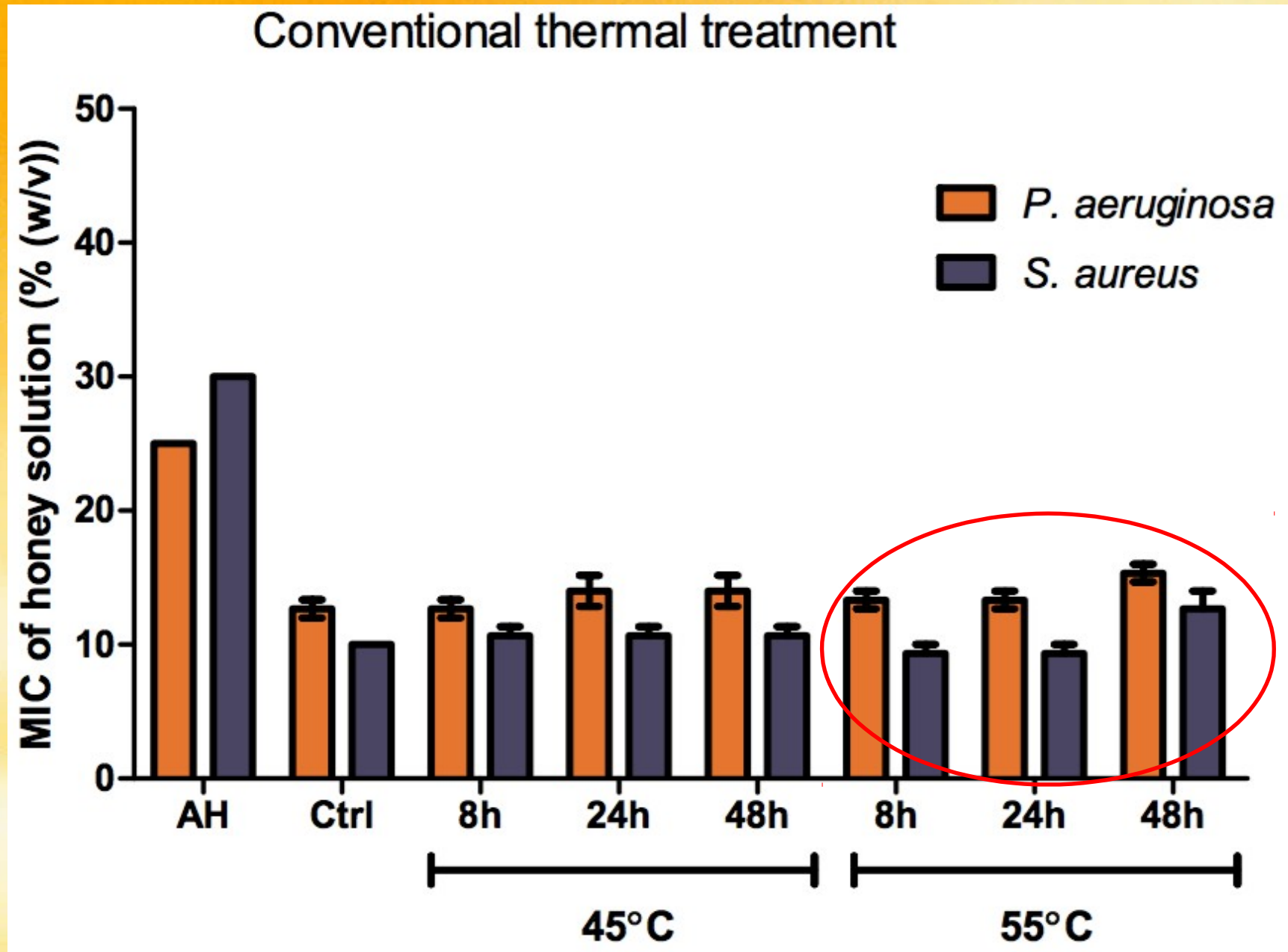
Antibacterial activity ?



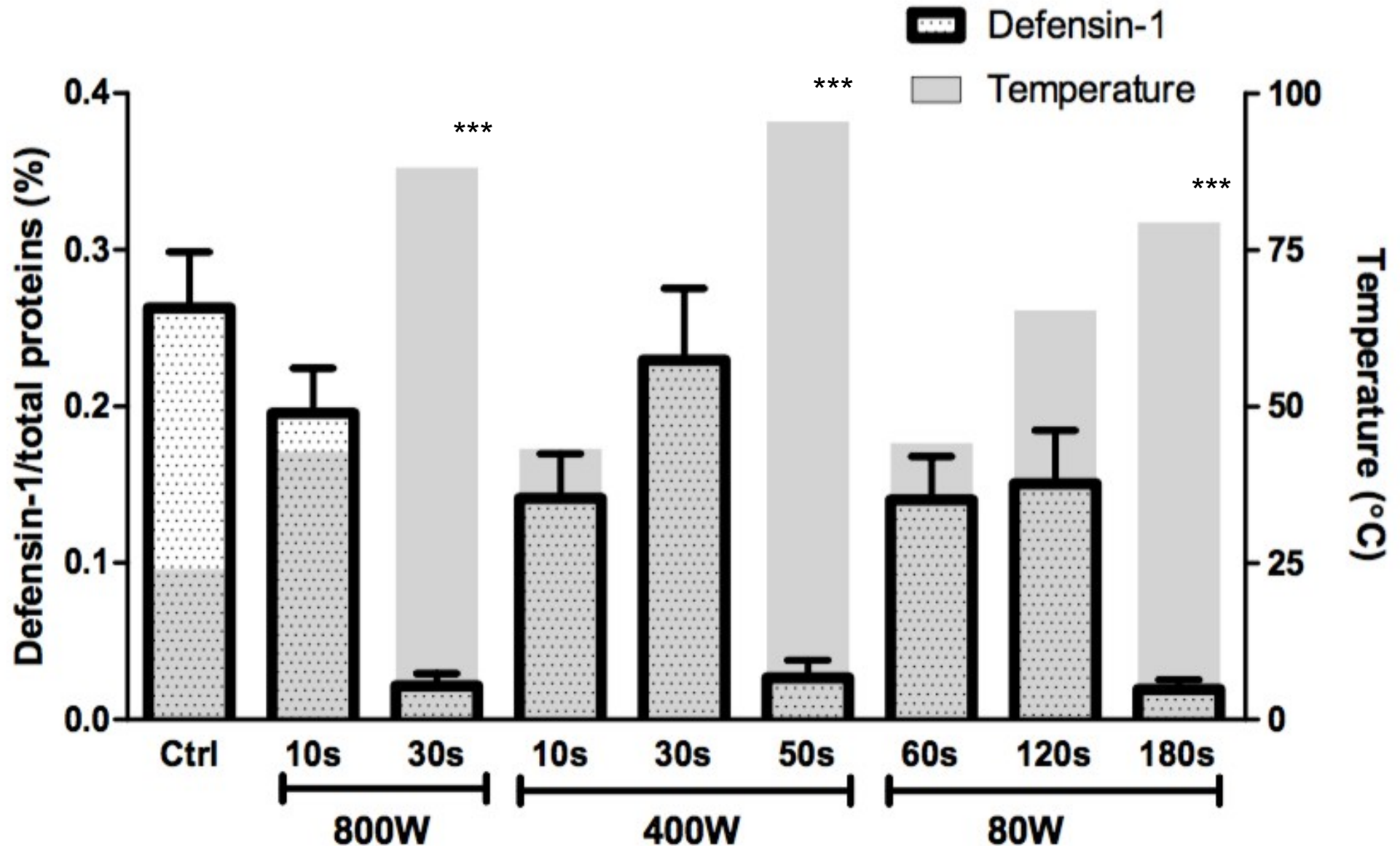
Antibacterial activity of honey after microwave processing



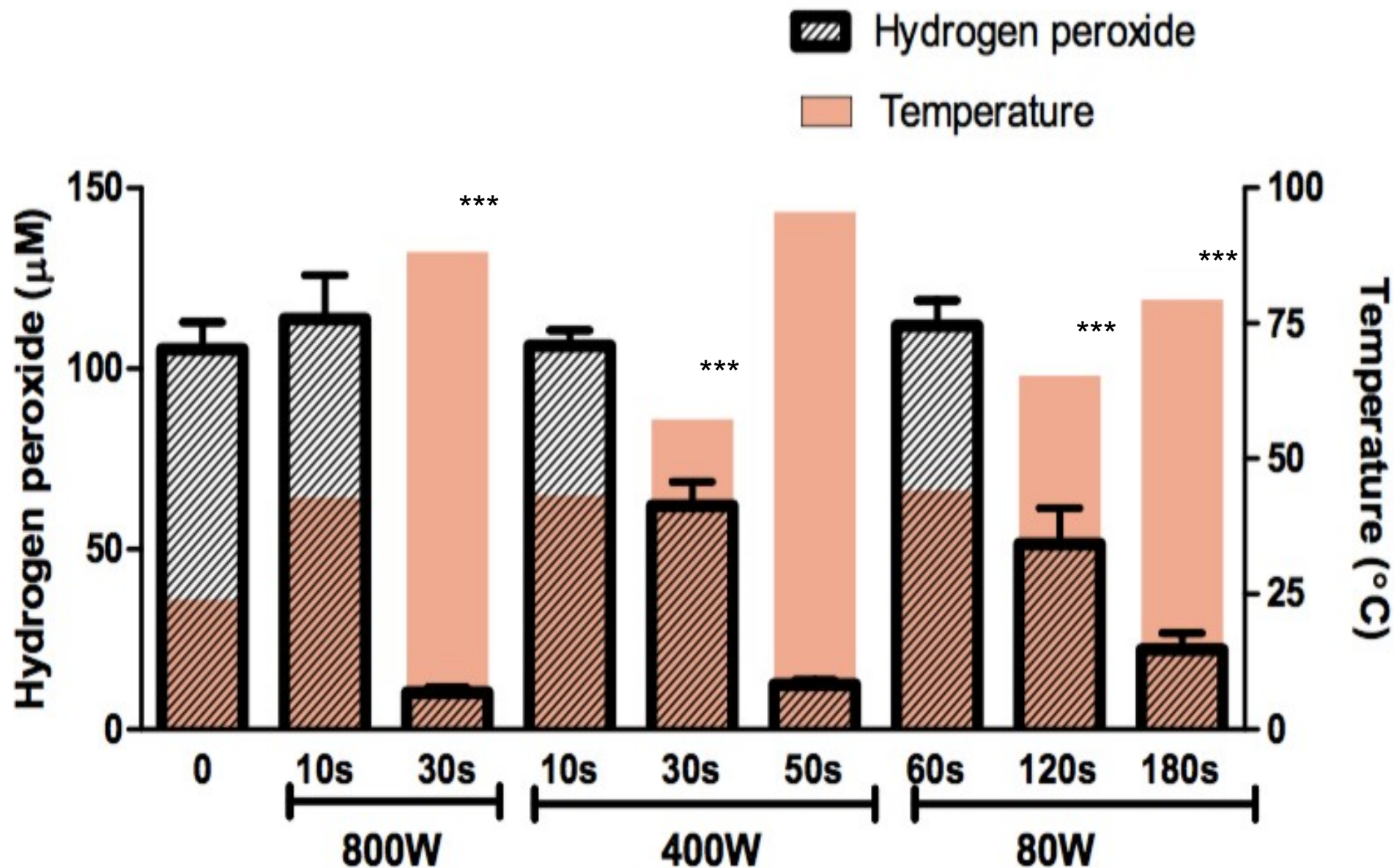
Antibacterial activity of honey after conventional heat processing



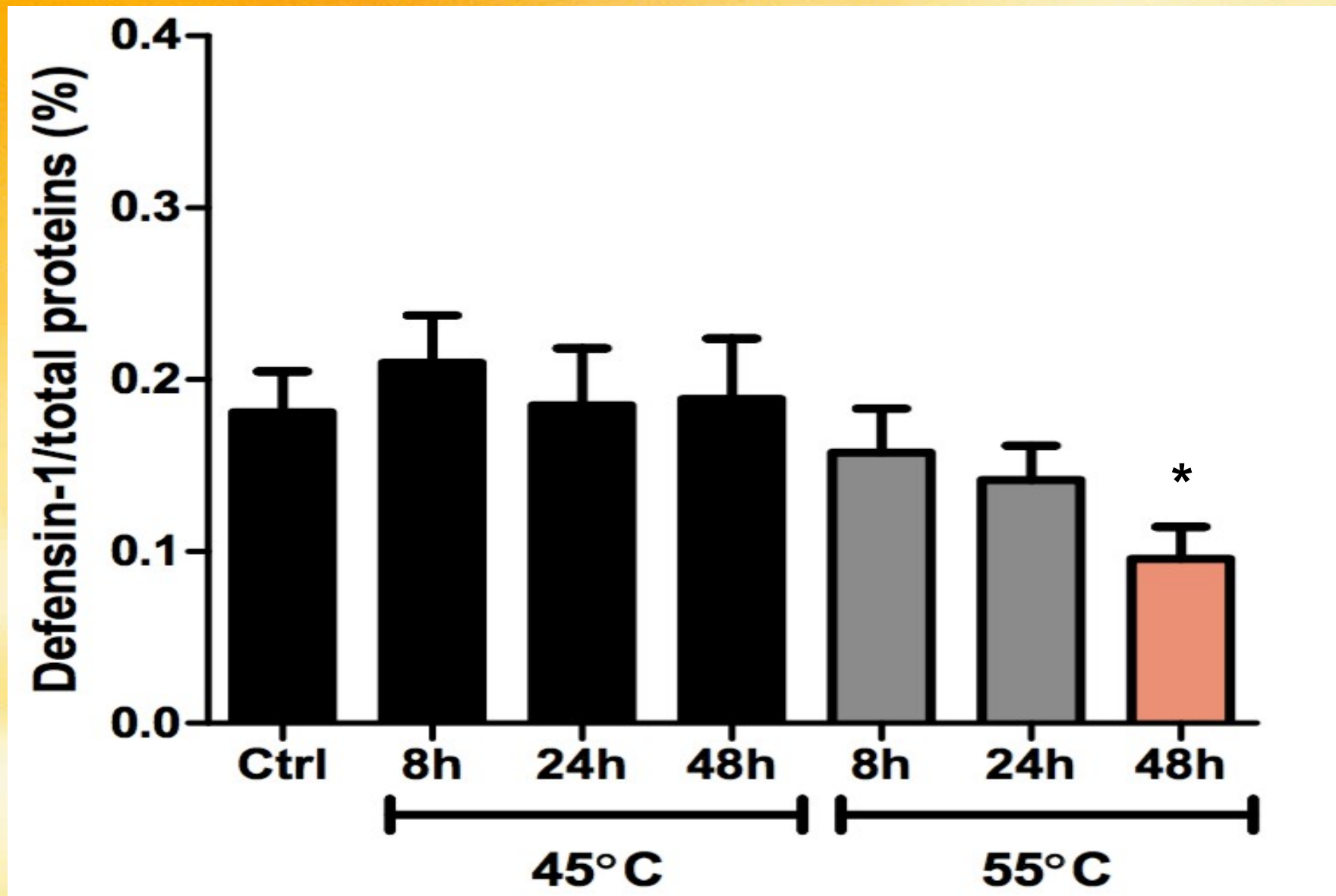
Defensin-1 content in microwave treated honey samples



H₂O₂ content in microwave treated honey samples



Defensin-1 content in conventional heated honey samples



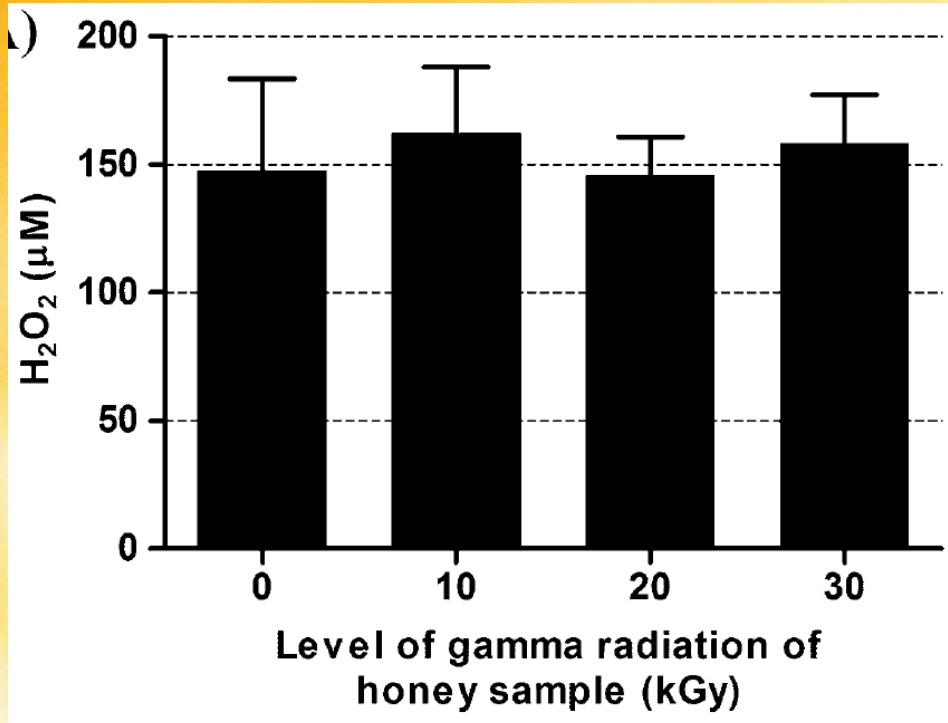
Effect of Gamma irradiation on honey major antibacterial components

Gamma radiation and honey

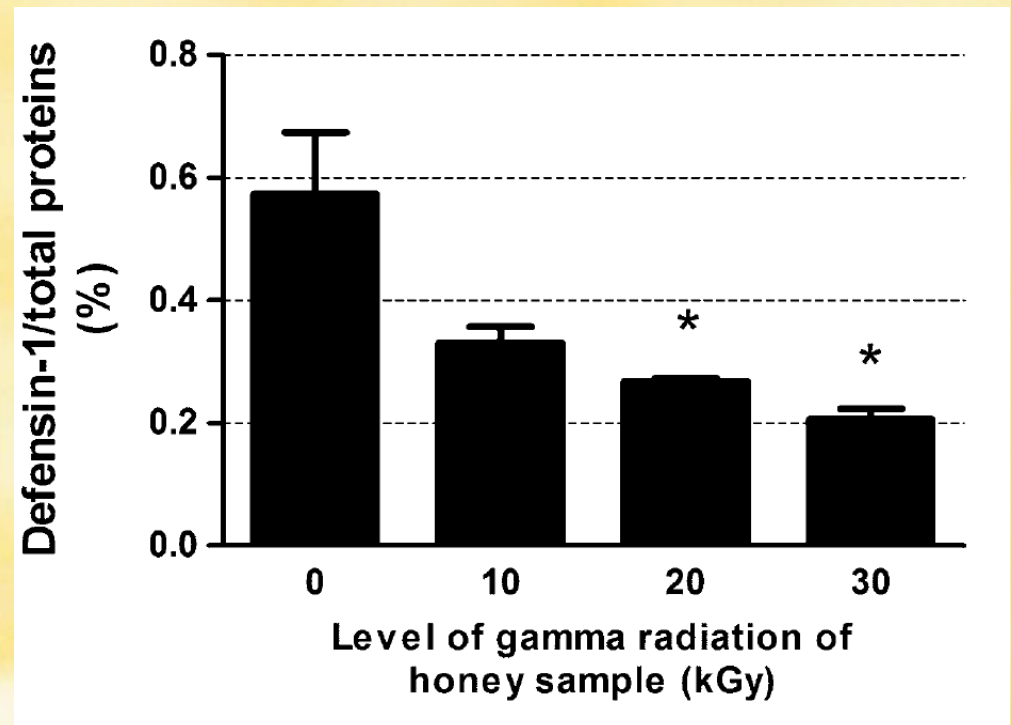
- Elimination of vegetative bacteria and bacterial spore
- Effective and low-cost process of sterilisation without heat-mediated denaturation
- Medical –grade honeys – essential processing
- No negative effects on antibacterial activity

Gamma radiation and major honey antibacterial components in clinically used honeydew honey

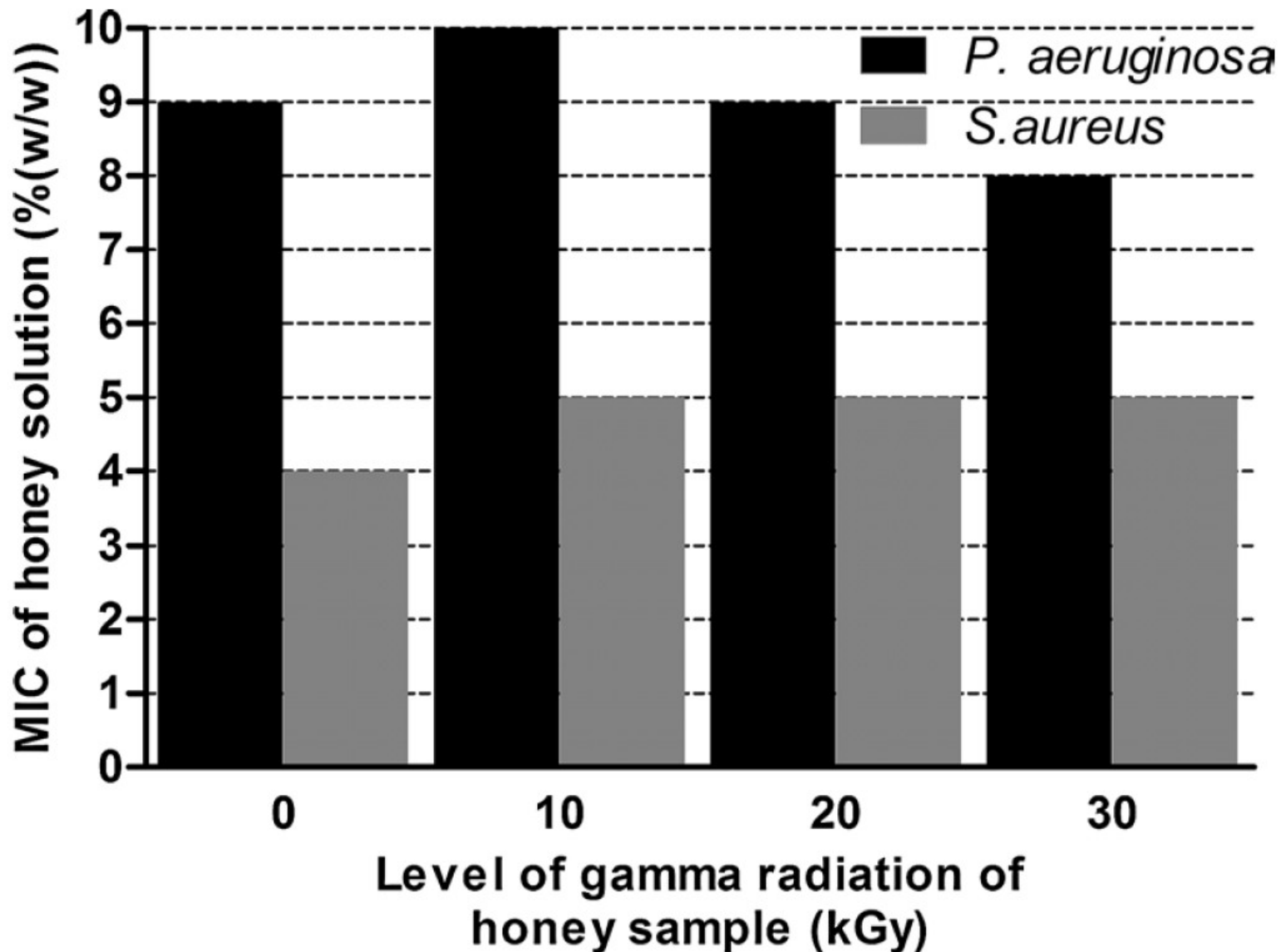
Hydrogen peroxide



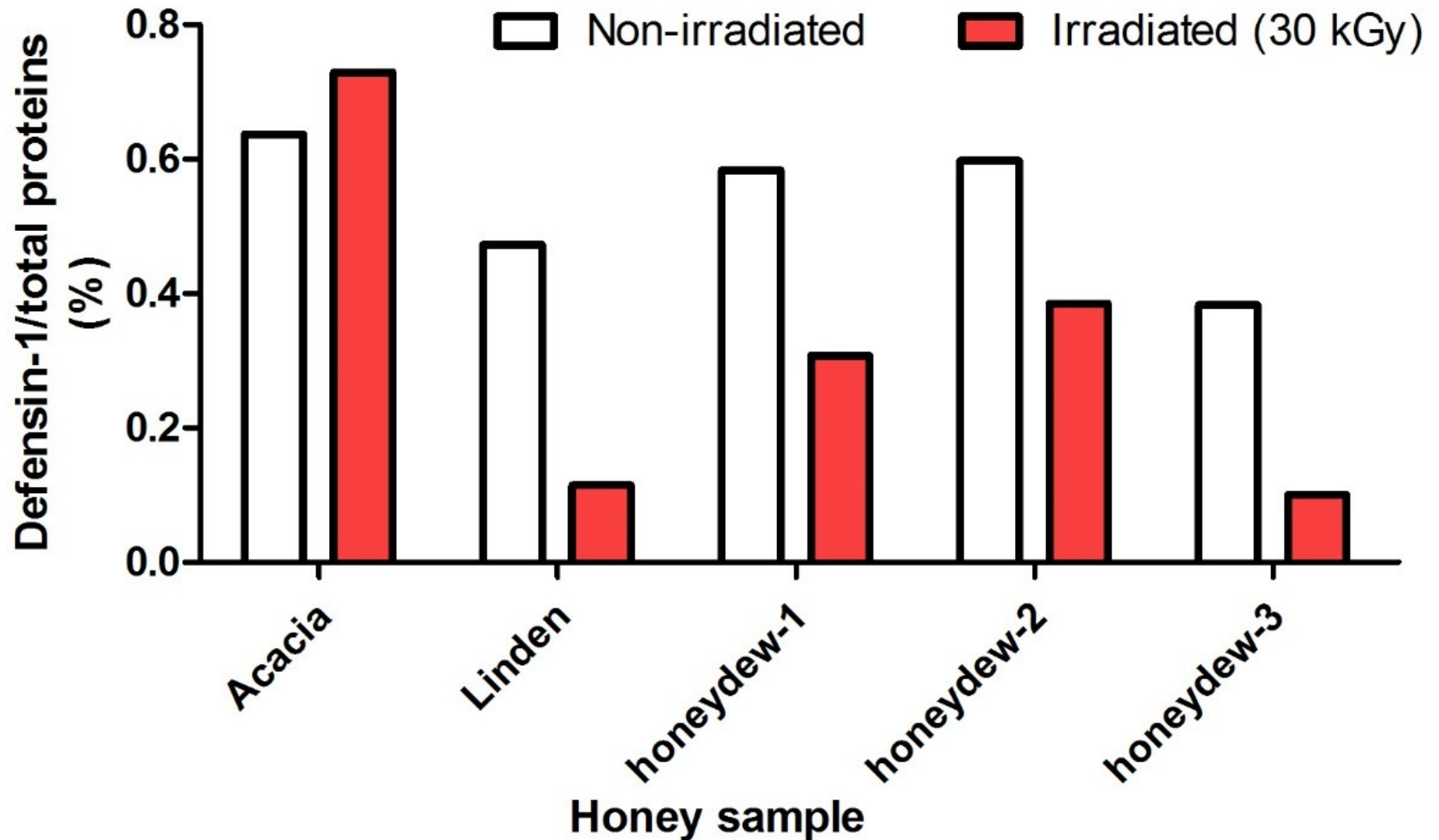
Defensin-1



Antibacterial activity of Gamma irradiated honeydew honey



Defensin-1 content in gamma irradiated honey samples



Conclusions

- A new developed competitive ELISA test for defensin-1 could be suitable method to characterise the quality of natural honey
- Microwave-mediated honey liquefaction is detrimental for its biological activity and conventional heating is still first option (up to 55 °C for short time)
- Bee-defensin-1 is a sensitive marker and could be used to monitor honey processing (e.g. liquefaction, sterilisation)

Acknowledgements

Team members:

Dr. Ivana Valachova

Dr. Marcela Bucekova

Valeria Juricova

Funding: This work was supported by the Slovak Research and Development Agency under contract No. APVV-0115-11

Thank you

