OP-248 [Beekeeping Technology and Quality]
The utmost importance of removing interferences for the proper analysis of glucose oxidase and catalase in honey

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BACKGROUND: Honey enzymes play an important role as authenticity, quality and potentially functional parameters. Glucose oxidase and catalase are related to antibacterial and antioxidant properties of honey. The former releases hydrogen peroxide, whereas the later breaks it down. Both enzymes are particularly sensitive to heat and storage time and conditions.

Problem description: Despite being of paramount importance, analyses of honey glucose oxidase and catalase are not common, because a tedious and time-consuming dialysis step that guarantees interferences removal is required before determination.

PURPOSE: The purpose of this research was to compare the activities of glucose oxidase and catalase of six honeys, carrying out the assays without interferences' removal, as well as after removing interferences with the conventional dialysis procedure and with a film-shaped polymer designed to selectively and quickly extract catechol derivatives that had proven to be the main interferents.

METHODOLOGY: With and without interferences' removal, enzymatic activities of both glucose oxidase and catalase were measured at 400 nm, quantifying the product formed by the reaction of hydrogen peroxide with o-dianisidine and peroxidase.

Results and CONCLUSION: Activities of glucose oxidase and catalase were significantly higher in purified samples. As for glucose oxidase activity [µg H2O2 h−1 g(honey)−1], values ranged between 364.4 and 998.7 (average: 569.4) without interferences' removal, between 530.4 and 1181.7 (average: 871.0) after removing interferences by dialysis, and between 523.7 and 1219.0 (average: 854.9) after removing interferences with the film-shaped polymer. With regard to catalase activity [g(honey)−1 10−3 min−1], the difference between the data of non-purified and purified samples was much more noticeable. Results ranged between 2.2 and 14.4 (average: 7.1) without interferences' removal, between 14.4 and 82.0 (average 29.9) after removing interferences by dialysis, and between 12.9 and 81.3 (average: 28.8) after removing interferences with the film-shaped polymer. No significant differences were found between the enzymatic activities in samples purified by dialysis and using the film-shaped polymer. In conclusion, for the proper analysis of honey glucose oxidase and catalase a purification step is absolutely compulsory. Practical implications for beekeepers: Undoubtedly, having the accurate values of the activities of glucose oxidase and catalase will help improve honey commercialization.

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THE UTMOST IMPORTANCE OF REMOVING INTERFERENCES FOR THE PROPER ANALYSIS OF GLUCOSE OXIDASE AND CATALASE IN HONEY

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UNIVERSIDAD DE BURGOS (SPAIN)
UNIVERSIDADE DE COIMBRA (PORTUGAL)

BACKGROUND

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FA: JCyL BU028G24

Honey enzymes

- Authenticity
- Quality
- Potentially functional

PARTICULARLY SENSITIVE
- Heat
- Storage
  - Time
  - Conditions

Glucose oxidase — RELEASES → **Antibacterial**

H_2O_2

Catalase — BREAKS DOWN → **Antioxidant**

GLUCOSE OXIDASE (GOx)

- Mainly animal origin → Hypopharyngeal glands of worker bees
  - Some flowers
  - Some honey microorganisms

- It is added to nectar and honeydew

  ↳ Glucose → Gluconic acid + H_2O_2

- GOx production depends on several factors
  - Stage of bee life
  - Bee nutrition
  - Other factors

- Activity inhibited by
  - High glucose concentration
  - High osmolarity
  - Low water activity
  - Light
  - Other factors

Brudzynski. 2020. DOI: 10.1016/j.foodchem.2020.127229
Grabek-Lejko & Hyrchel. 2023. DOI: 10.3390/antibiotics12111640
Mansour et al. 2025. DOI: 10.1016/j.jgeb.2025.100559
Osés et al. 2024. DOI: 10.3390/foods13091344
Strelec et al. 2018. DOI: 10.17508/CJFST.2018.10.1.06
White et al. 1963. DOI: 10.1016/0006-3002(63)90359-7

CATALASE (CAT)

- Mainly vegetal origin → Flower pollen
  - Some microorganisms
- CAT { regulates the activity of glucose oxidase; controls the equilibrium of H_2O_2
  - H_2O_2 → $H_2O + O_2$
  - Interaction with some polyphenols
- CAT → more resistant than other enzymes { low; room } temperature
- Activity { dependant on { plant sources; storage conditions }; inhibited by flavonoids }

Brudzynski. 2020. DOI: 10.1016/j.foodchem.2020.127229
Bucekova et al. 2018. DOI: 10.1038/s41598-018-27449-3
González-Ceballos et al. 2023. DOI: 10.1016/j.foodchem.2022.134789
Gruznov et al. 2024. DOI: 10.12944/CRNFSJ.12.2.27
Krych & Gebicka. 2013. DOI: 10.1016/j.ijbiomac.2013.03.070
Weston. 2000. DOI: 10.1016/S0308-8146(00)00162-X

PROBLEM DESCRIPTION

Analyses → ACTIVITIES → GOx, CAT → Interesting: **POTENTIALLY FUNCTIONAL PARAMETERS**

but...

Seldom carried out

Most methods are based on H_2O_2 quantification before and after an incubation period

Microbiological procedures → tedious

Kiermieier & Köberlein, 1954. DOI: 10.1007/BF01882933

Titration methods → not appropriate because of secondary reactions

Dold and Witzenhausen, 1955. DOI: 10.1007/BF02149974

Spectrophotometric analyses

- directly on honey solutions → **interferences**

Schepartz & Subers. 1964. DOI: 10.1016/0926-6569(64)90243-3

Alshareef et al. 2022. DOI: 10.3390/app12157584
Bucekova et al. 2018. DOI: 10.1038/s41598-018-27449-3
Grabek-Lejko & Hyrchel. 2023. DOI: 10.3390/antibiotics12111640
Gruznov et al. 2024. DOI: 10.12944/CRNFSJ.12.2.27
Strelec et al. 2018. DOI: 10.17508/CJFST.2018.10.1.06

- **after interferences removal** → **purification step**

6 l of phosphate buffer ← **tedious**
> 22 hours/sample ← **time-consuming**

DIALYSIS → sugars, polyphenols, other compounds

Schepartz & Subers. 1964. DOI: 10.1016/0926-6569(64)90243-3
Schepartz & Subers. 1966. DOI: 10.1080/00218839.1966.11100130

optimisation ↓

Huiodbro et al. 2005. DOI: 10.1093/jaoac/88.3.800

GOx and CAT ANALYSES: INTERFERENCES REMOVAL

FILM-SHAPED POLYMER

González-Ceballos et al. 2023. DOI: 10.1016/j.foodchem.2022.134789

- **Phenylboronic acid groups** ← COMPOSITION
- **Solid phase extraction** ← INTERFERENCES REMOVAL
- **1,2-Dihydroxybenzenes** ← SPECIFIC INTERACTION

→ **REAL INTERFERENT SUBSTANCES**

PHENOLICS

- Flavonoids: Luteolin, Quercetin, Catechin
- Phenolic acids: Gallic acid, Caffeic acid, Chlorogenic acid

PURPOSE

To **compare the activities of GOx and CAT** of six honeys { 2 Ling-Heather (*Calluna vulgaris* (L.) Hull); 2 Honeydew; 2 *"Forest"* } from Castilla-León (Spain)

↓ carrying out the assays in triplicate

- without interferences' removal
- after removing interferences with { the conventional dialysis procedure; the film-shaped polymer designed to { selectively; quickly } extract **catechol** derivatives }

↓

MAIN INTERFERENTS

This presentation summarises the results of two already published papers

❖ González-Ceballos et al. 2023. DOI: 10.1016/j.foodchem.2022.134789
❖ Osés et al. 2024. DOI: 10.3390/foods13091344

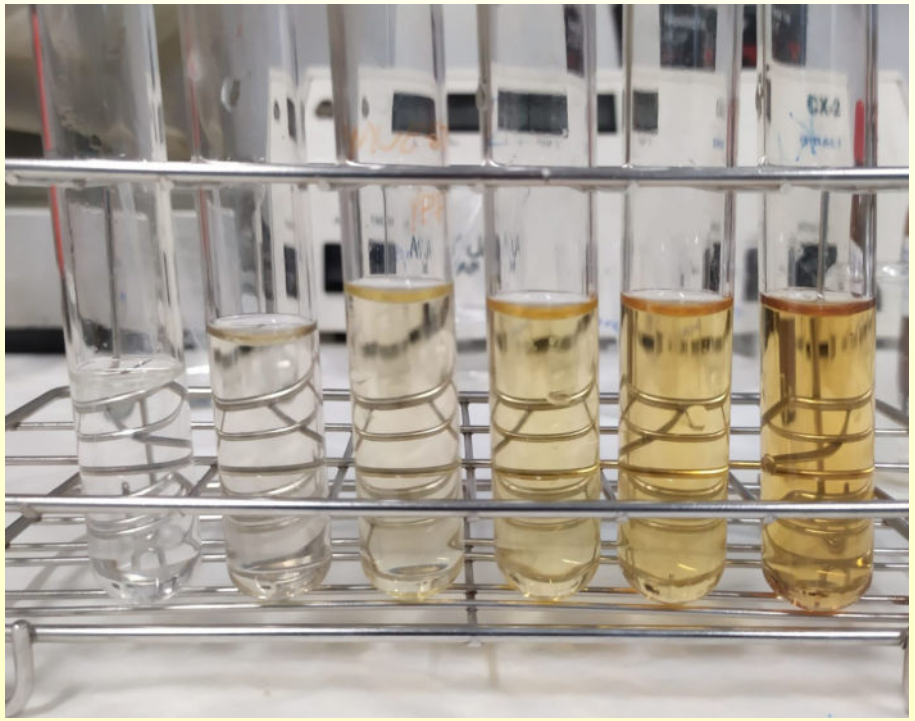
METHODOLOGY

THE UTMOST IMPORTANCE OF REMOVING INTERFERENCES FOR THE PROPER ANALYSIS OF GLUCOSE OXIDASE AND CATALASE IN HONEY

FA: JCyL BU028G24

With / Without → interferences' removal → enzymatic activities: GOx, CAT →

Absorbance **400 nm**

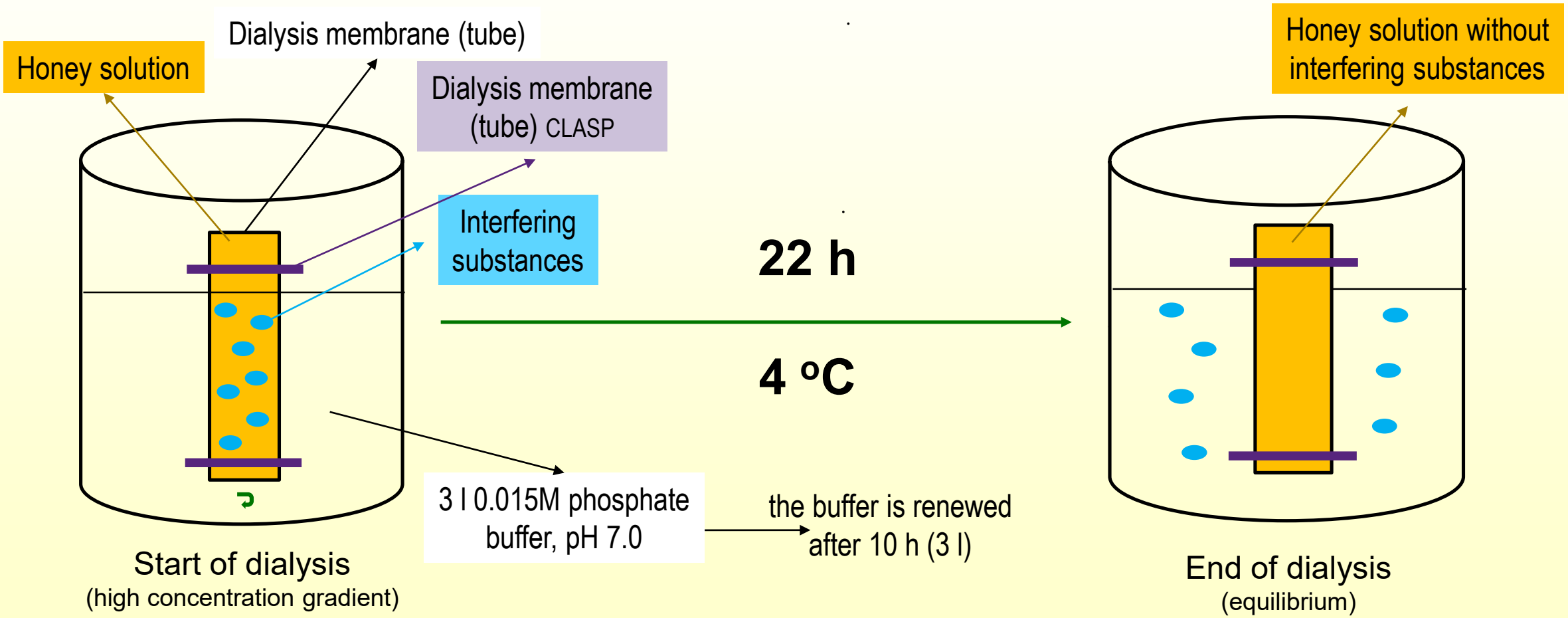


H_2O_2 → STANDARD for the calibration curve

→ Coloured product: *o*-dianisidine peroxidase H_2O_2

Interferences removal by DIALYSIS

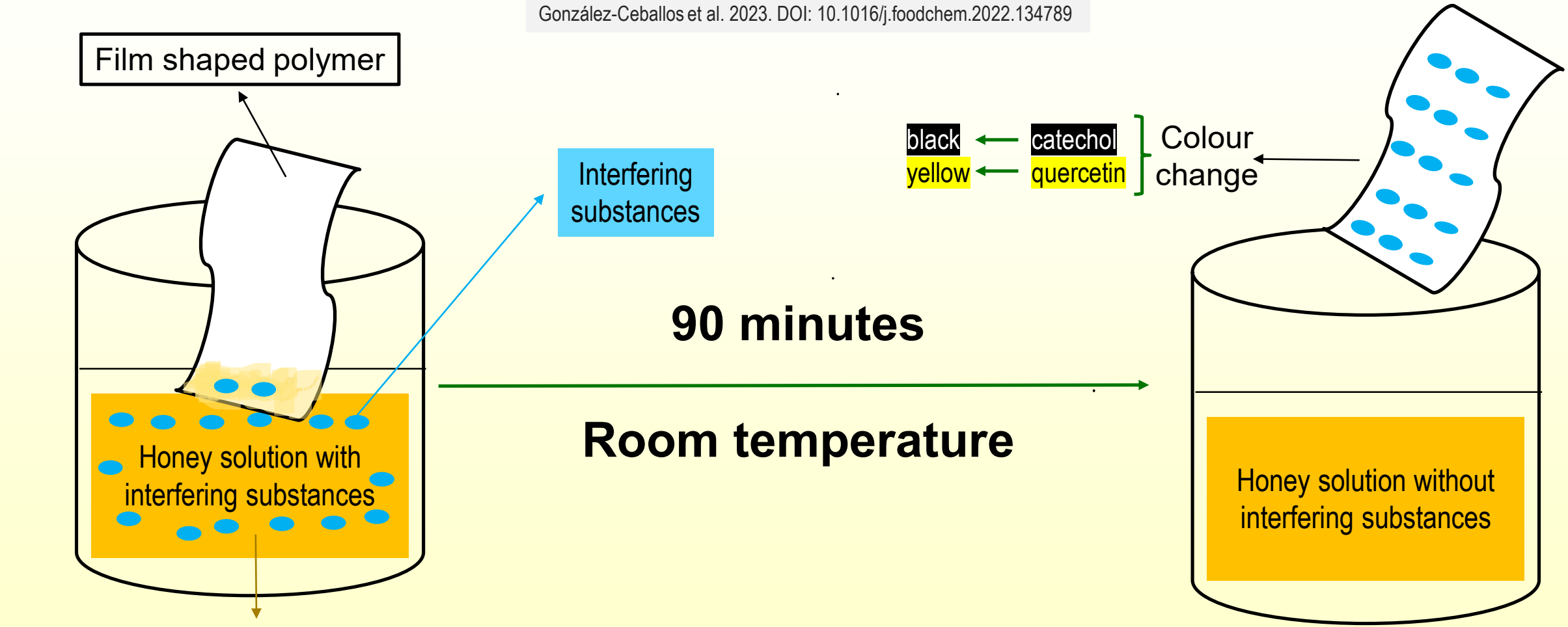
Huiodbro et al. 2005. DOI: 10.1093/jaoac/88.3.800



Honey solution: 7.5 g honey + 4 ml 0.015M phosphate buffer pH 7.0

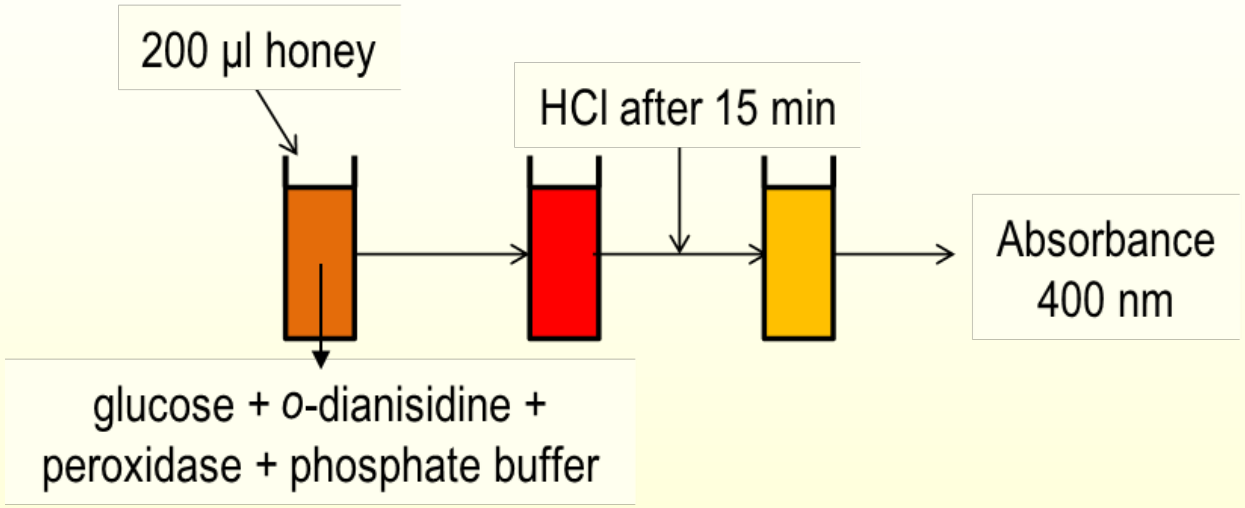
Interferences removal by USING A FILM SHAPED POLYMER

González-Ceballos et al. 2023. DOI: 10.1016/j.foodchem.2022.134789



Good value for money: less tan 1.50 €

GOx ANALYSIS

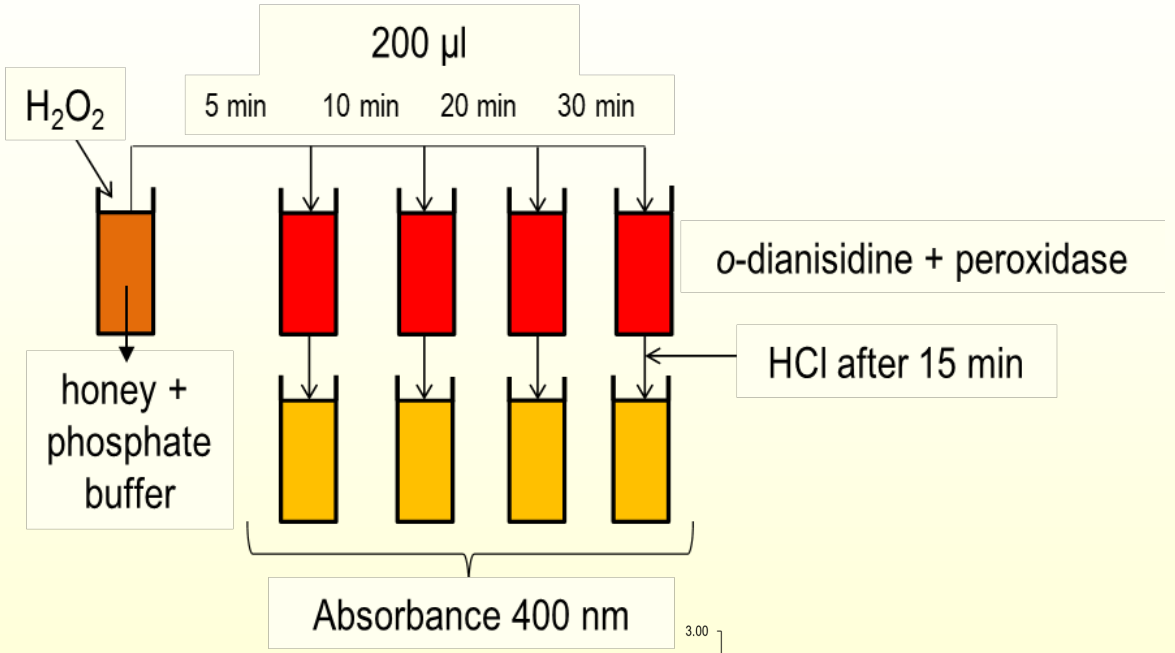


GLUCOSE OXIDASE ACTIVITY

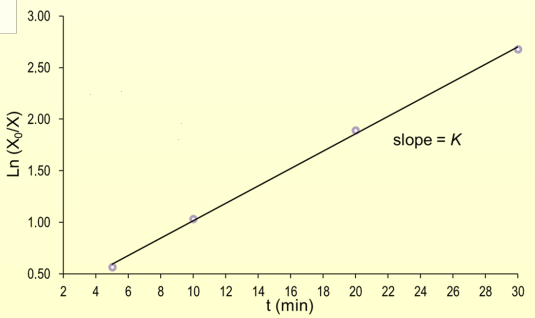
$$\frac{\mu g\ H_2O_2}{g\ honey/h}$$

Osés et al. 2024. DOI: 10.3390/foods13091344

CAT ANALYSIS



CATALASE ACTIVITY



$$K_f(min^{-1} * g^{-1}) = k * dilution\ factors$$

Huidobro et al. 2005. DOI: 10.1093/jaoac/88.3.800

RESULTS AND CONCLUSION

- Activities of GOx and CAT were **significantly higher in purified samples**

- **No significant differences** were found between the enzymatic activities in samples purified by **dialysis** and using the **film-shaped polymer**

Statistical analyses → GraphPad Prism v8

↓

Carried out with a significant number of samples of different botanical origins

↓

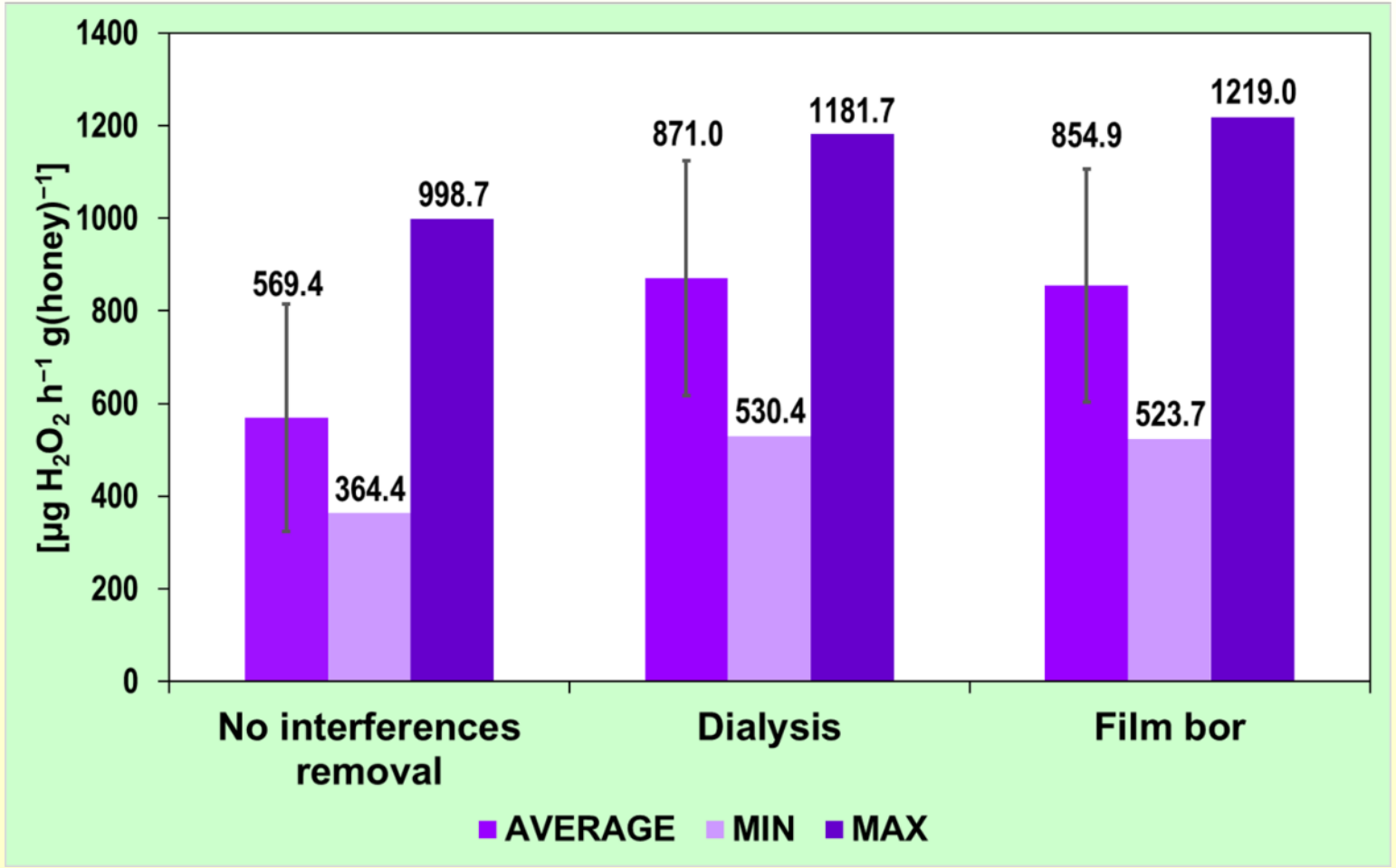
Assumptions for ANOVA were verified

↓

Two-way (ANOVA) followed by Uncorrected Fisher's least significant difference (LSD) test ($p<0.05$)

GLUCOSE OXIDASE ACTIVITY

[µg H_2O_2 h^{-1} g(honey)$^{-1}$]

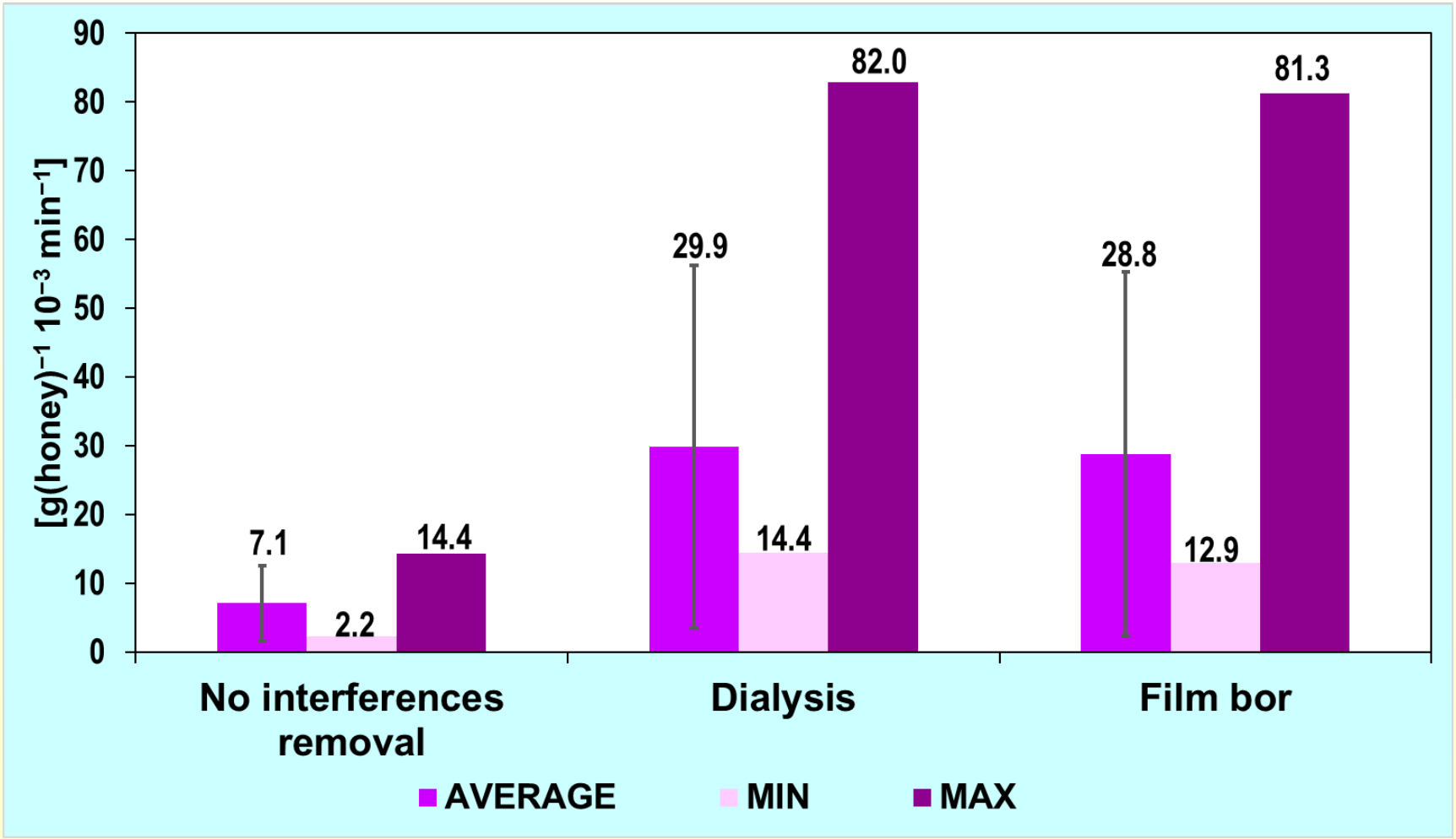


CATALASE ACTIVITY

[g(honey)$^{-1}$ 10^{-3} min^{-1}]

The **difference** between the data of non-purified and purified samples

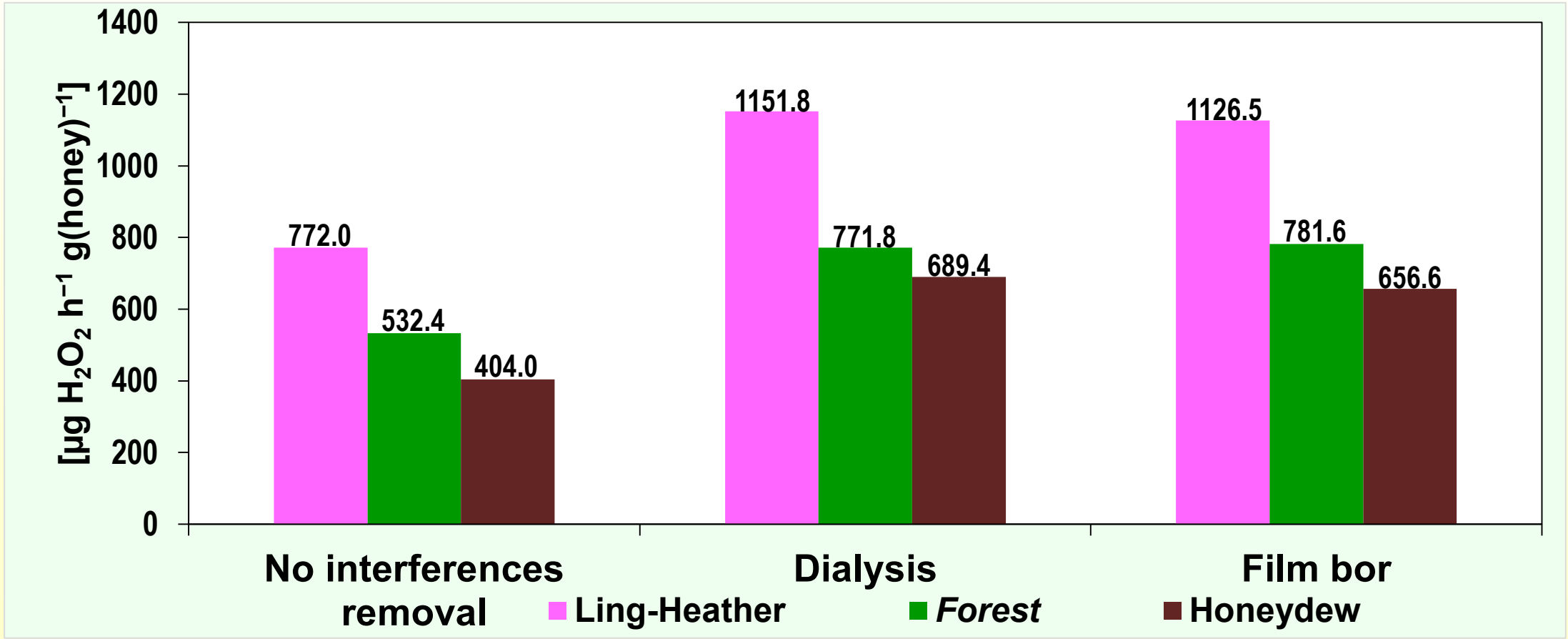
much more noticeable



Botanical origin: AVERAGES

GLUCOSE OXIDASE ACTIVITY

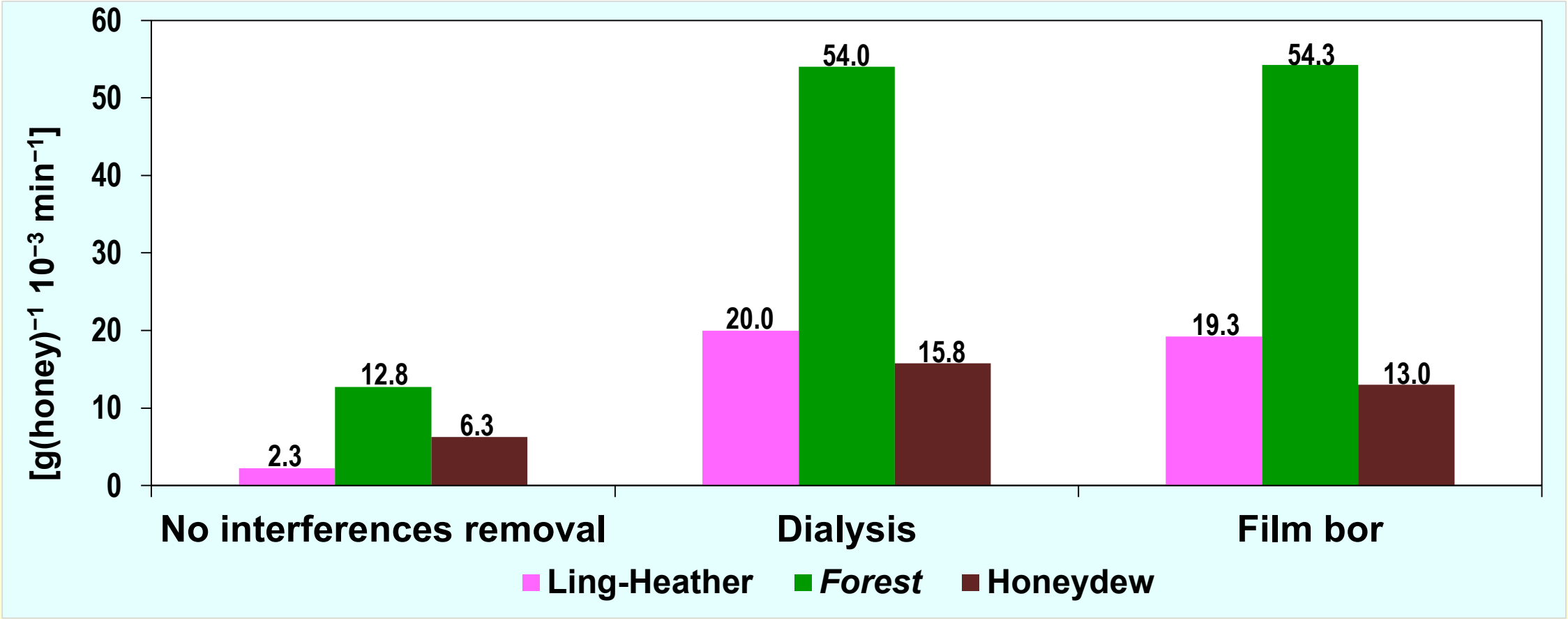
[µg H_2O_2 h^{-1} g(honey)$^{-1}$]



Botanical origin: AVERAGES

CATALASE ACTIVITY

[g(honey)$^{-1}$ 10^{-3} min^{-1}]



In conclusion:

1. Glucose oxidase and, particularly, catalase activities of honeys are significantly lower when the analyses are carried out in non-purified samples.

For the proper analysis of honey glucose oxidase and catalase a purification step is absolutely compulsory

2. For the purification step, a film-shaped polymer that selectively extracts catechol derivatives that are the main interferents for the determination of honeys' glucose oxidase and catalase, is proposed as a reliable, rapid, and low-cost alternative to the traditional dialysis procedure.

PRACTICAL IMPLICATIONS FOR BEEKEEPERS

Undoubtedly, the knowledge of the real values of glucose oxidase and catalase activities will help improve honey commercialization, because this enzymes are related to potentially functional activities that have a positive impact on the consumers' purchasing decision.

MANY THANKS FOR YOUR ATTENTION

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